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The Second National Festival & International Congress on Stem Cell & Regenerative Medicine

13-15 July, 2017

I.R Iran International Conference Centre (Saran Convention Hall), Tehran, Iran

Organizers

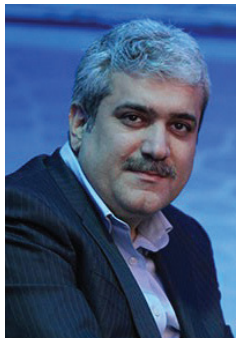
Vice - Presidency for Science and Technology

Council for Development of Stem Cell Sciences and Technologies

Council for Development of the Culture of Science, Technology and Knowledge-Based Economy

Tehran University of Medical Sciences

Layout and Graphic Group
Dr. Jalil Khalil azar
Narges Choopani dolatabad
Neda Borjinia
Directorkha@Gmail.com Mob: 0912- 704- 3072



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General Secretary of Festival:

Dr. Amir Ali Hamidieh

Executive Secretary of Festival:

Dr. Alireza Daliri

Scientific Secretary of the Congress:

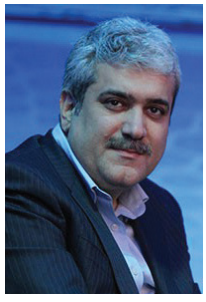
Dr. Nasser Aghdami

Executive Secretary of the Congress:

Dr. Mohammad Amir Amirkhani

Chairman Welcome Message

In the name of God
Mastering the advanced sciences and technologies and moving toward the frontiers of knowledge have always been one the most recommended policies by the Supreme Leader of Iran, Ayatollah Seyed Ali Khamenei, which will guarantee the country's development and prosperity. With a focus on strategic science and technologies, the Iranian Vice-Presidency for Science and Technology has provided a suitable platform for establishing the knowledge-based economy to meet the country's needs and having an effective presence in the global market.
For years, the oil-based economy of our country had shaped the attitude of the decision makers of this country. Hence, the absence of the main country's wealth, namely young and educated human resources, who are master in advanced and efficient sciences and technologies, was less sensed. Nonetheless, in the recent years, in order to change this attitude and employ the maximum scientific capacity of the country, based on the macro strategies of the country's comprehensive scientific plan, the Vice-Presidency for Science and Technology has taken the country's top talents, researchers and educated university graduates into account, treating them as the main capital of this country.
Researchers and graduates have always been one of the valuable assets of each country and of particular interest to policymakers and planners. Much of the development and achievement in science, technology and economy is carried out by this sector of the society. Over the past few years in our beloved homeland Iran, because of the inappropriate approach to national revenue in general and oil revenue in particular, the capabilities of this sector have been neglected.
Meanwhile, the field of stem cell sciences and technologies is one of the strategic areas, which has very widespread capacities in different aspects, and now it is directed by a good planning, with the efforts of my colleagues in the Council for Development of Stem Cell Sciences and Technologies. Becoming proficient in the knowledge of stem cells and regenerative medicine, not only attains honor for the country at the international level, but also offers ample opportunities for occupation and establishment of the new knowledge-based companies, and provides appropriate answers to treat refractory diseases. Therefore, in addition to the development of various technologies, this field of science would achieve the goals of the modern knowledge-based economy and would have a respectable position in this regard.
Cultural practices for promoting the knowledge-based economy, commercialization of the scientific ideas, supporting innovation and technology among students, researchers, professors, and business owners in the form of festivals, scientific congresses and Olympiads will facilitate the process of "translating ideas into product" as well as the identification of superior talents, and finally achieving effective outputs.
Furthermore, providing forum for technology development and commercialization in the area of stem cell sciences, in the form of organizing "the second National Festival and the International Congress of stem cell sciences and regenerative medicine" and awarding the national prize, can be a great basis for attaining the objectives mentioned above.
And here I would like to thank the organizers of the festival and I hope with the productive participation of all practitioners in this field, maintaining and improving the scientific status of the Islamic Republic of Iran would become possible.



Dr. Sorena Sattari

Chairman of the Festival

General Secretary Welcome Message

In the name of God

I and my colleagues in the Council for Development of Stem Cell Sciences and Technologies are proud to announce that stem cells sciences and regenerative medicine (SCRM), as a productive field of knowledge, is developing at a very rapid pace in our country, with the dedicated efforts of our prominent professors, young and passionate researchers, emerging knowledge-based companies and futuristic decision makers. In view of our achievements in this area of science, we are going to host the second scientific gathering "The 2nd National Festival and International Congress on Stem Cells and Regenerative Medicine" in Tehran in July 2017 with the participation of all students, scholars, researchers and other stakeholders in the field.

Stem cell sciences and technologies have growingly attracted the attention of university students, scientists and researchers. The advancements of SCRM as well as investments recently made by the major companies in this field, have paved way for hopeful future of lasting treatment for refractory and terminal diseases. These developments show that SCRM can play a significant role in future of the heath market. Hence, mastering this strategic knowledge can further boost the scientific status of our country at international levels.

Relying on the capabilities of its graduated scientists, Islamic Republic of Iran has launched extensive efforts to localize this knowledge during the past decade. These efforts have fortunately borne fruit and boosted the position of our country at a par with other advanced countries. The 2nd National Festival and International Congress on Stem Cells and Regenerative Medicine provides an opportunity for the general public to become familiar with the concepts of this strategic knowledge and for our scientists in the field to present and promote their ideas in a way which would be commercially viable and increase the competitiveness in the market.

This event also gives industries and investors to pick the most productive ideas in the field and help further developing and making them accessible to the public. It also opens window of hope for patients with refractory diseases. In addition, young students and entrepreneurs will grasp benefit from the business environment provided in this event to join this fast growing technology.

I would like to use this opportunity to invite all decision and policy makers in the health sector, professors, researchers and students in the medical, basic, technical and engineering sciences, and also, owners of industries and investors in the field of pharmaceuticals and medical equipment as well as all other relevant stakeholders to attend this scientific meeting. Additionally, to our international audience, I would like to invite them to visit the warm, welcoming, vibrant city of Tehran and to enjoy the scientific program of our festival.

Dr. Amir Ali Hamidieh

General Secretary of Festival

Scientific Secretary Welcome Message

In the name of God

Dear friends, colleagues, professors, and researchers,

Nowadays, topics related to stem cells and regenerative medicine are interesting, challenging and clinically promising; and due to the interdisciplinary nature of these fields of science, a good basis for cooperation of different scientists from medicine, biology, engineering, and even social sciences has been created. This, therefore, has led to promotion of this science and provision of enhanced treatments for refractory diseases. For this reason, scientific gatherings can help us update our information and exchange the latest developments of this field between various academic groups.

I am honored to invite all the scientific parties to attend the Second National Festival and International Congress on Stem Cell Sciences and Technologies which is going to be held on July 13-15, 2017.

The axes of this year's congress include: cell therapy, tissue engineering, gene therapy, immune cell therapy, cell banking, ethics and regulations, biomaterials and devices and other related sciences, and we tried to invite outstanding speakers in each field. We also tried to plan the congress program to be focused on clinical aspect of stem cell research.

With trust in the Almighty God, and with hope of a better future for the world and our dear country, we eagerly look forward to your participation in this valuable scientific event.

Dr. Nasser Aghdami

Scientific Secretary of the Congress

Executive Secretary Welcome Message

In the Name of God
The growth and development of stem cell sciences and technologies in our beloved Iran has advanced in parallel with the other developed countries and has reached a satisfactory pace at international level. The admirable efforts of senior Iranian scientists, researchers, business owners, manufacturers, university professors and students have led to significant production of knowledge, technology, cell-based products and clinical services in this field. As a result, the ranking of the Islamic Republic of Iran has increased significantly at international levels, during the recent years.
Over the past two years, the Council for Development of Stem Cell Sciences and Technologies, which is incorporated into the Iranian Vice Presidency for Science and Technology, has set up over 30 specialized expert committees and utilized the collective wisdom of more than 1200 faculty members, researchers, technicians, manufacturers, owners of knowledge-based companies and specialists in the health and technical and engineering section of the country. The Council, as the representative of all the stem cell activists in the country, has been working to develop the 10-year plan of development and a roadmap for this strategic knowledge, which has led to the organization of all the relevant resources at the national level.
The annual Festival, which is held this year under the title of "The Second National Festival and the International Congress on Stem Cell and Regenerative Medicine", is considered as the largest stem cell convention in the country, with the participation of all senior Iranian science and technology directors, professors, researchers, business owners and manufacturers, university and high school students. This festival is a great opportunity to review recent international advancements in this field, and to learn from the novel ideas of both national and international scientists to facilitate setting goals and making scientist progress in the country. The Festival is an important event for flourishing this knowledge at the national level, which by the grace of God, has been welcomed by many scientists and scholars of the field, as there would be over 2500 participants and over 600 paper presentations.
The most important components of the Festival include: "international Congress" which would be unique among other similar events with lectures by more than 60 international scientists, stem cell technology exhibition with the participation of over 50 Iranian Knowledge-based companies active in stem cell sciences and regenerative medicine, the startup competition with the participation of over 100 technicians in 10 teams, appreciation of the winners of the National Award and top students of the Olympiad and setting up 21 workshops for 300 researchers focused on sophisticated cutting edge technologies.
All in all, I would like to take this opportunity to thank all our colleagues in the Strategic Committee, the Executive Committee and the Scientific Committee of the Festival for cooperating to organize this valuable scientific event in the country.
I would like to invite all interested scientists to participate in this scientific adventure, which hopefully contributes to the flourishing of this strategic knowledge in our beloved country, Iran.

Dr. Mohammad Amir Amirkhani

Executive Secretary of the Congress

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Invited Section

Is-001: Principles of Cell Therapy Products Manufacturing

Hamid Reza Aghayan

Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
Email: hr.aghayan@gmail.com

Cell based therapies provide new treatment modalities for chronic and incurable diseases. In most cases, large number of pure cell population are required, therefore the isolated cells need to be expanded and purified in vitro before transplantation. In vitro manipulation of cell products requires complex laboratory procedures that increase the risk of possibly adverse events for the recipient. To minimize the associate risks of cell transplantation, adhering to current standards for clinical preparation of cell based products is critical. According to the current international regulations and regulations of Iran Food and Drug Organization, cell therapy products should be manufactured under principles of Good Manufacturing Practice (GMP). The main focus of this presentation will be on principals of GMP compliant cell processing which defines optimal quality and safety for cell therapy products. Among different elements of GMP cell manufacturing, appropriate ancillary materials, and environmental condition of cell processing facility are the most challenging aspects. Therefore, I discuss about how to select appropriate ancillary materials for clinical grade cell manufacturing. However, different aspects of clean room facility with paying particular attention to facility design, classification, qualification and maintenance will be discussed.
Keywords: Cleanroom, Clinical Grade, Cell Therapy, GMP, Stem Cell

Is-002: Elastic Polymeric Scaffolds for Minimally Invasive Implantation of Engineered Tissues

Samad Ahadian

Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada
Email: samad_ahadian@yahoo.com

Background and Aim: Although tissue engineering is a well-established research field, the field is dealed with a critical limitation that prevents full adoption of engineered tissue constructs: engineering tissues in vitro need to be implanted in the body through complicated surgical operations followed by long postsurgical care and treatments. Simple and direct injection of cells alone or combined with hydrogels into damaged area in a minimally invasive manner does not give provide tissue-level connections and high organization that are required for immediate functionality of tissues. The retention of the cells at the delivery site may also be compromised. Therefore, there is still a major challenge to restore or repair tissues in vivo at a high successful rate and in a minimally invasive approach.
Methods: We have designed elastic polymeric scaffolds to develop injectable, yet fully functional engineered tissues. The elastic scaffold is poly (octamethylene maleate (anhydride) citrate) (POMAC), which can be synthesized via a polycondensation reaction between three different monomers (i.e., 1,8-octanediol, maleic anhydride, and citric acid). The Young's modulus of POMAC scaffold matches the stiffness of native tissues. Diamond-like structure of POMAC scaffold was able to restore its original shape upon injection.
Results: The injectability of cell-laden POMAC scaffold was also achieved without significant damage in the cellular viability and function. As a model system, we fabricated cardiac tissues using POMAC scaffold and successfully implanted them onto the heart of pig models. Flexibility of scaffold will allow the tissue collapse during injection, and subsequently regaining its original shape at the desired location while maintaining cell viability and tissue function. Moreover, different functional groups in the POMAC structure can be conjugated with angiogenesis-induced soluble factors to induce vascularization in vivo. Vascularization of engineered tissues is required for survival and proper function of cells within thick tissues.
Conclusion: The designed elastomeric scaffold is a great asset in translational studies of engineered tissues as to implant the tissues in the body without expensive, risky, and complicated surgical operations.
Keywords: Tissue Engineering, Scaffolds Shape Memory, Implantation In Vivo

Is-003: Regenerative Medicine Needs a Hand from Industry for Commercialisation of the Products

Mohammad Alavijeh[1], Alexander Seifalian[2]

1. Pharmidex, London, UK
2. NanoRegMed, London, UK
Email: Mo.Alavijeh@Pharmidex.com

Regenerative medicine has huge potential to provide a widespread improvement in healthcare and patient wellbeing via the delivery of therapies that can restore, regenerate or repair damaged tissue. However its progress has been slow, this is due to academic do not have the expertise in manufacturing and scaling up the products to the industrial standard as well as funding require to manufactured under GMP/GLP standard.
For industry, it could worth a multibillion dollar, which it is significant, contribute to economic growth if products are successfully commercialised. The pharma industry also can help with regulatory hurdles and commercialisation of the products. This talk will highlight the experience of an academic with translation from laboratory to patients of regenerative medicine products and solution by a successful industry been helping academic and pharmaceutical industry for their testing and commercialisation.

Is-004: Endogenous vs Exogenous Repair of Intervertebral Disc

Mauro Alini

AO Research Institute, Davos, Switzerland
Email: mauro.alini@aofoundation.org

Using an organ culture model of induced IVD degeneration, we have shown that human MSCs could migrate through the endplate of a bovine disc. Furthermore, the expression of certain chemokine receptors was significantly up-regulated in MSCs cultured with degenerative disc conditioned medium, confirming the responsiveness of the cells to the degenerative environment. The feasibility of MSC recruitment in the disc in vivo was also evaluated on a mouse tail model of induced disc degeneration, though only a limited number of bone-marrow cells were recruited in the disc.
The finding that MSCs could migrate through the endplate was further explored by injecting the chemokine SDF1 into the cavity of a partially nucleotomised disc in organ culture. SDF1 was delivered using a hyaluronan-based thermoreversible injectable hydrogel, which had previously been shown to support disc cell growth, matrix production and MSC differentiation towards the disc-like phenotype. SDF1-releasing hydrogel significantly increased MSC recruitment to the disc, demonstrating the potential of a chemokine delivery system to accelerate cell homing.
To identify disc-derived chemotactic factors, the proteomic profile of the conditioned medium of an IVD maintained under induced degenerative settings was analysed. Proteomic analysis revealed CCL5/RANTES and CXCL6 as two main chemotactic factors secreted by the degenerative disc. The presence of CCL5/RANTES and its receptors was also confirmed in histological sections of bovine and human degenerative discs, suggesting that this chemokine plays a role in cell recruitment.
In conclusion, there is clear evidence that homing of endogenous regenerative progenitor cells can occur in the disc and can be amplified by chemoattractant delivery system. In addition, recent studies have described the presence of progenitor cells at different locations in healthy and degenerative IVDs. Mobilisation, augmentation and activation of these endogenous progenitor cell populations represent attractive targets for future regenerative strategies.
Keywords: Stem Cell, Chemoattractants, Bioreactor, Hydrogels

Is-005: MSC and Complex Loading Pattern for Cartilage Repair

Mauro Alini

AO Research Institute, Davos, Switzerland
Email: mauro.alini@aofoundation.org

Research in the use of mesenchymal stem cells (MSCs) to enhance orthopaedic repair has dramatically increased over the last 20 years. The unique properties of MSCs and their natural presence within the bone mar-

row make them an attractive source of cells for novel therapeutics. When considering the natural repair environment, it is clear that the microenvironment the cells experience plays a major role in the repair response. Within the musculoskeletal system, one of the major drivers of repair is the mechanical load applied to the cells within the defect.

When developing new therapies in vitro, static culture is the most commonly used method. However, it is clear that due to the critical role mechanics plays in vivo, a more physiological loading regime in vitro would be most appropriate and this can be achieved by the use of bioreactors. Using a multiaxial load bioreactor system1, we have been investigating the effect of mechanical stimulation on human stem cell differentiation. Performing studies in the absence of growth factors, specifically Transforming growth factor β (TGFβ), allows the direct effect of the mechanical strain applied to be elucidated. Our bioreactor system allows for the application of shear, compression or a combination of both stimuli to establish the phenotypic changes induced within MSCs. In particular, the effect of the various mechanical stimuli on chondrogenic differentiation will be discussed and compared to responses seen in chondrocytes.

As a model system, human bone marrow derived MSCs are embedded in a fibrin gel, which is then retained in a macroporous biodegradable polyurethane (PU) scaffold. This system provides a naturally occurring support matrix (fibrin), while allowing for cyclical load to be applied due to the resilience of the PU scaffold. Neither compression alone, nor shear alone can induce a change in MSC phenotype within this system. However, we have demonstrated that a combination of compression and shear is able to induce chondrogenic differentiation and this is due to increased endogenous expression of TGFβ from the loaded cells2, 3. Finite Element modelling of the bioreactor system demonstrated that the degree of principle component strain was the main driving force in this system4.

Using this multiaxial load bioreactor system we are able to investigate novel treatments and therapies in vitro, under physiologically relevant kinematic load. In addition, potential rehabilitation protocols to be used after cell therapy in cartilage repair can also be investigated.

Keywords: Chondrogenesis, Scaffolds, Shear Load, Rehabilitation Protocols, Bioreactor

Is-006: New Technologies in Assembling Stem Cells for Skin Regeneration: 3D Skin Printer

Saeid Amini Nik[1-5]

1. Department of Surgery, University of Toronto, Canada
2. Department of Laboratory Medicine and Pathobiology (LMP), University of Toronto, Canada
3. Sunnybrook Research Institute, Toronto, Canada
4. Sunnybrook's Trauma, Emergency & Critical Care (TECC) Program, Toronto, Canada
5. Ross Tilley Burn Centre, Toronto, Canada

Email: saeid.amininik@sunnybrook.ca

With an aging population and skin loss due to trauma and burn, there is an immediate need for skin substitutes. Skin is the largest organ of the body and possesses a unique layered organization of cells and extracellular matrix components. Our team, composed of tissue engineers, clinician scientists, and skin stem cells biologists, has worked on wound healing and skin regeneration for many years and contributed to the growing data in this field. Specifically, our focus is on burn injury, which represents one of the most severe forms of trauma. Each year in the United States alone, more than two million people are burned with a high mortality in severe burns. This is more in developing countries. While prevention is the gold standard for reducing the morbidity (mostly associated with social scars) and mortality, burn still happens and there is a critical need for skin substitutes in burned patients. Stem cells and its promising potentials are changing the landscape for skin regeneration. However, skin is a 3-dimensional structure, composed of several cell types with well-defined extracellular matrices and stem cells, by itself, cannot reconstitute skin. The spatial composition of skin is in part responsible for organ function. Here, I showcase few projects in the lab which aims to use stem cells in a 3D configuration in order to provide 3D skin substitutes for regeneration. In particular, I introduce a 3D bioprinting strategies which aim at reconstituting aspects of native tissues by positioning different cell types and extracellular matrix components. The provided microenvironment and spatial organization influence cell migration, elongation, clustering, proliferation, differentiation, and function.

Is-007: Transgenic Animals, Cell Lineage Studies for Fate Mapping of Stem Cells During Healing and Regeneration

Saeid Amini Nik[1-5]

1. Department of Surgery, University of Toronto, Canada
2. Department of Laboratory Medicine and Pathobiology (LMP), University of Toronto, Canada
3. Sunnybrook Research Institute, Toronto, Canada
4. Sunnybrook's Trauma, Emergency & Critical Care (TECC) Program, Toronto, Canada
5. Ross Tilley Burn Centre, Toronto, Canada
Email: saeid.amininik@sunnybrook.ca

When the protective barrier of the skin is damaged, an intricate process of tissue repair is set in motion that involves multiple cell types and signaling pathways. Three percent of the population suffers from disordered wound repair. Insufficient or excessive healing responses result in either a nonhealing wound or formation of a hypertrophic scar, respectively. Both conditions have major deleterious effects, resulting in morbidity from loss of function, negative psychosocial effects from disfigurement, or even mortality from the loss of the skin's barrier function. Understanding the mechanism of tissue healing is essential for mapping a paradigm for skin regeneration. Skin is a large reservoir of stem cells. Once activated, skin stem cells produce shorter-lived progenitors that divide rapidly several times, but then progress to terminally differentiated cells in order to reconstitute the skin. Understanding stem cell plasticity will enlighten light on the new approaches to direct their differentiation in favor of regeneration. To understand how a stem cell chooses its path, we have taken several approaches, in particular, cell lineage study by using different fluorescent proteins under the control of various skin promoters (reporter animals). We can activate them at different stages of skin healing and observe their fate. Here, I showcase projects that aimed to find new sources of cells in the skin by developing transgenic animals. I will discuss their origin, their fate during skin healing and their contribution into the reconstruction of skin.

Is-008: Probing the Involvement of Immune Responses in the Efficacy of Cord Blood Cell Therapy with Biomarkers for Cerebral Palsy

Seong Soo An[1], MinYoung Kim[2], Mino Kang[1]

1. Gachon University, Seongnam, South Korea
2. CHA Bundang Medical Center, CHA University, Seongnam, South Korea
Email: seongaan@gachon.ac.kr

Background and Aim: This study evaluated the efficacy of umbilical cord blood (UCB) cell for patients with cerebral palsy (CP) in a randomized, placebo-controlled, double-blind trial and also assessed factors and mechanisms related to the efficacy.
Methods: Thirty-six children (ages 6 months to 20 years old) with CP were enrolled and treated with UCB or a placebo. Muscle strength and gross motor function were evaluated at baseline and 1, 3, and 6 months after treatment. Along with function measurements, each subject underwent (18)F-fluorodeoxyglucose positron emission tomography at baseline and 2 weeks after treatment. Cytokine and receptor levels were quantitated in serial blood samples.
Results: The UCB group showed greater improvements in muscle strength than the controls at 1 (0.94 vs. -0.35, respectively) and 3 months (2.71 vs. 0.65) after treatment (Ps<0.05). The UCB group also showed greater improvements in gross motor performance than the control group at 6 months (8.54 vs. 2.60) after treatment (P<0.01). Additionally, positron emission tomography scans revealed decreased periventricular inflammation in patients administered UCB, compared with those treated with a placebo. Correlating with enhanced gross motor function, elevations in plasma pentraxin 3 and interleukin-8 levels were observed for up to 12 days after treatment in the UCB group. Meanwhile, increases in blood cells expressing Toll-like receptor 4 were noted at 1 day after treatment in the UCB group, and they were correlated with increased muscle strength at 3 months post-treatment.
Conclusion: In this trial, treatment with UCB alone improved motor outcomes and induced systemic immune reactions and anti-inflammatory changes in the brain. Generally, motor outcomes were positively correlated with the number of UCB cells administered: a higher

number of cells resulted in better outcomes. Nevertheless, future trials are needed to confirm the long-term efficacy of UCB therapy, as the follow-up duration of the present trial was short.
Keywords: Cerebral Palsy, Biomarkers, Cord Blood, Cell Therapy

Is-009: Improved Fibronectin-immobilized Fibrinogen Microthreads for the Attachment and Proliferation of Fibroblasts.

Seong Soo An

Gachon University, Seongnam, South Korea
Email: seongaan@gachon.ac.kr

Background and Aim: The aim of this study was to fabricate fibrinogen (Fbg) microfibers with different structural characteristics for the development of 3-D tissue-engineering scaffolds. Fabricated Fbg microfibers were investigated for their biomolecule encapsulation, cell adhesion, and proliferations.
Methods: Microfibers with three different concentrations of Fbg (5, 10, and 15 wt%) were prepared by a gel solvent-extraction method using a silicone rubber tube. Fbg microfibers were covalently modified with fibronectin (FN) by using water-soluble 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as the cross-linking agent. Fbg microfibers were characterized by their FN cross-linking properties, structural morphology, and in vitro degradation. Furthermore, FN/Fbg microfibers were evaluated for cell attachment and proliferation. The bio-compatibility and cell proliferation of the microfibers were assessed by measuring adenosine triphosphate activity in C2C12 fibroblast cells. Cell attachment and proliferation on microfibers were further examined using fluorescence and scanning electron microscopic images.
Results: FN loading on the microfibers was confirmed by fluorescence and infrared spectroscopy. Surface morphology was characterized by scanning electron microscopy, and showed highly aligned nanostructures for fibers made with 15 wt% Fbg, a more porous structure for fibers made with 10 wt% Fbg, and a less porous structure for those made with 5 wt% Fbg. Controlled biodegradation of the fiber was observed for 8 weeks by using an in vitro proteolytic degradation assay. Fbg microfibers with highly aligned nanostructures (15 wt%) showed enhanced biomolecule encapsulation, as well as higher cell adhesion and proliferation than another two types of FN/Fbg fibers (5 and 10 wt%) and unmodified Fbg fibers.
Conclusion: The promising results obtained from the present study reveal that optimal structure of Fbg microfibers could be used as a potential substratum for growth factors or drug release, especially in wound healing and vascular tissue engineering, in which fibers could be applied to promote and orient cell adhesion and proliferation.
Keywords: Fibronectin, Fibrinogen Microthreads, Cell Proliferation

Is-010: In vitro Production of Human T-cell Progenitors for the Treatment of Immune Deficiency after Hla-partially Incompatible Hematopoietic Stem Cell Transplantation

Isabelle André-Schmutz

French Institute of Health and Medical Research, Paris, France
Email: andre.schmutz@gmail.com

Exploiting the Notch signaling pathway for the in vitro generation of T cell precursors provides a promising approach to fasten T cell reconstitution after hematopoietic stem cell transplantation (HSCT). Here, we tested the T lineage differentiation capacity of adult hematopoietic stem and progenitor cells (HSPCs) – the most common source of stem cells for HSCT- in a feeder cell-free culture based on the use of a modified Delta-like-4 (DL-4) Notch ligand and T cell cytokines. Within 7 days, adult HSPCs were able to produce $CD7^+$ T cell precursors expressing T lineage master genes and with high in vitro T cell differentiation potential. Compared to cord blood (CB), adult HSPC DL-4 cultures were associated with lower rate of proliferation, increased apoptosis and lower expression of NOTCH1. Most importantly, DL-4 T cell precursors derived from adult HSPCs expressed chemokine receptors implicated in thymus homing and efficiently produced polyclonal T cells upon transplantation in NOD/SCID/$\gamma c^{-/-}$ (NSG) mice. These results provide insights into pathways of Notch-based T cell differentiation and demonstrate that

adult HSPCs provide an effective and available source of in vitro cultured T cell precursors in the context of future clinical applications directed to shorten T cell recovery after HSCT.

Is-011: Gene Therapy of Hematopoietic Disorders: Where Do We Stand?

Isabelle André-Schmutz

French Institute of Health and Medical Research, Paris, France
Email: andre.schmutz@gmail.com

More than 20 years ago, the seminal X-linked severe combined immunodeficiency (SCID-X1) clinical studies, based on first-generation gammaretroviral vectors, demonstrated good long-term immune reconstitution in most treated patients despite the occurrence of vector-related leukemia in a few of them. This gene therapy has successfully enabled correction of the T cell defect. Natural killer and B cell defects were only partially restored, most likely due to the absence of a conditioning regimen. The success of these pioneering trials paved the way for the extension of gene-based treatment to many other diseases of the hematopoietic system, but the unfortunate serious adverse events led to extensive investigations to define the retrovirus integration profiles and to the development and implementation of new generations of safer vectors such as self-inactivating gammaretroviral or lentiviral vectors. This approach has been tested in several inherited disorders of the hematopoietic system since then.
When considering inherited diseases that can be treated by gene transfer into hematopoietic stem cells (HSCs), there are only two in which the HSC and progenitor cell distribution inside the bone marrow and its microenvironment are exactly the same as in a healthy subject: metachromatic leukodystrophy (MLD) and adrenoleukodystrophy (ALD). In all other settings [X-linked severe combined immunodeficiency (X-SCID), adenosine deaminase deficiency, Wiskott-Aldrich syndrome, and β-hemoglobinopathies], the bone marrow content of the different stem and precursor cells and the cells' relationship with the stroma have very specific characteristics. These peculiarities can influence the cells' harvesting and behavior in culture, and the postgraft uptake and further behavior of the gene-modified hematopoietic/precursor cells. In the present mini-review, we shall briefly summarize these characteristics and outline the possible consequences and challenges.

Is-012: Reproducible and Stable Therapeutic Gene Expression with UCOE®-Based Lentiviral Vectors

Michael Antoniou

Gene Expression and Therapy Group, Department of Medical and Molecular Genetics Faculty of Life Sciences and Medicine, King's College London, UK
Email: michael.antoniou@kcl.ac.uk

Reproducible and stable expression following delivery of transgenes via lentiviral or gammaretroviral vectors is essential when targeting stem cell populations in order to achieve long-term therapeutic benefit. A system that shows a great of deal of promise in meeting this crucial objective in an effective and safe manner exploits then use of a ubiquitous chromatin opening element (UCOE®). UCOE® elements are derived from housekeeping gene loci and consist of a methylation-free CpG island spanning one or more transcriptional start sites. Transcription either directly off one of the innate UCOE® element promoters or linking a UCOE® element upstream of a heterologous ubiquitous or tissue specific promoter confers unprecedented stable expression in somatic/embryonic/induced pluripotent stem cells and all lineages of their differentiated progeny. Thus UCOE® element regulated gene expression vectors have the potential to make major contributions in gene-stem cell based therapies.

Is-013: Pre-Natal Gene Therapy for Inherited Diseases using Lentiviral Vectors

Michael Antoniou

Gene Expression and Therapy Group, Department of Medical and Molecular Genetics Faculty of Life Sciences and Medicine, King's College London, UK
Email: michael.antoniou@kcl.ac.uk

Delivery of gene therapy for inherited conditions at a pre-natal stage of development offers distinct advantages to standard post-natal treatments. This includes preventing early onset disease, targeting a rapidly expanding stem cell population, use of much smaller amounts of vector, negate immune reactions against therapeutic protein due to immune system naivety, and avoids dilemmas associated with termination of an affected pregnancy. As part of a long-term programme to develop pre-natal gene therapy we have shown that human foetal liver-derived haematopoietic stem cells (hflHSCs) can be readily transduced with lentiviral vectors in vitro. More significantly, hflHSCs transduced with a vector based on a ubiquitous chromatin opening element provide reproducible, long-term stable gene expression once cells are transplanted into mice. We have also shown that direct delivery of UCOE® element-based lentiviral vectors to the foetal liver in mice efficiently transduces both HSCs and especially hepatocytes leading to long-term post-natal gene expression. Our studies have also shown that delivery of a UCOE®-FIX lentiviral vector to the murine foetal liver results in long-term post-natal serum levels of FIX that would be curative of haemophilia B if reproduced in human subjects. We have also delivered a lentiviral vector harbouring a human β-globin gene transcription unit to the foetal liver in a humanised murine model of β-thalassaemia. Our initial results show that this gives rise to the post-natal cure of the thalassaemia intermedia phenotype of heterozygous animals. Studies are ongoing to improve this approach to rescue the thalassaemia major condition of homozygous animals following pre-natal treatment.

Is-014: Generation and Tissue Engineering of Human Stem Cell-Derived Cardiomyocytes

Hossein Baharvand[1,2]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
Email: baharvand@royaninstitute.org

Efficient and reproducible generation of human stem cell-derived cardiomyocytes (CMs) is crucial for biomedical applications. Recently, we reported a scalable, robust, and integrated differentiation protocol for large-scale production of virtually pure (~90%) functional contracting CMs in a stirred suspension bioreactor in the presence of an optimized cocktail of small molecules under defined conditions within 10 days. Here, I describe the development of different applications that require large numbers of cells such as high-throughput screening and drug discovery and in vitro organ development. In addition, this will also be beneficial for cardiac tissue engineering as we used for recellularization of growth factor tethered rat hearts resulted in improved migration of cells and the differentiation.

Is-015: Stem Cell Transplantation in Difficult Ocular Surface Disorders

Alireza Baradaran-Rafii

Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: alirbr@gmail.com

Patients with limbal stem cell deficiency (LSCD) are poor candidates for conventional corneal transplantation. The only definitive treatment for LSCD is limbal stem cell transplantation (LSCT). The surgery type depends on: the extent of involvement (partial or total), laterality (uni or bilateral), severity of ocular surface inflammation, presence of symblepharon, tear status, ocular surface keratinization, and systemic factors such as age and general health of the patient. Strategies in partial stem cell deficiency include conservative therapy, sequential sector conjunctival epitheliectomy (SSCE), and amniotic membrane transplantation (AMT). Surgical strategies in total unilateral cases include conjunctival-limbal autograft (CLAU), cultivated limbal epithelial transplantation (CLET), simple limbal epithelial transplantation (SLET), and in vivo cultivation of limbal stem cells. In bilateral total LSCD surgical strategies include keratolimbal allograft (KLAL), living-related conjunctival-limbal allograft (lr- CLAL), CLET (If the LSCD is bilateral, but there are some remaining unaffected regions), and cultivated oral mucosal epithelial transplantation (COMET). AMT is complementary

and can be used in conjunction with or preceding to any type of limbal transplantation. For final visual rehabilitation subsequent corneal transplantation may be necessary.

Is-016: Functional Dissection of the Enhancer Repertoire in Human Embryonic Stem Cells by Chip-Starr-Seq

Stefan Barakat[1,2], Florian Halbritter[3], Ian Chambers[1]

1. MRC Centre of Regenerative Medicine, University of Edinburgh, Edinburgh, UK
2. Department of Clinical Genetics, Erasmus MC, University Medical Centre, Rotterdam, the Netherlands
3. CeMM, Vienna, Austria
Email: t.barakat@erasmusmc.nl

Enhancers are genetic elements that regulate spatio-temporal gene expression. Enhancer function requires transcription factor binding, with active enhancers also marked by H3K27ac and H3K4me1. However, the extent to which histone modifications and transcription factor binding identify active enhancers' remains unclear. Here we combine chromatin-immunoprecipitation with a massively parallel reporter assay to identify functional enhancers genome-wide quantitatively in human embryonic stem cells. Active enhancers associate with specific transcription factors and enhancer activities change upon human embryonic stem cell conversion to naive pluripotency. However, only a small minority of regions marked by NANOG, OCT4, H3K27ac and H3K4me1 function as strong enhancers. Large tracts within super-enhancers lack activity with small sub-domains harboring enhancer function. Similarly, only some putative enhancers associated with transposable elements have activity. Our analysis also reveals a novel enhancer set associated with housekeeping genes. This catalogue of validated enhancers in different states of human pluripotency represents a valuable resource for further functional dissection of the non-coding genome.
Keywords: Massively Parallel Reporter Assay, Naive Pluripotency, Genome-wide Functional Enhancer Map, Housekeeping Enhancers, Super-enhancers, Transposable Elements, NANOG, OCT4, H3K27ac, H3K4me1

Is-017: Mechanisms of X Chromosome Inactivation and Reactivation in Embryonic Stem Cells

Stefan Barakat[1], Joost Gribnau[2]

1. Department of Clinical Genetics, Erasmus MC, University Medical Centre, Rotterdam, The Netherlands
2. Department of Developmental Biology, Erasmus MC, University Medical Centre, Rotterdam, The Netherlands
Email: t.barakat@erasmusmc.nl

In placental mammals, evolution has resulted in females with two X chromosomes and males with a single X chromosome, and a tiny Y chromosome. To achieve an equal dosage of X-linked genes in both sexes, one of the two X chromosomes in female cells becomes transcriptionally silenced during early development by epigenetic means in a process called X chromosome inactivation (XCI). The faithful execution of this process is of crucial relevance for females, as abnormalities of this process can result in the manifestation of X-linked disorders, and even failure of female embryonic development. A valuable model system to study XCI are pluripotent stem cells. Female mouse embryonic stem cells undergo XCI upon differentiation, whereas the silent X chromosome in female somatic cells becomes reactivated upon iPS cell reprogramming. The XCI status of human embryonic stem cells is still a matter of debate, with some human embryonic stem cells showing two active X chromosomes, whereas other cell lines represent a post-XCI state with a silenced X chromosome. Here we will first present a short story of the trans-and cis-acting factors involved in the regulation of mouse XCI, including the crucial role of the X-linked RNF12 protein as a dose-dependent, *trans*-acting regulator of XCI as determined in heterokaryon experiments. This will be followed by a second story aiming to clarify the XCI status in human iPS cells upon reprogramming, by using reprogramming of female fibroblasts heterozygous for large X-chromosomal deletions. These fibroblasts show completely skewed XCI of the mutated X chromosome, enabling precise monitoring of X reactivation and XCI using allele-specific single cell expression analysis. The role of various culture conditions, including the recently discovered naïve pluripotent stem cell state will be discussed.

Keywords: X Chromosome Inactivation, X Chromo-Some Reactivation, Human iPS Reprogramming, Human Model Systems for X Chromosome Inactivation

Is-018: Mesenchymal Stromal Cell Therapy: Progress and Obstacles to Translation

Frank Barry

Regenerative Medicine Institute National University of Ireland Galway, Ireland
Email: frank.barry@nuigalway.ie

Background and Aim: The use of mesenchymal stromal cell transplantation for tissue repair has received a great deal of attention in recent years and these cells stand at the forefront of successful strategies in regenerative medicine. Recent data from clinical trials has lead to some optimism that MSC therapy has the potential to be a transformative technology in medicine. Despite this, there are questions still unanswered relating to the biology, logistics and translation of MSC therapy.
Methods: Although clinical proof of concept may be forthcoming there are still important questions in a biological context that need a much deeper understanding. These are potency, mechanism of action and immunomodulation. In the logistical sense, issues of manufacturing, supply and delivery need to be addressed. These include the use of more efficient and specific methods of isolation, scalable and closed bioreactor configurations, the incorporation of fully xeno-free growth media, improved methods of storage and the development of tissue-specific devices for delivery of cell products to patients.
Results: A complete understanding of mechanism of action is needed in order to optimise cell dose, develop next generation products involving disease-specific and personalized therapies. This understanding will only emerge if the activity of cells in situ is understood, i.e. the molecular and cellular impact that transplanted MSCs has on the host within the injury niche. This impact will relate possibly to differentiation of the delivered cells and their structural contribution to repair, but more likely to paracrine effects mediated through one or many secreted factors. A further concept in paracrine signaling is that transplanted MSCs become activated or "licensed" by exposure to host cytokines, immunomodulatory factors or cells.
Conclusion: In the event that MSC therapy becomes a widely used or routine aspect of medical practice then significant additional research is needed to answer these questions regarding the biology of the cell product and the logistics of manufacturing and delivery. It will then be possible to optimize efficiency, minimize costs and develop improved personalized strategies.
Keywords: Mesenchymal Stromal Cell, Cell Therapy,-Mechanism of Action, Potency

Is-019: MSC Therapy for Arthritic Diseases

Frank Barry

Regenerative Medicine Institute, National University of Ireland Galway, Ireland
Email: frank.barry@nuigalway.ie

Background and Aim: Osteoarthritis (OA) is a complex joint disease, often characterized as a biomechanical condition associated with abnormal joint loading resulting from obesity, joint instability or trauma. Damage to the articular cartilage is a consistent feature, along with changes to subchondral bone and synovium. Despite many years of investigation there is today no approved pharmacological intervention, biological therapy or procedure that prevents the progressive destruction of the OA joint. All current approved treatments produce symptomatic rather than regenerative results. It has been considered that cellular therapies may deliver solutions for OA and in this context there has been a considerable focus on the use of mesenchymal stromal cells.
Methods: Much of the early experimental investigation into the therapeutic potential of MSCs involved surgically created chondral or osteochondral defects in small animal models. More direct and ultimately more successful approaches involved the treatment of post-traumatic OA in caprine or ovine models. In these studies, resection of the anterior cruciate ligament, sometimes combined with complete medial meniscectomy in the stifle joint resulted in substantial joint degeneration with cartilage fibrillation, osteophyte formation and subchondral sclerosis typical of advanced OA. These

preclinical studies have in turn leaded to human trials that have to date focused on OA of the knee.

Results: Evidence from a wide array of preclinical studies has suggested that MSCs elicit a regenerative response when delivered to the OA knee. Generally, levels of engraftment were seen to be quite low, giving rise to important questions surrounding cytokinetics and cytodynamics. The synovium may be the primary responder tissue in joint repair following MSC transplantation with contact between MSCs and synovial cells an essential element of the response. In human studies there has been evidence of functional recovery and improvements in pain outcome. Careful assessment using X-Ray and MRI protocols will determine the structural response.

Conclusions: Clinical testing of MSC therapy is well underway and the results of large, randomized, multicentre, double-blinded, placebo-controlled trials will be eagerly awaited. In parallel with clinical testing, elements of the mechanism of action need to be assessed.

Is-020: True de Novo Whole Genome Sequencing Iis Here: What is Next?

Nezih Cereb

CEO & Co-Founder, Histogenetics, USA
Email: nezih@histogenetics.com

PacBio RS II platform had been considered the Gold Standard for long range sequencing (up to 40 kb) that enables de novo assembly of the genomes. Recently PacBio introduced a new instrument, Sequel, that uses the SMRTcells with 1,000,000 ZMW as compared to 150,000 on PacBio RSII® platform, that means more a than 6-fold increase in capacity and multiplexing ability. We wanted to use this new breakthrough in sequencing technology to sequence the whole genome of a known human subject. All HLA genes of this subject been sequenced at whole gene level for class I and long range (exon 2 - 3'UTR) for class II. KIR gene content was determined using in house method on Illumina platforms. Full or partial sequences of KIR genes were determined with amplicon and/or fosmid cloning. We prepared genomic DNA libraries with average lengths of 41 kb to with 53 kb. Sequencing performed on PacBio Sequel® platform using v2 chemistry and v4 software. Movie time for these large fragments were set to 600 minutes and secondary analysis was performed with SMRTLink's (v4.0.0) SMRT Analysis Application: de novo Assembly (HGAP 4). We have had 118x coverage (355GB by sequencing 3 different libraries in 73 SMRT® Cells.

We obtained 2.9 Gbp in the final assembly (DNANexus) with just under 4500 contigs. N50 contig length was over 4.2 Mbp with maximum contig length of 19.7 Mbp. Over 50 % of bases were contained under 190 contigs. This level of coverage and length is unprecedented by using just one technology and achieving these in 30 days.

With this quality of data, we can get the whole MHC and KIR genes and haplotypes of this individual that is not possible with other short or long read sequencing technologies. Similarly, we are currently analyzing other complex genetic regions to take advantage of this quality data and enhance the Human Genome database. We anticipate that soon the throughput of Sequel system will increase 20 to 30-fold to complete this quality of genome data in less than 2 days with much lower cost. Then whole genome sequence could be one of the main tests of medical practice. Meanwhile bioinformatics teams will work on further automating the data analysis and annotation.

Is-021: Whole Gene Sequencing of HLA Class 1 Genes in a Large Sample Set Unravel Distinct Evolutionary Pattern for each Gene

Nezih Cereb

CEO & Co-Founder, Histogenetics, USA
Email: nezih@histogenetics.com

We have been sequencing whole gene HLA class I on a large scale at Histogenetics on PacBio RS II® platform since the beginning of July 2016. To date we have sequenced 211,701 samples for whole gene class I. We observed 1770, 1814 and 1792 unique sequences in HLA-A, -B and -C, respectively. We compared inter-allelic variation for each HLA class I gene (HLA-A, -B, -C) for 106,377 that were sequenced as of December 31st 2016. Mean values of non-synonymous and synonymous divergence (dN and dS, respectively) for all codons for all pairwise comparisons among alleles

were determined using SNPGenie, an implementation of the Nei-Gojobori method (Nei and Gojobori, 1986) for use with next-generation sequencing data (Nelson and Hughes, 2015; Nelson et al., 2015). Here we present a summary of our findings.

In summary regarding positive selection, the following exons give signatures of positive selection: (1) HLA-A exons 2, 3, and 5 (weak); (2) HLA-B exons 2 and 3 (weak); and (3) HLA-C exons 1, 6, and 7. Regarding HLA-C, this seems quite revolutionary, and may help to explain previous studies that failed to identify evidence of positive selection specifically favoring amino acid charge differences in the PBR residues of HLA-C only. Teasing out the exact targets of selection and its strength is underway. However, regional differences can be clearly seen in the dN/dS sliding window figures (not shown).

The main observation in support of the recombination/homogenization and subsequent drift of introns would be dS higher in exons than d in introns. All loci fit this pattern: for HLA-A, dS-exons=0.0404 while d-introns=0.0236; for HLA-B, dS-exons=0.0347 while d-introns=0.0094; and for HLA-C, dS-exons=0.0305 while d-introns=0.0186.

Keeping in mind that the PBR residues are encoded by exons 2 and 3, we'd more specifically expect to see d in introns 1, 2, and 3 being lower than dS in exons 2 and 3. This indeed seems to hold for HLA-A and HLA-C, but not for HLA-B—and with the new caveat that exons 2 and 3 don't show overall positive selection in HLA-C. Since intron 3 is the longest, and therefore contains the most sites that are farthest from the PBR, we'd also expect d to be lower in intron 3 than introns 1 and 2; this is indeed the case for all loci.

Given that previous studies show evidence for recombination within intron 2, especially in HLA-B, we can make some sense of the specific values of d observed: values for introns 2 and 3 are always more similar to one another than either is to intron 1, and the difference is most substantial for HLA-B.

Overall values for dN at HLA-A, -B, and -C are 0.0305, 0.0306, and 0.0240, respectively. Overall values for dS are 0.0395, 0.0344, and 0.0300, respectively. Therefore, in terms of selectively neutral diversity, diversity is greatest in HLA-A, less in -B, and least in –C.

Is-022: BioPearl: A Breakthrough Technology for High Throughput Miniaturized 3D Micro-Tissue Fabrication

Noushin Dianat[1], Raif Eren Ayata[1], Pablo Ibanez[2], Léo Chamayou[1], Anne Perreard Dumaine[3], Magalie Pardon[3], Gérard Sanderink[3], Nicolas Bremond[1], Andrew Griffiths[2], Jérôme Bibette[1]

1. ESPCI ParisTech, PSL University, Laboratoire de Colloïdes et Matériaux Divisés (LCMD), Paris, France
2. ESPCI ParisTech, PSL University, Paris, France
3. Disposition Safety and Animal Research (DSAR), Sanofi, Paris, France

Email: noushin.dianat@espci.fr

Background and Aim: Today it is well known that cells behave structurally and functionally different when seeded on thin 2D coated substrates compared to 3D organized conformations, which mimic more closely their natural environment. 3D cultures exhibit a higher degree of structural complexity and homeostasis analogous to tissues and organs and provide more reliable closer to vivo responses.

Methods: BioPearl is a millifluidic based technology for 3D cell spheroid fabrication in alginate microcapsules developed in the Laboratory of Colloids and Divided Materials (LCMD) lab at EPSCI Paris. BioPearl allows scaffold-free 3D micro-tissues to be grown or maintained in the liquid core of alginate microcapsules under physiological conditions. The alginate shell allows oxygen and nutrient exchange while allowing scaffold-free 3D micro-tissues growth.

Results: This technology is already validated on different mammalian cell types, and demonstrate significant advantages to the traditional 2D culture methods. We believe that BioPearl represents a technological breakthrough and is a powerful tool in basic research, drug discovery and development, and biomedical applications, such as tissue engineering and regenerative medicine.

Conclusion: In this talk, BioPearl technology, different 3D cellular models established through this technology and our new achievements of their application in drug development will be presented.

Keywords: Spheroid, 3D Cell Culture, Micro-Tissue, Encapsulation, Alginate

Is-023: Next-Generation Microfluidics Systems for High-Throughput Cell Sorting

Majid Ebrahimi Warkiani

School of Mechanical and Manufacturing Engineering, University of New South Wales (UNSW), Sydney, Australia
Email: m.warkiani@unsw.edu.au

Cell sorting is critical for many applications ranging from stem cell research to cancer therapy. Isolation and fractionation of cells using microfluidic platforms have been flourishing areas of development in recent years. The need for efficient and high-throughput cell enrichment, which is an essential preparatory step in many chemical and biological assays, has led to the recent development of numerous microscale separation techniques. Size-based passive particle filtration using inertial microfluidics have recently received great attention as a promising approach for particle focusing, filtration and fractionation due to its robustness and high rates of operation. The main advantage of inertial-based microfluidics approaches is that continues-flow separation without clogging can be realized using relatively large microchannels with relatively high resolution. In this seminar, I will describe our recent efforts in development of ultra-high throughput microfluidics systems for separation of rare cells (e.g., circulating tumour cells (CTCs), malaria parasites and fetal cells) from blood for diagnostic and therapeutic applications. Further, I will show that how inertial microfluidics enables efficient sorting of Mesenchymal stem cells (MSCs) as a function of cell diameter, and show that this enables selection and sorting of osteoprogenitor cells from marrow for applications such as bone regeneration. I will also present some of our recent efforts for commercialization of these microfluidic systems in biotechnology and bioprocessing industries. Eventually, I will discuss how simple micro-engineered tools (i.e., fabricated using 3D printing and MEMS techniques) can be combined with fluid mechanics concepts in order to develop functional devices for both basic and applied research.

Is-024: Stem Cell Research in the United Kingdom Ethical Legal and Regulatory Background

Peter Ellis

Law Chambers of Collingwood Thompson QC, London, UK
Email: PEllis@7br.co.uk

Background and Aim: It is seventy years since those involved in the prosecution of Nazi doctors formulated the Nuremberg Code The Nazi doctors were found guilty of conducting medical experiments on prisoners without their consent Since then numerous guidelines and regulations have emerged in the developed world in order to ensure that clinical research has an ethical basis and is properly conducted and regulated This paper seeks to review the modern ethical legal and regulatory background to stem cell research in the UK within its historical context and to consider whether the system is adequate to protect trial subjects
Methods: Review of relevant national and international ethical legal and regulatory schemes as the basis for scrutiny of the UKs system
Results: Stem cell research in the UK is conducted within a tight ethical legal and regulatory framework both at the level of individual doctors and their research clinics
Conclusion: The UK provides a robust ethical legal and regulatory system for the protection of clinical trial subjects The system is also sufficiently flexible to allow innovative medical scientific research to flourish
Keywords: Clinical Ethics Helsinki Declarations

Is-025: Acellular Dermal Matrix Revitalized with Allogenic Dermal Fibroblasts as a Biological Wound Coverage; Do We Need Fibroblasts?

Ali Farrokhi[1], MohammadReza Rahavi[2], Mohammadreza Pakyari[1], Victoria McCann[1], Reza Jalili[1], Gregor Reid[2], Aziz Ghahary[1]

1. Burn & Wound Healing Research Group, Division of Plastic Surgery, Department of Surgery, International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Vancouver, Canada
2. Division of Hematology, Oncology, and BMT, Department of Pediatrics, Faculty of Medicine, University of British Columbia, Vancouver, Canada
Email: farrokhi_a@hotmail.com

Background and Aim: Acute and chronic wounds contribute to increased morbidity and mortality in affected people and impose significant financial burdens on healthcare systems. Currently, allogeneic cell-based skin substitutes as ready-to-use wound coverage have been proposed as an alternative to conventional split-thickness skin grafts, but the survival and usefulness of such cells after transplantation into an immunocompetent host remain controversial. Here, we hypothesized that the application of an indoleamine 2,3-dioxygenase (IDO) expressing allogeneic dermal fibroblast populated within an acellular dermal matrix (ADM) is sufficient to create an immune-privileged area within the wound to protect fibroblasts from rejection and help the graft to restore its function by synthesizing extracellular matrix components and growth factors by these cells.
Methods: In this study, ADMs were prepared using a new detergent–free method, recellularized with IDO-expressing or control fibroblasts from C57/B6 mice, and were transplanted on splinted full-thickness skin wounds in Balb/c mice.
Results: In our study, ADM significantly enhanced the wound-healing process within three weeks but there was no more improvement when we recellularized it with fibroblasts. Application of IDO-fibroblasts reduced infiltration of CD4+IL-17+TH-17 and CD4+IFN-G+TH-1 immune cells to the grafts, but it was not statistically significant (*p >0.05). One week after transplantation, analysis of grafts for presence of MHC-I antigen of donor cells revealed that only 2% of the total cell were detectable as IDO-fibroblasts, while the control fibroblast were not. In weeks two and three, neither of those cells were detectable. To further investigate the transplanted cells' fate, we intradermally injected luciferase-labeled fibroblasts in wild type Balbc and immunodeficient NOD-SCID-Il2r gamma null (NSG) mice. Bioluminescence in vivo imaging showed that the survival of both type of fibroblasts in allogeneic recipients was significantly shorter compared to that exhibited in NSG recipients, revealing the role of immune rejection.
Conclusion: It is worth considering that our animal model represents an acute wound model, and the migration of host cells to ADM might outweigh those of the transplanted cells, questioning whether we need to repopulate ADMs with allogenic fibroblasts or not. Also, although it has been reported by other researchers that IDO-expressing fibroblasts exhibit strong immunosuppressive activity in vitro and in vivo, we documented rejection of these cells in our study, suggesting that the application of these cells in wound sites requires further improvements.
Keywords: Wound Healing, Acellular Dermal Matrix, Fibroblasts

Is-026: Therapeutic Cancer Vaccines

Farzin Farzaneh

Chair of Molecular Medicine, King's College London, UK
Email: farzin.farzaneh@kcl.ac.uk

A large number of different classes of tumour-associated antigens provide targets for immune recognition of malignantly transformed cells by the immune system, and for immune therapy of cancer. These antigens include viral gene products expressed by oncogenic viruses such as Human Papilloma Virus (HPV), as well as a whole array of mutated oncogenes, abnormally glycosylated proteins, as well as other proteins that are expressed ectopically or at elevated levels in different malignancies. In addition to these common antigenic targets, recent data has demonstrated the presence of other, entirely patient and tumour specific mutations. Both the common and the "private" tumour-associated antigens provide potential targets for the immune mediated therapy of cancer. Such immune therapy strategies are of particular relevance to the eradication of the residual cancer cells, most importantly the cancer stem cells, which can contribute to the relapse and recurrence of cancer, despite a successful initial response to therapy.
In this presentation the broad range of cancer immune therapy strategies, including some of the most exciting new developments in the field, such as the use of immune check-point inhibitors will be reviewed. New strategies for vaccination mediated induction of antigen-specific cellular immunity, against both the common oncogenic targets and the unique patient and tumour specific neo-antigens will be described.

Is-027: Cancer Gene Therapy – New Concepts and Novel Strategies

Farzin Farzaneh

Chair of Molecular Medicine, King's College London, UK
Email: farzin.farzaneh@kcl.ac.uk

With better understanding of the molecular pathology of cancer, and with the development of better strategies for the delivery of therapeutic genes, gene therapy of cancer has held the promise of a significant contribution to the clinical treatment of cancer for over two decades. However, until very recently this promise has been substantially unfulfilled. Some of the more recent innovations are now resulting in the rapid development of a host of novel therapeutic strategies. These include the delivery of tumour suppressor genes, anti-oncogenic regulatory RNA sequences, delivery of genes encoding drug converting enzymes for the *in vivo* synthesis of cytotoxic agents from their less-toxic precursors, and the development and clinical use of oncolytic viral vectors including those with selective replication and lytic activity within the tumour cells. Other applications of gene therapy, include the development of genetically engineered, tumour targeted, immune cells with cytolytic activity specifically against cancer cells (*e.g.* CAR-T cells). Recent developments in this array of gene therapy based strategies will be reviewed. Specific examples of the most promising new strategies, including recently licensed gene therapy drugs will be described.

Is-028: Cell-Based Therapies for Treatment of Pulmonary Diseases

Maliheh Ghadiri, Paul M. Young and Daniela Traini

Respiratory Technology, Woolcock Institute of Medical Research and Discipline of Pharmacology, School of Medicine, The University of Sydney, Australia
Email: mali_ghadiri@yahoo.com

Background: During the last few decades, cell-based therapies have shown great potential to treat patients with lung diseases. It has been proposed that the administration of cells into an injured lung could be considered as a therapeutic method to repair and replace injured lung tissue. Cell-based therapy aims to perform structural repair (engraftment of cells) and have an immunomodulation effect to treat the diseased lung. The ability to enhance endogenous stem cell to regenerate lung tissue is key for the treatment of a multitude of fatal lung diseases. Due to the challenges facing cell-based therapy, achieving success to find a safe and efficient strategy for treatment has been slow. There are few factors that need to be considered in cell-based therapies, including; cell type, routes of delivery, safety and efficiency of treatment.
Cell types used in the treatment: Various types of cells have been proposed as prospective source of novel treatment in pulmonary diseases for example, lung primary cells, alveolar epithelial type II, lung mixed epithelial cells and stem cells. Stem cell includes lung progenitor cells, mesenchymal stem cells (MSCs), induced pluripotent stem cells (iPSs), embryonic stem cells (ESCs) and adipose stem cells (ASCs). Endogenous alveolar epithelial cells and bone marrow mesenchymal cells have been the most widely studied in cell-based therapies of pulmonary diseases. MSCs are the most commonly studied stem cell and they have mainly originated from umbilical cord, bone marrow and adipose tissue. The therapeutic potential of MSCs is not only related to their multilineage differentiation capacity but to their potent anti-apoptotic, anti-inflammatory properties and, also to their ability to modulate immune responses and modify the microenvironment at the engraftment sites.
Routes of delivery: The most common routes of delivery are intravenous, intraperitoneal and intra tracheal routes. Spray aerosolisation of stem cells can be beneficial in the speeding up of healing processes by delivering cell *in situ* to the injured area; survival of the aerosolised cells is still an issue. Cells are very fragile and a suitable aerosolisation method must be established in order to assure that hydrodynamic stress due to liquid atomization will not damage their structure. Droplets size and spray speed should be considered to obtain an optimum bioaerosol.
Conclusion: Cell-based therapy for pulmonary diseases is in its infancy and still remains exploratory with many challenges that need to be addressed. These challenges include bioethical issues, safety of cell transplantation, ideal routes of delivery and the dose and timing of administration that would make cellular therapy effective. In spite of all hindrances and challenges, according to the data collected in both animal models and in pre-

clinical studies, cell-based therapies seem to have the potential to treat lung diseases.
Keywords: Stem Cell, Cell Therapy, Pulmonary

Is-029: Adipose-Derived Mesenchymal Stem Cells for Treatment of Airway Injuries in Patients after Long-Term Exposure to Sulfur Mustard

Mostafa Ghanei, Amir Nejad-Moghaddam, Eisa Tahmasbpour

Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
Email: mghaneister@gmail.com

Background and Aim: Sulfur mustard (SM) is a potent mutagenic agent that targets several organs, particularly lung tissue. Although numerous studies have demonstrated pathological effects of SM on respiratory system, unfortunately there is no effective treatment to inhibit further respiratory injuries or induce repair in SM-exposed patients. Due to the extensive progress in stem cell therapy, here we evaluated safety and potential efficacy of adipose derived mesenchymal stem cell (AdMSC) administration on SM-exposed patients with chronic lung injuries.
Materials and Methods: In this clinical trial study, 9 patients received 100×106 cells every 20 days for 4 injections over a 2-month period. After each injection, we evaluated the safety, pulmonary function tests (PFT), chronic obstructive pulmonary disease (COPD) Assessment Test (CAT), St. George's Respiratory Questionnaire (SGRQ), Borg Scale Dyspnea Assessment (BSDA), and 6 Minute Walk Test (6MWT). Sputum samples were also provided after each injection. Changes in expression of several oxidative stress genes (metallothionein 3, glutathione reductase, oxidative stress responsive 1, glutathione peroxidase 2, lacto peroxidase, forkhead box M1) and inflammation-related genes (matrix metallopeptidase 2, matrix metallopeptidase 9, transforming growth factor-b1, vascular endothelial growth factor, metallopeptidase inhibitor 1, metallopeptidase inhibitor 2) were also evaluated using real-time PCR. Two-lung epithelial-specific proteins including Clara cell protein 16 and Mucin-1 protein levels were measured using enzyme immunoassay method.
Results: There were no infusion toxicities or serious adverse events caused by MSC administration. Although the PFTs improvement was not significant, we found a significant improvement for 6MWT, BSDA, SGRQ, and CAT scores after each injection. No significant differences were found between serum levels of Clara cell protein 16 and serum Mucin-1 protein in patients before and after cell therapy. Most of the oxidative stress responsive genes, particularly oxidative stress responsive 1, were overexpressed after treatments. Expressions of antioxidants genes such as metallothionein 3, glutathione reductase and glutathione peroxidase 2 were also increased after cell therapy. Upon comparison of inflammation-related genes, we observed upregulation of vascular endothelial growth factor and matrix metallopeptidase 9 after mesenchymal stem cells therapy.
Conclusion: MSC administration appears to be safe in SM-exposed patients with moderate to severe lung injuries. It also mitigates oxidative stress and inflammation in sulfur mustard-exposed patients.
Keywords: Mesenchymal Stem Cells, Transplantation, Sulfur Mustard, Airway Remodeling, Oxidative stress

Is-030: Autophagy Inhibition Directs the Epithelial to Mesenchymal Transition in Non-Small Lung Cancer Cells

Saeid Ghavami, Javad Alizadeh, Thomas Klonisch, James Thliveris

Department of Human Anatomy and Cell Science Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Canada
Email: Saeid.Ghavami@umanitoba.ca

Background and Aim: Lung cancer is considered as one of the most important causes of cancer-related death worldwide and Non-Small Cell Lung Cancer (NSCLC) accounts for 80% of all lung cancer cases. Autophagy is a cellular process responsible for the recycling of damaged organelles and protein aggregates. Transforming growth factor beta-1 (TGFβ1) is involved in Epithelial to Mesenchymal Transition (EMT) and autophagy induction in different cancer models and plays an important role in pathogenesis of NSCLC. It is not clear how

autophagy can regulate EMT in NSCLC. In the present study, we have investigated the regulatory role of autophagy in EMT induction in NSCLC cells.
Methods: Human NSCLC cell lines (A549 and H1975) were treated with TGFβ1 (5ng/ml) for 24, 48, 72 hrs. Western blots were done on cell lysates to investigate autophagy (LC3β lipidation, p62 degradation), EMT (E-cadherin and vimentin), and TGFβ1 canonical signaling (SMAD2 phosphorylation). Phenotype of the cells were imaged upon TGFβ1 treatment by phase contrast microscopy. We confirmed autophagy and EMT upon TGFβ1 treatment using immunocytochemistry (ICC) and Transmission Electron Microscopy (TEM). We also used stable Atg7 knockdown and corresponding scramble NSCLC cells to investigate the role of autophagy inhibition in TGFβ1-induced EMT in NSCLC using immunoblotting and ICC.
Results: Our experiments showed that TGF?1 can simultaneously induce both autophagy [LC3? lipidation and p62 degradation using immunoblotting, autophagosome formation using TEM, and punctuated LC3 using ICC] and EMT [E-cadherin downregulation and vimentin upregulation]. Furthermore, TGF? 1 treatment caused cells to acquire mesenchymal phenotype. Our results showed that EMT marker vimentin was significantly downregulated upon TGF?1 treatment in Atg7 knockdown cells [downregulation of vimentin using immunoblotting and lower signal intensity in ICC] compared to corresponding scramble cells while E-cadherin was almost unchanged. Also, Atg7 knockdown cells treated with TGF?1 had less migration compared to scramble counterparts.
Conclusion: Our results highlight the important role of autophagy as a positive regulator of TGFβ1-induced EMT in NSCLC cells. New autophagy blocking drugs may be useful in reducing NSCLC motility and metastasis in NSCLC.
Keywords: Autophagy, Epithelial to Mesenchymal Transition, Non-Small Cell Lung Cancer, Metastasis

Is-031: Autophagy and the Unfolded Protein Response Promote Pro-fibrotic Effects of TGFbeta1 in Human Lung Fibroblasts

Saeid Ghavami[1], Behzad Yeganeh[2], Amir A Zeki[3], Shahla Shojaei[1], Nicholas J Kenyon[3], Afshin Samali[4], John Patterson[5], Javad Alizadeh[1], Adel Rezaei Moghadam[1], Ian M Dixon[6], Helmut Unruh[7], Darryl A. Knight[8], Thomas Klonisch[1], Andrew J Halayko[9]

1. Department of Human Anatomy and Cell Science Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Canada
2. Department of Physiology and Experimental Medicine, University of Toronto, Canada
3. Division of Pulmonary, Critical Care, and Sleep Medicine, University of California, Davis, USA
4. Apoptosis Research Centre, National University of Ireland, Ireland
5. Mannkind Corporation, California, USA
6. St. Boniface Research Centre, Canada
7. Department of Internal Medicine, University of Manitoba, Winnipeg, Canada; and Section of Thoracic Surgery, University of Manitoba, Winnipeg, Canada
8. School of Biomedical Science and Pharmacy, University of Newcastle, Australia
9. Department of Physiology and Pathophysiology, University of Manitoba, Canada
Email: Saeid.Ghavami@umanitoba.ca

Background and Aim: Idiopathic pulmonary fibrosis (IPF) is a lethal fibrotic lung disease in adults with limited treatment options. Autophagy and the unfolded protein response (UPR), fundamental processes induced by cell stress, are dysregulated in lung fibroblasts and epithelial cells from humans with IPF.
Methods: Human primary cultured lung parenchymal and airway fibroblasts from healthy and lung IPF donors were stimulated with TGFβ1 with or without the inhibitors of autophagy or UPR (IRE1 inhibitor). We monitored temporal changes in abundance of protein markers of autophagy (LC3betaβ, Atg5-12), UPR (BIP, IRE1 alpha, cleaved XBP1), and fibrosis (collagen 1A2, fibronectin) using immunoblotting. Using fluorescent immunohistochemistry we profiled autophagy (LC3beta) and UPR (BIP, XBP1) markers in human non-IPF and IPF lung tissue.
Results: TGFβ1-induced collagen1A2 and fibronectin protein production was significantly higher in IPF lung fibroblasts compared to lung and airway fibroblasts from non-IPF donors. TGFbeta1 induced the accumulation of LC3β in parallel with collagen 1A2 and fibronectin, but, autophagy marker content was significantly lower in lung fibroblasts from IPF subjects. Inhibition of

autophagy flux significantly reduced TGFβ1-induced collagen and fibronectin in fibroblasts from the lungs of non-IPF and IPF donors. Conversely, only in lung fibroblasts from IPF donors did TGFbeta1 induce UPR markers. IRE1 alpha inhibitor decreased TGFβ1-induced collagen 1α2 biosynthesis and fibronectin in IPF lung fibroblasts, but not those from non-IPF donors.
Conclusion: The IRE1 pathway of the UPR is uniquely induced by TGFβ1 in lung fibroblasts from human IPF donors, and is required for excessive biosynthesis of collagen and fibronectin in these cells.
Keywords: IRE1 Inhibitor, Fibrosis, Autophagy Inhibition, Spliced XBP1, Transforming Growth Factor beta 1

Is-032: Bio-based Materials for Regenerative Medicine Applications

Samaneh Ghazanfari

Maastricht University and Aachen University, Institute for Biobased, Sciences, Faculty of Humanities and Sciences, the Netherlands
Email: samaneh.ghazanfari@maastrichtuniversity.nl

Background and Aim: All biological systems are well organized. Everything from molecules embedded in our genomes to nano-scale structures synthesized from molecules, micro-scale tissue structures formed by cells and finally organs created from tissues have a controlled structure.
Methods: The aim of regenerative medicine field is to create biological systems at different length scales which mimic some of these complexities in vitro. For instance, using the proper material in which molecules can be derived using different methods, such as bioprocessed techniques. Then, modifying the material chemically and fabricating it into different structures using techniques like textile technologies, and finally using it for different applications. One of the major scientific challenges in creating in vitro tissues is to create constructs with a native-like structure and functionality using a right material. An ideal material should be biomimetic, possess appropriate physical, mechanical and chemical characteristics, degrade over time (especially for young patients) and preferably has autofluorescence properties for imaging purposes.
Results: Among all polymeric materials, bio-based polymers have recently gained a huge momentum to be used for medical applications since they are intrinsically bio-active.
Conclusion: Therefore, our research is focused on how different bio-based materials designed for specific regenerative medicine applications have to be fabricated at different length scales to provide appropriate architecture and mechanical properties, how they have to be functionalized and most importantly how they interact with the biological systems.
Keywords: Bio-Based Materials, Fiber-Based Scaffolds, Regenerative Medicine

Is-033: Dynamics of Salivary Gland Stem Cells during Development, Homeostasis and Regeneration of Salivary Glands

Mingyu Kwak, Ninche Alston, Kevin Shan and Soosan Ghazizadeh

Department of Oral Biology and Pathology, School of Dental Medicine, Stony Brook University, NY, USA
Email: Soosan.Ghazizadeh@stonybrookmedicine.edu

The mammalian salivary gland is composed of several differentiated cell lineages including acinar, ductal and myoepithelial cells. A pivotal question in salivary gland biology is the cellular mechanism maintaining these differentiated cell lineages during tissue homeostasis and regeneration. Using mouse submandibular gland (SMG) as a model, we have identified a population of long-lived, yet cycling ductal stem cells residing in a spatially defined region in the intercalated ducts. Inducible lineage tracing studies using Cre-Lox system showed that these stem cells are established from an embryonic multipotent K14+ progenitor population during branching morphogenesis, are restricted in their lineage and contribute to the formation and maintenance of granular ducts throughout life. In response to injury, K14+ ductal stem cells display remarkable plasticity and multi-lineage regenerative capacity to regenerate the entire secretory complex, including acini and their contiguous intercalated and granular ducts. Our findings uncovered a major source of cell renewal and regeneration in salivary glands, and provide new insights into salivary gland pathobiology.

Is-034: Induced Pluripotent Stem Cell Core Facility of the ErasmusMC Stem Cell and Regenerative Medicine Institute

Mehrnaz Ghazvini and Joost Gribnau

Erasmus MC iPSC core facility, Erasmus Medical center, Rotterdam, The Netherlands
Email: m.ghazvini@erasmusmc.nl

The discovery of induced pluripotent stem cells (iPSC) in 2006 had an enormous impact biomedical research providing unlimited resources for disease models, in regenerative medicine and for drugs screening[1]. Several methodologies are published to generate iPSC lines, nevertheless the process remains labor intensive and requires defined standard operating procedures to maintain consistency and reproducibly. The iPSC & ES core facility at Erasmus Medical Center was established in 2010 with the mission to generate iPS cell models to accelerate research on stem cells, disease, and regenerative medicine. As a core facility, we aim to deliver state of the art iPS cells with the best available reprogramming protocols and facilitate the iPS related research of customers by on side training of tissue culture practice.The Erasmus MC iPSC & ES core facility has generated more than eighty iPS lines of controls and patients with a wide spectrum of diseases including: bipolar syndrome, schizophrenia, depression, autism, Angelman syndrome, Rett syndrome, congenital neutroprenia, Parkinsons disease, immune dificiencies, fragile X syndrome, cardiomyopathies, X linked mental retardation, RNF12 syndrome, poly-cystic kidney disease, cancer. IPS cell lines were derived from a wide variety of sources including fibroblasts of skin biopsies, peripheral blood, bone marrow, kidney epithelial cells, and epithelial cells from urine. Quality control involves characterization of our iPSC lines by morphology, cell surface markers, expression of pluripotency and differentiation markers, and karyotyping. Recently we added whole genome methylation profiling to our quality control pipeline by applying the MeD-seq technology which is based on a methyl dependent restriction enzyme LpnPI. In my seminar I will discuss our findings to characterize our iPSC lines with this MeD-seq technology in relation to DNA methylation profiles in donor cells and human ESCs.

Is-035: Genome Wide DNA Methylation Profiling Uusing the Methylation Dependent Restriction Enzyme LpnPI

Joost Gribnau

Department. Developmental Biology, Erasmus MC, Rotterdam, The Netherlands
Email: j.gribnau@erasmusmc.nl

DNA methylation is a well-known epigenetic modification that plays a crucial role in gene regulation, but genome-wide analysis of DNA methylation remains technically challenging and costly. DNA methylation-dependent restriction enzymes can be used to restrict CpG methylation analysis to methylated regions of the genome only, which significantly reduces sequencing depth required, and simplifies subsequent bioinformatics analysis. Unfortunately, this approach has been hampered by over-digestion of DNA in CpG-methylation dense regions, resulting in fragments that are too small for accurate mapping. We recently identified a different DNA methylation-dependent enzyme, LpnPI, and found that its activity is blocked by a fragment size smaller than 32 base pairs. This unique property prevents over-digestion of methylation-dense DNA and allows accurate genome-wide analysis of CpG methylation at single nucleotide resolution, at a sequencing depth <1/10th required for whole genome bisulfite sequencing (WGBS). MeD-seq identified a high number of patient and tissue-specific differential methylated regions (DMRs), and revealed that patient-specific DMRs observed in both blood and buccal samples predict DNA methylation in other tissues and organs. In this seminar I will discuss the many applications of this MeD-seq technology in stem cell and cancer research.

Is-036: Activation of X Inactivation, and the Loss of Pluripotency

Joost Gribnau

Dept. Developmental Biology, Erasmus MC, Rotterdam, The Netherlands
Email: j.gribnau@erasmusmc.nl

All biological differences between women and men originate from the sex chromosomes. Some 160 million years ago, the X and Y chromosomes were very similar, but since then the Y chromosome has lost most of its genes, whereas the present X chromosome contains more than 1000 genes. Hence, the dosage of X-encoded genes needs to be equalized between female (XX) and male (XY) cells. This is achieved by random inactivation of one of the X chromosomes in female embryonic cells. Activation of XCI is tightly coupled to development and embryonic stem cell differentiation, and many pluripotency factors, including Rex1, Oct4, Sox2 and Nanog, participate in the regulation of XCI by repression of the key XCI regulator Xist. X-encoded Xist produces a functional long non-coding RNA that is up-regulated on the inactive X chromosome, kicking off a plethora of events that ultimately results in stable X-linked gene repression, which is then faithfully transmitted to all daughter cells. Our research has revealed new insights in the interplay between stem cell specific transcription factors and Xist regulatory elements and genes, which will be discussed extensively in my seminar.

Is-037: Stem Cell Sciences and Technologies in Iran: Investment for a Bright Future

Amir Ali Hamidieh[1,2]

1. Council for Development of Stem Cell Sciences and Technologies, Tehran, Iran
2. Pediatric Stem Cell Transplant Program, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran

Email: aahamidieh@tums.ac.ir

Since the inception of stem cell research in almost 60 years ago, the world has seen a collection of rises and falls in this field of science. Nowadays, emerging from the shadows of unclarity and failures, stem cell sciences and technologies have been recognized as one of the most productive and promising areas of basic and clinical sciences.

Hematopoietic stem cell transplantation (HSCT) started over 50 years ago and up to now over 1.2 million people have benefited from this kind of treatment across the globe. HSCT has been carried out for near 3 decades in Iran for over 8,000 patients. Beginning with the use of intact HSCs and by the succedent discovery of other types of stem cells in the body, further stem cell-based approaches have been developed to treat chronic refractory diseases. Today, we stand at the verge of the next generation of stem cell-based treatments, when in addition to the high quality intact stem cells; manipulated engineered cells are used for lifelong cure of many kinds of refractory diseases and cancers. In this context, exosome-activated immune cell therapy, CAR T cell immunotherapy and correction of genome defects by application of gene editing tools such as CRISPR/Cas9 have revolutionized this field of science. The translation of basic stem cell science into medically and commercially meaningful outcomes is the cornerstone of the cell therapy industry. It is hoped that in the near future these sciences and technologies become a clinical routine.

Prospects of stem cell sciences and technologies look bright with new funding boost. In Iran, with investments backed by the Iranian Council for Development of Stem Cell Sciences and Technologies, in the forms of supporting grants given to researchers and academic faculty members as well as finance given to knowledge-based companies, the stem cell sciences have grown steadily, so that in 2016, Iran stood in the first rank among the middle eastern and eastern Mediterranean countries considering the number of publications in reputable journals. Moreover, the number of knowledge-based companies directly working in stem cell sciences and regenerative medicine increased from 3 in 2013 to 58 in 2017. Only in 2016, two comprehensive institutes for stem cell and regenerative medicine have been established to bridge basic medical sciences, medicine and engineering disciplines in a way that they address the knowledge gaps and research obstacles and ultimately deliver new therapeutic products.

Our duty at the Council is to support young scientists, to accelerate provision of suitable academic and laboratory platforms and to prompt cutting edge research projects in order to provide scientific advance for the country and eventually bring hope to Iranian patients taking advantage from cellular therapeutics.

Keywords: Hematopoietic Stem Cell Transplantation; Stem Cell Research; Immunotherapy; Gene Therapy

Is-038: The Role of the Long Non-Coding RNA Wintrlinc1 in Homeostasis and Carcinogenesis of the Intestine and Beyond

Pantelis Hatzis

Biomedical Sciences Research Center 'Alexander Fleming', 16672 Vari, Greece
Email: hatzis@fleming.gr

The canonical Wnt pathway plays a central role in stem cell maintenance, differentiation and proliferation in the intestinal epithelium. We have identified several novels Wnt-regulated long non-coding RNAs relevant to intestinal homeostasis and carcinogenesis. One, termed WiNTRLINC1, is a direct target of TCF4/β-catenin in colorectal cancer cells. We show that WiNTRLINC1 acts by positively regulating the expression of its genomic neighbor ASCL2, a transcription factor that controls intestinal stem cell fate. WiNTRLINC1 interacts with TCF4/β-catenin to mediate the juxtaposition of its promoter with the regulatory regions of *ASCL2*. ASCL2, in turn, regulates WiNTRLINC1 transcriptionally, closing a feedforward regulatory loop that controls stem cell-related gene expression. This regulatory circuitry is highly amplified in colorectal cancer and correlates with increased metastatic potential, decreased patient survival and other clinical features. The WiNTRLINC1-ASCL2 axis is also relevantly amplified in other cancers.

Is-039: The Role of the Lysine Methylase PR-SET7 in Transcriptional Regulation and Hepatic Carcinogenesis

Kostas C Nikolaou[1], Panagiotis Moulos[1], Vagelis Harokopos[1], George Chalepakis[2], Haruhiko Oda[3], Reinberg Danny[4], Pantelis Hatzis[1], Iannis Talianidis[1]

1. Biomedical Sciences Research Center Al. Fleming, Vari, Greece
2. Department of Biology, University of Crete, Herakleion, Greece
3. Medical Institute of Bioregulation, Kyusyu University, Fukuoka, Japan, Gastrointestinal and Oncology Division, National Kyusyu Cancer Center, Fukuoka, Japan
4. HHMI, Department of Biochemistry, New York University School of Medicine, New York, NY, USA
Email: hatzis@fleming.gr

PR-SET7-mediated histone 4 lysine 20 methylation has been implicated in mitotic condensation, DNA damage response and replication licensing. We show that PR-SET7 function in the liver is pivotal for maintaining genome integrity. Hepatocyte-specific deletion of PR-SET7 in mouse embryos results in cell division-dependent DNA damage and hepatocyte necrosis after 3-4 months of age. This is accompanied by inflammation, fibrosis and compensatory growth induction of neighboring hepatocytes and resident ductal progenitor cells. Prolonged necrotic regenerative cycles coupled with oncogenic STAT3 activation lead to the spontaneous development of hepatic tumors composed of cells with cancer stem cell characteristics. These include a capacity to self-renew in culture or in xenografts and the ability to differentiate to phenotypically distinct hepatic cells. Hepatocellular carcinoma in PR-SET7-deficient mice displays a cancer stem cell gene signature specified by the co-expression of ductal progenitor markers and oncofetal genes. At earlier post-natal stages, we show that H4K20Me1 turnover in gene bodies positively correlates with gene activity and that loss of H4K20Me1 primarily affects RNAPII release from promoter-proximal regions. Most sensitive to this promoter escape regulation are genes involved in glucose and lipid homeostasis, whose defect resulted in a widespread metabolic reprogramming and genome damage. The results suggest that H4K20Me1 also safeguards genome integrity in non-dividing cells through controlling the transcription of metabolic genes at the post-initiation steps.

Is-040: Pluripotent Stem Cells for Screening and Therapy

Jürgen Hescheler

Institut for Neurophysiologie, Robert-Koch-Str. 39, 50931 Koln, Germany
Email: J.Hescheler@uni-koeln.de

Embryonic Stem cell-based novel alternative testing strategies aims at developing a toxicity test platform based on embryonic stem cells (ESC), especially hu-

man ESC, to accelerate drug development, reduce R&D costs, propose a powerful alternative to animal tests and increase human safety. To this end, a battery of toxicity testing using human ESC-based systems subject to standardized culture and differentiation protocols were built up. The test battery focuses on selected reference compounds of pharmaceutical interest and unknown prenatal toxicity with emphasis on the nervous system and covers reproductive toxicity, neurotoxicity, hepatotoxicity, cardiotoxicity, toxicogenomics as well as metabolism and toxicokinetics. Transcriptomics data were combined with phenotypic and functional readouts. The gene expression signatures were used to establish classifiers allowing the identification of compounds that act by a certain toxic mechanism or induce a specific phenotype. In a proof of principle study, the transcriptomics data revealed that the test compounds such as valproic acid (VPA) and methylmercury chloride induce a "common response" which can be distinguished from "compound-specific" responses. Thus assay battery approach allows classification of human developmental neurotoxicants, reproductive toxicants, cardiotoxicants and hepatotoxicants on the basis of their transcriptome profiles. All data have been successfully uploaded onto the diXa data infrastructure and subjected to its standardized quality control protocol; toxicological data are thus sustained. The potential use of the cell test systems can be further improved after replacing of ESC with iPS cells, introduction of 3D models and utilizing the cell systems established from both healthy and diseased donors. To demonstrate the ability of pluripotent stem cells for regenerative medicine and tissue repair, CMs differentiated from iPS and ES cells were injected into the cryoinfarcted left ventricular wall of adult wild type mice. Translation from the laboratory into the clinic will be one of the future key problems of stem cell research. Although proof of principle for the therapeutic use of iPS cells in cardiac diseases has been shown both at the laboratory scale and in animal models, the methods used today for generation, cultivation, differentiation and selection are not yet suitable for the clinic.

Is-041: Pluripotent Stem Cells and Personalized Medicine

Jürgen Hescheler

Institut for Neurophysiologie, Robert-Koch-Str. 39, 50931 Köoln, Germany
Email: J.Hescheler@uni-koeln.de

Due to their ability to reproduce the embryonic, neonatal and adult differentiation of all different organotypic cellular phenotypes, pluripotent stem cells represent an ideal tool to study physiological processes of embryogenesis under in vitro conditions as well as to provide the basis of cellular therapeutics, to build up test assay systems for drug discovery or toxicology and to develop novel disease models for companion diagnostics within personalised medicine. In particular embryonic (ES) and induced pluripotent stem (iPS) cells can reproduce all organotypic electrophysiology, signalling cascades and genes involved in the development (functional genomics). This is spontaneously occurring within three dimensional cell aggregates - embryoid bodies (EBs) – which we developed 25 years ago. Induction of pluripotency by reprogramming allows to obtain individual iPS cells of patients with his/her specific genetic background. This provides a unique new tool to build up test systems for representing the patient for personalized medicine. Moreover novel disease models can be generated. To demonstrate the proof of principle, reprogramming of fibroblasts from patients with LQT3 or CPVT syndrome by ectopic expression of the Yamanaka's transcription factors was performed resulting in generation of iPS cells for disease modelling. This novel approach may also enable patient-specific cell replacement therapies which appears an indispensable prerequisite for a later use in clinics. iPS cells from patients may also represent a new diagnostic tool to precisely analyse the pathophysiology and to develop personalised strategies for an optimized therapy.

Is-042: Proteome Alterations and Mechanisms of Aging in the Cellular Network of Human Bone Marrow

Anthony Ho[1], Anne-Claude Gavin[2]

1. Department of Medicine, University of Heidelberg, Germany
2. European Molecular Biology Laboratories, Heidelberg, Germany
Email: sekretariat.ho@med.uni-heidelberg.de

Background and Aim: To acquire a systems understanding of the human bone marrow cellular network during the aging process, we have studied the dynamic alterations in proteome landscapes of human hematopoietic progenitor and stem cells (HPC) as compared to those of five other cellular elements comprising the bone marrow niche.
Methods: The primary cells were derived from 59 healthy human subjects with ages ranging from 20 to 60 years. To simultaneously measure the molecular alterations associated with aging at both spatial (cell population) and temporal (aging) resolution, we combined two complementary MS-based quantification methods. The age-associated changes were measured by isobaric labeling using tandem mass tag (TMT) and the cell type specific ones by a label free technique adapted from Schwanhausser et al.
Results: The most prominent finding was a significant up-regulation of proteins and enzymes associated with glycolysis, glycogen catabolism, fatty acid oxidation (FAO), as well as acetyl-CoA metabolism in old HPC as compared to young HPC. In addition, enzymes involved in the elimination of nitrous oxide (NO) were increased significantly in aged human HPC, indicating the prominent role of accumulation of NO in aged human HPC. There was also a significant decrease in IRF-8 in aged HPC versus young HPC. All these changes were remarkably found only in HPC and not in other cell types, including the mesenchymal stem cells. Unbiased, comprehensive proteome analysis has also provided unequivocal proofs that, upon senescence, human HPC showed a reduced propensity to differentiate into lymphoid cells and are myeloid- and especially megakaryocyte-biased.
Conclusion: Provided with this depository of primary bone marrow cells from healthy human subjects of different age groups, we have captured for the first time the proteomic signatures of the aging process in human bone marrow, with a remarkable and complex re-organization of the central carbon metabolic pathways, amino acid, and lipid metabolisms, as well as accumulation of NO, lineage skewing and decrease in IRF8 activity. Our data represent an invaluable resource for further in-depth mechanistic analyses, as well as validation of knowledge derived from diverse animal models.
Keywords: Proteomics, Hematopoietic Stem Cells, Bone Marrow Niche, Aging

Is-043: Hematopoietic Stem Cell Transplantation, 32 Years Old, Learning New Tricks

Anthony Ho, Michael Schmitt

Department of Medicine, University of Heidelberg, Germany
Email: sekretariat.ho@med.uni-heidelberg.de

Background and Aim: Bone marrow transplantation (BMT) has developed from a risky and experimental treatment in the 1960s into a routine life-saving procedure for patients with specific types of acute leukemia, lymphomas, and other cancers. Only in the mid-1980s did BMT become an accepted treatment procedure.
Methods: In 1985, our group in Heidelberg has discovered that stem cells derived from peripheral blood could be used successfully for transplantation in lieu of the bone marrow. Within the 30 years, hematopoietic stem cells harvested from the peripheral blood instead of the bone marrow have become the preferred and standard source for transplantation because they induce a more rapid recovery of the hematopoietic and immune systems. Harvesting peripheral HSC is also more comfortable for the donor.
Results: In these 30 years, we have shown that specific diseases might derive benefit from autologous HSCT whereas others only from allogeneic HSCT. In the allogeneic setting, the most powerful weapon against residual cancer cells and therefore responsible for long-term cure are the functional T lymphocytes, especially the T natural killer cells derived from the healthy donor. Recently, it has been demonstrated that T natural killer cells derived from the patient could be genetically manipulated in the laboratory to target cancer specific antigens like CD19, or CD20. Technologies have also been developed to enhance the proliferative activity of such cancer specific T cells such that they become more proliferative upon exposure to tumor antigens. This concept has been proven to be effective in early clinical trials, even in the face of refractoriness to chemotherapy or allogeneic transplantation.
Conclusion: These successful applications of genetically manipulated, cancer specific T cells (chimeric antigen receptor, CAR, T-cells) have aroused enthusiasm in the oncology community. In Heidelberg, preclinical preparations have been completed. This presentation will provide a review on the development of cell ther-

apy for cancer patients in Heidelberg, starting from HSCT to CAR T-cell Therapy.
Keywords: Bone Marrow Hematopoietic Stem Cell Transplantation CART Therapy

Is-044: Acute Inflammation is Required for Muscle Regeneration

Ping Hu, Xin Fu, Lele Yang

1211 New Biochemistry Building, Shanghai Institute of Biochemistry and Cell Biology, 320 Yueyang Road, Shanghai, 200031, China
Email: Hup@sibcb.ac.cn

Background and Aim: Skeletal muscle regeneration involves a series of physical responses after injury or disease, including activation of quiescent satellite cells (muscle stem cells), proliferation of satellite cells and myoblasts, differentiation of myoblasts, and formation of new myofibers. In recent years, more and more evidences suggested that inflammation plays important roles during muscle regeneration process. However, how inflammation affects muscle regeneration remains to be elusive.
Methods: We used in vitro culturing and cell transplantation to study the functions muscle stem cells.
Results: Upon muscle injury, we observed large amount of T cell infiltrated at injury site. In immunodeficient mice, where the T cell infiltration is diminished while other liphocytes such as macrophage infiltration remains normal, reparation of muscle injury was dramatically delayed. To further investigate the mechanism of T cell promoting muscle regeneration, we characterized the protein profile of activated T cells. A combination of four factors was identified to be able to promote satellite cell proliferation and long term expansion dramatically in culture. The cultured expanded satellite cells continue to express muscle stem cell marker, and were able to regenerate functional myofiers in vivo. Furthermore, muscular injection of the four factor cocktail could rescue the muscle regeneration defects caused by T cell deficiency.
Conclusion: Our results demonstrate that T cell mediated inflammation is required for muscle stem cell proliferation at early stage of post-injury regeneration.

Keywords: Muscle Stem Cells, Acute Inflammation, Muscle Regeneration

Is-045: Msi2 Regulates Myogenesis by Repressing Microrna Processing

Lele Yang, Yan Liu, Sheng Li, Jingyi Hui, and Ping Hu

State Key Laboratory of Cell Biology, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, 320 Yueyang Road, Shanghai 200031, China
Email: Hup@sibcb.ac.cn

Myogenesis is a well-orchestrated multi-step process. Several transcription factors such as MyoD, Myf5, myogenin have been shown to be critical for this process. Recently, the so called myomiRs including miR1, miR133, and miR206 have also been demonstrated to be critical for myogenesis. More layers of regulation of myogenesis need to be investigated.
We reported that the RNA binding protein Msi2 displayed special functions in muscle stem cell differentiation. Msi2 is usually highly expressed in stem cells and considered to be an oncogene. Here we show that Msi2 is required for muscle stem cell differentiation by repressing the processing of miR7. miR7 targeted Rb1 during myogenesis to inhibit muscle differentiation. Msi2 mediated miR7 processing inhibition reduced the miR7 level upon differentiation cues and allowed the differentiation to process. In muscle stem cells, Msi2 promotes muscle differentiation by increasing the expression of the tumor suppressor Rb1.

Is-046: Tumor Microenvironment and Potential Therapies

Reza Izadpanah

School of Medicine Heart and Vascular Institute, Tulane University, New Orleans, LA, USA
Email: rizadpan@tulane.edu

Tumors develop following genetic and epigenetic alterations in cells. Tumor growth is strongly influenced by non-malignant cells that comprise the tumor microenvironment (TM). In addition to malignant cells, the TM in malignancies consists of many cell types, including in-

filtrative inflammatory cells, cells with stem-like properties, as well as some cells undergoing necrosis. Rapid tumor growth results in hypoxia and aberrant vascular proliferation as well as the infiltration of immune cells including macrophages, eosinophils, neutrophils, and T lymphocytes. The nonmalignant cells in TM become involved in tumor development via several mechanisms, including: (i) cell–cell and cell–matrix interactions that confer tumor cell resistance to apoptosis, (ii) local release of soluble mediators that promote survival and growth of tumor cells (crosstalk between stroma and tumor cells), (iii) direct cell-cell interactions within the tumors (crosstalk or oncologic trogocytosis), (iv) generation of specific niches within the TM that facilitate the acquisition of drug resistance, (v) conversion of the tumor cells to tumor-initiating cells or tumor stem cells. In these studies we further elucidated how the interactions among various cell types involve numerous cytokines that activate the release of inflammatory mediators, which in addition to promoting tumor proliferation, angiogenesis, and invasion, likely contribute to the molecular evolution of the tumor cells. In light of the tumor-tropic attribute of stem cells, we studied the effects of TM on the biological characteristics of stem cells recruited to TM. Specifically, we discuss how these effects are mediated via a strong crosstalk between malignant cells and stem cells by means of elements within the TM. Understanding the contribution of TM in cancer progression will enhance our knowledge of the reciprocal signaling among different cells in TM that promotes cancer growth and invasion, and potentially lead to the development of novel therapeutics.

Is-047: The Hematopoietic Potential of Mesenchymal Stem Cells and Therapeutic Possibilities

Reza Izadpanah

School of Medicine Heart and Vascular Institute, Tulane University, New Orleans, LA, USA
Email: rizadpan@tulane.edu

Mesenchymal and hematopoietic tissues are important reservoirs of adult stem cells. The potential of tissue resident mesenchymal stem cells (MSCs) to differentiate into cells of mesodermal and ectodermal lineages has been reported previously. Here we studied the potential of adherent MSCs in generating cells with hematopoietic characteristics. When cultured in differentiation media, clonally isolated MSCs develop into cells with hematopoietic attributes. The hematopoietic differentiated cells (HD) express early hematopoietic (c-kit, PROM1, CD4) as well as monocyte/macrophage markers (CCR5, CD68, MRC1, CD11b, CSF1R). Additionally, HD cells display functional characteristics of monocyte/macrophages such as phagocytosis and enzymatic activity of a-Naphthyl Acetate Esterase. HD cells are also responsive to stimulation by IL-4 and LPS as shown by increased CD14 and HLA-DRB1 expressions and release of IL-2, IL10, and TNF. Considering the presence of profuse amounts of MSCs in different tissues, these findings suggest the possible role HD cells derived from MSCs play in a number of hematopoietic diseases such as HIV-1 infection. We showed that MSCs differentiation into HD cells confers permissivity to HIV-1 infection. Also, our data indicate that HIV-1 exposure increased the expression of some hematopoietic lineage related genes in undifferentiated MSCs. These findings signify the importance of MSCs in HIV-1 research and facilitate the understanding of the disease processes. Taken together, these studies characterize the potential of MSCs to generate functional hematopoietic cells and therefore pave way for their possible use in cell therapy applications.

Is-048: The Dream of Functional Organ Engineering and Preclinical Transplantation

Abdolmohamad Kajbafzadeh

Pediatric Urology and Regenerative Medicine Research Center, Tehran University of Medical Sciences, Tehran, Iran (IRI)
Email: kajbaf105@gmail.com

This lecture contributes to the advancement of regenerative medicine with functional organ engineering and preclinical transplantation. Specifically, it will highlight that the extracellular matrix (ECM) scaffolds may be used to produce functional tissues and organs and eventually alleviate the severe demand for transplanted organs. The use of natural scaffolds to direct the stem cells toward the formation of functioning tissues have

pave the road for tissue engineering and regenerative medicine during the last two decades at our center. These natural scaffolds have promising results compared to synthetic biomaterial scaffolds used in the field of regenerative medicine. From September 1995 we designed our center based on production of different natural scaffolds and application of autologous stem cells /adult progenitor cells. The effect of more than 150 tissues such as decellularized pericardium, appendix, colon, prepuce, and gallbladder in bladder augmentation cystoplasty was evaluated to find applicable strategies to comfort shortage of donor tissues as the major limitation of the transplantation and offer novel therapies for patients with serious injuries. The role of non-immunologic matrices of urethral and corpous cavernosum was also investigated.

ECM has been considered as an ultimate scaffold which can preserve the active protein architecture for successful cell adhesion, migration, proliferation, differentiation, and mediates signal transduction. Presently, due to a severe kidney organ shortage, an adequate intracorporeal replacement for a well-designed and functional kidney organ with promising advancements in renal tissue engineering is of great value.

Production of an ideal artificial bladder for total bladder transplantation was a major challenge in the field of urology in the last two decades. A wide variety of biologic, nonbiologic, autologous, nonautologous alternatives have been recommended for partial bladder replacement. However, urinary tracts infection, stone formation, mucous formation, and absorption of excretory urinary products stomal complications, are among the complications following the application of these substitutes which have led the urologist to continue their research for other options. Furthermore, due to failure of different types of bladder augmentation in the last decades, finding promising techniques are required to replace the whole organ as a functioning bladder. However, the whole bladder engineering for transplantation is yet to be considered. Scaffold-less autologous smooth muscle cell sheet was used in our center for bladder reconstruction in rabbit model with promising long-term outcomes. Therefore, it is our hypothesis that the tissue engineered bladder by the application of seeded natural scaffolds would serve as the best option for bladder tissue regeneration.

Embryonic stem (ES) cells with their pluripotential nature, has been considered as a source for the multitude of cells that must organize to form practical renal structures and these cells have the ability to differentiate into renal cells and integrate into primordial kidney culture. In addition, natural ECM has been shown to cause the directed differentiation of ES cells. Extraction of the kidney whole-organ ECM, using the best detergent-based decellularization technique, and further cell seeding of these scaffolds with ES cells, can have promising results which can support embryonic cell attachment, proliferation, and differentiation.

Current treatment options focus on reducing the symptoms of fecal incontinence (FI) and improving quality of life through therapeutic and surgical strategies. Various interventions are used to improve continence ranging from dietary changes to surgery. There are several well-known surgical methods such as sphincter repair, artificial sphincter implantation, and sphincteroplasty that can enhance the maintenance of anal sphincter tone and closure pressure. These procedures are often not recommended unless in cases of severe FI, since they might be associated with numerous complications and a high failure rate. However, long-term satisfactory results have been obtained in other processes such as injection of biomaterials. More recently, the application of decellularized ECM and acellular dermal matrix (ADM) in closure of anal fistula has been considered as a more effective mean for treating external anal sphincters (EAS) muscle deficiencies. Various studies have shown that this method requires minimal healing period with less pain and more notable results. In our previous modality, the recapitulation of a myogenic program after auto-grafting of satellite cells and the improvement of electromyography profile were successfully demonstrated in FI rabbit models after 6 months of follow-up. Researchers have built the first functional anal sphincters in the laboratory, suggesting a potential future treatment for both fecal and urinary incontinence. We have demonstrated the regaining histological characteristics of bioengineered EAS in rabbit fecal incontinence model.

In conclusion, there is cautious optimism that the field of tissue engineering can play a crucial role in the management of several system diseases. In addition, safety and effectiveness of engineered tissues with or without cell seeding are the most critical issues to be addressed

in more experimental studies as a platform prior to and during clinical trials.
Keywords: Tissue Engineering, Organ Engineering, Organ Transplantation, Combined Bioreactor, Stem Cells, Tissue Augmentation, Organ Augmentation

Is-049: Gene Therapy of β-Globinopathies: An Overview

Carsten Werner Lederer, Marina Kleanthous

Department of Molecular Genetics Thalassaemia, the Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus & Cyprus School of Molecular Medicine, Nicosia, Cyprus
Email: lederer@cing.ac.cy

Background and Aim: This presentation will summarize the current state of play in the gene therapy of β-globinopathies, sickle cell anemia and β-thalassemia.
Results: In preclinical research, substantial success has been achieved in the correction of critical disease parameters by all three principal therapeutic approaches of (i) gene addition of β-globin-like transgenes, (ii) repair of the primary mutation by genome editing and (iii) functional correction of β-globin deficiency by re-activation of the primarily fetal γ-globin chain. Gene addition is the longest-established of these approaches and is the only one as yet applied in the clinic, with encouraging results for sufferers of both types of β-globinopathies. Repair of the primary mutation is still hampered by low efficiencies in primary cells but is based on still nascent genome editing technology with substantial scope for improvement. Finally, the greatest diversity of strategies is dedicated to the activation of fetal hemoglobin, including genome editing, expression of synthetic transcription factors and RNA-interference-mediated knockdown of γ-globin repressors.
Conclusion: Approaches (i) through (iii) have all been vindicated by extant data and, pending optimization of safety and efficacies, may lead to therapeutic outcomes if applied to patients. Their long-term prospects for clinical application will moreover depend on additional factors, including cost and market size for corresponding medical products, further technical improvements and changing policies relating to advanced therapies, and are therefore hard to gauge at this juncture.

Keywords: Anemia, Sickle Cell, beta-Thalassemia, Genetic Therapy, Gene Editing, Fetal Hemoglobin

Is-050: Gene Therapy of β-Thalassemia: Getting Personal

Petros Patsali[1,2], Constantinos Loucari[1,3], Panayiota Papasavva[1,3], Coralea Stephanou[1,2], Claudio Mussolino[4], Thamar B. van Dijk[5], Giandomenico Turchiano[4], Soteroulla Christou[6], Maria Sitarou[6], Sjaak Philipsen[5], Michael N Antoniou[2], Toni Cathomen[4], Carsten Werner Lederer[1,3], Marina Kleanthous[1,3]

1. Department of Molecular Genetics Thalassaemia, the Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
2. Department of Medical and Molecular Genetics, King's College London, London, SE1 9RT, UK
3. Cyprus School of Molecular Medicine, Nicosia, Cyprus
4. Institute for Cell and Gene Therapy, University Medical Center Freiburg, Freiburg, Germany
5. Erasmus University Medical Center, Rotterdam, the Netherlands
6. Thalassaemia Centre, Ministry of Health, Cyprus
Email: lederer@cing.ac.cy

Background and Aim: Thalassemia is caused by deficient production of α- or β-globin. The latter form, β-thalassemia, has particular clinical relevance and is common in many regions historically affected by Malaria, with 6% carrier rate in Iran and with 12% as the worldwide highest national rate in Cyprus. As a severe monogenic disease of the hematopoietic system, β-thalassemia is an ideal target for gene therapy, either by gene addition or gene correction, and here we present three different strategies pursued in our laboratory towards that goal. Two of those strategies are specific for the common β-thalassemia mutation, *HBB*$^{IVS1\text{-}110(G>A)}$ (relative carrier frequencies of 78% in Cyprus and 10% in Iran), which introduces an aberrant splice acceptor site in intron 1 of the β-globin gene and which in homozygotes leads to severe, chronically transfusion-dependent thalassemia.
Methods: We performed functional analyses in hematopoietic cell models and in primary β-thalassemia patient-derived erythroid precursor cells, to evaluate treatment by β-globin gene addition, by RNA interference against aberrant mRNA species and by genome

editing using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 or transcription activator-like effector nucleases (TALENs).
Results: (i) We applied RNA interference against aberrant transcripts produced from the *HBB*$^{IVS1-110(G>A)}$ mutant locus in order to reduce their interference with normal β-globin expression. As monotherapy this achieves extremely significant induction of β-globin expression from the mutant locus in humanized murine erythroleukemia cells transgenic for the human mutant gene (MEL-*HBB*$^{IVS1-110(G>A)}$) and in *HBB*$^{IVS1-110(G>A)}$-homozygous HSPCs. In combination with β-globin gene addition using the GLOBE vector, RNA interference achieves significant improvement of disease parameters in *HBB*$^{IVS1-110(G>A)}$-homozygous HSPCs compared to GLOBE treatment alone.
(ii) Based on CRISPR/Cas9 or TALENs, we established designer nucleases targeting the *HBB*I$^{VS1-110(G>A)}$ mutation, in order to achieve high-efficiency functional correction of β-thalassemia through non-homologous end joining and its disruption of the aberrant splice acceptor site. Plasmid delivery in MEL-*HBB*$^{IVS1-110(G>A)}$ achieved extremely significant induction of β-globin expression, and DNA-free delivery in HBB$^{IVS1-110(G>A)}$-homozygous HSPCs likewise achieves extremely significant induction of β-globin expression and correction of erythroid differentiation, at transfection efficiencies of immediate clinical relevance.
(iii) Based on CRISPR/Cas9 nucleases, we targeted the XL isoform of the γ-globin repressor BCL11A for disruption, towards functional analysis of its conserved sequence motifs and towards a universal therapeutic approach to β-globinopathies. Conclusion: Our research exemplifies the diverse field of β-thalassemia gene therapy and establishes innovative mutation-specific gene-therapy approaches as promising strategies for clinical translation.
Keywords: Beta-Thalassemia, RNA Interference, Genetic Therapy, Gene Editing, Fetal Hemoglobin, Erythroid Precursor Cells

Is-051: Application of the Human Liver Organoid Platform to Study HBV Infection, Pathogenesis and Tumorigenesis

Tokameh Mahmoudi

Department of Biochemistry, Erasmus University Medical Center, Ee-634 PO Box 2040 3000CA Rotterdam, the Netherlands
Email: t.mahmoudi@erasmusmc.nl

The liver organoid platform is a game changing stem cell-based in vitro 3D culture system that allows expansion, banking and differentiation of hepatocytes. Although persistent Hepatitis B virus (HBV) infection remains the leading cause of liver cirrhosis and hepatocellular carcinoma, the lack of a relevant primary untransformed model system has greatly hampered the study of the molecular events that occur as consequence of HBV infection and which mediate onset of hepatocellular carcinoma. We have applied the human liver organoid technology to the study of HBV infection and HBV-induced tumorigenesis. We have generated several HBV infected patient and healthy liver organoid culture lines that were seeded from surgically explanted tissue. Human liver organoids were ex vivo infected with both recombinant virus as well as HBV infected patient serum. Ex vivo infected liver organoids expressed HBV proteins HBS Ag and HBV core, and produced infectious virus. We have also generated transgenic liver organoid lines including those that exogenously express the HBV receptor NTCP or the viral gene HBX. Insights from our studies using the HBV infected or patient-derived organoid platforms as to the determinants of viral infection and replication, pathogenesis and pathways towards tumorigenesis will be discussed.

Is-052: Advanced Polysaccharide-Based Hydrogels for Tissue Engineering and Regenerative Medicine

João F Mano

Department of Chemistry, CICECO, University of Aveiro, Aveiro, Portugal
Email: jmano@ua.pt

The organization and dynamics of the stem cell niche provides valuable cues for the development of biomimetic environments that could have potential to stimulate the regenerative process. In vitro such highly hydrated 3D environments can be partially recreated using

hydrogels. We propose the use of polysaccharide-based biomaterials to produce hydrogels able to encapsulate different cell types. In fact, due to their hydrophilic nature and richness in chemically active groups, such polymers can be used to produce a variety of crosslinked structures fabricated using aqueous-based or other environmental-favorable procedures. Examples are shown on such hydrogels obtained with distinct shapes, internal organization and sizes. Such biomaterials encapsulating mesenchymal stem cells may be used as implantable devices to regenerate tissues. Alternatively, in order to avoid diffusion limitation of nutrients to the cells location sites, hydrogel particles may be used to support cellular organization over their surface, acting as cells supports for injectable scaffolds. By decorating the surface with antibodies these particles are able to recruit specific cell populations, enhancing the therapeutic potential of such system. Polysaccharides may be also used to coat liquefied capsules that may entrap viable cells, using the layer-by-layer technology. The presence of solid microparticles inside such capsules offers adequate surface area for adherent cell attachment increasing the biological performance of these hierarchical systems, while maintain both permeability and injectability. The liquid environment allows for a free-organization in the space of the cells towards the formation of new microtissues. The compartmentalization of distinct cell types (including mesenchymal stem cells and endothelial cells) may enhance the osteogenic capability of this system that could be useful in bone tissue engineering applications.

Keywords: Hydrogels Scaffolds Compartments Stem Cells Polymers

Is-053: Nanostructured Films to Control Cell Behaviour

João F Mano

Department of Chemistry, CICECO, University of Aveiro, Aveiro, Portugal
Email: jmano@ua.pt

Tissue Engineering has been integrating principles of engineering, chemistry, materials science, biology and health sciences in order to develop regenerative-based therapeutic strategies combining stem cells and biomaterials. The development of hybrid devices for tissue engineering are often inspired by the composition and complexity of native tissues. At the lowest level of such organization, one should select the adequate biomaterials to be used as the building block of the structure that will support cells and control their behaviour towards the production of new tissue. Nanostructured multilayered films have been often fabricated using the layer-by-layer technology, where consecutive layers of macromolecules are well stabilized by electrostatic interactions or other weak forces. Such multilayered could be then integrated in more complex porous macroscopic devices, often exhibiting a multi-scale organization. Using adequate templates, non-flat multilayers can be fabricated with tuned compositions along the build-up assembly, including patterned membranes or porous devices. This enables the production of very well controlled multifunctional and structural devices using mild processing conditions that could be useful in biomedicine, including in tissue engineering. In particular we have been interested in developing more complex/hierarchical porous structures using natural-based polymers that could fulfil specific requirements in such kind of applications. Methodologies developed in our group will be exemplified, permitting the production of (i) 3-dimensional (open) porous nanostructured scaffolds for tissue engineering, enabling the support of cells, by combining LbL and rapid prototyping technology; and (ii) free-standing films featuring patterns to control cell orientation or micro-wells to provide local three-dimensional environments to the cells.

Keywords: Nanobiomaterials Films Scaffolds Biopolymers

Is-054: Improved Tissue Cryopreservation using Radio Frequency Inductive Heating of Magnetic Nanoparticles

Navid Manuchehrabadi[1,4], Zhe Gao[2], Jinjin Zhang[3], Hattie L. Ring[2], Qi Shao[4], Feng Liu1, Michael McDermott[2], Alex Fok[5], Kelvin G. M. Brockbank[6], Michael Garwood[7], Yoed Rabin[8], Christy L. Haynes[2], John C. Bischof[1,4]

1. Department of Mechanical Engineering, University of Minnesota, Minneapolis, MN 55455, USA

2. Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA
3. Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN 55455
4. Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455, USA
5. Minnesota Dental Research Center for Biomaterials and Biomechanics, University of Minnesota, Minneapolis, MN 55455, USA
6. Department of Bioengineering, Clemson University, Clemson, SC 29634, USA. and Tissue Testing Technologies LLC, North Charleston, SC 29406, USA
7. Department of Radiology, University of Minnesota, Minneapolis, MN 55455, USA
8. Department of Mechanical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213, USA
Email: manuc001@umn.edu

Background and Aim: Vitrification, a kinetic process of liquid solidification into glass, poses many potential benefits for tissue cryopreservation including indefinite storage, banking, and facilitation of tissue matching for transplantation. To date, however, successful rewarming of tissues vitrified in VS55, a cryoprotectant solution, can only be achieved by convective warming of small volumes on the order of 1 ml. Successful rewarming requires both uniform andfast rates to reduce thermal mechanical stress and cracks, and to prevent rewarming phase crystallization.
Methods: We present a scalable nanowarming technology for 1- to 80-ml samples using radiofrequency-excited mesoporous silica–coated iron oxide nanoparticles in VS55. Advanced imaging including sweep imaging with Fourier transform and microcomputed tomography was used to verify loading and unloading of VS55 and nanoparticles and successful vitrification of porcine arteries. Nanowarming was then used to demonstrate uniform and rapid rewarming at >130°C/min in both physical (1 to 80 ml) and biological systems including human dermal fibroblast cells, porcine arteries and porcine aortic heart valve leaflet tissues (1 to 50 ml).
Results: Nanowarming yielded viability that matched control and/or exceeded gold standard convective warming in 1- to 50-ml systems, and improved viability compared to slow-warmed (crystallized) samples. Last, biomechanical testing displayed no significant biomechanical property changes in blood vessel length or elastic modulus after nanowarming compared to untreated fresh control porcine arteries.
Conclusion: In aggregate, these results demonstrate new physical and biological evidence that nanowarming can improve the outcome of vitrified cryogenic storage of tissues in larger sample volumes.
Keywords: Vitrfication, Organ Banking, Magnetic Nanoparticle, Nanowarming

Is-055: New Insights in Cell Therapy of Diabetes using Pluripotent Stem cells

Mohammad Massumi, Andras Nagy

Institute of Medical science University of Toronto, Toronot, M5T 3H7
Email: mohammad.massumi@utoronto.ca

Background and Aim: Type 1 Diabetes (T1D) is characterized by the autoimmune destruction of pancreatic beta-cells and the need for insulin therapy to control hyperglycemia. According to the World Health Organization, more than 350 million people worldwide suffer from diabetes. In some cases, pancreatic islet cell transplantation can reverse hyperglycemia in T1D and negate the use of insulin therapy. Unfortunately, donor islet scarcity, ultimate graft failure and the required use of potentially harmful immune-suppressive drugs have restricted their use for the treatment of T1D. Insulin-producing beta-like cells generated from embryonic stem (ES) cells or induced pluripotent stem (iPS) cells offer potential regenerative medicine approach that could be used instead of primary islet cell transplantation. We and other scientists from Harvard and University of British Columbia have shown that functional Beta cells can be generated in vitro. In this talk, I will go over of the recent achievements in the generation of pancreatic beta cells and the use of the new niches in hosting of insulin-producing cells.
Methods: In the first study, we used the pluripotent stem cells for generation of pancreatic Beta cells through a five-stage protocol. In the second study, we used safe embryonic stem cells to generate a niche to host the insulin-producing cells.
Results: Our result showed that 35% of differentiated cells are mono-hormonal insulin positive cells, 1% insulin and glucagon-positive cells and 30% insulin and

NKX6.1 co-expressing cells. Functionally, ES-derived beta cells were responsive to high glucose in static incubation and perfusion studies and could secrete insulin in response to successive glucose stimulations. In the second we showed that the generated niche from safe stem cells could host insulin-producing cells properly and deliver the produced insulin into the whole body and reverse hyperglycemia in diabetic mice
Conclusion: In conclusion, we have developed an abbreviated and simplified in vitro protocol for the generation of glucose-responsive, ES-derived beta-like cells. The majorities of the insulin-producing cells were mono-hormonal and demonstrated many key characteristics of mature beta cells.
Keywords: iPS Cells, Beta Cells, Pancreas

Is-056: Cell Based Therapies in Gastrointestinal and Liver Disorders: Current Status and Future Prospects

Mehdi Mohamadnejad

Digestive Diseases Research Institute, Tehran University of Medical Sciences, Tehran, Iran
Email: mehdi.nejad@gmail.com

Progress in the field of stem cell research and the identification of organ-specific stem cells provides hope for the use of stem cells in the treatment of gastrointestinal and liver disorders. Embryonic stem cells and induced pluripotent cells have the potential to give rise to any cell type in the human body, however their therapeutic application in human remains challenging.
There are several potential mechanistic approaches to benefit from cell-based therapies in gastrointestinal and liver disorders. First, stem cells may be used to regenerate and replace damaged cells in disorders like acute liver failure, or liver-based hereditary metabolic disorders. Second, stem cells or their products may ameliorate inflammatory cascade and help to treat disorders like inflammatory bowel disease, peri-anal fistulas, or acute liver failure. Third, manipulated immune cells could exert anti-tumor properties and help to treat solid tumors like colon cancer.
Here, we describe recent advances on the therapeutic potentials of cell-based therapies in gastrointestinal and liver disorders.

Is-057: Stem Cell Therapy in Multiple Sclerosis: Advances and Hopes

Seyed Massood Nabavi[1,2]

1. Neurology group, Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Center for Neuroscience and Cognition, MS Clinic and Research Unit, Royan institute, Tehran, Iran
Email: nabbvi@gmail.com

The advances in technology of stem cells in the recent decade opened an opportunity window to consider different types of these cells as potential therapy for some chronic disabling neurological diseases like multiple sclerosis (MS).
There are some safety results in application of Mesencheymal stem cells (MSCs) in MS. some other limited open label trials emphasis to the efficacy of them.
MSCs in these trials are autologous and have been obtained from Bone marrow or Placenta.
There is also some promising data of application of autologous hematopoietic stem cells (AHSC) in MS which seems to be promising.
In this review presentation we try to address the promises of stem cell therapy in MS and also we discuss some new clinical trials in cell therapy in MS as sponsoring investigator or as a co-investigator.
Keywords: Stem cell, MS, AHSC, Therapy

Is-058: Personalized Stem Cell Medicine

Karim Nayernia[1,2]

1. International Center for Personalized Medicine (P7Medicine),
Dusseldorf, Germany/ London, UK/ Toronto, Canada/ Istanbul, Turkey/ Tehran, Iran
2. International Stem Cell Academy,
Dusseldorf, Germany/ Cologne, Germany
Email: prof.nayernia@gmail.com

In recent development in stem cell research, it was shown that every cell in our body can be turned to stem cells, so called induced pluripotent stem cells (iPSC). Induced pluripotent stem cells allow generation of

stem cells capable of making all cell types in the body from adult cells of any human, including patients with genetic diseases. Such cell lines allow modelling the genetic disease progression in the Petri dish. iPSCs could be made for a patient, then used to create a laboratory culture of, for example, cardiac cells, in the case of a patient with myocardial diseases. The efficacy and safety of various drugs could then be tested on the cultured cells, and doctors could use the results to help determine the best treatment. This approach could move much of the trial-and-error process of beginning a new personalized treatment. Among the most exciting breakthroughs driving personalized medicine is stem cell therapy, or more specifically, autologous stem cell treatments using patient-specific stem cells. Several studies are showing that stem cell treatments conducted using patient specific stem cells, for example iPSC or mesenchymal stem cells, can effectively treat, and even in some cases reverse disease. This makes stem cell therapy combined with gene therapeutic approaches is a highly attractive alternative to current drug-based and physical therapy treatments, which tend to only temporarily manage symptoms.
The objective of the talk is to explain different applications of stem cells in personalized medicine.

Is-059: Personalized Stem Cell Therapy of Male Infertility

Karim Nayernia[1,2]

1. International Center for Personalized Medicine (P7Medicine),
Dusseldorf, Germany/ London, UK/ Toronto, Canada/ Istanbul, Turkey/ Tehran, Iran
2. International Stem Cell Academy,
Dusseldorf, Germany/ Cologne, Germany
Email: prof.nayernia@gmail.com

Infertility refers to an inability to conceive after having regular unprotected sex. Infertility can also refer to the biological inability of an individual to contribute to conception, or to a female who cannot carry a pregnancy to full term. In many countries infertility refers to a couple that has failed to conceive after 12 months of regular sexual intercourse without the use of contraception. About 20% of cases of infertility are due to a problem in the man. Many cases of apparent infertility are treatable. Infertility may have a single cause in one of the partners, or it could be the result of a combination of factors. Significant progress has been made in several fields of medicine towards personalizing treatment recommendations based on individual patient genotype. As the number of clinical and genetic biomarkers available to physicians has increased, predictive models able to integrate the contributions of multiple variables simultaneously have become valuable tools for medical decision making. Leveraging genotype information and multivariate predictive models holds the promise of bringing greater efficiency to, and reducing the costs of, fertility treatments. To follow this approach, we developed a precision medicine strategy, *FERTILITY ASSAY*$^{\text{MALE}}$, and a personalized medicine technique, *FERTITHERA*, for personalized diagnostics and therapy of male infertility. *FERTILITY ASSAY*$^{\text{MALE}}$ is a genomic diagnostic test for more accurate cellular characterization of male infertility. The test will support the physician to identify the cellular causes of the male infertility, avoid unnecessary treatment and provide a personalized therapy of male infertility. Based on the precision diagnostics of male infertility, a personalized cell therapy will be designed for the patient using his own stem cells.
This revolutionary stem cell treatment offers significant results in certain cases of Azoospermia.

Is-060: Fabrication of Biocompatible Organic-Inorganic Hybrid Hydrogels to Reconstruct Ligament to Bone Interface

Ali Negahi Shirazi, Fariba Dehghani

School of Chemical and Biomolecular Engineering, the University of Sydney, Sydney, Australia
Email: alinegahi@gmail.com

Background and Aim: The organic-inorganic hybrids fabricated by the sol-gel method are intrinsic bioactive materials with extensive applications in bone tissue engineering. The brittleness and limited water uptake capacity of these monoliths, however, restrict their applications for engineering the soft tissues and their interfaces with bone. To address these challenges, a unique class of organic-inorganic hybrid was developed

in which polymer crosslinking ceased the over-condensation of a bioactive glass component and eradicated the formation of brittle structure.
Methods: In this study, an organosilane-functionalized gelatin methacrylate (GelMA) was covalently bonded to a bioactive glass during the sol-gel process, and the condensation of silica networks was controlled by photocrosslinking of GelMA. The physicochemical properties and mechanical strength of these hybrid hydrogels were then tuned by the incorporation of secondary crosslinking agents such as poly (ethylene glycol diacrylate) (PEGDA).
Results: The resulting bioresorbable hydrogels displayed elastic properties with ultimate elastic compression strain above 0.2 (mm/mm) and tuneable compressive modulus in the range of 42-530 kPa. The swelling ratio of these hybrids, however, was suitable for tissue engineering applications. In addition to remarkable enhancement in the mechanical properties of gelatin-based hydrogels, their structural integrity was significantly increased. As an example, these hybrid hydrogels kept their structures for more than 28 days, and only 30% of gelatin was released during this period in simulated body fluid. The presence of homogeneously distributed bioactive glass in these hydrogels, moreover, promoted the precipitation of calcium phosphate particles as the main inorganic compositions of the bone extracellular matrix. The continuous increase of alkaline phosphatase activity of bone progenitor cells for at least 28 days post-culture confirmed the osteoconductive properties of these hybrid hydrogels. The in vivo mice-subcutaneous implantation, moreover, confirmed the biocompatibility and bio-resorption of these hydrogels. A bioactive hydrogel with a gradient of mineralisation was also fabricated to confirm the feasible application of these hybrid hydrogels in interface tissue engineering.
Conclusion: In summary, an organic-inorganic hybrid was developed that has favourable swelling properties and higher mechanical strength compared to ceramic based scaffolds. These hybrids were also bioactive, cytocompatible and bioresorbable. These gelatin-bioactive glass hydrogels can be used for regeneration of bone defects. It can also be used for the fabrication of gradient bioactive hydrogels for enhancing the integration of soft to hard tissue interfaces such as ligament and tendon.

Keywords: Bone Tissue Engineering, Organic-inorganic Hybrid, Hydrogel, Scaffold, Biocompatible

Is-061: Optimisation of an Explant Technique for Initiation and Propagation of Limbal Mesenchymal Stromal Cell (L-MSC) Cultures from Human Cadaveric Tissue

Elham Nili[1], Damien G. Harkin[1], Neil Richardson[1], Rebecca Dawson[2]

1. Queensland Eye Institute, School of Biomedical Sciences, Institute of Health and Biomedical Innovation, Faculty of Health, Queensland University of Technology, Brisbane, Queensland 4001, Australia
2. School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Brisbane, Queensland 4001, Australia
Email: elham.niliahmadabadi@hdr.qut.edu.au

Background and Aim: There is convincing evidence that limbal mesenchymal stromal cells (L-MSC) contribute to the maintenance and repair of the corneal stroma. Nevertheless, currently there is no consensus regarding optimal protocols for the isolation and cultivation of L-MSCs for therapeutic use. This study reports a comparison of two attachment techniques for isolation of L-MSCs using explanted limbal tissue: 1) Attachment Factor (AF) or, 2) immersion in a collagen I gel. Additionally, the potential of two different culture media; 1) DMEM containing 10% fetal bovine serum or 2) serum free "knockout" stem cell medium (SCM), to promote L-MSC expansion, were determined. The results of this study suggest that the combination of collagen immersion and DMEM serum supplemented medium offers the greatest potential to establish and expand L-MSC cultures from explanted human donor tissue.
Method: 1- Explant technique: 2mm explants were prepared from limbal stroma excised from 5 cadaveric donors (n=5). Explants were then adhered to tissue culture plastic using Attachment Factor (AF) or immersion in a collagen I gel. Culture success was assessed according to % explants with outgrowth after 7, 10 and 14 days. 2- Culture medium: L-MSC cultures were initiated from six donor limbal rims using the collagen immersion technique then cultured with either DMEM

or "knockout medium" for 5, 10, 15 and 20 days. A Resazurin assay was conducted to measure the metabolic activity for each culture at 20 days.
Results: 1- Explants established through use of collagen immersion consistently produced a significantly higher number of successful cultures per donor that using AF ($p<0.05$). Moreover, the morphology of L-MSCs cultured in collagen was more characteristic of L-MSCs in vivo. 2- Significantly ($P<0.5$) higher growth of L-MSCs was observed in in SCM after 20 days.
Conclusion: Our results suggest that the combination of collagen immersion and use of DMEM serum supplemented medium has potential for generating MSC cultures from limbal stromal explants.
Keywords: Cornea, Limbus, Stroma, Stem Cells, MSC

Is-062: HDAC7 is a Repressor of Myeloid Genes Whose Downregulation is Required for Transdifferentiation of Pre-B Cells into Macrophages

Maribel Parra

Cancer Epigenetics and Biology Program Bellvitge Biomedical Research Institute, Barcelona, Spain
Email: mparra@idibell.cat

Background and Aim: B lymphopoiesis is the result of several cell commitment, lineage choice and differentiation processes. Every differentiation step is characterized by the activation of a new, lineage-specific, genetic program and the extinction of the previous one. To date, the central role of specific transcription factors in positively regulating these distinct differentiation processes to acquire a B cell specific genetic program is well established. However, the existence of specific transcriptional repressors responsible for the silencing of lineage inappropriate genes remains elusive.
Methods: Here we addressed the molecular mechanism behind repression of non-lymphoid genes in B cells. We report that the histone deacetylase HDAC7 was highly expressed in pre-B cells but dramatically down regulated during cellular lineage conversion to macrophages.
Results: Microarray analysis demonstrated that HDAC7 re-expression interfered with the acquisition of the gene transcriptional program characteristic of macrophages during cell transdifferentiation; the presence of HDAC7 blocked the induction of key genes for macrophage function, such as immune, inflammatory and defense response, cellular response to infections, positive regulation of cytokines production and phagocytosis. Moreover, re-introduction of HDAC7 suppressed crucial functions of macrophages, such as the ability to phagocytose bacteria and to respond to endotoxin by expressing major pro-inflammatory cytokines. To gain insight into the molecular mechanisms mediating HDAC7 repression in pre-B cells, we undertook co-immunoprecipitation and chromatin immunoprecipitation experimental approaches. We find that HDAC7 specifically interacted with the transcription factor MEF2C in pre-B cells and was recruited to MEF2 binding sites located at the promoters of genes critical for macrophage function.
Conclusion: In B cells HDAC7 is a transcriptional repressor of undesirable genes. Our findings uncover a novel role for HDAC7 in maintaining the identity of a particular cell type by silencing lineage inappropriate genes.
Keywords: Histone Deacetylases HDAC B Llymphocytes Ttranscriptional Repression

Is-063: Gene Silencing Mechanisms in Hematopoietic Stem Cells and B Lymphocyte Progenitors

Maribel Parra

Cancer Epigenetics and Biology Program Bellvitge Biomedical Research Institute, Barcelona, Spain
Email: mparra@idibell.cat

Background and Aim: Class IIa Histone Deacetylases (HDACs) subfamily members are tissue-specific gene repressors with crucial roles in development and differentiation processes. A prominent example is HDAC7, a class IIa HDAC that shows a lymphoid-specific expression pattern within the hematopoietic system.
Methods: We have explored its potential role in B cell development by generating a conditional knockout mouse model.
Results: Our study demonstrates for the first time that HDAC7 deletion dramatically blocks early B cell development and gives rise to a severe lymphopenia in peripheral organs, while leading to pro-B cell lineage

promiscuity. We find that HDAC7 represses myeloid and T lymphocyte genes in B cell progenitors, through interaction with myocyte enhancer factor 2C (MEFC2). In B cell progenitors HDAC7 is recruited to promoters and enhancers of target genes and its absence leads to increase enrichment of histone active marks. More preliminary results from our laboratory demonstrate that HDAC7 is also essential for proper hematopoietic stem cells haemostasis.
Conclusion: Our results prove that HDAC7 is a bona fide transcriptional repressor essential for HSC function and B cell development.
Keywords: HDAC B Cell Development Hematopoietic Stem Cells Gene Transcriptional Repression

Is-064: A New Biomimetic Nanocomposite Material for Bone Regeneration

Shirin Parsno[1,2], Alexander Seifalian[3]

1. Centre for Nanotechnology and Regenerative Medicine, UCL, London, UK
2. Nova Dental Care Centre, London, UK
3. Centre for Nanotechnology and Regenerative Medicine Commercialisation (Ltd), the London BioScience Innovation Centre, London, UK
Email: shirin.pa@gmail.com

Background and Aim: Losing teeth is not just an aesthetic issue; it effects facial bone also and could cause long-term structural bone problems. Bone is similar to the rest of human tissue in that it requires stimulation in order to maintain strength such as muscle or density such as bone. During chewing and talking, teeth make contact with each other. Through contact, stresses are transmitted via the periodontal ligament that surrounds the teeth, stimulating the bone to remodel and regenerate continually. Hence, missing teeth do not just effect the immediate surrounding bone, i.e. alveolar, but affect the entire maxillofacial bone. The aim of this research is to repair and regenerate degenerative bone by repairing the missing teeth with teeth implants.
Methods: 12% nanodiamond nanoparticles were added to the polyhedral oligomeric silsesquioxane poly (carbonate-urea) urethane to enhance the hydrophobicity and mechanical properties. Then, the materials were printed to make the 3D scaffold. The scaffold was filled with nanohydroxyapatite via a multichannel in order to promote osseointegration and at the same time, the nitric oxide (NO) donor was incorporated with the nanocomposite materials for eluting NO in order to enhance the healing of the bone with the surrounding structure.
Results: The material's characterisation, NO eluting will be presented and the preliminary toxicology in vitro showed that materials are not toxic.
Conclusion: The preliminary data showed that materials are promising for bone regeneration. Current focus are assessment of preclinical model studies on osseointegration and bone regeneration.

Is-065: A Novel Method for the Delivery of Therapeutic Oligonucleotides in Muscle

Styliana Philippou[1, 2], Nicolaos P. Mastroyiannopoulos[1, 2] and Leonidas A. Phylactou[1,2]

1. Department of Molecular Genetics, Function and Therapy, the Cyprus Institute of Neurology and Genetics, P O Box 23462, 1683 Nicosia, Cyprus
2. The Cyprus School of Molecular Medicine, the Cyprus Institute of Neurology and Genetics, P O Box 23462, 1683 Nicosia, Cyprus
Email: laphylac@cing.ac.cy

A successful gene therapy protocol for muscular dystrophies requires systemic delivery to reach all affected muscles throughout the body. Up until now, there have been no successful attempts able to overcome this difficulty and therefore, current applications are limited due to poor specificity and large diversity in efficacy. As a result, a substantial amount of the therapeutic molecules does not reach the affected muscles, but instead travels to other tissues or clears quickly from the circulation. By coupling the sequences with a target-specific vehicle the tissue specificity during delivery could be improved. Aptamers are a new class of small, synthetic, single stranded nucleic acids that fold into unique secondary structures. Results will be presented which show the identification of the first RNA aptamer that specifically recognizes and enters skeletal muscle cells. This aptamer may serve as a muscle specific delivery vehicle for a wide spectrum of therapeutic sequences thus opening a new era of safe and targeted aptam-

er-mediated therapies for the broader group of muscular dystrophies.
Keywords: Aptamer, Muscle, Muscular Dystrophy, Gene Delivery

Is-066: Novel Oligonucleotides for the Therapy of Muscular Dystrophy

Nikolas Mastroyiannopoulos[1, 2], Melina Christou[1, 2], Jesper Wengel[3], Michaella Georgiadou[1], and Leonidas A. Phylactou[1, 2]

1. Department of Molecular Genetics, Function and Therapy, The Cyprus Institute of Neurology and Genetics, P O Box 23462, 1683 Nicosia, Cyprus
2. The Cyprus School of Molecular Medicine, the Cyprus Institute of Neurology and Genetics, P O Box 23462, 1683 Nicosia, Cyprus
3. University of Southern Denmark, Biomolecular Nanoscale Engineering Center, Department of Physics, Chemistry and Pharmacy, Campusvej 55, 5230, Odense M, Denmark
Email: laphylac@cing.ac.cy

Muscular Dystrophy is a group of inherited diseases which are characterise by muscle weakness and wasting. Duchenne Muscular Dystrophy (DMD) and Myotonic Dystrophy (DM) are among the most common muscular dystrophies and to date they remain still with no radical therapy. The most promising approach towards both of these diseases is the application of chemically modified antisense oligonucleotides (ASOs) that directly target the mutations. Several studies have tested the efficacy of different chemically modified ASOs to restore muscle function in mouse models and clinical trials. Result will be presented showing for the first time the use of ASOs that incorporate two commonly used, high binding affinity modifications in a single antisense construct: the locked nucleic acid (LNA) chemistry, which exhibits unprecedented duplex stability, and the nontoxic, naturally occurring 2'-O-Methyl (2'OMe) chemistry, which shows high RNA binding affinity and inherent resistance to endonuclease degradation. Our results demonstrate correction in mutant cells and animal models for both DMD and DM. The combination of the two chemistries in a single ASO holds promise for the future of DMD and DM and follow up studies will reveal its potency as gene therapy candidates.

Keywords: Antisense Oligonucleotides, Muscle, Duchenne Muscular Dystrophy, Myotonic Dystrophy

Is-067: Immunocell Therapy for Pediatric Malignancies

Matthew Porteus

Department of Pediatrics, Stanford University, USA
Email: mporteus@stanford.edu

The transformation of many pediatric cancers, including acute lymphoblastic leukemia (ALL), from something was nearly universally fatal to one that can have a cure rate of over 90% has been transformative for both the individuals and families that are affected by ALL but also demonstrates how rigorous, cooperative and iterative approaches to a disease can cure the disease. Nonetheless, even for pediatric ALL our current best therapy still fails a large number of patients and their families. One of the most exciting recent developments is the recent findings that using gene therapy to deliver a chimeric antigen receptor (CAR) to T-cells can arm those T-cells (CAR-T) to attack and eradicate tumor cells. It is expected that this form of the therapy might become approved for non-experimental use in the United States in 2017. I will review the basic principles of CAR-T therapy and the published findings. These published results highlight both the promise of the approach and also some of the future challenges, including significant toxicities, the initial lack of efficacy in some and the long-term lack of efficacy in others. I will discuss how genome editing might be used to solve some of these problems by reviewing the published literature and discussing some of our own unpublished results.
Keywords: Gene Therapy, Pediatric Acute Lymphoblastic Leukemia, CAR-T therapy, Genome Editing

Is-068: Genetic Engineering of Somatic Stem Cells to Cure Disease

Matthew Porteus

Department of Pediatrics, Stanford University, USA
Email: mporteus@stanford.edu

There are thousands of diseases caused by mutations in single genes (monogenic diseases) that affect hundreds of millions of people around the world. Examples of such diseases include sickle cell disease, beta-thalassemia, severe combined immunodeficiency, cystic fibrosis for genetic diseases of the blood and immune system, allogeneic hematopoietic stem cell transplantation (allo-HSCT) can be curative. Many patients do not have an appropriate donor for allo-HSCT and the process is associated with significant immunologic toxicities. Instead of using allogeneic hematopoietic stem cells (HSCs) that do not contain the pathologic variant, we have been developing genome editing in which the patient's own HSCs could be corrected and used to cure the disease.

Our method is to combine the delivery of the CRISPR/Cas9 nuclease system as a ribonucleoprotein complex by electroporation with AAV6 transduction into CD34+ hematopoietic stem and progenitor cells (HSPCs). We have optimized this manufacturing process.

Using this method we achieve 30-60% genome editing by homologous recombination at several different loci including the *HBB* gene (associated with sickle cell disease and beta-thalassemia) and the *IL2RG* gene (associated with the X-linked form of severe combined immunodeficiency). The edited cells retain their stem cell properties as demonstrated by reconstituting long-term hematopoiesis after transplantation into immunodeficient mice and generating differentiating into appropriate progeny. Using patient derived CD34+ HSPCs, we can show that the corrected cells generate the mature progeny that retain full functional capabilities and are phenotypically corrected for the underlying defect. Finally, we see no evidence of pathologic toxicity of the manufacturing process.

In conclusion, genome editing of patient derived CD34+ HSPCs is now highly efficient and is achieving correction frequencies that would cure disease. We are now moving our laboratory based proof-of-concept studies into a GMP grade process in order to initiate first-in-human clinical trials in the next 18-24 months.

Keywords: CRISPRCas9, Genome Editing, Homologous Recombination, Hematopoietic Stem Cell, Gene Therapy

Is-069: Stem Cell-derived Alveolar-like Macrophages for Pulmonary Cell Therapy

Martin Post

Translational Medicine Program at the Hospital for Sick Children, Toronto, Canada
Email: martin.post@sickkids.ca

In recent years, directed differentiation of pluripotent stem cells (PSCs) has rapidly become a major focus of regenerative medicine to help address the shortcomings of pulmonary therapeutics or transplantation. Specific efforts have focused on endoderm-derived lung epithelium tissue regeneration, while mesoderm-derived tissues in the lungs -such as non-circulating hematopoietic lineages- have received minimal attention. This oversight in pulmonary stem cell regenerative medicine has led to a failure to appropriately address the importance of the innate immune system of the lungs; particularly it's most abundant population of airway cells, the alveolar macrophage (AM). We have established a protocol for generating AMs from murine PSCs under serum-free, feeder free, factor defined conditions (AMs are highly adapted phagocytic cells of the pulmonary innate immune system that represent the primary hematopoietic cells of the airways and are distinct of myeloid cells). The identity of PSC-AMs was phenotypically confirmed via co-expression F4/80: CD11c: Siglec F and functionally characterized by their ability to phagocytose and to remain functionally active in healthy, injured and injury-resolving mice lungs, without an obvious compromise in immune response. Furthermore, the PSC-AM remained viable in culture for several months using expansion and maintenance media. The PSC-AMs display bactericidal effects to various common pathogenic bacteria – including *E. coli, S. aureus,* and *P. aeruginosa*. Moreover, in a rodent model of *E. coli*-derived pulmonary sepsis, intratracheal-delivered PSC-AMs internalize live *E. coli* and reduce the number of airway neutrophils. Noting the therapeutic value of these functional macrophages, we also genetically modified the cells to constitutively express the anti-inflammatory cytokine interleukin-10 and/or the protease inhibitor alpha-1 antitrypsin. These advances in therapeutically scalable and functionally active stem PSC-AMs for pulmonary disease represent a novel non-pharmacological approach addressing the unmet needs of a variety of chronic airway diseases where innate immunity is insufficient or compromised including

chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and bronchopulmonary dysplasia (BPD).
Keywords: Embryonic Stem Cells, Alveolar, Macrophages, Lung, Cell Therapy

Is-070: Building a Lung using Acellular Lung Scaffolds

Martin Post

Translational Medicine Program at the Hospital for Sick Children, Toronto, Canada
Email: martin.post@sickkids.ca

Differentiation of functional lung epithelial cells from pluripotent stem cells holds the potential for applications in regenerative medicine. However efficient differentiation to proximal and distal lung epithelial cell populations remains a challenging task. The three-dimensional extracellular matrix scaffold is a key component that regulates the interaction of secreted factors with cells during development by often binding to and limiting their diffusion within local gradients. The development of matrices that can recapitulate the in vivo environment is key for directing lung lineage-specific differentiation. Here we examined the role of the lung ECM in differentiation of pluripotent cells in vitro and demonstrate the robust inductive capacity of the native matrix alone using decellularized adult lung scaffolds. The decellularization procedure was optimized and the scaffolds generated were carefully characterized to ensure complete removal of resident cells and preservation of the ECM. Lung scaffolds were recellularized with mouse and rat embryonic stem cell-derived endoderm and maintained for up to three weeks of culture at air liquid interface, in defined, serum-free medium conditions. Recellularization of lung scaffolds with endodermal cells resulted in differentiation to early NKX2-1+/SOX2+ proximal lung progenitor cells and a heterogeneous basal epithelial cell population, within seven days of culture. Extended culture resulted in robust differentiation to mature airway epithelia, complete with FOXJ1+/TUBB4A+ ciliated cells and SCGB1A1+ secretory club cells, with morphological and functional similarities to native airways. Differentiated day 21 cells contained beating ciliated cells in culture and exhibited functional CFTR protein expression. Addition of growth factors resulted also in differentiation into alveolar SFPTC+ type 2 epithelial cells on the scaffolds. This work demonstrates the importance of a 3-dimensional matrix environment and the role of site-specific cues for directing differentiation of pluripotent stem cells to lung epithelial cells. This is a valuable step towards uncovering ECM-mediated signaling during lung specification and offers a platform for modeling lung development and airway-related diseases using pluripotent stem cells.
Keywords: Embryonic Stem Cells, Acellular Lung Scaffolds, Extracellular Matrix, Lung Cell Differentiation

Is-071: The Effect of GABA in Trans-Differentiation of Human and Mouse Pancreatic Islets, a Promising Approach to Treatment of Diabetes Type1 and 2

Farzaneh Pourasgari, Michael Wheeler

University Health Network, University of Toronto, Toronto, Canada
Email: farzaneh.pourasgari@gmail.com

Background and Aim: Diabetes is hyperglycemia originated from insulin deficiency and/or insulin resistance. In Type 1 diabetes (T1D), reduction of beta cells through autoimmune destruction causes Insulin deficiency. In type-2 diabetes (T2D) this reduction is through stress-induced apoptosis of beta cells. In both of them, insulin therapy or transplantation of pancreas or islets can be considered as a cure. Recent studies implies that beta cells retrieval can be a promising alternative for diabetes research. It was reported GABA molecule can do this retrieval. In our study, we studied the role of GABA molecules in trans-differentiation of α cells into β cells in mouse and human pancreatic islets through GABA receptors.
Methods: Mouse and Human pancreatic islets were treated with GABA for 8 hours. Quantitative PCR was used to detect expression levels of genes involved in the differentiation of beta cells. Then, to detect which GABA receptors are getting involved in trans-differentiation, we treated the islets with GABA-A and -B receptors (GABA-AR and GABA-BR) agonists (Musimol and Baclophen) and antagonists (SR95531and Pha-

clophen). Also, droplet digital PCR (ddPCR) of whole islets and sorted α and β cells by flow cytometry from MIP-GFP mice was performed to demonstrat expression level of 19 subunits of GABA-AR and -BR. Immunofluorescent (IF) staining and confocal microscope imaging was employed to confirm the GABA-AR subunits expression in α and β cells.
Results: GABA treatment revealed up-regulation of β cells-related genes and down-regulation of α cells specific genes. Treatment of human and mouse islets by GABA-AR and -BR agonists and antagonists showed GABA-AR agonist (Musimol) induces and GABA-AR antagonist (SR95531) inhibits insulin gene expression. Whereas GABA-BR agonist and antagonist did not indicate any significant change in the level of insulin and glucagon gene expression. Quantative GABA-AR subunits gene expression showed α2, α4, α5 and α6 subunits are the most expressed α subunits in human and mouse whole pancreatic islets. Furthermore, ddPCR results proved β3 subunit is the most expressed β subunit in both mouse and human whole islets. When sorted cells were tested for the same experiment, α2 and α5 showed to be the most expressed α subunits in β cells and α cells, respectively. IF results confirmed the same results as gene expression pattern at the protein level.
Conclusion: Long-term treatment of pancreatic islets with GABA showed that GABA induces trans-differentiation of α cells into β cells. However, GABA-BR is important in insulin secretion affected by GABA, this magic molecule makes its effect on the trans-differentiation through GABA-AR receptors. Most likely, α5 subunit of GABA-AR is a key subunit in the trans-differentiation and potentially it could be a target for future studies for retrieval of β cells in diabetic patients.
Keywords: GABA, Trans-Differentiation, Diabetes, Pancreas, Islets

Is-072: Mesenchymal Stem Cells Suppress T Cell Immune Responses by Transcriptome Modulation

Rajesh Ramasamy, Shalini Vellasamy, Chih Kong Tong, Atiqah Azhar, Radha Kodiappan, Soon Choy Chan, Abhi Veerakumarasivam

Immunology Unit, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, 43400, Selangor Darul Ehsan, Malaysia
Email: rajesh@upm.edu.my

Allogeneic transplantation has become more feasible with immunosuppressive regimens such as radio and chemotherapy. Although such generalised immunosuppression is crucial and lifesaving at times; however prolonged immunosuppression often leads to severe adverse effects. Hence, stem cell therapy emerged as partial substitutes for the immunosuppression therapy whereby their therapeutic effect is governed by specific mechanisms towards tissues regeneration and balanced immune responses. In line with this, mesenchymal stem cells (MSCs) have been identified as pan-immunosuppressant in various in vitro and in vivo inflammatory models. Most of these studies have addressed the immunosuppression mechanisms at cellular and protein levels yet failed to decipher such effect at the gene and transcriptional levels. By using global gene expression profiling, this study aimed to unravel the molecular pathways associated with umbilical cord-derived MSC (UC-MSC) mediated immunosuppression of activated T cells. UC-MSCs exerted a dose-dependent inhibitory effect on T cell proliferation. This inhibition was mainly mediated through a direct cell-cell contact rather than soluble factors. The treated T cells did not appear to undergo apoptosis but were significantly arrested at the G0/G1 phase. The expression of many genes in the activated T cells was found to be dysregulated by UC-MSCs. For example, IFNG, CXCL9, IL2, IL2RA and CCND3 were downregulated while IL11, VSIG4, GJA1, TIMP3 and BBC3 were upregulated. Dysregulated gene clusters that were associated with lymphocyte proliferation/activation, apoptosis, and cell cycle and immune response ontologies were selected for further analysis. Using the Ingenuity Pathway Analysis, 13 canonical pathways were identified as enriched with these dysregulated genes. These pathways include T helper cell differentiation, cyclins and cell cycle regulation as well as gap/tight junction signalling. In conclusion, this study indicates that MSCs-mediated immunosuppression is multifactorial that involve modulation of many genes via specific transcriptomic changes.

Is-073: Characterisation of Human Mesenchymal Stem Cells Derived from Normal and Osteoarthritis Cartilages

Rajesh Ramasamy, Satar Jabbar Rahi Al-Qraittee, Ling King Hwa, Fahrudin Che Hamzah

Immunology Unit, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, 43400, Selangor Darul Ehsan, Malaysia
Email: rajesh@upm.edu.my

Osteoarthritis (OA) is a degenerative disease which affects millions of people in the world. Although the leading cause of OA is still elusive yet increasing body of data indicates ageing, trauma and injuries are often associated with the onset of OA. Although, the regeneration of cartilage due to physical and traumatic injuries governed by the local tissue resident stem cells, however, certain circumstances could hinder the full regeneration. This could be a result of aberrant stem cell nature of the diseases condition which later leads to organ based degenerative diseases. Mesenchymal stem cells (MSCs) have been implicated in the pathogenesis of OA and, in turn, the progression of the disease therapeutically modulated by MSCs. Whether the defective MSCs causes the destruction of articular cartilage in OA with depleted stem cell pool or loses differentiation that does not allow the proper execution of stem cell functions remains unclear. Thus, this study compared the characteristics of healthy human cartilage and OA-derived MSCs. A small fraction of non-weight bearing human articular cartilage from normal and osteoarthritic patients was harvested during the arthroscopy session. Patients were selected based on grade 4 osteoarthritis according to the Kleegren and Lawrence score system, and 5 normal cartilage sample were obtained from healthy donors undergoing knee surgery or arthroscopy due to sports injuries. An enzymatic digestion method and adherent culture system were employed to generate cartilage-derived MSCs. Upon in vitro culture, adherent cells from OA and normal cartilages showed a fibroblastic morphology with standard growth kinetics. Although not much of differences were noted between OA and normal cartilage MSCs in term of morphology and immunophenotyping yet OA-MSCs possessed a lower growth rate and prolonged doubling time when compared to the normal cartilage-derived MSCs. Moreover, the magnitude of immunosuppression is much lower in OA-MSCs. The normal and OA cartilage MSCs were both able to differentiate into adipocytes, osteocytes and chondrocytes upon induction. However, in OA cartilage MSCs showed a downregulation of the chondrogenic genes with an up-regulation of adipogenic and osteogenic genes. The quality of OA-MSCs is compromised when compared to the normal cartilage-derived MSCs. The growth rate, the ability to exert immunosuppression and differentiation towards chondroblasts were declined in OA-MSCs which may explain the inability of OA-MSCs to fail to regenerate cartilage.

Is-074: Effect of TLR Activation on Treg Induction by Mesenchymal Stromal Cells

Iran Rashedi

University of Toronto University Health Network, Toronto, Canada
Email: i.rashedi@utoronto.ca

Background and Aim: Mesenchymal stromal cells (MSCs) are the subject of numerous clinical trials and investigations for the treatment of a variety of conditions, mainly due to their immunomodulatory properties via (largely unknown) interactions with a variety of cells within the immune system. MSCs promote the generation of cells with regulatory function including regulatory T cells (Treg). However, the effect of environmental stimuli on this process remains unknown. Several Toll-like receptors (TLRs), especially TLR3 and TLR4, are highly expressed on MSCs and their activation by danger signals, released during inflammation and cellular damage, can modulate the immunosuppressive and anti-inflammatory functions of MSCs.
Methods: We investigated the effect of TLR 3 and 4 activation on the induction of Treg cells by human MSCs.
Results: We showed that pre-conditioning MSCs with TLR3 or TLR4 agonists enhances MSC-mediated Treg induction. The augmentation is cell contact-dependent and involves Notch signaling in co-culture with CD4+ lymphocytes.

Conclusion: These findings suggest new means to enhance the potency of MSCs for treating disorders with an underlying immune dysfunction.
Keywords: MSC TLR Treg Immunomodulatory

Is-075: Combining Renewable Human Liver Tissue and Novel Extracorporeal Devices to Deliver Mammalian Liver Support

Hassan Rashidi[1], Maaria Ginai[2], Salamah Alwahsh[1], Nguyet Thin Luu[3,] Philip Newsome[4], Stuart Forbes[5], Mark Bradley[6], Anthony Callanan[7], David Hay[8]

1. MRC Centre for Regenerative Medicine, Little France Drive, University of Edinburgh, Edinburgh, United Kingdom
2. University of Edinburgh, Edinburgh, United Kingdom
3. Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT, United Kingdom
4. NIHR Birmingham Liver Biomedical Research Unit, University Hospital Birmingham, Birmingham, United Kingdom
5. University/BHF Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland, United Kingdom
6. EaStCHEM, School of Chemistry, University of Edinburgh, David Brewster Road, EH9 3FJ Edinburgh, United Kingdom
7. Institute for Bioengineering, School of Engineering, University of Edinburgh, Edinburgh, United Kingdom
8. Medical Research Council Centre for Regenerative Medicine, University of Edinburgh, 5 Little France Drive, Edinburgh, EH16 4UU, Scotland, United Kingdom
Email: hrashidi@exseed.ed.ac.uk

Background and Aim: Liver disease is the fifth most common cause of death in the UK and the death toll is rising. Liver transplantation is an effective procedure to treat end-stage liver disease and organ failure, however, donor organ shortage represents a significant problem. Therefore, there is a clear imperative to develop novel and scalable alternatives to transplant to treat human liver disease. We have developed an interdisciplinary approach to this problem, drawing upon engineering, chemistry and biology to build a novel liver support device for application.

Methods: Pluripotent stem cells were differentiated to hepatospheres using existing methodology and characterised in vitro and in vivo. Following this, hepatosphere attachment to four polycaprolactone surfaces was studied. The optimal surface was selected and incorporated within a novel bioartificial liver device. Post device assembly, stem cell-derived hepatospheres were exposed to fluid shear stress with cell performance and longevity examined using appropriate biochemical techniques.
Results: Hepatospheres produced from research and GMP grade human embryonic stem cell lines were metabolically active and stable for over 100 days in culture. To assess hepatosphere capacity to support liver function, spheres were implanted intraperitoneally after 30% partial hepatectomy in immune compromised mice. Notably, hepatospheres supported animal weight recovery better than the control animal group, demonstrating that stem cell-derived tissue was fit for purpose. Following their robust characterisation, hepatosphere binding to different polycaprolactone surfaces was examined. After we identified the optimal polycaprolactone surface, the bioartificial liver device was assembled and charged with stem cell derived hepatospheres. The populated bioartificial liver devices were studied in-depth, with a focus on their ability to perform basic liver functions.
Conclusion: Stem cell derived tissue can be produced at scale from research and clinical grade hESC lines using a GMP ready process. Following specification, stem cell-derived hepatospheres can populate a novel bioartificial liver device and scaffold, displaying appropriate levels of liver function. We believe that these studies represent an important advance for the field and highlight importance of interdisciplinary research.
Keywords: Liver, Hepatocyte, Pluripotent Stem Cells, Extracorporeal Devices

Is-076: Mouse Paternal-RNAs Initiate Pattern of Metabolic Disorders in a Strain Dependent Manner

Gul Basaran[1], Yagut Akbarova[1], Kezban Korkmaz[1], Kursad Unluhizarci[2], François Cuzin [3,4,5], Fahrettin Kelestimur[1] and Rassoulzadegan Minoo

1. Erciyes Üniversitesi Gen-Kok, Kayseri, Turkey

2. Department of Endocrinology, Erciyes University Medical School, Kayseri, Turkey
3. Inserm, U1091, Nice, F-06108, France
4. CNRS, UMR7277, F-06108, France
5. University of Nice-Sophia Antipolis, UFR Sciences, Nice, F-06108, France
Email: Minoo.RASSOULZADEGAN@unice.fr

Several instances of RNA-mediated inheritance of phenotypic variation have been reported, among them metabolic disorders. We and others have observed that microinjection into naive fertilized mouse eggs of total sperm RNAs of obese and diabetic males maintained on high-fat diet induce the same pathological variation in the offspring. A role of noncoding RNAs is currently the prevailing hypothesis. The sperm RNA content is complex. It depends on environmental conditions, but another level of variability is illustrated by the distinct pathological features reported by different groups, a variability that we tentatively attributed to the use of different inbred strains. We investigated the effect of different genotypes on the response to high-fat diet and its transgenerational maintenance. To that purpose, we maintained in the same housing and diet conditions mice of two inbred strains, C57BL/6 and Balb/c and B6/D2 F1 hybrids. From F0 males raised on high-fat diet we derived three generations by sexual mating (F0, F1 and F2) and two generations (G1 and G2) by microinjection into fertilized mouse eggs (Balb/c) of sperm RNAs of different founders. All mice other than the F0 males were maintained on a normal diet and followed for body weights and metabolic health up to 22 weeks. We observed very little difference among founders of the three genotypes fed on high fat diet. During this period strain dependent difference of gain of weight are observed. Variations in body weight were noticeable in the F1 and perpetuated to F2 generation (follow-up so far). Glucose and insulin tolerance tests evidenced a pathology prominent in founder animals and showed stronger differences in a strain dependent manner in generations F1 and F2. These results suggests that the initial signals that program offspring health are differentially perceived in the germ line of the different genotypes and start a genetic anticipation process. Furthermore, strain dependent phenotypic variations of the disease spectrum (GTT and ITT) are transferable by microinjection into fertilized eggs (Balb/c) of total sperm RNAs from C57/BL6, B6/D2 and Balb/c males. Together, these findings uncover a novel area of RNA-mediated epigenetic hereditary variation. Work is still in progress, up-dated results will be presented in the meeting.
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Is-077: Transgenerational Control of Telomere Complex

Minoo Rassoulzadegan, Jafar Kiani [4] and François Cuzin [2,3,4]

1. Inserm, U1091, Nice, F-06108, France
2. CNRS, UMR7277, F-06108, France
3. University of Nice-Sophia Antipolis, UFR Sciences, Nice, F-06108, France
4. Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
Email: Minoo.RASSOULZADEGAN@unice.fr

Recent studies of our laboratory and others pointed to the sperm RNAs as transgenerational vectors of epigenetic inheritance. To inquire into a possible function of TERRA in development and transgenerational effect, we sought to determine whether TERRA is present in sperm. In fact, short fragments of TERRA are present in the Trizol extracted sperm RNAs fraction. However, we have found that majority of the sperm TERRA is associated with telomeric DNAs. On the way, we have settled experiments that extended the notion of TERRA and the telomeric DNA that are engaged in R-loop structures at all developmental stages from early embryo to adult sperm. Next, to inquire into a possible function of TERRA in development and transgenerational effect, we sought to determine whether an excess of TERRA in the one-cell embryo generate a specific phenotype. Microinjection of short oligo fragments of TERRA RNAs into the fertilized mouse eggs was toxic at standard concentration of RNAs, we had to adjust dosage to hundred time lower amount. These results suggest that too much of TERRA RNAs fragments are not tolerated with normal development. Mice born after microinjection of lower amounts of TERRA develop normally and are fertile. We show that introducing an excess of TER-

RA molecules at the earliest developmental stage results in the establishment of a new regime of telomeric function stably maintained during development including in germ cells and thereby extended to the progeny, characterized by (i) increased TERRA accumulation in complexes with chromosomal DNA, (ii) increased size of the telomeres.

Is-078: Pathogen Reduction of Cellular Blood Components

Paolo Rebulla

Foundation Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milan, Italy
Email: paolo.rebulla@policlinico.mi.it

In spite of a progressive development of significantly improved procedures for blood donor screening, current recipients of blood components are still at measurable risk of developing infectious complications of blood transfusion. Although the risk is very low for a number of known viruses, such as HCV, HBV and HIV, which can be detected with good accuracy in the donor blood by using well standardized, commercial laboratory assays, the risk can nether be quantified a priori nor avoided when novel infectious agents enter the blood supply. Recent examples of the latter occurrence are the Zika and Chikungunya epidemics, which are currently affecting large numbers of individuals in different regions worldwide. Measures of risk reduction include deferral of donors travelling to endemic areas, which can be the only effective procedure until specific donor screening assays are developed, validated and distributed by industry. Deferral of donors travelling to endemic areas for selected infections and use of novel donor screening tests are collectively termed 'reactive measures'. In spite of their recognized efficacy, implementation of reactive measures takes time, significantly reduces the available donor pool and requires significant economical and organizational resources. To overcome the above limitations, a preventive approach has been developed based on the use of a number of procedures, collectively termed 'pathogen reduction technologies' (PRT). PRT for platelets include the exposure to UV light with or without the addition of photosensitizers (amotosalen or riboflavin). PRT for plasma include procedures similar to those used for the platelets plus the 'sovent-detergent' method and a procedure based on visible light exposure in the presence of methylene blue. Whole blood PRT uses UV light illumination in the presence of riboflavin, while PRT for red blood cells uses a frangible anchor-linked effector (S303) and glutathione. This presentation will focus on specific PRT that have been developed and approved for commercial distribution for platelets and plasma. Moreover, the current stage of PRT for whole blood and red blood cells, which are in an advanced experimental phase, will be described.

Keywords: Pathogen Reduction, Blood Transfusion, Infectious, Complications

Is-079: Multicomponent Cord Blood Banking

Paolo Rebulla

Foundation Ca' Granda Ospedale Maggiore Policlinico, Via Francesco Sforza 35, 20122 Milan, Italy
Email: paolo.rebulla@policlinico.mi.it

Historically, newborn's blood left in the placenta after delivery (usually termed 'placental blood' or 'umbilical cord blood' - CB) has been used as a source of hematopoietic stem cells for bone marrow replacement therapies in myeloablated patients affected by hematologic malignant and non-malignant conditions. Worldwide, about 700,000 UCB units generously donated with informed consent for community use by volunteer, non-remunerated, healthy parents are currently stored in public CB banks, which have facilitated the performance of more than 30,000 allogeneic CB transplants during the last 30 years. The clinical outcome of CB transplant is directly related to the cell dose administered to patients. Therefore, in consideration of the high cost of CB cryopreservation, most public CB banks have recently selected high thresholds of total nucleated cell (TNC) count (e.g. 1.5 billion) and/or CD34+ cells for units to be processed and placed into the hematopoietic transplant inventory, as those with lower TNC counts could have reduced chances for future therapeutic use. This has increased the CB unit discard rate, which has now settled at about 90% of the collections in most large public banks. Although supported by valid economic analyses on the appropriate use of resources, the high

discard rate associated to the high TNC threshold for banking may disappoint parents keen to offer their generous gift to the community, which, in turn, could reduce the chance for patients to find effective units needed for their treatment. All the above prompted some investigators to explore alternative uses of CB units donated to the public banks and showing TNC counts below the threshold for cryopreservation and hematopoietic transplant use. This presentation will discuss the operational, regulatory and clinical issues related to the development of multicomponent (RBC, Platelets, Plasma) CB banking programs aimed at fully exploiting the potential applications of CB through the cooperation between CB banks, blood transfusion services and industry interested in manufacturing novel laboratory reagents and blood derivatives disclosure. Paolo Rebulla, a co-inventor of patented procedures and devices for the preparation of blood components from cord blood, is founding member and equity holder of Episkey srl.
Keywords: Cord Blood, Platelet Lysate, Platelet Gel, Eye Drops

Is-080: Human 3 Dimensional Testicular Organoid System: A Novel Tool for In Vitro Spermatogenesis and Toxicity Assay

Hooman Sadri-Ardekani[1,2]

1. Wake Forest Institute for Regenerative Medicine, North Carolina, USA
2. Department of Urology, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA
Email: hsadri@wakehealth.edu

Current methods for determining gonadotoxicity of pharmaceutical compounds and environmental toxins rely heavily on in vivo functional assays in animals; however these assays cannot accurately predict toxicity in humans. Furthermore, existing in vitro two-dimensional culture of human testicular cells do not maintain the germ cell niche and the complex signaling interactions that existed in vivo. Therefore an effective in vitro model of human testis is desirable. We established a three-dimensional human testis organoid by combining isolated and propagated spermatogonial stem cells, Sertoli, Leydig cells and extracted human extracellular matrix. These 3D organoids maintained viability in culture, produced androgens, and went through cell differentiation from spermatogonia to post-meiotic germ cells. These organoids were frozen and thawed successfully and showed a dose-dependent response to gonadotoxic chemotherapeutic drugs. This three-dimensional human testis organoid model can be used for high throughput screening of drugs and environmental chemicals as well as study in vitro spermatogenesis.

Is-081: Spermatogonial Stem Cell Transplantation to Preserve Fertility in High Risk Patients

Hooman Sadri-Ardekani[1,2]

1. Wake Forest Institute for Regenerative Medicine, North Carolina, USA
2. Department of Urology, Wake Forest School of Medicine, Winston-Salem, North Carolina, USA
Email: hsadri@wakehealth.edu

Male infertility management has made significant progress in past three decades. However, many patients still suffer from primary testicular failure due to acquired or genetic causes. Spermatogenesis originates from spermatogonial stem cells (SSCs) that reside in the testis. Many of these men lack SSCs or have lost SSCs over time as a result of specific medical conditions or toxic exposures. Loss of SSCs is critical in prepubertal boys who suffer from cancer and are going through gonadotoxic treatments, as there is no option of sperm cryopresrvation due to sexual immaturity. The development of SSC transplantation in a mouse model to repopulate spermatozoa in depleted testes has opened new avenues of research in other species, including non-human primates. Recent advances in cryopreservation and in vitro propagation of human SSCs offer promise for human SSC autotransplantation in the near future. Ongoing research is focusing on safety and technical issues of human SSC autotransplantation.

Is-082: Innovative Chimeric Antigen Receptor Designs to Target Oncofetal Glycan's in Human Cancer

Gholamreza Safaee Ardekani, Mads Daugaard

Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada
Email: safaee@mail.ubc.ca

Background and Aim: Enhancing patients' own immune system to react against cancer has been the fantasy of cancer research for decades. The recent successful cases of B-cell leukemia eradication using chimeric antigen receptors (CARs) have brought new hopes for cancer immunotherapy. In this innovative approach we have used our novel recombinant malaria protein (rVAR2), which broadly targets oncofetal glycosaminoglycan modifications in human tumors, to build a VAR2-CAR fusion construct. The use of VAR2 in CAR fusions could enable immunotherapeutic targeting of oncofetal chondroitin sulfate in a wide range of malignancies.
Methods: Primary T-cells were isolated from healthy volunteers and were artificially stimulated in-vitro. Lentiviral transduction was utilized to introduce two separate CAR sequences (VAR2-CAR and SPY-CAR) into T-cells. Cytotoxic effect of CAR T cells on cancer cell lines were measured by the level of Caspase 3 activity in target cells. Finally, tumor luminescence and volume of subcutaneous tumors in SCID mice were measured for in-vivo efficacy assessment.
Results: T cells were isolated with 99.7% purity and CAR constructs were successfully expressed in 61.4% and 64.82% of T-cells (for VAR2-CAR and SPY-CAR respectively). In addition, we were able to successfully activate and arm SPY-CAR T-cells with VAR2-SPY-catcher construct in-vitro and in-vivo. More importantly, both CAR T-cells showed very significant cytotoxic effects on malignant cell lines in-vitro in a dose dependent manner (p value= 0.0049 SPY-CAR and 0.0047 VAR2-CAR). Animal study also revealed strong effect in reducing tumor volume compared with control group.
Conclusion: Our data provide proof of concept for successful expression of broadly effective VAR2-CAR constructs and their cytotoxicity towards cancer cells in both in-vitro and in-vivo conditions.
Keywords: Chimeric Antigen Receptor (CAR), Cancer Immune Therapy, Oncofetal Chondroitin Sulfate, Glycosaminoglycan, Malaria

Is-083: Marketing of Advanced Therapy Medicinal Products in Europe: Overview, Future Prospects, Bottlenecks

Ralf Sanzenbacher

Paul-Ehrlich-Institut Federal Institute for Vaccines and Biomedicines, Langen, Germany
Email: Ralf.Sanzenbacher@pei.de

Realizing the strength of the research progress and increasing translational developments in regenerative medicine, cell and gene therapy, the European Union (EU) adopted the Regulation (EC) 1394/2007 on Advanced Therapy Medicinal Products (ATMP) which came into force in 2008. The ordinance covered tissue engineered-products together with gene therapies and somatic cell therapy as pharmaceuticals and provides a common framework for marketing of these products for all EU member states. This presentation aims to provide an overview for which ATMPs a centralized EU marketing approval was granted so far, as well as a summary on the setup and possibilities of the regulatory approval processes, together with current challenges and bottlenecks. Recently, the EC has initiated an initiative calling GMP provisions specially tailored for ATMPs which is currently in the final consultation phase. Alternatively, in situations of medical need and when no authorized product might be available the so-called hospital exemption clause can be applied to enable individual patients to receive "non-routine" ATMPs. Additionally, the concept of offering patients unapproved cell therapies directly at bedside gains momentum and will be tackled.
Keywords: ATMP

Is-084: Manufacturing of (Stem) Cell-Based Medicinal Products: How to Set Up and Control a Process?

Ralf Sanzenbacher

Federal Institute for Vaccines and Biomedicines, Langen, Germany
Email: Ralf.Sanzenbacher@pei.de

Background and Aim: This presentation aims to summarize important aspects in the quality development for production of cell-based medicinal products. Necessary time and efforts are frequently undervalued, especially at the transition phase from a lab scale prototype to an early investigational product to be released for patient treatment within clinical trials, but also beyond with a view to an economically viable commercial manufacturing setup. In first line, depending on the type of product, all steps may be critically addressed with a view on product safety, integrity, and functionality, starting from donor evaluation and procurement of cells or tissues, as well as the following manufacturing process including isolation, expansion, modification, formulation, storage, distribution and transport and reconstitution. Second, meaningful, reliable and robust characterization and control measures should to be established. Third, as legally requested, manufacturing has to be conducted in compliance with the requirements of good manufacturing practice (GMP), posing another challenge. For a better grasp practical examples will be discussed.
Keywords: Manufacturing Characterisation and Control under GMP

Is-085: Nanotechnology and Stem Cell Therapy for Cardiovascular Diseases

Alexander Seifalian

Nanotechnology and Regenerative Medicine Commercialisation Centre (Ltd), London, UK
Email: a.seifalian@gmail.com

Background and Aim: Nonmaterial and stem cells are the next generations of biotechnology have been used for development of cardiovascular implants as well as treatment of cardiac diseases. In my lecture I will talk about a number of nanoparticles potentially can be used for delivery of drugs or stem cells into heart such as for regeneration of ischemic myocardium.
Methods: Commercially available devices are prone to calcification, thrombogenicity and structural failure. To overcome these problems we have developed and patented a family of biomaterials with surface nano-topography. The material has been fabricated as cardiovascular implants.
Results: Then these devices have been functionalized with peptides and antibodies such as CD133 and CD34 to promote endothelisation of these implants from circulating progenitor stem cells from peripheral circulating blood. I will also talk about the development of cardiovascular implants including coronary artery bypass graft; heart valves and stents using above new biotechnology tool. Currently, coronary artery bypass grafts completed its preclinical trial and undergoing to clinical trial, transcatheter heart valves and stents are at preclinical trial. Data on manufacturing and evaluation as well as in vitro and preclinical in vivo testing will be presented. The route to translational from laboratories to clinical setting will be highlighted.
Conclusion: In conclusion application of nanotechnology, nanocomposite materials and stem cells are next generation tool under development for the treatment of cardiovascular diseases.
Keywords: Cardiovascular Stem Cells, Nanotechnology

Is-086: A Central Mediator of Neurodegeneration Common among Alzheimer's, Diabetes and Macular Degeneration That Is Blocked by Antibody

Koorosh Shahpasand[1], Leila Sattarian[1], Yaser Tahmtani[1], Aysun Farhadi[1], Ensiyeh Hajizadeh-Saffar[1], Xiao Zhou[2], and Kun Ping Lu[2]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Division of Translational Therapeutics, Department of Medicine, Cancer Research Institute, Harvard Medical School, Boston, MA
Email: shahpasand9@gmail.com

Background and Aim: Tauopathies are a group of neurodegenerative disorders common in abnormal tau aggregation and hyperphosphorylation. However, it remains uncertain that how tau gets pathogenic upon tauopathy process since tau abnormal hyperphosphorylation takes quite a while to happen, sometimes over a decade, makes it very hard to track the pathogenic phosphorylation event. We herein identify a neurotoxic cis conformation of phosphorylated tau at Thr231 as a

major early driver of tauopathy and neurodegeneration that is effectively blocked by the conformation specific monoclonal antibody.
Methods: We immunostained control and human Alzheimer's disease (AD) as well as traumatic brain injury (TBI) brains. Also, we studied age-related macular degeneration (AMD) retina and diabetic brains in mouse models with our cis/trans monoclonal antibodies. Moreover, we examined TBI mouse models treated with either control IgG or cis mAb employing immunostaining and electron microscopy. Furthermore, we studied risk-taking behaviour of those TBI mice.
Results: We found robust cis, but not trans, p-tau after sport- and military-related TBI as well as AD human brains. Also, we observed prominent cis p-tau accumulation in AMD retina and diabetic brain stem in mouse models. Acutely after TBI in mice, neurons profoundly produce cis p-tau, which disrupts axonal microtubule network, spreads to other neurons, and leads to apoptosis, a pathogenic process, which we termed "cistauosis" that appears long before known tauopathy. Also, while TBI causes abnormal risk-taking behaviour in mouse models, cis antibody treatment restores the phenomena.
Conclusions: Treating TBI mice with cis antibody prevents tauopathy development and spread, and restores brain histopathological and functional outcomes. These results uncover cistausosis as an early driver of tauopathy and neurodegeneration upon AD, TBI, Diabetes and AMD. We anticipate that cis p-tau will be a new early biomarker and that cis p-tau antibody may be used to treat or even prevent neurodegeneration upon various devastating tauopathies like AD.

Is-087: Designing Selective Competitive Antagonists for Inhibition of Specific Transcription Factor-Promoter Interactions

Ali Shariati

Stanford University, California, USA
Email: sam.shariati@gmail.com

Background and Aim: Transcription factors can regulate expression of hundreds of genes. While "omics" approaches have depicted the global organization of these transcriptional networks, the functional contribution of specific transcription factor-promoter interaction to cellular phenotype is unclear.
Methods: Here, we propose a method for investigating the functional role of specific transcription factor-promoter interaction by site-specific inhibition of transcription factor binding to their cognate DNA site. Our strategy is to prevent specific transcription factor-promoter interaction by targeting an RNA-guided nuclease deactivated Cas9 (dCas9) to the binding site of the specific transcription factor.
Results: We show that the binding of dCas9 will compete for binding site of the transcription factor, essentially functioning as a selective competitive antagonist. We will use this approach to investigate the role of autoregulatory loop formed by Nanog, Oct4 and Sox2 in maintaining pluripotency of embryonic stem cells.
Conclusion: We present a broadly applicable method for precise perturbation of transcriptional networks
Keywords: ESCs Transcription CRISPR

Is-088: Recent International Regulatory Trends in Stem Cells and Regenerative Medicine

Douglas Sipp[1–4]

1. RIKEN Center for Developmental Biology, Kobe, Japan
2. Keio University School of Medicine, Tokyo, Japan
3. Keio University Global Research Institute, Tokyo, Japan
4. RIKEN Center for Advanced Intelligence Project, Tokyo, Japan
Email: sipp@cdb.riken.jp

The pathway to commercialization of advanced biotherapeutics involves regulatory oversight, rules-making and enforcement. These are intended to protect the public, establish consensus minimum evidence standards for product qualities such as safety and efficacy, and to provide a level playing field for competing firms. Premarket requirements for medical products (drugs) were first introduced more than 50 years ago, with the Kefauver-Harris amendments to the FDCA in the United States in 1963. These requirements have remained controversial ever since, as they are accused of impeding innovation and harming patients by delaying access to products that might ultimately prove to be beneficial. These complaints have found a positive reception in

many countries, leading to calls for deregulation. Due to various economic and political factors, several countries have sought to implement health products deregulation by focusing on stem cell and regenerative medicine products. I will give a brief historical overview and explanation of the present global regulatory situation in this field, followed by discussion of the implications of deregulation for the health care system, the scientific field, and for individual patients.

Is-089: The Growth of the Stem Cell "Dark Economy"

Douglas Sipp[1–4]

1. RIKEN Center for Developmental Biology, Kobe, Japan
2. Keio University School of Medicine, Tokyo, Japan
3. Keio University Global Research Institute, Tokyo, Japan
4. RIKEN Center for Advanced Intelligence Project, Tokyo, Japan
Email: sipp@cdb.riken.jp

Therapeutic uses of stem cells and their derivatives in regenerative medicine and tissue engineering have attracted a great deal of scientific interest, media attention, and public funding. While precise global figures on public spending in stem cell R&D are difficult to obtain, clearly a number of major economies, including the United States, China and Japan have spent billions of dollars each in this field. Despite that large investment, however, approved stem cell therapeutics remain scarce, and to date there are no pluripotent stem cell-based medicinal products on any market in the world. However, a penumbral market sector has emerged over the past decade, which aggressively promotes unproven uses of supposed stem cell-based interventions for a great many serious medical conditions. Recent work has begun to show the extent of this industry, which surprisingly operates unimpeded in supposedly well-regulated nations such as Australia, Japan and the US. However, many fundamental details about this shadowy stem cell economy remain unknown. I will discuss how the industry has developed, the current state of knowledge, and the large gaps in our understanding of this "dark economy."

Is-090: Gene-Corrected Human CD8+ T-Cells for the Treatment of an Immune Deficiency

Tayebeh Soheili[1], Julie Rivière[1], Ida Ricciardelli[2], Amandine Durand[1], Els Verhoeyen[3], Geneviève de Saint Basile[4], Isabelle André-Schmutz[1], Marina Cavazzana[5]

1. Human Lymphohematopoiesis laboratory, Imagine Institute, Necker children hospital, Paris, France
2. Molecular and Cellular Immunology Unit, Institute of Child Health, London, UK
3. Centre International de Recherche en Infectiologie, Enveloped Viruses, Vectors and Innate Responses Research Group, Lyon University, Lyon, France
4. Normal and Pathological Homeostasis of the Immune System Laboratory, INSERM U1163, Paris, France
5. Biotherapy Clinical Investigation Centre, Necker Children's Hospital, Paris, France
Email: tayebeh-shabi.soheili@inserm.fr

Background and Aim: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome, characterized by severe hyperinflammation and immunopathological manifestation in several tissues. HLH is due to low or absent CD8+ cytotoxic lymphocyte cytotoxicity which leads to impared regulation of the immune response. HLH is fatal when untreated but even with hematopoietic stem cell transplantation still has a high mortality rate. Among the genes involved in this pathology, mutations in UNC13D gene cause type 3 familail HLH which represents 35% of all familial HLH cases. As Munc13-4's function is to allow proper cytotoxic activity in mature cytotoxic CD8+ T cells we proposed that these laters may constitute target for gene correction.
Methods: We constructed a self-inactivating HIV-1 derived lentiviral vector encoding human Munc13-4 in two different pseudotypes, the high tropism VSV-G and the measles virus glycoproteins (H/F) envelope which target more efficiently lymphoid cells through the signaling lymphocyte activation molecule (SLAM).
Results: We demonstrated that both vectors are able to stably transduce FHL3 CD8+ T cells resulting in correction of defective degranulation capacity of these cells. Furthermore T memory stem cells (TSCM) were also successfully transduced and maintained their stem

characteristics all along the culture period. However comparative analysis showed that H/F pseudotyped vector was more efficient than VSV-G vector to transduce FHL3 T cells. Adoptive transfer of the gene-corrected FHL3 T cells in SCID mice bearing autologous B-LCL lymphoma led to significant tumor regression due to an efficient homing into the tumor mass and long persistence of corrected T cells in peripheral blood as compared to non-corrected T cells receiving mice.
Conclusion: Our study shows for the first time that a lentiviral mediating gene transfer in T cells could be proposed to treat a HLH disorder.
Keywords: T Cell Gene Therapy Hemophagocytic, lymphohistiocytosis, Lentiviral Vectors

Is-091: Gene Transfer into Hematopoietic Stem Cells Improves Immune Deficiency in a Murine Model of Hemophagocytic Lymphohistiocytosis (HLH)

Tayebeh Soheili[1], Amandine Durand[1], Fernando Sepulveda[2], Geneviève de Saint Basile[2], Marina Cavazzana[3], Isabelle André Schmutz[1]

1. Human Lymphohematopoiesis laboratory, Imagine Institute, Necker children hospital, Paris, France
2. Normal and Pathological Homeostasis of the Immune System Laboratory Imagine Institute, Necker children hospital, Paris, France
3. Biotherapy Clinical Investigation Centre, Necker children hospital, Paris, France
Email: tayebeh-shabi.soheili@inserm.fr

Background and Aim: Patients with mutations in UNC13D gene, coding for Munc13-4 protein, suffer from type 3 Familial hemophagocytic lymphohistiocytosis (FHL3), a life-threatening disorder of the immune system which represents 25% of all FHLs. Munc13-4 controls docking of lytic granules before they fused with the plasma membrane in cytotoxic T and NK lymphocytes and it's defect results in defective cytotoxic function of these cells. Hematopoietic stem and progenitor cell (HSPC) transplantation, which is the only curative treatment for FHL3 to date, is partially successful even when a compatible donor is available because of the important inflammatory background of patients. In this context gene therapy, which has emerged as a convincing therapy for primary immunodeficiencies during the last decade, could be a promising therapeutic option especially for those patients without any compatible donor.
Methods: In this study, we generated and used a self-inactivated lentiviral vector to complement HSC from Unc13d -/- (Jinx) mice and transplanted them back into the irradiated Jinx recipients. This transplantation led to the complete reconstitution of the immune system at levels comparable to that of control mice. The recipients were then challenged with lymphocytic choriomeningitis virus (LCMV).
Results: While Jinx mice reconstituted with GFP expressing HSPC developed leukopenia, anemia and body weight loss, characteristic of hemophagocytic lymphohistiocytosis (HLH) in this murine model, gene corrected Jinx recipients developed only mild or no HLH manifestations. This reduction in HLH manifestation correlated with a significant reduction of virus titer in the liver and serum level of IFN-g and inflammatory cytokines. All these ameliorations might be explained by the restoration of cytotoxic function of CTLs as demonstrated in an in-vitro degranulation assay.
Conclusion: Overall, this study provides data supporting the potential of HSC gene therapy in a FHL immune dysregulation such as UNC13D deficiency.
Keywords: Gene Therapy Hematopoietic Stem Cell Hemophagocytic Lymphohistiocytosis

Is-092: Regulation of the Adult Muscle Stem Cells in Homeostasis and Resgeneration

Shahragim Tajbakhsh

Stem Cells and Development, Developmental and Stem Cell Biology Department, CNRS UMR 3738, Institute Pasteur, 75015, Paris, France
Email: shahragim.tajbakhsh@pasteur.fr

Background and Aim: The microenvironment is critical for the maintenance of stem cell populations, and it can be of cellular and non-cellular nature, including secreted growth factors and extracellular matrix (ECM). Skeletal muscle stem cells are quiescent during homeostasis and they are mobilised to restore tissue function after muscle injury. Although certain signalling pathways that regulate quiescence have been identified, the

composition and source of niche molecules that regulate stem cell properties in a variety of tissues remain largely unknown.
Methods: We have used genetically modified mice combined with ChIP-sequencing, immune detection and histological and imaging techniques to interrogate how muscle stem cells execute their functions.
Results: We identified Notch signalling as a major regulator of muscle stem cells in the embryo and postnatally. Specifically, Notch/RBPJ-bound regulatory elements are located adjacent to specific collagen genes in adult muscle stem cells (MuSCs). These molecules are linked to the ECM and constitute putative niche components. Using a variety of genetically modified mice, we show that the expression of these collagens is controlled by Notch activity in vivo. Notably, we find that MuSC-produced collagen V (COLV) is a critical component of the quiescent niche, as conditional deletion of Col5a1 leads to anomalous cell cycle entry and differentiation of MuSCs. Strikingly, COLV, but not collagen I and VI, specifically regulated MuSC quiescence through receptor mediated activity.
Conclusion: Our findings unveil a Notch/COLV signalling cascade that cell-autonomously maintains the MuSC quiescent state, and raises the possibility of a similar reciprocal mechanism acting in diverse stem cell populations. Given that other stem cells are maintained by Notch signaling, we anticipate that this model could be widely applicable to other tissues and organs.
Keywords: Muscle Stem Cells, Quiescence, Regeneration, Asymmetric Cell Division

Is-093: Induced Pluripotent Stem Cells (Hipsc) as a Model for Congenital Thrombopenia

Marten Hansen[1], Tatjana Wust[1], Alexander B Meijer[2], Rinske van Oorschot[3], Anna E. Marneth[3], Bert A. van der Reijden[3], Emile van den Akker[1], and Marieke von Lindern[1]

1. Sanquin Research, Department of. Hematopoiesis, Amsterdam, The Netherlands, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands
2. Sanquin Research, Department of. Plasma Proteins, Amsterdam, The Netherlands, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, the Netherlands
3. Department of. Hematology, Radboudumc, Nijmegen, The Netherlands
Email: m.vonlindern@sanquin.nl

Congenital defects in megakaryopoiesis can result in thrombocytopenia (shortage of platelets) with severe bleeding problems. Even when de mutation is known, it is not always easy to study the molecular and cellular characteristics of the megakarypoiesis defect because animal models do not always phenocopy the human disease, and because patient material is scarce. The recent advances in the generation of induced pluripotent stem cells (iPSC) present novel opportunities to generate in vitro models for megakaryopoiesis defects.
One issue of concern with respect to the use of iPSC as a model is the possibility that epigenetic memory from the somatic cell of origin may interfere with the lineage differentiation capacity of iPSC derived from that cell. Therefore we decided to generate iPSC from megakaryoblasts, and after full reprogramming we re-differentiated the iPSC to magakaryoblasts and megakaryocytes. In comparison to CD34+ hematopoietic stem and progenitor cells (HSPC), the iPSC cells yielded more mature megakaryocytes, the cells were multinuclear and expressed megakaryocyte surface markers. Proteome analysis by mass spectrometry indicated that iPSC derived megakaryocytes cluster together with megakaryocytes differentiated from CD34+ HSPC.
The heterozygous Q287 mutation in Growth Factor Independence 1B (GFI1B) causes an autosomal-dominant bleeding disorder characterized by gray platelets as a result of reduced α-granule content. Affected individuals exhibited macro-thrombocytopenia, increased megakaryocyte numbers and expression of the HSPC marker CD34 on platelets. GFI1B functions as transcriptional repressor by recruiting the histone modifying enzyme LSD1/KDM1A. The C-terminally truncated GFI1B-Q287 mutant has lost its repressive function and inhibits the function of wild type GFI1B in a dominant-negative manner.
We generated iPSC from patient-derived megakaryoblasts. The resulting iPSC were fully reprogrammed, and the karyotype was normal. Upon differentiation into the megakaryocytic lineage, the cells acquired megakaryocyte surface markers, but retained the HSPC

marker CD34, similar to patient platelets. In addition, the cells failed to become multinuclear. Mass spectrometry indicated that the proteome of megakaryocytes derived from [GFI1B-Q287]-iPSC resembled patient platelets more than wt iPSC. Thus, it seems that iPSC-derived megakaryopoiesis can be used as a faithful in vitro model for congenital thrombopenia to further identify the molecular and cellular consequences of GFI1B-Q287 in megakaryopoiesis.

Is-094: Differentiation of Human Induced Pluripotent Stem Cells (Hipsc) to Transfusion Ready Red Blood Cells

Patrick Burger, Marten Hansen, Eszter Varga, Esther Heideveld, Steven Heshusius, Tatjana Wust, Marijke Thiel, Erica Sellink, Jesse Eernstman, Francesca Aglialoro, Emile van den Akker, and Marieke von Lindern

Sanquin Research, Department of. Hematopoiesis, Amsterdam, the Netherlands, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands
Email: m.vonlindern@sanquin.nl

Donor-derived red blood cells (RBC) are the most common form of cellular therapy. However the source of cells is dependent on donor availability with a potential risk of allo-immunization and blood borne diseases.
In 2015, the Dutch blood supply foundation Sanquin provided 430.000 erythrocyte transfusion units to Dutch hospitals. The majority of all transfusions require standard matching for ABO and RhD blood group antigens. Additional blood group systems are matched for groups that are at risk for allo-immunisation: women in the reproductive age and patients with chronic anaemia. Nevertheless, allo-immunisation is a frequent problem in patients that are recurrently transfused such as patients with Sickle Cell Disease (SCD) or Thalassemia. In 2015, 7291 transfusion units with specified blood group antigens other than ABO or RhD were requested of which 6535 could be delivered “off the shelf”. The remaining 756 transfusion units were mostly acquired after Sanquin performed additional typing of available units, and 180 units were donated on invitation by donors from the rare donor cohort. Aging of the population reduces the donor population and is expected to increase the demand of transfusion products. Globalisation causes a larger ethnic diversity both in the patient and donor population, which increases the risk for allo-immunisation and augments the requirement for extensively typed erythrocytes. Erythrocytes cultured in vitro from a defined progenitor cell source that expresses few potentially immunogenic blood group antigens could supply matched cells to all patients and minimise complications due to immunisation.
We aim to produce unlimited numbers of cultured RBC with a defined `universal donor´ phenotype for transfusion purposes. To this end we prepare for a clinical test using autologous cultured RBC to test their in vivo stability. In parallel we develop methods for unlimited production of cultured RBC. An immortal source to produce in vitro cultured RBC (cRBC), such as induced pluripotent stem cells (iPSC) would allow selection of `universal donor` RBC, or provide an autologous end product with the absence of immune reactions. The production of cRBC has proven to be successful; however there are barriers to overcome prior to clinical application. e.g: xeno-free culturing methods, scale up cultures to obtain transfusion units (1-2*1012 erythrocytes), and for iPSC we need virus- and transgene-free reprogramming protocols.
To solve the above mentioned issues a customized humanized GMP-grade medium (Cellquin) was generated in order to control erythroid culture parameters and to reduce culture costs. This medium allowed 1*108 times erythroid expansion from peripheral blood mononuclear cells (PBMC) to pure adult erythroblast (EBL) cultures within 25 days, comparable to non-GMP commercial media. To generate iPSC, a non-integrative polycistronic episomal vector containing (OCT4-SOX2-KLF4-cMYC-LIN28) was used to reprogram PBMC-expanded EBL to iPSC, displaying pluripotency potential and normal karyotype. iPSC were adapted to single cell passage allowing directed colony differentiation using a feeder-free monolayer approach. From day 6 of differentiation Cellquin was applied with lineage-specific growth factors, resulted iPSC differentiation to EBL which was initiated by the appearance of haemogenic endothelium following hematopoietic specification. Our differentiation method resulted in ~150*106 CD41- CD34- CD71+ CD235+ CD36+ expanded EBL from 1200 iPSC within 21 days (12 days of iPSC diff. + 9 days of expansion). Further matura-

tion of iPSC-EBL yielded CD71+ CD235+ CD36- pure orthochromatic normoblasts expressing mainly gamma globin chains (foetal) and small amount of beta globins (adult) in agreement with literature. Currently we are testing enucleation potential of matured iPSC-EBL. Our monolayer approach is simple, highly controlled and compatible with upscaling. Avoiding virus-, integrative reprogramming, and feeders in combination with our GMP-grade media enables us to establish cost effective cRBC production and is an important step toward clinical application.

Is-095: Stem Cell-Based Organoid Culture System: the Science

Robert GJ Vries

Hubrecht Organoid Technology foundation (HUB), Yalelaan 62, 3584CM, Utrecht, the Netherlands
Email: ln.cmsumsare@egrebnet.d

The last couple of decades' major advances were made in stem cell research. Building on the isolation and study of embryonal stem cells, Haematopoietic stem cells were identified, iPSCs were developed and stem cells of adult organs were identified. The discovery of adult stem cells was in a large part spearheaded by the lab of Hans Clevers with the identification of LGR5 as a marker of intestinal stem cells. Subsequently, the lab found that LGR5 is a marker of many epithelial stem cells such as liver and pancreas. The identification of the adult stem cells greatly increased our understanding of stem cell biology. Among others, the Clevers group was able to demonstrate great plasticity in stem cells and their off spring in maintaining the stem cell population, the mechanism of symmetric stem cell renewal, and novel components of the Wnt signaling pathway. Importantly, the identification of the adult LGR5 stem cells resulted in the development of a culture system that allowed for the virtually unlimited expansion of these cells. The organoids are genetically and phenotypically stable, which makes them an ideal source of cells for therapy or, alternatively, as a model for development of regenerative drug therapies.

Is-096: Organoid Technology in Diagnostics and Disease

Robert GJ Vries

Hubrecht Organoid Technology foundation (HUB), Uppsalalaan 8, 3584CT, Utrecht, the Netherlands
Email: ln.cmsumsare@egrebnet.d

HUB (Hubrecht Organoid Technology) is a non-profit company that was founded by the Hubrecht institute (KNAW) and the UMCU with the aim to translate the Organoid Technology, invented in the lab of Hans Clevers, to preclinical and clinical applications. Key to the development of the Organoid Technology was the discovery of LGR5+ intestinal adult stem cells by the Clevers lab. When provided with the appropriate growth factors, LGR5+ cells were found to form a polarized epithelium in which stem cells, dividing daughter cells and differentiated cells maintain their natural hierarchical and functional role. Importantly, organoids proved to be both genetically and phenotypically stable during prolonged periods of cell culture and are amenable to all standard experimental manipulations.
After the discovery of the method for intestinal cells we developed methods for many other organs such as liver, lung and pancreas. Proprietary protocols for in vitro expansion of these stem cells from patient biopsies constituted the basis for the creation of a 'Living Biobank'. Because patient relevant models systems are arguably the biggest problem in drug development, the Organoids, which maintain the patient and disease characteristics, have the potential to greatly improve drug development and target identification. At the same time, the Organoids can be used as a next generation personalized medicine model to test effective drug treatments for individual patients in the clinic.
Since the start of HUB, we greatly expanded the Organoid portfolio in collaboration with a great number of Pharmaceutical and Biotech partners. In addition, we have started trials to validate the Organoids as a companion diagnostic and predictive tool for treatment of patients together with Dutch health insurance companies and hospitals.

Is-097: Treatment of Acute GVHD by MINI ECP an Alternative to Classical Photopheresis

Volker Witt

St. Anna Kinderspital, UKKJ MUW, Vienna, Austria
Email: volker.witt@stanna.at

Background and Aim: Extracorporeal photopheresis consists of 4 steps, 1) harvesting Leukocytes by apheresis, 2) adding 8-MOP, 3) irradiation with UVA 2.5 J/cm², and 4) the reinfusion to the patient. There are worldwide to main technical options, one is the inline method (all steps are performed by one system) and the offline system (each step is done separately). The limiting step for some patients is the harvesting of the Leukocytes by apheresis. Due to the systems in use under a bodyweight of 30 kg or 20 kg respectively the systems have to be primed with blood to prohibit extreme volume shifts in the patients. To address this a few groups are harvesting the Leukocytes by drawing peripheral blood. We here describe our in-house method so called MINI ECP.
Methods: Patients with a bodyweight under 20 kg and/or not sufficient venous access or in a critical care situation are entered in the MINI ECP program. We draw 10 ml/kg bw blood from the patient, performed a MNC purification, took the buffy coat in an irradiation bag, added 8-MOP and performed the irradiation with a standalone irradiation device (Macogenic™, Macopharma, Lilly, France), and reinfused the product to the patients. We compared the results with inline and offline method. Patients: 23 patients with > grade 2 acute GVHD refractory to SIT were treated with ECP (median age 6.9y (1.5 – 12), median bw 31 kg (7 – 60), 11 f, 12 m). Patients chart were analysed for GVHD, SIT, GVHD treatment, side effects, number of ECP and outcome of acute GVHD. The schedule was performed to our inhouse standards. ECP started by a frequency of 2 to 3 reinfusions/week, with individualized tapering due to response of the GVHD and the ability to reduce the immunosuppressive therapy (IT).
Results: In total 338 procedures in 23 patients were enroled (123 inline in 4 patients (bw median 40 kg, median 32ECP(10-49)/patient); 174 offline in 13 patients (bw median 24 kg, median 10 ECP(3-55)/patient); 38 MINI in 6 patients (bw median 10 kg, median 7 ECP(3-9)/patient). No severe side effects were observed in either method. 21/23 patients improved (20 CR, 1 PR (gut), 1 SD (skin+liver), 1 PD (skin, liver, gut)). Log-rank-sum-test showed no statistically significant difference (p = 0.98) between the used methods for outcome and survival. The groups were not comparable to age and bodyweight, therefore a bias has to be claimed. In 22/23 IT could have been reduced, especially corticoid dosage could be tapered.
Conclusion: ECP is in our hands an effective second line therapy in acute GVHD. Neither the method used nor the individualized schedule applied seems to influence the outcome. Due to the small patient number, this report could be only a step forward to prospective randomized trials, bringing hopefully an answer to these questions

Is-098: Erythrocyte Depletion of Bone Marrow in Stem Cell Transplantation in a Pediatric Center Comparison of Two Systems

Volker Witt, Herbert Pichler, Elisabeth Beiglböck, Robert Ritter

St. Anna Kinderspital, UKKJ, Vienna, Austria
Email: volker.witt@stanna.at

Background and Aim: ABO incompatible stem cell transplantation has an incidence of about 20 - 50%. To avoid side effects from hemolysis bone marrow is erythrocyte depleted. One possible procedure is the erythrocyte depletion with conventional apheresis systems. In our center we use this approach for ABO incompatible bone marrow transplants, but also for volume reduction if the bone marrow amount is inadequately to high (>30 - 40 ml/kg bw) for unmanipulated transfusion. Recently the OPTIATM System was introduced with a predefined program for bone marrow volume and erythrocyte depletion. We compared the data from our old system (Cobe spectraTM) with the new OPTIATM System in the framework of our JACIE quality system.
Methods: We retrospectively analysed our erythrocyte depletion procedures from 2008 to 2014. In total 32 procedures were performed in 32 patients with various diagnosis undergoing allogeniec stem cell transplantation with HPC-BM as the stem cell source. 21 were performed with the COBE SpectraTM and 11 with the OPTIATM system. The procedures were performed according to the manufacturer's instructions. In 8 cases the bone marrow was prediluted with recipient and donor compatible pRBCs due to the very small volume of the original harvested bone marrow. The mean age of

the recipients for both systems was comparable (n= 21, 8,64 +/- 2,58y COBE Spectra; n = 11, 6,24 +/- 2,45y OPTIA). Samples were taken pre and post procedure to perform HPC, leukocyte, erythrocyte, platelet, and lymphocyte counts. For all this parameters the collection efficiency was calculated. The charts were reviewed for engraftment data. For comparison of the groups the T-Test was applied.
Results: In both groups the preprocedure counts were comparable and not statistically different. The calculated collection efficiencies were for the most cell counts statistically not different between COBE spectra and OPTIA (HPC 98% versus 103%, p=0.57; Monocytes 96% versus 83%, p=0.25; Granulo 21% versus 34%, p=0.1; B-Lymphocytes 98% versus 94%, p=0.67; T-lymphocytes 104% versus 99%, p=0.5; platelets 49% versus 70%, p=0.08; total nucleated cells 40% versus 50%, p=0.14) but a highly significant difference in the collection efficiency for erythrocytes (5.5% versus 1.4%, p=0.001) resulting in a significant lower erythrocyte volume after the OPTIA procedures (pre Hct 30% versus 29%, p=0.58; post Hct 15% versus 4%, p<0.001; Erythrocyte volume in the product after the procedure 14.5 ml versus 3.7 ml, p< 0.001). The erythrocyte volume pre procedures was not different (291ml versus 257ml, p>0.05). 17/21 patients (1 died before, 3 data are missing) in the COBE Spectra group showed regular engraftment and 10/11 in the (1 died before engraftment). The day to reach > 1 G/l leukocyte count in the peripheral blood was not statistically different (d +20 versus d +19, p=0.56).
Conclusion: The erythrocyte depletion of bone marrow with OPTIATM system is regarding the leukocytes and leukocyte subpopulations as efficient as the procedure with the COBE spectraTM, even in pediatric patients. The OPTIATM system showed lower erythrocyte contamination, which is superior to the older system and also superior to Fenwal CS 3000 plus and AMICUS if we compare our former published data with this data.

Is-099: Bone Marrow Reconstitution with Young Sca-1+ Stem Cell, Rejuvenate The Heart

Azadeh Yeganeh, Ren Ki Li

Division of Cardiovascular Surgery, Toronto General Research Institute, University Health Network, Toronto, ON, Canada
Email: bayeganeh1@gmail.com

Background and Aim: Aging, inevitable concern for many centuries, associated with various complications including obesity, diabetes, muscle dystrophy and cardiovascular diseases (CVD). One of the suggested treatments for CVD specifically myocardial infarction (MI) is stem cells therapy. However, aged stem cell is one of the limitations that cause stem cells therapy not a very successful approach. During aging, stem cells regenerative capacity altered significantly. Thus the cardiac stem cells of the aged heart have a limited regenerative ability to repair the heart at the time of injury such as MI. Therefore, we studied the possibilities to restore the regenerative capacity of aged heart using young bone marrow (BM) reconstitution and rejuvenate the aged heart.
Methods: Old (20-22 month-old) mice (C57BL/6J) were reconstituted using bone marrow (BM) cells with Sca-1+ and Sca-1- phenotype from young and old GFP mice. Three months after reconstitution, coronary artery occlusion was performed. Cardiac function was measured at different time points. Mice were terminated 3 days after MI. Western blotting and immunofluorescent staining were applied to determine the level and localization of proteins of interest. Flow cytometry was used to confirm the purity of donor cells.
Results: This study revealed that the Sca-1+ cells of young BM donors were the most common cells that were homed into the heart and lungs. In addition, the young-Sca-1+ chimeras showed the greatest ability to improve healing of the aged heart after MI and rejuvenate the heart by activating different pathways. The young Sca-1+ cells had higher ability to differentiate into cells expressing Surfactant protein C (p-SPC), Clara Cell Secretory Protein (CCSP) and Aquaporin 5 (AQP5) in the lungs.
Conclusion: Sca-1+ cells from BM contribute to regeneration of the old heart after injury. Differentiation of BM-Sca-1+ cells into the lung's specific cell type suggests the significant of cell therapy in the lung diseases such as Chronic Obstructive Pulmonary Disease (COPD) and asthma. Thus, this cell type could be an

important target for the future stem cell therapy to regenerate and rejuvenate the aged heart and lungs.
Keywords: Heart, Sca-1+ Cells, Stem Cell Therapy

Is-100: The Role of Bone Marrow-Derived Stem Cells in a Transgenic Mouse Model of Lung Cancer

Behzad Yeganeh

Hospital for Sick Children Research Institute, University of Toronto, Canada
Email: behyeganeh@gmail.com

Background and Aim: The bone marrow (BM) is an extremely rich source of stem cells. Donor-derived BM cells have been shown to give rise to colonic mucosa, renal parenchyma, gastric mucosa, and pulmonary epithelium following total-body irradiation and allogeneic BM transplantation in humans. Furthermore, BM-derived stem cells are frequently recruited to sites of tissue injury and inflammation, and may undergo various stages of histological transformation that lead to the development of cancer. Here, we present a transgenic mouse (TGM) with high incidence of lung cancer in which Calreticulin (CRT, an endoplasmic reticulum resident chaperon protein) is overexpressed in both endothelial and hematopoietic stem cells (HSCs).
Methods: We performed PCR, Western blot, histology, immunohistochemistry (IHC) and immunofluorescence (IF) confocal microscopy to characterize this TGM. Flow cytometry analysis using specific markers of HSCs was used to investigate mobilization of BM-derived stem cells into the blood stream. Anchorage-independent growth was used to evaluate tumorigenicity in vitro.
Results: The histology and IHC analysis of lung cancers in this animals confirmed lung adenocarcinoma with a Surfactant Protein-C positive (SP-CPos) and Clara Cell Protein negative (CCPNeg) phenotype. At early stages of tumor formation, the lungs show signs of increased inflammation as evidenced by congestion, reddish discoloration and the accumulation of inflammatory cells. Our results show that the early stage tumors contain cells that express exogenous CRT and HSC markers including CD133, Sca-1, and c-Kit. As the tumor progresses to a fully developed adenocarcinoma, these cells lose the expression of exogenous CRT and HSCs markers and gain an alveolar type II phenotype (SP-CPos). Using flow cytometry and soft agar assay, we also demonstrated that mobilization of HSCs is increased in TGM as compared to wt controls and that these HSCs are more proliferative and potentially tumorigenic as they are able to form colonies under anchorage-independent growth conditions. In vitro evaluation of tumor cell phenotype following isolation and characterization of lung tumor cells demonstrated a differentiation dependent expression of HSC markers by tumor cells.
Conclusion: The results from this study provide evidence that lung tumors of this TGM are non-epithelial in origin and that undifferentiated populations of tumor cells have HSC characteristics. After differentiation, these cells lose their stem cell phenotype and acquire an epithelial phenotype.
Keywords: Bone Marrow Derived Stem Cells, Trans-Genic Mice, Lung Cancer

Oral Section

Os-001: Adult Autologous Adipose Derived Stem Cell Treatment for Chronic Spinal Cord Injury

Aftab Ahmad Chattha[1*], Rizwan Masud Butt[2], Faiza Munir Qazi[1], Shahzad Anwar[1]

1. Iffat Anwar Medical Complex, Lahore, Punjab, Pakistan
2. Department of Neurosurgery, Services Hospital, Lahore, Pakistan
Email: aftab.nays@gmail.com

Background and Aim: Spinal cord injury (SCI) occurs in the most productive part of life. Treatment options for the treatment of chronic SCI are few and have limited impact on clinical outcomes. Central nervous system (CNS) including brain and spinal cord has limited intrinsic regeneration capability. The study was done to evaluate the safety and efficacy of adult autologous adipose derived stem cells in treating patients suffering from chronic SCI.
Methods: 15 patients were selected with spinal cord injuries at levels from T6 to T10; a written and informed consent was explained to all patients, who signed the consent form. The study included patients with chronic SCI duration of injury was at least 3 years. PRP injections were given twice at specific acupuncture points at 2 weeks interval. Freshly harvested and activated adult autologous adipose derived stem cells were administered at the site of injury with fluoroscopic guidance, intravenous infusion and at some specific acupuncture points. Follow-up was done by a neutral examiner not involved in the treatment every 3 months for the following 1-year.
Results: All these patients were assessed by the neutral examiners who were not involved in the treatment using American Spinal Cord Injury Association's "International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) and Asia Impairment Scale (AIS). 9 patients showed improvement in motor function from grade 2 to grade 4 and their power improved from grade 2 to 4. These patients had improvement in pinprick sensation below the level of injury. 3 patients showed patchy improvement in pinprick sensation below the level of injury.
Conclusion: The results in this study show that Adult Autologous Adipose Derived Stem Cell treat is safe and effective in patients with chronic SCI.
Keywords: Stem Cells, Autologous Stem Cells, Spinal Cord Injury, Cell Therapy

Os-002: The Potential Therapeutic Effect of Melatonin on Human Ovarian Cancer by Inhibition of Proliferation, Invasion and Migration of Human Ovarian Cancer Stem Cells

Maryam Akbarzadeh[1], Nasser Samadi[2*], Mohammad Nouri[1*]

1. Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Biochemistry and Clinical Laboratories, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Email: maryamakbarzadehbio@gmail.com

Background and Aim: Ovarian cancer is the fifth most common cancer in women. Metastasis and recurrence are the main reason for high mortality rate in these patients. Cancer stem cells are thought to contribute to tumor recurrence and metastasis in many cancers. In this study we have investigated the role of anti-invasive effects of melatonin as a natural hormone in ovarian cancer stem cells (CSCs).
Methods: Isolation of CSCs was performed by MACS. Self-renewal ability was evaluated by spheroid formation and SOX2 protein expression. MTT assay and flow cytometry of ki67 expression were applied to determine CSCs proliferation rate under melatonin treatment. Invasion was assessed by matrix metalloproteinase 2 and 9 and EMT-related gene expressions by real time RT-PCR. The activity of MMP2 and MMP9 also were assessed by zymography. Cell migration and related signaling pathways were analyzed by Transwell migration assay.
Results: Isolated CSCs showed the ability of spheroid formation along with SOX2 overexpression. Melatonin (3.4 mM) inhibited proliferation of CSCs by 23% which was confirmed by a marked decrease in protein expression of Ki67 ($p<0.05$). Applying luzindole abolished anti-proliferative effect of melatonin by 12.33% and 17.59% in SKOV3 and CSCs respectively. Melatonin

also decreased EMT related gene expressions increase in E-cadherin gene ($p<0.05$). Incubation of CSCs with melatonin showed a marked decrease in MMP9 expression and activity without any significant change in MMP-2 activity ($p<0.05$). Melatonin also inhibited CSCs migration in a partially receptor dependent manner. Melatonin induced suppression in migration of CSCs with no effect on PI3K and MAPK signaling pathways.
Conclusion: Our results suggest that melatonin as a natural compound, can be considered as an important adjutant to control invasion, metastasis and proliferation of CSCs.
Keywords: Cancer stem cells, Ovarian Cancer, Melatonin, Invasion, Metastasis

Os-003: Use of Umbilical Cord Blood Crude Serum to Treat Skin Injuries: A Study on Animal Models

Intesar AL Ramahi, Abdulsamed Rahman*, Hanan Jalal kassab

AL Razi center for Research and production of Diagnosis kits, Baghdad, Iraq
Email: dr.intesar.alramahi2017@gmail.com

Background and Aim: There is evidence that umbilical cord blood (UCB) crude serum has healing effects on skin wounds and burns. The aim of this study was to investigate the possible effect of UCB crude serum on accelerating the wounds & burns healing in mice and rabbits.
Methods: The present study was carried out in the in Stem cell Research Unit (SCRU) of Al-Razi Center for Research & Medical Diagnostic Kits Production, Corporation for Research and Industrial Development, Ministry of Industry & Minerals, Baghdad, Iraq. Sixty to eighty ml UCB was obtained from human donors after delivery at Al-Karkh hospital. The donors were negative regarding the human immunodeficiency, hepatitis B and C viruses. Crude serum from UCB was collected and kept in a freezer at -20℃ until being used. Animals were randomly divided into three groups (controls and treatment), after causing the classic wound in the back area of each mouse and rabbit. In the first group, the wound was covered with UCB crude serum, wound of the second group was covered with cetrimide cream the third group received no coverage (control group).
Results: It was found that the wounds healed very well during 6 to 15 days and the average length of treatment was 1.23 days. Wounds of the group treated with UCB crude serum healed more rapidly than the other two groups. UCB crude serum can reduce the skin wound healing duration.
Conclusion: UCB crude serum is a good preparation for skin injuries and wounds.
Keywords: Umbilical Cord Blood, Skin, Wound, Serum, Regenerative Medicine

Os-004: Type 2 Diabetes Inhibited Human Mesenchymal Stem Cells Angiogenic Response by Over-Activity of the Autophagic Pathway

Omid Cheraghi*

Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
Email: omidcheraghi7070@gmail.com

Background and Aim: understanding the angiogenesis mechanisms, as de novo formation of blood vessels from pre-existing vessel beds, is one of the most interesting issues in the domain of regenerative medicine, peculiarly during physiological, and pathological conditions. Continuous in vitro and in vivo investigations to obtain endothelial cells (ECs) have contributed to several studies indicating the possibility of mesenchymal stem cell (MSC)-derived ECs that can restore blood supply in ischemic tissues. Noteworthy, accumulating data support the implication MSCs could also initiate neovascularization by paracrine manner through the secretion of multiple pro-angiogenic factors.
Methods: Human primary stem cells were enriched and incubated with serum from diabetic and normal subjects for 7 days.
Results: Compared to data from the control group, diabetic serum was found to induce a higher cellular death rate ($P<0.001$) and apoptotic changes ($P<0.01$). We also showed that diabetic condition significantly abolished angiogenesis tube formation on Matrigel substrate, decreased cell chemotaxis ($P<0.01$) in response to SDF-1a, and inhibited endothelial differentiation rate

(P<0.0001). Western blotting showed autophagic status by high levels of P62 (P<0.0001), beclin-1 (P<0.0001), and increase in LC3II/I ratio (P<0.001). In vivo Matrigel plug assay revealed that supernatant conditioned media prepared from cells exposed to diabetic serum caused a marked reduction in the recruitment of VE-cadherin- (P<0.01) and a-SMA-positive (P<0.0001) cells 7 days after subcutaneous injection.
Conclusion: we noted a crosstalk network between DM2, angiogenesis, and autophagy signaling. DM2 could potently modulate angiogenesis by the interaction of IL-1b with downstream insulin receptor and upstream androgen receptor. Corroborating to data, diabetic serum led to abnormal regulation of P62 during the angiogenic response.
Keywords: hMSCs, Diabetes Type 2, Angiogenesis, Autophagy, Differentiation, Paracrine Activity

Os-005: Transdifferentiation of Gliosis to Oligodendrocyte Progenitor Cells

Samaneh Dehghan[1], Sangita Biswas[2], Mohammad Javan[1], Wenbin Deng[2*]

1. Department of Physiology, School of Medicine, Tarbiat Modares University, Tehran, Iran
2. Department of Biochemistry and Molecular Medicine, University of California, Davis, California, USA

Background and Aim: Oligodendrocyte death or loss of oligodendrocyte precursor cell (OPC) pool in the CNS results in incomplete remyelination in many demyelinating disorders, leading to disabilities. As an alternative to exogenous oligodendrocyte replacement therapy via embryonic or induced pluripotent stem cells derived oligodendrocyte precursor cells (OPCs), in vivo direct conversion of reactive astrocytes to OPCs in situ is of considerable interest. Most CNS demyelinating injuries are associated with profound astrocyte activation. Reactive astrocytes may be more amenable to reprogramming to OPC in the diseased CNS.
Methods: Therefore, RFP- Oct4 lentiviral particles were injected into corpus callosum two days after Lysolecithin injection as a demyelinating agent. Expression of Oligodendrocyte progenitor cells markers in transduced cell was assessed by Immunohistoflourocence. In all experimental group, Valproic acid was administrated one week before the lentiviral injection.
Results: Here we demonstrate that reactive astrocytes expressing Oct4, can directly trans-differentiate into PDGFR-α positive OPCs in a context dependent manner. Specifically, valproic acid (VPA) pretreatment followed by the ectopic expression of the single transcription factor, Oct4, in reactive astrocytes, is sufficient to convert them into induced OPCs in the adult mouse brain in presence of a lysolecithin induced (LPC) demyelinating lesions.
Conclusion: As a proof of principle, we also show that primary mouse astrocytes pretreated with VPA, and virally transduced to express Oct4, can transdifferentiate into PDGFR+ OPCs and result in greater remyelination in corpus callosum than in untreated mice.
Keywords: Reactive Astrocyte, Oct4, VPA, OPC, Transdifferentiation

Os-006: Tissue Engineering of Stem Cells and Wharton's Jelly for Damage Repair

Bahaa Fakhri Hussein[1], Khayreia Kazzum Habeeb[1], Ibrahim A.H. Al-Zubaidy[2*]

1. University of Baghdad, College of Veterinary Medicine, Department of Anatomy & Histology, Iraq
2. University of Baghdad, College of Veterinary Medicine, Department of Internal Medicine, Iraq
Email: bahaafakhri1961@yahoo.com

Background and Aim: Wharton's jelly is a mucous connective tissue, which originates in the extraembyonic mesoderm and is composed of myofibroblast-like stromal cells (umbilical cord stroma cells; UCSCs), collagen fibers, and proteoglycans. It also contains some fibroblasts and macrophages, and is a postanatal source of fetal stem cells.
Methods: The in vitro developmental potential embryonic tissue was derived from three-dimensional Wharton's Jelly scaffold of human umbilical cord which was seeded with mesenchymal stem cells that has been investigated as a step prior to clinical investigation. Thus, Wharton's jelly is a gelatinous substance within the umbilical cord, which an attractive candidate to forming the embryonic tissue for repairing the damage that depend on the differentiation of stem cells.

Results: Wharton's jelly which converted to the inert material scaffold after added some factors. Therefore, many of histologicaly result observed were similar to embryonic tissue in 5- to 10-days of the egg-embryo divisions. In advance stages, from 15 days of culture there are many of the embryonic structures were created containing the myocardiac muscle, fibrous and adipose tissue in addition to blood vessels.
Conclusion: Ideally, these scaffolds have following characteristics: biocompatible and biodegradable, suitable surface chemistry and highly porosity, with an interconnected pore network.
Keywords: Umbilical Cord, Mesenchymal Stem Cells, Wharton's Jelly Scaffold

Os-007: Effect of Trichostatin A on MHC, histone deacetilaze and DNA Methylteransferase Gene Expression in Somatic Cell Nucleus Transfer Stem Cells

Fattaneh Farifteh[1], Mohammad Salehi[2], Elham Fazeli[1], Mojgan Bandehpour[2], Marefat Ghafari Novin[1], Mohsen Nourozian[1], Ahmad Hosseini[2*]

1. Department of Cell Biology and Anatomical Sciences, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Science, Tehran, Iran
Email: elhamfazeli15@yahoo.com

Background and Aim: Embryonic stem cells (ESCs) can differentiate into whole cell of the body, for this reason ESCs can be used for therapeutic goals in regenerative medicine. Somatic cell nuclear transfer (SCNT) is an alternative approach for produce ESCs without fertilization. Trichostatin A (TSA) is a HDACi that increase the histones acetylation and thus enhance expression transcriptionally silent allele of imprinted genes. TSA might promote the reprogramming process and improve cloned embryo development. The aim of this study was determination Effect of Trichostatin A on MHC, histone deacetilaze and DNA methylteransferase gene expression in somatic cell nucleus transfer stem cells.
Methods: In this experimental study, mature oocytes were recovered from BDF1 [C57BL/6×DBA/2) F 1 mice] mice and enucleated by micromanipulator. Cumulus cells were injected into enucleated oocytes as donor. Reconstructed embryos were activated in the presence or absence of TSA and cultured for 5 days. Blastocysts were transferred on inactive mouse embryonic fibroblasts (MEF), so ESCs lines were established. We selected 15 genes which consisted of MHC- (Qa-1, Qa-2, CIITA, H2db, H2dd , H2KB, H2KD, H2-IE-bb, H2-IE-bd), DNA DNA methylation - (Dnmt-1, Dnmt3a, Dnmt3b), Histone deacetylase- (Hdac1, Hdac2, Hdac3), genes in embryonic stem cell derived from blastocyst with in vitro treated with and without TSA blastocysts (group 1 and 2)and in vivo blastocysts (control group). For Measurement of gene expression were used real-time PCR (RT-PCR). All statistical analysis was performed using SPSS 11.5 software with $p < 0.05$ indicating significance.
Results: The blastocyst formation rate of the SCNT embryos treated with 100 nM TSA was higher than that of untreated embryos and control group. Stem cells in group 1 displayed up-regulated expressions of genes including Qa-2and H2-IE-Bd ($P<0.05$), whereas Dnmt1 and Hdac2 gene displayed down-regulated expressions ($P<0.05$). Stem cells, Dnmt3b, Hdac1, Hdac3 and H2-IE-Bd gene were significantly up-regulated ($P<0.05$), while Qa-1and Hdac2 , H2db, H2dd , H2kb and H2kd gen was down-regulated ($P<0.05$). Compared with treated embryos with TSA, stem cells in untreated group (GROUP 1) displayed up-regulated expressions of genes including Dnmt3b, Dnmt3a, Dnmt1 and Hdac2 ($P<0.05$), whereas H2kb ,H2dd, and H2-IE-Bd gen displayed down-regulated expressions ($P<0.05$)
Conclusion: TSA have positive effect on growth and development of SCNT embryos but TSA lead to abnormal changes in MHC, Dnmt, HDAC gene expression in ESCs cell lines.
Keywords: Somatic Cell Nuclear Transfer, Trichostatin A, Epigenetics Modification

Os-008: Production of Knockout Mice with FAM83H Gene Modifications by CRISPR/Cas9 Mediated Genome Engineering

Fardin Fathi[*]

Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran

Email: farfath@gmail.com

Background and Aim: Fam83h gene encodes a non-secreted protein known as Fam83h which is targeted for the nucleus and it predicted to play a role in the structural development and calcification of tooth enamel. In humans, Defects in gene FAM83H cause autosomal dominant hypocalcified amylogenesis imperfecta (ADHCAI). In this study we applied CRISPR/Cas9 system to target Fam83h gene in mice.
Methods: We directly microinjected the Cas9D10A mRNA (50ng/µl) mixed with sgRNAs (25 ng/µl) into pronucleus. Then two cell embryos Transferred to 0.5 day psudopregnant mouse and pups were born after 19 days. In order to screen newborn pups DNA extraction was performed from tail biopsies and PCR was conducted using specific primer for targeted region of Fam83h. Target region for FAM83H gene disruption by Crisper-Cas9-mediated cleavage were identified by DNA sequenceing.
Results: Our results showed that after Microinjection of mixed cas9/sgRNA into 135 zygotes, 75 zygotes were survived, 55 zygotes were developed to 2cell stage and then transferred to 0.5 day psudopregnant mice. 11 Pups were born and results of sequencing analysis showed that 7 mice have mutations in interested region. Among 7 knockout mice, 6 mice (85.7%) have deletion, 5 mice (71.4%) have nucleotide substitution, 3 mice (42.8%) have insertion along aimed genomic location. analysis phenotype of mutant mice showed the Mice carrying mutation in targeted region, in compare with control mice have small size and scruffy coat phenotype and one of them have defect in tooth formation.
Conclusion: It can be concluded that injection of zygotes with Cas9 mRNA and sgRNA is an efficient and reliable approach for generation of fam83h gene-modified mice.
Keywords: Amelogenesis Imperfecta, Hair Defects, Knockout Mouse, FAM83H Gene, Truncation, Mutation

Os-009: Human Leukocyte Antigen E Polymorphisms Influence Graft-Versus-Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation

Mehran Ghasemzadeh[1*], Ehteramolsadat Hosseini[1], Anthony P Schwarer[2]

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
2. Department of Hematology and Oncology, Eastern School, Monash University, Melbourne, Australia
Email: mehran1476@yahoo.com

Background and Aim: Hematopoietic-stem-cell transplantation (HSCT) is complicated by histocompatibility-dependent immune responses such as graft-versus-host disease, relapse, and graft rejection. The severity of these common adverse effects is directly related to the degree of human leukocyte antigen (HLA) incompatibility. In addition to the key role of classic HLA matching in influencing HSCT outcome, several lines of evidence suggest an important role for nonclassic major histocompatibility complex class I molecule, HLA-E.
Methods: The interaction of HLA-E with NKG2A, its main receptor on natural killer cells, modulates cell-mediated cytotoxicity and cytokine production, an important role in innate immune responses. In addition, the HLA-E molecule can present peptides to different subtypes of T cells that may either support graft-versus-leukemia effects or be involved in bridging innate and acquired immunity. To date, the role of HLA-E and its polymorphisms in HSCT outcomes such as graft-versus-host disease, transplant-related mortality, and improved survival has been published by a number of groups.
Results: These data suggest an association between HLA-E polymorphisms and relapse.
Conclusion: Whether the engagement of the HLA-E molecule in the modulation of donor T cells is involved in the graft-versus-leukemia effect, or whether a different mechanism of HLA-E dependent reduction of relapse is involved, requires further investigation.
Keywords: Hematopoietic Stem Cell transplantation (HSCT), HLA-E, Polymorphisms, Relapse, NKG2A

Os-010: Immunomodulatory Effects of Mesenchymal Stem Cells on B-Cells and B-Cell Activating Factor Receptor

Arezoo Gowhari Shabgah[1], Mojgan Mohammadi[1*], Jalil Tavakol Afshari[1], Zhaleh Shariati-Sarabi[2], Ali Ghasemi[3]

1. Immunology Research Center, Avicenna Research Institute, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Internal Medicine Department, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Bone marrow stem cell transplantation, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: goharia931@mums.ac.ir

Background and Aim: Human mesenchymal stem cells (hMSCs) suppress function of T-cells and dendritic cells and also represent a promising strategy for cell therapy in the autoimmune diseases. Due to their differentiation capacity to specific cell types and abundant growth factors and cytokines secretion, these cells have drawn much more interest in many fields like regenerative medicine, immunotherapy and etc. Moreover, MSCs can hold potent immunomodulatory and anti-inflammatory effects through cell- cell interactions with lymphocytes or via secretion of soluble factors. There is insufficient and controversial data regarding to the immunoregulatory effects of MSCs on B-cells. B cell-activating factor receptor (BAFF-R) is a receptor for BAFF, an important regulator for the B-cell function. In this study the correlation of the expressions of BAFF-R Was evaluated in patients with refractory rheumatoid arthritis, before and after administration of autologous MSCs.
Methods: Five adult patients with refractory rheumatoid arthritis received autologous MSCs intravenously. Expressions of BAFF-R were detected by multicolor flow cytometry technique. IRCT code: IRCT2015102824760N1.
Results: MSCs-based therapies have shown successful pain relief. No serious adverse events were detected during bone marrow harvesting and administration. The number of B-cells and also the expression of BAFF-R in peripheral blood mononuclear cells (PBMCs) of patients with RA were significantly decreased at the end of first month of the intervention.
Conclusion: Administration of autologous bone marrow derived MSCs seems to be safe and practical in the treatment of refractory rheumatoid arthritis. However there is still much to learn about the interaction between B-cells and MSCs and their influence on the immunopathogenesis of RA following the MSC therapy.
Keywords: Mesenchymal Stem Cell, Rheumatoid Arthritis, BAFF-R, B Cells

Os-011: NGAL Protects Bone Marrow-Derived Mesenchymal Stem Cells under Sub-Lethal Doses of Oxidative Stress Conditions by Regulation of Senescence

Mehryar Habibi Roudkenar[1*], Amaneh Mohammadi Roushandeh[2]

1. Medical Biotechnology Research Center, Faculty of Paramedicine, Guilan University of Medical Science, Rasht, Iran
2. Anatomical Sciences Department, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
Email: mhr376@yahoo.com

Background and Aim: Background; The regenerative potential of mesenchymal stem cells (MSCs) is impaired by cellular senescence, a multi factorial process that has various functions. However, pathways and molecules involved in senescence have not been fully identified. Lipocalin 2 (Lcn2) has been the subject of intensive research, due to its contribution to many physiological and pathophysiological conditions. The implication of Lcn2 has been reported in many conditions where senescence also occurs. Aim: In the present study, we evaluated the role of Lcn2 in the occurrence of senescence in human bone marrow derived mesenchymal stem cells (hB-MSCs) under oxidative conditions.
Methods: Lcn2 was cloned into pcDNA3.1 vector. The recombinant vector transfected to hB-MSCs. Expression of Lcn2 in hB-MSCs was evaluated by RT-PCR, ELISA and Western blot analysis. The MSCs cells were exposed to various stresses and cell proliferation was performed by WST kit. Senescence was evaluated by senescence-associated β-galactosidase staining.
Results: When hB-MSCs were genetically engineered to over-express Lcn2 (MSC-Lcn2) and exposed to H2O2, the proliferation rate of the cells increased. However, the number of colonies and the number of cells that made up each colony in both MSC-V and

MSC-Lcn2 cells decreased compared to those cultivated under normal conditions. Our results revealed that over-expression of recombinant Lcn2 in hB-MSCs decreases senescence induced by H2O2 treatment. Senescent cells were observed in aged hB-MSCs; however, no alteration in the expression level of Lcn2 was detected compared to earlier passages. Finally, a higher amount of Lcn2 protein was detected in the plasma of the elderly than in young people.
Conclusion: Our results suggest that Lcn2 regulates cell survival through senescence under harmful conditions. Our findings might also provide new insight into how cells react to stress and how this cellular response can affect complex processes such as aging in an organism. Our results also suggest that application of Lcn2 would be beneficial to retain the fitness ofMSCs for cell therapy purposes in elderly people, who are susceptible to many diseases in which autologus MSC-based therapy is convenient.
Keywords: MSC, Oxidative Stress, Senescence, NGAL/Lcn2

Os-012: IL-7 Engineered Mesenchymal Stem Cells Increased Fusion Potential in vitro and Improved Cardiac Function in vivo

Kanwal Haneef

National Center for Proteomics, University of Karachi, Pakistan
Email: kanwal.haneef@gmail.com

Background and Aim: Mesenchymal Stem Cells (MSCs) have the ability to differentiate into various types of lineages which make them attractive candidates in the field of regenerative medicine. MSCs hold significant promise as potential therapeutic candidates following cardiac tissue damage. Engrafting stem cells consistently experience programmed cell death and a large number of MSCs die in the ischemic area of heart after transplantation. To maintain the survival of engrafted stem cells against apoptosis, it is beneficial to use number of preconditioning procedures for stem cells prior to their engrafting into injured heart. The aim is to investigate the myogenic potential of virally transduced MSCs in co-culture and after transplanted into infarcted myocardium.
Methods: Rat bone marrow derived MSCs and neonatal rat heart derived cardiomyocytes were characterized on the basis of the presence of cell surface markers by immunostaining, RT-PCR, flow cytometry and western blotting. MSCs were transfected with IL-7 in order to elucidate the roles of this cell survival growth factor in fusion process. Analysis of myogenic potential of IL-7 trasnfected MSCs with cardiomyocytes were done by fluorescence microscopy and flow cytometry. The IL-7 transduced MSCs were than transplanted to the rat model of myocardial infarction. Cardiac functional studies were performed by echocardiography while histological analysis was performed to analyze fibrotic area.
Results: Mesenchymal stem cells expressed CD29, CD90 CD44 surface markers while cardiac specific proteins GATA4, Actin, CTT were present in the isolated cardiomyocytes. The co-culture of IL-7 transfected MSCs with CMs have shown increased number of fused cells as compared to normal MSCs. These fused cells may proliferate and contribute to the regeneration of damaged myocardium. Transplantation of IL-7 transfected MSCs into rat infarcted myocardium model showed improvement in left ventricular cardiac function possibly by enhancing the survival and fusogenic properties of MSCs.
Conclusion: Engineered cells (or tissues) may have an expended clinical applicability in future and may represent a viable therapeutic option for those who require tissue replacement or repair. This study would aid in designing a therapeutic strategy in which MSCs over expressed with growth factors can be used for the treatment of ischemic diseases.
Keywords: Myocardial Infarction, Growth Factors, Co-culture, Fusion

Os-013: Application of a Novel Bioreactor for In Vivo Engineering of Pancreas Tissue

Javad Hashemi[1], Parvin Pasalar[1], Abdolmohamad Kajbafzadeh[2*]

1. Department of Clinical Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Pediatric Urology and Regenerative Medicine Research Center, Section of Tissue Engineering and Stem Cells Thera-

py, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
Email: j.hashemii@yahoo.com

Background and Aim: Type 1 diabetes is characterized by autoimmune destruction of pancreatic cells. Insulin therapy represents the current treatment for this disease, but exogenous insulin cannot imitate the physiology of insulin secretion. Organ transplantation is an acceptable treatment for native organ failure. However, it is associated with several problems due to a number of reasons, such as lack of an appropriate donor and immunosuppression. Herewith, in our present study a novel model has been presented for in vivo recellularization of acellular pancreas by implanting between the host pancreas and adjacent omental flap.
Methods: The pancreases were harvested and cannulated via the common bile duct. The scaffolds were acellularized by a detergent based protocol and infused with methylene blue to turn blue before implantation. 35 rats were anesthetized and their abdomens opened with midline incision. The spleen was extracted with adjacent omentum and placed outside the abdomen. The acellularized scaffold was stretched over the host pancreas and the omentum was wrapped around it to make a sandwich-like structure, which was then fixed with Chromic Sutures 6-0 and marked with Prolene 4-0 at four sides.
Results: All the samples were biopsied at 14, 30, 60, 90, and 120 days post transplantation. The samples were recognized by Prolene sutures and the omentum was easily separated by the tissue but the distinction between host and implanted tissue was performed under a surgical microscope. The bluish color of implanted tissue was changed to a yellow color that indicates the implanted tissue. Histopathological analysis showed marked recellularization of acellularized pancreas with visible neovascularization and neoβ-cells with minimal inflammatory response.
Conclusion: It is a novel strategy to produce a normal-like pancreas by allograft transplantation for pancreas tissue engineering. We observed that in vivo transplantation of acellularized pancreas can promote recellularization, proliferation, and differentiation by blood circulation. These findings support that in vivo studies can contribute to finding faster solutions for treatment of diabetes.

Keywords: Acellularization, Pancreas, Bioscaffold

Os-014: The effect of Human Umbilical Cord Wharton's Jelly Stem Cells Cultured on amniotic membrane in Chronic Wound Healing (Clinical control randomize trial)

Seyedeh Sara Hashemi[1], Ali Akbar Mohammadi[2*], Hamed Kabiri[2]

1. Burn and Wound Healing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Plastic and Reconstructive Surgery, Burn and Wound Healing Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Email: sara_hashemi@sums.ac.ir

Background and Aim: Wound healing is a complex and dynamic process which includes restoration as soon as possible cellular structures and tissue layers are damaged. Due to the excellent properties of mesenchymal stem cells and amniotic membranes, we decided that combination of Wharton's jelly stem cell, acellular amniotic graft to use a perfect dressing for chronic wounds and scar less wound treatment.
Methods: After getting the written consent of the patient, they divided randomly into 3 groups: Group I: Wounds covered by usual dressings and usual care given. Group II: Wounds covered by acellular amniotic graft dressing. Group III: Wounds covered by acellular amniotic graft dressing, mesanchymal stem cell dressed every 3 days with the same method. Data are recorded by a physician who is unaware of the classification. In this study percentage of wound healing, time of wound healing and time of the admission at the hospital are compared by capturing photo and planimetry.
Results: On day 6, a statistically significant difference ($p < 0.01$) was observed in mean wound size, with wound size in the group III than in the group I and II. At 2 weeks after cell transplantation, more than 80% of patients exhibited obvious improvements in Wound Healing.
Conclusion: Human umbilical cord stem cells cultured on amniotic scaffolds could be an alternative source instead bone marrow stem cells for cell therapy and tissue engineering.

Keywords: Mesenchymal Stem Cells, amniotic scaffolds, Chronic Wound Healing, Clinical trial.

Os-015: In Vivo Effects of Allogeneic Mesenchymal Stem Cell in a Rat Model of Acute Ischemic Kidney Injury

Shahrzad Havakhah[1], Abolfazl Khajavi Rad[2*], Ahmadreza Bahrami[3], Mojtaba Sankian[4], Gholam Hossein Kazemzadeh[5]

1. Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Biology, Faculty of Sciences, Ferdowsi University of Mashhad
4. Immunobiochemistry Laboratory, Immunology Research Center, Bu-Ali Research Institute, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
5. Vascular and Endovascular Surgery Research Center,1 Faculty of Medicine, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
Email: havakhah.sh@gmail.com

Background and Aim: Kidney diseases highly prevalent in general population and resulted in significant morbidity and mortality. Acute kidney injury (AKI) occurred in one in five adults and one in three children hospitalized with acute illness. Mesenchymal stem cell (MSC) can ameliorate renal IRI, glycerol model of AKI, cisplatinum model of AKI, glomerulonephritis model, Alport's syndrome, diabetic nephropathy and rhabdomyolysis-associated AKI. The aim of this study was to test the therapeutic potential of MSCs administered via suprarenal aorta, immediately after reflow in a rat model of IRI/AKI.
Methods: MSCs were generated by standard procedures, from the femur and tibia of 6 weeks old male Wistar rats. Cells had a typical spindle-shaped appearance, and the MSCs phenotype was confirmed by differentiation into osteocytes and adipocytes with specific differentiation media. Culture expanded MSCs were negative for CD45, CD11b, CD34 and positive for CD44, CD166, CD73 and CD90 expression, determined by FACS analysis. Passage 3-4 was used in all experiments. Adult male Wistar rats weighing 250–300 g were used. The rats were randomly assigned to the following groups: vehicle treated, IRI with vehicle-treated, and IRI with cell treated. After laparotomy, renal pedicles were clamped for 40 min and the suprarenal aorta was cannulated and ~2×106 MSCs/animal were given. Blood samples were collected at baseline and days 1 post-IRI and were determined by autoanalyzer.
Results: MSCs were generated by standard procedures and were morphologically defined by a fibroblast-like appearance, confirmed by their ability to differentiation and flow cytometry analysis. 40 min of clamping in animals led to severe renal insufficiency, as evidenced by a rise in serum Cr to 3.41±0.23 mg/dl and serum BUN to 86.8±6.7 mg/dl in vehicle-treated IRI rats at 24 h postischemia. Animals infused with MSC, had significantly lower serum Cr, 1.05±0.09 mg/dl and BUN levels, 58.92±3.96 mg/dl at 24 h after cell injection compared with vehicle-treated IRI animals.
Conclusion: The present study provides further evidence that MSC has significant renoprotection effects in rats with IRI/AKI. Current thoughts as to how they might ameliorate renal IRI are as follows: transdifferentiation/de-differentiation/homing of SC, paracrine or endocrine effects of grafted SC, the role of resident stem/progenitor cell expansion and proliferation. MSCs are known to have immunomodulatory properties and secrete various growth factors and cytokines. Accordingly with our results, there might not be any transdifferentiation events of MSC within the first day of administration, and the protective effects occur via differentiation-independent mechanisms, due to various cytokines secreted by MSCs, and expression of several growth factors such as HGF, VEGF, and IGF-I, or their antiapoptotic, mitogenic and other cytokine actions such, renoprotection outcomes are not obtained by infused fibroblasts. In conclusion, our data showed the promising kidney-protective effect of MSC in IRI/AKI experimental model in intra-arterial injection route. More investigations are required to determine the mechanism responsible for this recovery.
Keywords: Mesenchymal Stem Cells, Acute Kidney Injury, Cell Therapy

Os-016: A New Approach to Provide Functional NK Cells Applicable for Cellular Therapy

Ehteramolsadat Hosseini[1*], Mehran Ghasemzadeh[1], Anthony P Schwarer[2]

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
2. Department of Hematology and Oncology, Eastern School, Monash University, Melbourne, Australia
Email: e.hosseini10@yahoo.com.au

Background and Aim: NK cells ability to eradicate either infected or malignant cells without prior sensitization make them as a proper candidate for adoptive immunotherapy. Considering umbilical cord blood (UCB) as a rich source of hematopoietic stem cells, we introduced a cost-effective approach to expand CD3depleted-UCB-MNCs into the functional NK cells.
Methods: CD3depleted-UCB-MNCs were expanded in the presence or absence of a feeder (BM-MSCs or osteoblasts), with or without cytokines for 2 weeks. Cell differentiation into NK cells was determined by flowcytometry-based assay. NK cells functional activities quantified by LAMP-1/CD107a expression and intracellular release of TNF-α and IFN-γ as well as LDH release and PI staining in targets.
Results: Higher fold expansion of NK cells was observed after two weeks in the presence of BM-MSCs and cytokines (104±15 folds) compared to osteoblasts and cytokines (84±29 folds, $p<0.05$). On CD3depleted-UCB-MNCs in the presence of BM-MSCs and cytokines, lower expression of CD3, CD19, CD14 and CD15 (on day 14 compared to day 0) and CD69 (on day 14 compared to day 7) as well as higher expression of CD2 and CD7 were suggestive for cell differentiation into mature NK cell lineage. A strong cytolytic potential of expanded cells against K562 was also identified with higher LDH release and PI % of targets. We also observed significant upregulation of LAMP-1 with decreased release of IFN-γ and TNF-α from effectors.
Conclusion: We demonstrate an effective expansion of UCB-NK cell progenitors to large numbers of NK cells maintaining their functional capabilities applicable for cellular therapies.
Keywords: CD3depleted Mononuclear Cells, Cytokine, Cytotoxicity, Mesenchymal Stem Cells, NK Cell, Osteoblast, Umbilical Cord blood

Os-017: NCR Overcomes the Inhibitory Effect of NKG2A and Supports Functional Recovery of NK Cells after Allogeneic Hematopoietic Stem Cell Transplantation

Mehran Ghasemzadeh[1], Ehteramolsadat Hosseini[1*], Anthony P. Schwarer[2]

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
2. Department of Hematology and Oncology, Eastern School, Monash University, Melbourne, Australia
Email: e.hosseini10@yahoo.com.au

Background and Aim: NK cell cytolytic potential against residual leukemic cells is crucial for immune system reconstitution after hematopoietic stem cell transplantation (HSCT). NK cell function is regulated by a delicate balance of signals induced by activatory and inhibitory receptors. Since immune recovery after transplant still remains a major concern, in this study we studied the counterbalance of different NK cell receptors after HSCT and its importance in NK cell functional recovery.
Methods: We investigated NK cell reconstitution in 27 AML and ALL patients at 1st, 2nd, 3rd, 6th, 9th and 12th months following HLA-matched allogeneic HSCT. Patients' profiles were then compared to those of relevant donors as the control population. NK cells were evaluated for their cytolytic activity in a standard 51Cr release assay against different target cells and also analyzed for their receptors expression using flow cytometry method.
Results: We found an elevated expression of natural cytotoxicity receptors (NCRs) and NKG2A, a decreased expression of CD16 and killer Immunoglobulin-like receptors (KIRs) along with an impaired cytotoxicity of post- HSCT NK cells. All the abnormalities were normalized by one year after HSCT.
Conclusion: Collectively, we confirmed an early recovery of NCRs as well as a gradual functional reconstitution on post-transplant NK cells, the phenomenon that is also along with the significant decrease in NKG2A as a major inhibitory receptor. These results suggest an important role for the kinetics of reconstituted receptors in NK cell functional recovery, the finding that may be considered as a predictive value for HSCT outcome.

Keywords: HSCT, NCRs, NK cell, NKG2A, Reconstitution

Os-018: Deregulation of Molecular Signalling Pathways in Leukaemia Cells Affected by Mesenchymal Stem Cells in Medicating the Growth Inhibition of Leukemic Tumour Cells

Vahid Hosseinpour Sarmadi, Rajesh Ramasamy*, Salma Ahmadloo, Seow Heng Fong, Ling King Hwa

Universiti Putra Malaysia, Serdang, 43400, Selangor Darul Ehsan, Malaysia
Email: vahidsarmadi@gmail.com

Background and Aim: Leukaemia is a hematopoietic malignancy in which conventional treatments may be associated with chemotherapy resistance, resulting in tumour relapse and thereby causing poor prognosis. Mesenchymal stem cells derived from umbilical cord (UC-MSCs) have shown to have an impact on the growth of various type of tumour cells such as leukaemia. However, there is little or no information on the molecular mechanism involved by which UC-MSCs inhibits the growth of leukaemic tumour cells. Thus the present study was designed to conduct gene expression profiling of Leukaemia cells grown in the presence of UC-MSCs.
Methods: The isolated cells from human umbilical cord Wharton's Jelly were characterised by immunophenotyping and differentiation capacity to adipocyte and osteocyte. Subsequently, the effect of UC-MSCs on leukaemic tumour cell viability and proliferation were analyzed by MTS assay and tritiated thymidine incorporation assays. Furthermore the inhibition of overall cell growth was assessed by transwell assay, apoptosis assay and cell cycle analysis. Finally, we conducted microarray gene expression profiling of leukaemia cells grown in the presence of UC-MSCs to gain further molecular insight for the growth inhibiting activity of UC-MSCs on HL-60 and BV173 cell lines. The microarray results were further validated by RT-qPCR of selected genes and also validated by Western blot assay for corresponding protein expression.
Results: The results showed that UC-MSCs passed the minimal criteria to define human MSCs, as proposed by ISCT. Moreover, the MTS assay and tritiated thymidine incorporation assay showed that UC-MSCs was able to attenuate leukaemic cell viability and proliferation in a dose dependent manner. Further analysis revealed that the UC-MSCs-induced inhibition was mediated by direct cell to cell contact, without apoptosis induction, and the growth inhibition was in part due to G0/G1 phase cell cycle arrest of leukaemia cells. Furthermore the microarray results identified 502 and 3019 differentially expressed genes (DEGs) with fold-change (more than 2 fold and p-value<0.05) in HL-60 and BV173 cells, respectively. Further analysis showed that HL-60 and BV173 share 35 DEGs (same expression direction) in the presence of UC-MSCs. In silico analysis indicated that the most of DEGs are implicated in the cell cycle and cell cycle related biological processes and signalling pathways. Finally, the microarray results of selected genes were validated at the mRNA level and protein levels which showed a good correlation.
Conclusion: The present study revealed that UC-MSCs could exert anti-proliferative activity on Leukaemia cells via direct cell to cell contact, and perturbation in cell cycle, without apoptosis induction, which was correlated with dysregulation of tumour suppressor and oncogenes and several DEGs with unknown or unrelated function. Our results will open new door for further cutting-edge molecular investigation.
Keywords: Umbilical Cord Mesenchymal Stem Cells, Leukaemia, Gene Profilling, Signalling Pathways

Os-019: Evaluation of Intra-articular Injection of Platelet Rich Plasma in Athletes with TMJ pain and Dysfunction

Shefaa Hussain[1], Abd Al-Razaq Al-Majedy[2], Samira Jameel Al-Obaidi[2*]

1. Al-Isra University, Amman, Jordan
2. Thi Qar University, Nasiriyah, Iraq
Email: dr.shefaahusain@gmail.com

Background and Aim: To evaluate the effect of intra-articular injection of platelet rich plasma (PRP) in athletic people suffering pain and dysfunction in temporomandibular joint (TMJ).
Methods: Thirty patients with an age range of 18 to 25 years were included. Clinical manifestations of the patients were limited mouth opening, joint click sound,

and joint pain and dislocation. The patients were referred to radiographic assessments with CT scan and Cone Beam CT in Al-Karkh general hospital and Al-Esraa University where their diagnosis was confirmed. All patients received PRP injection in different doses using PRP kit. The patients were categorized in 3 groups based on the number and interval of injections (1 ml PRP): group I (10 patients) received 3 injections at 0, 3rd and 6th month, group II (15 patients) received 3 injections at 0,1st and 3rd month, group III (5 patients) received one injection only.
Results: TMJ pain and dysfunction of the patients was shown to be improved. The mean percentage of clinical improvement based on pain relief and joint function was 45% in group 1, 40% in group 2 and 15% in group 3 during follow up visits.
Conclusion: Intra-articular injection of PRP is effective to treat TMJ dysfunction and pain. Multiple injections are more effective than single injection of the PRP.
Keywords: Platelet Rich Plasma, Temporomandibular Joint, Regenerative Medicine

Os-020: Nanoporous Alumina Based Localized Drug Delivery System for Bone Implants

Alireza Jahangiri*, Mehdi Tavakoli, Yousef Seyed Jalili

Skeykh Bahaee Research Complex (SBRC), Science and Research Branch, Islamic Azad University, Tehran, Iran - Plasma Physics Research Center, Science and Research Branch, Islamic Azad University, Tehran, Iran
Email: jahangiria@chmail.ir

Background and Aim: Advances in drug delivery had led to increased quality of life and average life expectancy. Breakthroughs in emerging technologies such as regenerative medicine (bioprinting), stem cell biology (iPS) and microfluidics (point of care diagnostics) show a positive outlook. However, conventional drug delivery suffers from problems, and may prove to be incompatible with these technologies. In order to correctly differentiate Mesenchymal stem cells into the desired cell, cell differentiation must be achieved via cell signaling over a long period of time. Incorrect cell differentiation is painful: consider what would happen if instead of cartilage cells, bone cells were induced. In this talk, we present a novel nanoporous alumina based localized drug delivery system, which can deliver drugs over a period of time to the desired tissue.
Methods: Briefly, commercial aluminium sheets were cut up into square pieces (1.5cm x 1.5cm). After achieving a mirror-finish with P2000 sandpaper, the aluminium substrate was fixed in a home-made two electrode electrochemical cell. To reduce the roughness further, the substrate was subjected to chemical polishing in a mixture of Phosphoric and Nitric Acid. Two step anodization was performed by anodizing in a 0.1M oxalic acid electrolyte solution, in a ice-water bath for 8 hours at 40V. Afterwards, the oxide layer was removed via treatment with hot chromic acid for 1 hour. The second anodizing step was performed as the initial step, but the time was reduced to 2 hours. Based upon the data obtained from AFM and SEM analysis, a COMSOL simulation was performed, to evaluate the drug releasing capabilities.
Results: Prior to imaging, the pore widening was performed in dilute phosphoric acid. AFM and SEM confirm the synthesis of nanoporous alumina, with sub-100nm pores. COMSOL simulation shows sustained drug release over a long period of time.
Conclusion: In our research, we have demonstrated a novel nanoporous alumina based localized drug delivery system for bone implants. This system may be used to for sustained release of genes as well as cartilage inducing drugs such as glucosamine directlt to the cartilage. In addition, previous studies have shown that the porous structure leads to a increased mechanical durability.
Keywords: Nanoporous Alumina, COMSOL, Mesenchymal Stem Cells, Drug Delivery

Os-021: Myc Decoy Oligodeoxynucleotide Inhibits Growth and Modulates Differentiation of Mouse Embryonic Stem Cells as a Model of Cancer Stem Cells

Behrooz Johari[1], Mina Ebrahimi-Rad[1], Faezeh Maghsood[1], Majid Lotfinia[1], Zohreh Saltanatpouri[1], Ladan Teimoori-Toolabi[2], Zahra Sharifzadeh[3], Morteza Karimipoor[2], Mehdi Kadivar[1,*].

1. Biochemistry Department, Pasteur Institute of Iran, Tehran, Iran

2. Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran
3. Hybridoma Laboratory, Immunology Department, Pasteur Institute of Iran, Tehran, Iran
Email: kadivar@pasteur.ac.ir

Background and Aim: Myc (c-Myc) alone activates the embryonic stem cell-like transcriptional module in both normal and transformed cells. Its dysregulation might lead to increased cancer stem cells (CSCs) population in some tumor cells.
Methods: Because of the technical limitations, difficulties of keeping CSCs in culture and their similarities with mouse embryonic stem cells (mESCs), we used the latter as a model cell of study to analyze the potential of differentiation therapy mediated by Myc decoy approach. To our best of knowledge this is the first report outlining the application of Myc decoy in transcription factor decoy "TFD" strategy for inducing differentiation in mESCs. A 20-mer double-stranded Myc transcription factor decoy and scrambled oligodeoxynucleotides (ODNs) were designed, analyzed by electrophoretic mobility shift (EMSA) assay and transfected into the mESCs under 2 inhibitors (2i) condition. Further investigations were carried out using fluorescence and confocal microscopy, cell proliferation and apoptosis analysis, alkaline phosphatase and embryoid body formation assay, real-time PCR and western blotting. All experiments were performed in triplicate for Myc decoy and controls.
Results: EMSA data showed that Myc decoy ODNs bound specifically to c-Myc protein. They were found to be localized in both cytoplasm and nucleus of mESCs. Our results revealed the potential capability of Myc decoy ODNs to decrease cell viability by (16.1±2%), to increase the number of cells arrested in G0/G1 phases and apoptosis by (14±3.1%) and (12.1±4.2%), respectively regarding the controls. Myc decoy could also modulate differentiation in mESCs despite the presence of 2i/LIF in our medium the presence of 2i/LIF in our medium.
Conclusion: By approval of the long term effects of Myc decoy ODNs, the optimized approach might be considered as a promising alternative strategy for differentiation therapy investigations.

Keywords: Mouse Embryonic Stem Cells, Decoy ODN, Myc Transcription Factor, Cancer Stem Cells, Differentiation Therapy

Os-022: Microfluidic Environment for Efficient Differentiation of iPSCs to Hepatocytes

Mousa Kehtari[1], Bahman Zeinali[1], Masoud Soleimani[2*]

1. Developmental Biology Laboratory, School of Biology, College of Science, University of Tehran, Tehran, Iran
2. School of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: mousakehtari@gmail.com

Background and Aim: In recent years, microfluidic cell culture devices have been used for applications such as tissue engineering, diagnostics, drug screening, immunology, cancer studies and stem cell proliferation and differentiation. Microfluidic technology allows dynamic cell culture in microperfusion systems to deliver continuous nutrient supplies for long term cell culture. Here, we present a microfluidic bioreactor for culturing and differentiating of iPSCs to hepatocytes in a tissue-like microarchitecture. The microfluidic environment mimicked physiological liver mass transport, enabling sustained culture of high density cells without nutrient limitation for over 4 weeks. The key feature of this design was dynamic flow of medium and mimicking physiological parameters such as shear stress.
Methods: Photomask or desired pattern was designed by Corel Draw (CorelDraw Graphics suite X7) and before fabrication of microfluidic device COMSOL Multiphysics (COMSOL Inc.) was used to estimate mechanical factors such as shear stress. The microfluidic cell culture array was fabricated by softlithography and replicate molding with PDMS.IPSCs were cultured in this device and nutrient depletion within the cell mass was avoided by maintaining a continuous flow of hepatic differentiation medium (100µl/h) that diffused across the bioreactor. Hepatic differentiation of iPSCs and their functionality in this system was evaluated by flow cytometery and secretion of albumin and hepatic gene markers expression.
Results: Flow cytometery analysis showed that more than 97% of iPSCs after treatment with differentiation

medium in this microfluidic bioreactor are albumin positive. Albumin secretion after step2 of differentiation protocol gradually increased and was continued until day 33. Real- Time RT-PCR analysis showed increased expression of albumin, TAT, cytokeratin19 and Cyp7A1 genes during the course of differentiation program.
Conclusion: In conclusion, our results demonstrated that our microfluidic bioreactor could be a proper platform to significantly increase the hepatogenic differentiation potential of iPSCs and could also be introduced as a promising candidate for liver tissue development and drug screening studies.
Keywords: Microfluidic Bioreactor, iPS Cells, Hepatocytes

Os-023: A Novel Scaffold-Free Tissue Engineering Method for Skin Regeneration

Neda Keyhanvar[1], Mohammad Nouri[2], Peyman Keyhanvar[3*]

Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran
Email: nedakeyhanvar@gmail.com

Background and Aim: Given that after degradation and absorption of the tissue engineering scaffolds, cells start to proliferation and migration for compensation of the empty spaces of polymer degradations. This fact can lead to the fibrosis and in some complex situations to the necrosis in that place. And also, enzymatic isolation of the cells from the bottom of the culture plate will end in cell destruction and ECM damage. To overcome this issue, the aim of this study is to use both cell sheet engineering technology, which does not need the degradable scaffolds, and topographically guided stem cell differentiation, which help the mesenchymal stem cells to differentiate better and faster to the skin cells. In 2004, Okano et. al, developed a novel method for isolation of the cells from the culture plates and named it cell sheet engineering. The basis of the method was based on the thermoresponsive smart polymers which shift from the hydrophobic state to the hydrophilic state (and vice versa) following the change in temperature. In 2013, Cerqueira et. al, could develop cell sheets for restoration of the skin wounds using Adipose Derived Stem Cells (ADSCs). In 2014, in another study, Cerqueira et. al, could also develop a homotypic and heterotypic cell sheets to recover the skin wounds. Moreover, different studies were done for evaluation of the topography effect on proliferation of the keratinocytes, and also differentiation of the stem cells have been done. Recently, Mahmoudi et al, developed a cell imprinted substrates for directing the ADSCs differentiation to the keratinocytes.
Methods: After isolating and characterizing the skin cells, the silicon substrate having a skin cell-imprinted topography developed and the morphology of the substrate was evaluated by SEM microscopy. Then, using plasma device, the previously developed silicon substrate has been coated with a layer of smart polymer. FTIR spectroscopy has been done to confirm the coating procedure. Mesenchymal Stem Cells (MSCs) was then seeded on the developed substrate and their differentiation to skin-like cells has been evaluated. Gene expression profiling by quantitative real-time PCR using marker specific primers has been done to evaluate the stem cell differentiation.
Results: Skin cells have been isolated and characterized. The cell imprinted silicon substrate was successfully developed and evaluated using SEM electron microscope. FTIR analysis confirmed that the smart monomer was successfully coated on the substrate. Gene expression analysis confirmed the differentiation of MSCs to skin-like cells seems successful.
Conclusion: Scaffold free tissue engineering methods are among favorite methods of the regenerative medicine. In our study, the cell sheet engineering as a good example of the aforementioned method has been used to overcome the shortcomings of the scaffold based methods in dermatology. Moreover, to help better differentiation of the stem cells, we used cell imprinted topographical clues to develop skin-like cells. After further evaluation and protein expression analysis of the differentiated cells, we can harvest them in a sheet like structures.
Keywords: Mesenchymal Stem Cells (MSCs), Cell Imprinting, Skin Cells, Topography

Os-024: The Potential Role of Mesenchymal Stem cells in Asherman Syndrome Treatment

Masoumeh Majidi Zolbin[1], Hugh S Taylor[2*], Gulcin Sahin Ersoy[2]

1. Tehran University of Medical Sciences, Tehran, Iran
2. Yale School of Medicine, New Haven, USA
Email: masoumeh.majidizolbin@gmail.com

Background and Aim: Asherman's Syndrome (Ash) results from trauma to the endometrium and healing process is orchestrated by complex actions within different cells in which specific molecular pathways have emerged. It is accompanied by the formation of fibrotic tissue and adhesions. The atrophic endometrium is generally a symptom of Asherman's Syndrome, which results from the obliteration of the uterine lining leading to infertility.
Methods: In a current study, we established the mouse model of Asherman's syndrome. Systemic injection of adipose-derived mesenchymal stem cell (AD-MSCs) has been done to target fibrosis pathway in the uterus through different aspects. Engraftment of MSCs evaluated with Flow cytometry, histologic evaluation (IHC, IF, Trichrome and SHG), proliferation, and statistical analysis consequently have been done to figure out the reconstructive role of MSCs in the fibrotic uterus.
Results: Our results demonstrated that MSCs can efficiently remove the uterine fibrosis through the ECM degradation and regain the normal function. As shown by reduced matrix content that mainly produced by myofibroblast through the deposition of alpha smooth muscle actin (αSMA) and Collagen also with fibrosis PCR array to evaluate 84 prominent genes that involved in fibrosis. Atrophic endometrial epithelial cells rapidly regenerated and formed a normal uterine epithelial layer, indicating a robust epithelial-regenerating capacity of treatment with PCNA and CK staining. Also, flow cytometry data demonstrated the engraftment of GFP+ MSCs in the uterus Based on second harmonic generation study collagen deposition will reduce and IHC staining showed the extensive decrease in αSMA content of stroma. Higher expression of PCNA in epithelium also is strong evidence for epithelial regeneration. the significant decline in profibrotic and inflammatory genes are confirmed immunomodulatory of injected stem cells.
Conclusion: Clinical application of stem cell therapy could extensively alleviate patients suffering from impairment of the endometrium and infertility through uterine regeneration. Ultimately with removing extracellular components, have the ability to regrow and repair the uterine lining and to improve functionality to the point of implantation and pregnancy.
Keywords: Asherman Syndrome, MSCs, Adhesion, Fibrosis, Stem Cell

Os-025: Direct Conversion of Mouse Embryonic Fibroblasts into Renal Lineage Like Cells By Establishment of an Initial Epigenetic Activation

Zohre Mansoori Moghadam, Reza Moghadasali[*]

Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute, ACECR, Tehran, Iran
Email: zmansouri70@yahoo.com

Background and Aim: Objective or background considering the increasing prevalence of kidney diseases while current treatments are ineffective; cell therapy has been introduced as a promising treatment. Pluripotent stem cells are one of the available sources for cell therapy; however, their usage is limited by ethical problems, immunological rejection and the ability to form teratoma in vivo. In principle, lineage reprogramming which can be defined as the direct conversion of functional cell types from one lineage to another without passing through an intermediate pluripotent stage could become an alternative to produce the desired cell types. In this study we aimed to convert primary mouse embryonic fibroblasts into renal lineage like cells.
Methods: Materials & methods we made epigenetic instability in mouse embryonic fibroblasts by transducing Yamanaka factors (Oct4, Sox2, Klf4, C-Myc) in medium for generating intermediate cells. Afterwards, we hijacked intermediate cells to renal lineage like cells using defined nephrogenic medium including small molecules CHIR99021 (glycogen synthase kinase 3b inhibitor) and TTNPB (retinoic acid receptor (RAR) agonist).
Results: Four days of transgenic expression of Yamanaka factors in reprograming medium was enough for generating SSEA1+, NANOG- intermediate cells. To confirm that mouse intermediate mesoderm (IM) cells

(renal lineage like cells) induced by this method have an IM signature, we used RT-PCR analysis and immunocytochemistry to measure the expression of IM markers.
Conclusion: Conclusion our findings suggest that direct conversion of mouse embryonic fibroblasts into renal lineage cells is feasible, make it possible to use in renal tissue repair and regeneration.
Keywords: Direct Conversion, Renal Lineage Like Cells, Small Molecules

Os-026: Scaffold-Free Labell-Free Magnetic Levitation of Tissue Spheroids

Vladislav Parfenov[1], Elena Bulanova[1], Elizaveta Koudan[1], Frederico Pereira[1], Alisa Knyazeva[1], Pavel Karalkin[1], Anna Gryadneva[1], Oleg Petrov[2], Michael Vasiliev[2], Maxim Myasnikov[2], Yusef Hesuani[1], Vladimir Mironov[1]

1. 3D Bioprinting Solutions, Moscow, Russia
2. Russian Academy of Science, Moscow, Russia
Email: vladimir.mironov54@gmail.com

Tissue spheroids have been proposed to use as building blocks in biofabrication technologies. Label-based magnetic forces-driven 2D patterning of tissue spheroids requires cell labelling by magnetic nanoparticles. Here we report a first time rapid assembly of 3D tissue construct using label-free magnetic levitation of tissue spheroids. Tissue spheroids (so-called chondrospheres) of standard size and shape capable to tissue fusion have been biofabricated using non-adhesive cell culture flasks from primary culture of ovine chondrocytes. Label-free magnetic levitation have been performed using experimental set with permanent magnets in presence of gadolinium in cell culture media which enables magnetic levitation. Potential toxic effect of gadolinium have been systematically evaluated. Mathematical modeling and computer simulations have been used for modeling of magnetic field and kinetics of tissue spheroids assembly into 3D tissue constructs. Plastic beads have been initially used as physical analogs of tissue spheroids for determining an optimal regime of magnetic levitation in presence of gadolinium. It have been shown that chondrospheres were able to rapidly assemble into 3D tissue construct in the permanent magnetic field in presence of gadolinium in cell culture media. Thus, label-free magnetic levitation of tissue spheroids is a perspective approach for rapid scaffold-free 3D biofabrication and attractive alternative to label-based magnetic tissue engineering.

Os-027: Bioinformatics Estimate of the miR-372 Effect on Genes Involved in Stem Cells Division and Reprogramming

Masoumeh Mohamadian Namaqi, Zarrin Minuchehr*

National Institute of Genetic Engineering & Biotechnology, Tehran, Iran
Email: masume.mohamadian@gmail.com

Background and Aim: The organization of embryonic tissues and the renewal of adult tissues rely on stem cells. Embryonic stem cells have the capacity to divide for long time and maintain their ability to make all cell types within the organism. These are named pluripotent stem cells. Although numerous studies have been done, the mechanisms that tightly regulate stem cell division are still poorly understood. MicroRNAs (miRNAs) are key players in various critical cellular processes such as proliferation, cell cycle progression, and differentiation in many tissue types including stem cells. Hsa-miR-372 regulate human embryonic stem cell division, enhance human iPSC production and human somatic cell reprogramming. MiR-372 is potent inhibitors of TGF-β–induced EMT as well as promoters of MET during reprogramming. It is valuable and affordable that first, we predict target genes and then examine experimentally like the use of the microarray techniques. So the use of bioinformatics methods can be a suitable option for predicting mRNA targets for miRNAs. To accomplish this goal, there are several software and algorithms. Thus, the purpose of this theoretical study was to estimate the effect of miR-372 on genes involved in stem cells division and reprogramming by using different soft wares and databases.
Methods: The potential targets of miR-372 were recognized by use of different algorithms in TargetScan and DIANA databases. Then, a score table was prepared from the candidate genes, based on the affinity of the seed region of miR-372 and the number of targets in the 3`-UTR region of genes. DIANA database evaluates

targets based on miTG score (from 0 to 1, the closer to 1, the greater the confidence) that is a general score for the predicted interaction. Target Scan evaluates targets based on aggregate Pct (Between 0 and 1, the higher the score, the greater the conservation) Ultimately, Candidates were selected (for experimental analysis) from targets that possess the highest score.
Results: we found that the MLLT6, NFIB, DYRK2, UBE2B, E2F7, YOD1, RSF1, and ZBTB41 genes are the most potential targets that might be affected by miR-372 during human embryonic stem cell division.
Conclusion: As was mentioned previously, these genes are under the control of the miR-372 and play a crucial role in human embryonic stem cell division. So, they can be considered as suitable new candidates for experimental evaluation.
Keywords: Bioinformatics, MicroRNA, Human Embryonic Stem Cell, miR-372

Os-028: The Effects of Intravenous Injection of Conditioned Medium of Human Amniotic Membrane-Derived Mesenchymal Stem Cell on Heart Failure

Sarah Allahverdy[1], Maryam Naseroleslami[2], Nahid Aboutaleb[3*]

1. Department of Physiology, Iran University of Medical Sciences, Tehran, Iran
2. Department of Biology, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran
3. Physiology Research Center, Iran University of Medical Sciences, Tehran, Iran
Email: naseroleslami@gmail.com

Background and Aim: Heart failure is one of the most important causes of death in the world. In recent decades, the researchers have focused mainly on using the stem cells for treatment of many illnesses; Due to side effects of stem cell, recently the paracrine secretion instead of stem cells have been focused by researchers. Since, the purpose of this study was to investigate the effects of intravenous injection of conditioned medium of human amniotic membrane-derived mesenchymal stem cell on heart failure.
Methods: Male Wistar rats (n=35, 180 g) were randomly divided into five groups: control, heart failure, heart failure + conditioned medium, heart failure + culture media and heart failure + PBS. At the first day, heart failure was induced by Subcutaneous injection of Isoproterenol (170 mg/kg/d), four consecutive days. After 28 days, induction of heart failure was evaluated by Echocardiography procedure (EF and FS were determined); the day after echocardiography, 50 µg culture media/5 ml PBS in heart failure + culture media group, 50 µg MSC-CM/5 ml PBS in heart failure + conditioned medium group and 5 ml PBS in heart failure + PBS group were injected through the tail vein, two times for 4 days. At last, approval echocardiography procedure were used 4 weeks after the last injection of isoproterenol, and then the heart tissue were isolated and paraffinized. For evaluating the fibrosis level and the number of vessels, Trichrome Masson's staining; for nuclei and texture morphology Hematoxylin and Eosin staining and cardiac function echocardiography was used.
Results: Conditioned medium significantly increased fractional shortening and ejection fraction ($P<0.05$). As well as, it significantly increased the number of euchromatin nuclei and decreased number of heterochromatic nuclei ($P<0.05$). There were also decreased fibrosis level and increased angiogenesis significantly ($P<0.05$).
Conclusion: So, this findings indicated that intravenous injection of stem cells-CM of human amniotic membrane have therapeutic effects on heart failure by reducing the lesion level and preventing the progression of failure due to its paracrine effects
Keywords: Heart Failure, Stem Cells, Conditioned Medium

Os-029: The Novel Method in Explant Culture of Limbal Stem Cells on Human Amniotic Membrane and Fibrin Glue as an Appropriate Scaffold

Abolfazl Nosrati Tirkani[1], Nadia Boromand[2], Dina Nazirulhassan[2], Alireza Hosseinzadeh[3], Danial Taherzadeh[2], Siamak Zarei Ghanavati[4], Daryoush Hamidi Alamdari[5*]

1. Department of Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

2. Student Research Committee, Department of Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Biology, Faculty of Basic Sciences, Islamic Azad University, Sanandaj Branch, Iran
4. Eye Research Center, Khatam-Al-Anbia Eye Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
5. Department of Biochemistry, Biochemistry and Nutrition Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: nosratia9@mums.ac.ir

Background and Aim: Limbal stem cells (LSCs) locate in the limbal basal layer, the narrow zone encircled the cornea, comprise the constant source of the adult limbal epithelial progenitor/stem cells. They are responsible for the persistence of corneal cells homeostasis and corneal cells replacement. Thermal and chemical burn, physical injuries, and congenital disorder caused the limbal stem cell deficiency (LSCD). Corneal transplantation is the current therapeutic strategy that needs to find the appropriate donor and risk of rejection. The LSCs expanded on the epithelial surface of the human amniotic membrane (HAM) is the new therapeutic alternative. The aim of present study was the expanding the LSCs on the HAM. The immunohistochemistry (IHC) and immunofluorescent (IF) were conducted as a confirmatory test.
Methods: The corneal rim obtained from khatam-al-Anbia eye hospital. The rim washed, the conjunctival layer removed, and the rim divided into the 1.5 × 1.5 mm2 segment. Then, the segments fixed on the human amniotic membrane by fibrin glue separately. After two weeks incubation in 5% CO2 and 37 °C, the LSCs develop on the HAM. To verify the LSCs development on the HAM, the paraffin block prepared. The IHC and IF test was done by using ΔNp63 (p63 (A4A): sc-8431) antibody to verify the existence of expanded LSCs.
Results: The results of immunofluorescent confirmed qualitatively the ΔNp63 positive expanded cells on the epithelial surface of HAM. The results of immunohistochemical staining showed that the 3.242% - 6.37% of the explant fibrin-cultured limbal stem cells on human amniotic membrane contained ΔNp63 positive cells. Depends on the age and sex of limbal biopsy donors, the amount of ΔNp63 positive cells expanded on HAM were different.
Conclusion: The explant culture of limbal biopsy is a new method for expanding the LSCs. The fibrin glue used to fix the explant provide an appropriate scaffold for migration of LSCs. Notably, the limbal biopsy and fibrin glue can be obtained from patients themselves thereby the risk of rejection and find the HLA-matched donor has been eliminated. Interestingly, the HAM not only provide a biological carrier matrix but also it's a convenient membrane (surgeon hands friend) with a favorable characteristic such as anti-bacterial activity, anti-tumorigenic, anti-fibrosis, and anti-angiogenic properties. Use of minimally invasive therapeutic strategy along with simultaneous utilization of HAM and fibrin glue elevate the quality (stemness) and quantity of cultivated LSCs. Nevertheless, the transplantation of LSCs expanded on the HAM would be a new therapeutic strategy for management and treatment of patients suffered from LSCD.
Keywords: Limbal Stems Cell Deficiency, Explant Culture, Human Amniotic Membrane

Os-030: Potential of Emu Oil Based Electrospun Nanofibrous Scaffold Captured with ADSCs as an Innovative Bioengineered Cell-Scaffold Construct for Skin Tissue Regeneration

Younes Pilehvar-Soltanahmadi[1], Mohammad Nouri[1], Mikaël M. Martino[2], Amir Fattahi[1], Effat Alizadeh[1], Sepideh Jalilzadeh-Tabrizi[1], Nosratollah Zarghami[1*]

1. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Australian Regenerative Medicine Institute, Monash University, Clayton, Australia
Email: ypilehvar@gmail.com

Background and Aim: Electrospun nanofibrous scaffolds containing natural substances with wound healing properties such as Emu oil (EO) may be had a great potential for increasing the efficiency of stem cell-based skin bioengineering. For this purpose, EO blended PCL/PEG electrospun nanofibrous mats were fabricated and the efficiency of the scaffolds in supporting the adherence, proliferation, cytoprotection and differentiation of adipose tissue-derived stem cells (ADSCs) to keratinocyte was evaluated.

Methods: Fatty Acid and carotenoid composition of pure EO were analyzed using GC/MS and HPLC, respectively. (20wt%) EO-PCL/PEG nanofibrous mats fabricated by electrospinning and the scaffolds were characterized using FE-SEM, FTIR and Universal Testing Machine. MTT assay was used to determine cell viability and antioxidative potential of EO-loaded PCL/PEG nanofibers. Finally, the epidermal differentiation of ADSCs on EO blended nanofibrous scaffolds was assessed via immunocytochemistry and real time PCR.
Results: Adhesion and proliferation of ADSCs on EO-PCL/PEG nanofibers was higher than on PCL/PEG nanofibers and tissue culture polystyrene (TCP) control ($P \leq 0.05$), after 14 days of cell culture. In addition, EO-loaded PCL/PEG matrices with free radical scavenging properties conferred a protective effect against free radicals responsible for cell damage and death by necrosis, while the ability to support cell adhesion and growth was maintained or even improved. Immunostaining of ADSCs on EO-PCL/PEG nanofibers confirmed the change in morphology of ADSCs from spindle to polygonal shape indicating their differentiation into epidermal linage. Also, the expression levels of the keratin (early), filaggrin (intermediate) and involucrin (late) that are involved in epidermal differentiation were upregulated in a stage-specific manner.
Conclusion: In summary, EO-PCL/PEG nanofibrous scaffold appear suitable to support cell adhesion, survival and differentiation of ADSCs and could be convenient candidate for the fabrication of wound dressings and skin bioengineered substitutes with ADSCs.
Keywords: Emu oil, Nanofiber, PCL/PEG, Differentiation, ADSCs

Os-031: Neuroprotective Effect of Combined Treatment with Minocycline and Olfactory Ensheathing Cells after Spinal Cord Injury in Rats

Soheila Pourkhodadad[1*], Shahrbanoo Oryan1, Gholamreza Kaka[2]

1. Department of Biology, Kharazmi University, Tehran, Iran
2. Neuroscience Research Center, Baghiatollah University, Tehran, Iran
Email: s.porkhodadad@yahoo.com

Background and Aim: Spinal cord injury is a destructive disease that led to the severed of neurons and neuronal damage. Several mechanisms such as activation of astrocytes, the formation of glial scar, inflammatory cell invasion occur in the spinal cord injury that cause a lack of re-growth of axons. Inflammatory responses are a major component of secondary injury and play a central role in regulating the pathogenesis of acute and chronic SCI, and seem to play a pivotal role in nerve injury and contribute to the control of the regenerative response. Therefore, it is believed that reducing inflammation could decrease secondary degeneration and the functional deficit after SCI. Minocycline is an antibiotic with immunomodulatory activity that the neuroprotection afforded by minocycline is thought to be associated with its ability to inhibit microglial activation, thereby reducing the levels of cytotoxic factors released by microglia. Several strategies have been developed to rebuild the injured spinal cord in animals such as transplanting OECs cells. Several recent studies have highlighted the potential therapeutic role of olfactory ensheathing cells for the repair of cord injuries and functional recovery. The aim of this study is to reduce inflammation with minocycline and axonal regeneration after spinal cord injury by OEC cells.
Methods: Adult male Wistar rats (220-250 g) obtained from the Razi Vaccine and Serum Research Institute, Karaj, Iran. The rats were divided randomly into five groups of (n = 10 in each group), including: sham group, SCI group, minocycline group, OECs group and OECs + minocycline group. Rats received intraperitoneal injections of minocycline immediately after SCI and then 24 h after injury at a dose of 90 mg/kg. Transplantations were performed 7 days after the injury. Rats received DMEM medium in medium-injected group, transplants of OEC (1 × 106) in OEC group, and OEC + minocycline group by means of 5 µL Hamilton syringe. Locomotor function was evaluated using the Basso, Beattie and Bresnahan (BBB) open-field locomotor test using two independent observers. BBB assessments are performed 1 days after injury and 7, 14, 21, 28 and 35 days post transplantation.
Results: We investigated whether the effect of combination therapy affected on the functional recovery after SCI. These results of BBB showed that SCI could lead to descend BBB scores compared to that of sham group. As shown only OECs-treated or only minocy-

cline-treated effectively increased BBB scores in SCI rats, respectively ($p<0.05$). But there wasn't any significant difference between treatment groups.
Conclusion: The results shown in the present study may be another relevant step to develop a combined treatment for repairing the injured spinal cord.
Keywords: Spinal Cord Injury, Minocycline, Olfactory, Ensheathing Cells

Os-032: In Vitro Generation of Humanized Rat Heart Using hESC-Derived Cardiovascular Progenitor Cells and bFGF -Tethered Decelurized Heart Matrix

Sareh Rajabi Zeleti[1*], Sara Pahlavan[1], Hasan Ansari[1], Saeed Abbasalizadeh[1], Mohammad Kazemi Ashtiani[2], Forough azam Sayyahpour[1], Fahimeh Varzideh[1], Nasser Aghdami[1], Hossein Baharvand[1]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Cell Engineering, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: sareh.rajabi@gmail.com

Background and Aim: To date, many patients suffering from cardiovascular diseases are awaiting for heart transplantation which is largely limited due to donor shortage. However, there are still critical challenges for creating human heart in lab including large scale production of cell ingredients and their quality as well as poor functionality of recellularized organ in vitro due to low recellularization efficacy. In this study, we have developed a novel strategy for in vitro beating humanized rat heart employing a scalable culture system for production of cardiac progenitor cells (CPCs) and a bFGF-tethered whole heart matrix to improve recellularization efficacy.
Methods: The whole rat heart were decellulariezd with modified protocol. After characterization of decellularized heart with histology, DNA content, immunohistochemistry, the heart was immobilized with bFGF and recellularized with CPCs with an optimized dynamic perfusion system. After 12 days the hearts was analyzed for Electrophysiology assessments, histology, gene and protein expression.
Results: We showed that bFGF immobilization (RC-ECM/bFGF) combined with step-wise seeding strategy resulted in improved cell attachment and migration of CPCs into the heart scaffold and subsequently supported higher proliferation and differentiation efficacy as compared to natural scaffold. More importantly, migrated CPCs were successfully differentiated to cardiomyocytes, smooth muscle cells and endothelial cells to reconstruct the decellularized hearts. After 12 days of perfusion, RC-ECM/bFGF exhibited spontaneous and synchronous contractions. Moreover, transmission electron microscopy (TEM) micrographs of RC-ECM/bFGF showed more developed myofilament alignment and cell to cell junctions. Higher expression level of cardiac-associated genes in RC-ECM/bFGF confirmed histological and functional results.
Conclusion: Our study may provide a robust platform for in vitro generation of human heart employing a scalable culture system of CPCs and a functional heart scaffold.
Keywords: Decellularized Heart, Heart Bioengineering, Perfusion Bioreactor

Os-033: Acceleration of Full-Thickness Skin Defect Regeneration Using New Source of Acellular Scaffold with or without Bone Marrow Mesenchymal Stem Cells

Morteza Rashtbar[1], Jamshid Hadjati[1], Jafar Ai[1], Issa Jahanzad[2], Mahmoud Azami[1], Sadegh Shirian[2], Esmaeel Sadroddiny[3*]

1. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Pathology, Immunohistochemistry Laboratory, Cancer Institute of Iran, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: m.rashtbar@gmail.com

Background and Aim: Decellularized extracellular matrices (ECM) based materials are routinely used for a variety of clinical applications. Hereof, in vivo application of decellularized ovine small intestinal sub-mu-

cosal (DOSIS) layer as, a scaffold is yet to be investigated. In this study, the effectiveness of the DOSIS scaffold, with or without bone marrow mesenchymal stem cells (BM-MSCs), in full thickness wound healing of critical-sized defect was experimentally studied in a rat model. The experimental groups included; group I (control), group II (DOSIS) and group III (BM-MSCs-seeded DOSIS). Wound healing of all groups was examined and compared clinically and histopathologically on days 7, 14 and 21 post-operation. Our results represented BM-MSCs-seeded DOSIS accelerated wound contraction and healing compared to both the DOSIS alone and control groups. Epithelization was close to completion 21 days post-operation in DOSIS alone. In OSIS with BM-MSCs group, epithelization was faster and had fully taken place at the subsequent time points. DOSIS layer, as cell-free form with low substantially DNA content, accelerated healing of rat skin wound defects that was created at critical-size and full-thickness. In conclusion, decellularized OSIS alone and in combination with BM-MSCs has the potential to be used as a wound graft material in skin regenerative medicine.
Methods: Fresh OSIS layer was rinsed by tap water then cut longitudinally in 10cm pieces, the tunica mucosa were removed by erosion by wet gauze, and after that external tunica muscularis and serosa layers removed by a very thin knife. For decellularization 0.05% SDS reagent as chemical part, agitation (100rpm) and temperature (37°C) as physical part were used. Hematoxyline and eosin (H&E), DAPI, Masson's trichrome, Alcian blue staining, quantification of GAG content, residual DNA quantification, thickness and mechanical strength, light microscopy and SEM evaluation were used to determine properties of OSIS scaffold. In second part, as in vivo study, BM-MSCs were harvested and seeded (1×105) on scaffolds as allogeneic source. In vivo study was conducted on 12 healthy Specific pathogen-free Male Wistar and Lewis rats (8–12 weeks, weight 200–250 g). Efficacy of sole and MSC seeded OSIS in a rectangular critical-sized (2×2 cm2) full-thickness rat skin defect model were investigated. Planimetry, wound contraction and histological evaluations were done on 7, 14 and 21 days.
Results: Our results represented BM-MSCs-seeded DOSIS accelerated wound contraction and healing compared to both the DOSIS alone and control groups. Epithelization was close to completion 21 days post-operation in DOSIS alone. In OSIS with BM-MSCs group, epithelization was faster and had fully taken place at the subsequent time points. DOSIS layer, as cell-free form with low substantially DNA content, accelerated healing of rat skin wound defects that was created at critical-size and full-thickness.
Conclusion: Decellularized OSIS alone and in combination with BM-MSCs has the potential to be used as a wound graft material in skin regenerative medicine.
Keywords: Small Intestine Sub-Mucosal Layer, Ovine,- Decellularization, Skin Regeneration, Bone Marrow Mesenchymal Stem Cell

Os-034: Engineered Channels throughout a PLGA/ β-TCP Cylindrical 3D-Porous Scaffold Enhance MC3T3-E1 Osteoblast Proliferation in a Static-Flask Bioreactor

Alireza Saatchi, Ghassem Amoabediny*

School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
Email: alireza-saatchi@hotmail.com

Background and Aim: Cell density in 3D-porous scaffolds is important issue to create large engineered constructs for large bone defects. We presumed development of channeled, 3D-porous scaffolds would increase cell density through 3D-porous scaffold in a static-flask bioreactor. One of the critical bottleneck for current tissue engineering approaches is to create dense populated constructs. Here we aimed to employ PLGA/ β-TCP cylindrical 3D-porous scaffold with engineered channels to assess cell proliferation and distribution in a static flask bioreactor.
Methods: PLGA/ β-TCP cylindrical 3D-porous scaffolds (radius 2.2 cm, height 1 cm) were used without channels and with 12 channels (1 millimeter diameter each channel) throughout the 3D-porous scaffold in static flask bioreactor for cell culturing of MC3T3-E1 pre-osteoblast for 21 days. Cell viability on the 3D-porous scaffolds was determined using the Alamar Blue assay after 7, 14 and 21 days. The results of cell viability for scaffolds were normalized compare to day zero. ALP activity of MC3T3-E1 cells determined for each scaffolds after 14 days. Collagen networks were stained

with Picrosirius red staining for each scaffold after 14 days.
Results: After 7 days, the cellular activity increased for channeled and un-channeled scaffolds in static flask bioreactors. After 7 days, in static flask bioreactors, the cellular activity of cell/polymer constructs increased from channeled to un-channeled scaffolds by 15%, though this increase was not significant ($p < 0.05$). After 14 days, in static flask bioreactors, the cellular activity of cell/polymer constructs increased from un-channeled to channeled scaffolds by 13%, though this increase was not significant ($p < 0.05$) but After 21 days, in static flask bioreactors, the cellular activity of cell/polymer constructs increased significantly from un-channeled to channeled scaffolds by 14% ($p < 0.05$). By day 21, channeled scaffolds were expressed higher levels of the ALP activity than un-channeled scaffolds under static flask bioreactors, though it was not significant ($p < 0.05$). The alizarin-red staining on the channeled scaffolds in stirred flask bioreactors were clearly stronger than un-channeled scaffolds in stirred flask bioreactors at 21 days.
Conclusion: In conclusion, our data shows that using channels in 3D-porous scaffolds enhanced MC3T3-E1 pre-osteoblast cell proliferation, which could significantly improve cell viability and collagen networks in the 3D-porous scaffold in the static flask bioreactors. We have shown the channeled scaffold is a promising approach toward creating thick tissue-engineered constructs.
Keywords: Bone Tissue Engineering, 3D-Porous Scaffold, Channeled Scaffold, Cell Proliferation, Static Flask Bioreactor

Os-035: A New Method for Treating Fecal Incontinence by Implanting Stem Cells Derived From Human Adipose Tissue: Preliminary Findings of a Randomized Double-Blind Clinical Trial

Arash Sarveazad[1], Graham L. Newstead[2], Rezvan Mirzaei[1], Mohammad Taghi Joghatae[3], Mehrdad Bakhtiari[3], Asrin Babahajian[4], Bahar Mahjoubi[1*]

1. Colorectal Research Center, Iran University of Medical Sciences, Tehran, Iran.
2. Sydney Colorectal Associates, Prince of Wales Hospital, Randswick, NSW, Australia.
3. Cellular and Molecular Research Center, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.
4. Liver and Digestive Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran.
Email: arashsarveazad@gmail.com

Background and Aim: Anal sphincter defects are a major cause of fecal incontinence causing negative effects on daily life, social interactions, and mental health. Because human adipose-derived stromal/stem cells (hADSCs) are easier and safer to access, secrete high levels of growth factor, and have the potential to differentiate into muscle cells, we investigated the ability of hADSCs to improve anal sphincter incontinence.
Methods: The present randomized double-blind clinical trial was performed on patients with sphincter defects. They were categorized into a cell group (n=9) and a control group (n=9). Either 6×106 hADSCs per 3 ml suspended in phosphate buffer saline (treatment) or 3 ml phosphate buffer saline (placebo) was injected. Two months after surgery, the Wexner score, endorectal sonography, and electromyography (EMG) results were recorded.
Results: Comparing Wexner scores in the cell group and the control group showed no significant difference. In our EMG and endorectal sonography analysis using ImageJ/Fiji 1.46 software, the ratio of the area occupied by the muscle to total area of the lesion showed a 7.91% increase in the cell group compared with the control group.
Conclusion: The results of the current study show that injection of hADSCs during repair surgery for fecal incontinence may cause replacement of fibrous tissue, which acts as a mechanical support to muscle tissue with contractile function. This is a key point in treatment of fecal incontinence especially in the long term and may be a major step forward.
Keywords: Clinical Trial, Fecal Incontinence, Human, Stem Cells Derived From Human Adipose Tissue

Os-036: Safety and Efficacy of Allogeneic Mesenchymal Stem Cell Transplantation in Friedreichs Ataxia Patients

Bita Shalbafan[1], Mandana Mohyeddin[2*]

1. Iranian Social Security Organization, Tehran , Iran
2. Sinacell Company, Tehran , Iran
Email: shalbafan.b@gmail.com

Background and Aim: Friedreich ataxia (FA) is the most frequent cause of genetic ataxia. FA is one of the untreatable neurometabolic disorders that causes the progressive ataxia, developmental Diabetes Mellitus and cardiac involvement .Age of onset is teen ages. There are no effective drug for this disease. According to new studies, in degenerative disease, inflammation is highly suggested as an important cause of disease progression, so, several anti-inflammatory drugs have tried in these diseases. Based on anti –inflammatory effects of mesenchymal stem cells transplantation, it should be a new hope for FA patients to slow disease progression. Autologous mesenchymal stem cells should have lesser efficacy than their allogeneic types, because FA has genetic etiology.
Methods: In this research, we evaluate the safety and efficacy of allogeneic mesenchymal stem cell transplantation in 3 genetic proven FA cases with progressive Ataxia. After intravenous and intratechal injections of 3-4 million per kg, their disability will follow with several frequent quantitative test (SARA, MSFC) for 2 years.
Results: Preliminary reports show no serious side effects in time of injection till 7 months after that. Not only their safety is proven, but also, 2 case of them have significant improvement in neurological exams (quantitative tests) during to 7 months. Disease progression in the third patient was stopped in these 7 months.
Conclusion: Two-year follow up will demonstrate late efficacy and safety of this new hope .We should estimate the time for booster dose, based on their disease progression.
Keywords: Friedriech Ataxia, Allogeneic Mesenchymal Stem Cell, Efficacy, Safety, Disease Progression

Os-037: Naïve NKs Education against Neuroblastoma Tumor via NK Cell-Derived Exosomes from NKs Previously Exposed to Neuroblastoma Cells

Alireza Shoae-Hassani[1], Amir Ali Hamidieh1[2*], Maryam Behfar[2], Seyed Abdolreza Mortazavi-Tabatabaei[1], Rashin Mohseni[1], Jafar Ai[1], Shahab Asgharzadeh[3,4]

1. Applied Cell Sciences & Tissue Engineering Department, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Pediatric Stem Cell Transplant Program, Children's Medical Center, Tehran University of Medical Sciences, Tehran, Iran
3. Division of Hematology, Oncology and Blood & Marrow Transplantation, Department of Pediatrics, The Saban Research Institute, Children's Hospital Los Angeles, USA
4. Keck School of Medicine, University of Southern California, Los Angeles, California, USA
Email: cell.therapy@yahoo.com

Background and Aim: Immune cell-derived exosomes can increase immunity against tumors. On the other hand, tumor-derived exosomes can reduce the immunity and can change the tumor microenvironment to further develop and provide metastasis. These effects take place by an alteration in the innate and adoptive immune cell functions. In this experiment, we have studied the NK cell (NKs) effectiveness on tumor cells after expansion and then incubation it with exosomes.
Methods: The exosomes were derived from two populations of NKs: 1) Naïve NKs and, 2) NKs previously exposed to neuroblastoma cells. Also, we have studied the neuroblastoma derived exosomes (NB-Ex) on NK function. The molecular load of the characterized exosomes (by means of nanoparticle tracking analysis, flow cytometry, scanning electron microscopy and western blot) from NKs exposed to the neuroblastoma cell was revealed their expression of NCRs in addition to CD56, NKG2D, and KIR2DL2 receptors. These exosomes were used to treat NKs and then administered to NB tumor cells both in vitro and in vivo.
Results: Our results showed some kind of NK education by the exosomes. This education from NKs previously exposed to neuroblastoma cell-derived exosomes caused efficient and greater cytotoxicity against NB tumors, but NB-Ex act as tumor promoters by providing a tumor supporting niche.
Conclusion: This method of preparing the exosomes has a dramatic effect on activation of anti- NKs against neuroblastoma cells.
Keywords: Cancer Therapy, Exosome, Immune Cell Therapy, Natural Killer Cell, Neuroblastoma

Os-038: Conjunctiva Derived Mesenchymal Stem Cell (CJ-MSCS) As a Potential Platform for Differentiation into Corneal Epithelial Cells on Bioengineered Electrospun Scaffold

Fatemeh Soleimanifar[1], Yousef Mortazavi[1*], Samad Nadri[1], Masoud Soleimani[2]

1. Medical Biotechnology and Nanotechnology Department, Faculty of Medicine, Zanjan University of Medical Science, Zanjan, Iran
2. Department of Hematology and Blood Banking, Faculty of Medicine, Tarbiat Modaress University, Tehran, Iran

Background and Aim: With the aid of tissue engineering, the capability of nanofibrous scaffolds was examined for corneal epithelial differentiation of Conjunctiva mesenchymal stem cells as a new source. This study tried to provide a synthetic corneal sheet which could applied in corneal regeneration. The hybrid arrangement of fine Silk fibers and polyurethane (pu) fibers. The highest biocompatibility of Silk-PU hybrid nanofibrous scaffolds based on cell attachment and proliferation were proven using MTT assay. The differentiation morphology of cells was observed by SEM micrographs of cells seeded on the scaffold after 12 days treatment through the differentiation medium. Up-regulation of Cytokeratin (CK)3, CK8, CK12, Desmocollin (DSC)1 and Desmoglein (DSG)1were examined by real time PCR. Immunocytochemistry (ICC) analysis was used to characterize the protein expression of CK3 in CJ-MSCs seeded on hybrid scaffold. Finally, it was inferred that the hybrid PU and silk nanofibrous scaffold has the potential for the treatment of corneal epithelium.
Methods: Cell isolation(Isolation of conjunctiva mesenchymal stem cells (CJ-MSCs) Cell characterization: flowcytometry and differentiation scaffold fabrication and methanol and plasma treatment Mechanical testing Cell Seeding SEM morphology of scaffolds and differentiated CJMSCs on scaffolds Viability evaluation RNA Extraction, Reverse Transcription, Real-Time PCR Immunohistochemistry
Results: Hybrid arrangement of large and small fibers of PU and Silk resulted in large interconnected voids within the fibers that are responsible for a porous network interacted and integrated well on 12 days of culture. High specific area as well as suitable porosity of the fibers provide suitable environment for gas/nutrient exchange and cell interactions. CJMSCs cultured on treated hybrid Silk-PU nanofibrous scaffolds showed surprisingly higher expression levels of CK3, CK8 and CK12 suggesting the superiority of Scaffold. The protein level of epithelial differentiation was demonstrated by immunofluorescence staining for CK3 after 12 days of culturing. CK3 as a specific marker for epithelial cells was expressed at higher level. Thus corroborating the gene expression and the protein data, Similarly, CJMSCs seeded on hybrid scaffold had higher levels of corneal phenotype markers CK3 after differentiation.
Conclusion: Several favorable properties such as suitable water vapor and oxygen permeability, slow degradation rate, biocompatibility, and minimal inflammatory response could make it more beneficial in different forms of synthetic ECM. As well as, thermal and mechanical stability and silk fibers within the body condition made it as an ideal substrate. Polyurethane was widely used in different tissue engineering due to adequate mechanical and physical properties. Moreover, biocompatibility and biodegradability of scaffolds could simply adapt to specific tissue. Through applying hybrid fabrication of silk and PU scaffolds could achieve advantages of both polymers in one singular sheet. Silk and PU hybrid nanofibrous scaffolds contained fibers with large diameter range that is responsible for larger voids. It is clearly seen that optimum mechanical property between these two polymers was obtained by hybrid fabrication of PU and Silk.
Keywords: Polyurethane, Silk, CJ-MSCs, Corneal Epithelial Differentiation

Os-039: Long-Term Follow-up of Autologous Peripheral Blood Cell Transplantation Along with Platelet Rich Plasma in Treatment of Women with Vesicoureteral Reflux: A Clinical Trial Study

Salman Soltani[*]

Department of Urology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: soltanis@mums.ac.ir

Background and Aim: Vesicoureteral reflux is a common urological disease. Previously, the intervention for reflux was by replanting the ureter to the bladder (open re-implant). It was the only possible treatment. Of late, endoscopic injections containing bulking agent have been proposed for the treatment of reflux. Several agents have been used for endoscopic injection so far. This study aimed to evaluate the therapeutic efficacy of endoscopic sub ureteric injection of autologous peripheral blood cells along with platelet-rich plasma.
Methods: This clinical trial study was performed after in department of Urology of Imam Reza Hospital in Mashhad, 2015 A total of 17 vesicoureteral reflux patients, all of them 15–35 years old, who fulfilled our inclusion criteria, were evaluated. Endoscopic injection was administered by one surgeon in all patients at 6 o'clock position and 0.5 cm away from the ureteral orifice, followed by renal ultrasonography and radiologic VCUG, 3 and 18 months after the injection. The success of the injection was determined by remission of vesicoureteral reflux following VCUGs. If the reflux persisted, the injection was re-administered after three months. IRCT code: IRCT2016050926038N2
Results: The success rate was 89% for renal units after the first injection and 94% after the second injection. All patients were evaluated using sonography and cystography for any reflux recurrence. We did not encounter any late recurrence or obstruction during 18 months follow up.
Conclusion: This study suggests that endoscopic injection of total blood nucleated cells is an effective treatment method for vesicoureteral reflux and recurrent pyelonephritis. The endoscopic injection of total blood nucleated cells is simple, non-invasive, and non-antigenic with a good success rate.
Keywords: Vesico Ureteral Reflux, Blood Platelets, Cell and Tissue Based Therapy, Injections, Treatment Outcome

Os-040: Notch-1 Activated Leukemia Stem Cells through Upregulation of Wnt/B-Catenin Signaling Pathway: Role in AML and Resistance to Standard Treatments

Nasser Ghaly Yousif

Medical Oncology Department, Medical College, Muthanna University, Iraq
Email: yousif_ghaly@yahoo.com

Background and Aim: Leukemia stem cells (LSCs) in AML play important roles in leukemia initiation, progression, and were the root of chemotherapeutic drug resistance and disease relapse. Both preclinical and clinical investigations suggest that Notch signaling is critical for the development of many cancers and for their response to chemotherapy. We previously showed that Notch-1 ligand signaling pathway is activated in cervical cancer and associated with poor prognosis. Also, we presented that high-level of Notch-1/JAG-1 signaling pathway up regulated chemo-resistance of bevacizumab in colon cancer and induced metastasis and poor survival.
Methods: Human AML cell lines including U937, KG-1, THP-1 were cultured and used as the experiment cell lines. MTT assay was used for proliferation detection, flowcytometry was used to detect apoptosis and cell cycle arrest upon HHT functioning, western blotting was used to detect the protein level changes, viral shRNA transfection was used to suppress the expression level of the target protein candidate, and viral mRNA transfection was used for over-expression.
Results: We found both Notch and Wnt/β-catenin signaling pathways played important roles in increasing the stemness LSCs. Moreover, knocking down of Notch-1 with lentivirus N1ShRNA inhibits LSCs and downregulation of AML proliferation.
Conclusion: Our findings thus establish that potential Notch-1 target holds great promises for future development of therapeutic approaches for patients with AML treated with stem cells transplantation.
Keywords: Notch-1, Wnt/β-catenin, Stem Cell, Leukemia

Os-041: Long-Term Overexpression of Thap11 Gene in Fibroblast Cells Will Promote Expression of Key Pluripotency Genes

Saeid Ziaei[1], Mostafa Rezae[1], Masoud Soleimani[2*]

1. Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2. Stem Cell Technology Research Center, Tehran, Iran
Email: sziaee@gmail.com

Background and Aim: Despite remarkable efforts in reprograming somatic cells to stem cells via over expression of some transcription factors or using newly founded cell regulatory systems or nanotechnology methods, the molecular mechanisms underlying the stemness have only begun to emerge. although that we know three famous transcription factor essential for pluripotent state - Oct4, Nanog and Sox2 – that they work in the same regulatory network with similar or even the same DNA binding sites, in recent years some of researchers introduce THAP11 as the new key transcription factor essential for pluripotency which is working outside of known pluripotency regulatory network. They named THAP11 as Ronin – the word for a samurai warrior without a master – because they believed that Ronin has distinct role from other known pluripotency genes and it works lonely.
Methods: Thap11 gene were amplified with PCR by phusion DNA polymerase and cloned into PCDH lentiviral vector. Virus production was done with lipofectamine 3000 virus production protocol by using psPAX and PMD helper vectors. Low passage primary human fibroblast cells treated with viral particles (without virus concentration) and incubated for 48h, then antibiotic selection was done with 2 μg/ml puromycin after the 3rd day. to determine the effects of THAP11 overexpression on pluripotency key genes, The total RNA was extracted by Qiagen RNA extraction kit followed by cDNA synthesis with Fermentas RevertAid Reverse Transcriptase enzyme. the Real-time PCR analysis was done with Sox2, Oct4, Nanog, KLF4 and MYC primers with Applied Biosystems SYBR Green Master Mix with StepOnePlus Real-Time PCR System. Data analysis was done with Relative Expression Software Tool (REST).
Results: The fibroblast cells show slightly difference in proliferation rate according to MTT results in 24h and 48h post transfection and Real-time PCR on puromycin selected cells (long-term overexpression) showed that the Sox2, Oct4, Nanog, KLF4 and MYC over expressed 2, 9, 0.8, 5.9, 1.7 d respectively. Thap11 overexpression although was proved by real-time PCR with 2-fold overexpression results.
Conclusion: According to the articles it is proved that, THAP11 have a distinct role in stemness of the cell and In this research we found that Thap11 overexpression will promote the cells to pluripotency state. It is against of some articles which said that the THAP11 will promote the cells to apoptosis. Although It shows that we could have a look to this gene for generating IPSc from somatic cells or other aspects of stem cell researches.
Keywords: Ronin, IPS, Stem Cell, THAP11

Poster Section

Ps-001: 7SK Small Nuclear RNA Transcription Level Down-Regulates in Human Tumors and Stem Cells

Mozhgan Abasi[1*], Hossein Ghanbarian[2], Zahra Bazi[3]

1. Department of Molecular Biology and Genetic Engineering, Stem Cell Technology Research Center, Tehran, Iran
2. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: abasi.mozhgan2@gmail.com

Background and Aim: The small nuclear noncoding RNA (snRNA) 7SK is a highly conserved noncoding RNA of 331 nucleotides in animals, which is present in a nuclear ribonucleoprotein complex with proteins such as methylphosphate capping enzyme (MePCE), hexamethylene bisacetamide-inducible proteins 1 and 2 (HEXIM1 and HEXIM2) and La-related protein 7 (Larp7). Regulating the activity of the positive transcription elongation factor b (P-TEFb) is the key function of 7SK noncoding RNA. Recently, we have shown that 7SK snRNA over-expression reduces human embryonic kidney 293T cell line viability here, we attempt to monitor the expression level of 7SK snRNA in different human cell lines and cancer tissues.
Methods: cancer cell lines, stem cells and cancer stem cells were cultured. Cancer and normal tissue samples were kindly provided by tissue bank of Stem Cell Technology Research Center. Total RNA was isolated from these cells and tissue samples. quantitative real-time PCR was performed for 7SK snRNA
Results: Examination of 7SK transcription either in cell lines or in different malignant tissues including blood (CML), breast and colon showed that 7SK expression signi?cantly down-regulated in cancer. Similar to human cancer tissues and cell lines, 7SK transcriptional level decreased in stemcells in comparisonwith differentiated cell types.
Conclusion: In this regard, over-expression of 7SK snRNA might be a powerful tool for blocking cancer progression by controlling the activity of P-TEFb.
Keywords: 7SK Noncoding RNA Cancer P-TEFb Stem Cells

Ps-002: The Effect of Topography on Differentiation Fates of Matrigel-Coated Mouse Embryonic Stem Cells Cultured on PLGA Nanofibrous Scaffolds

Mozhgan Abasi[1*], Mohammad Massumi[2]

1. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Animal and Marine Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.
Email: abasi.mozhgan2@gmail.com

Background and Aim: Due to pluripotency of embryonic stem (ES) cells, these cells are an invaluable in vitro model that investigates the influence of different physical and chemical cues on differentiation/development pathway of specialized cells. We sought the effect of roughness and alignment, as topomorpholocial properties of scaffolds on differentiation of green fluorescent protein-expressing ES (GFP-ES) cells into three germ layers derivates simultaneously. Furthermore, the effect of Matrigel as a natural extracellular matrix in combination with poly (lactic-co-glycolic acid) (PLGA) nanofibrous scaffolds on differentiation of mouse ES cells has been investigated.
Methods: The PLGA nanofibrous scaffolds with different height and distribution of roughness and alignments were fabricated. Then, the different cell differentiation fats of GFP-ES cells plated on PLGA and PLGA/Matrigel scaffolds were analyzed by gene expression profiling.
Results: The findings demonstrated that distinct ranges of roughness, height, and distribution can support/promote a specific cell differentiation fate on scaffolds. Coating of scaffolds with Matrigel has a synergistic effect in differentiation of mesoderm-derived cells and germ cells from ES cells, whereas it inhibits the derivation of endodermal cell lineages.
Conclusion: It was concluded that the topomorpholocial cues such as roughness and alignment should be considered in addition to other scaffolds properties to design an efficient electrospun scaffold for specific tissue engineering.
Keywords: Stem Cells Nanofibrous Scaffolds Regenerative Medicine Differentiation

Ps-003: The Synergistic Effect of Beta-Boswellic Acid and Nurr1 Overexpression on Dopaminergic Programming of Antioxidant Glutathione Peroxidase-1-Expressing Murine Embryonic Stem Cells

Mozhgan Abasi[1*], Mohammad Massumi[2], Gholamhosein Riazi[3]

1. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Animal and Marine Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.
3. Department of Biochemistry, Institute of Biochemistry and Biophysics, Tehran University, Tehran, Iran
Email: abasi.mozhgan2@gmail.com

Background and Aim: Parkinson's disease (PD) is a neurodegenerative disorder in which the nigro-striatal dopaminergic (DAergic) neurons have been selectively lost. Due to side effects of levodopa, a dopamine precursor drug, recently cell replacement therapy for PD has been considered. Lack of sufficient amounts of, embryos and ethical problems regarding the use of dopamine-rich embryonic neural cells have limited the application of these cells for PD cell therapy. Therefore, many investigators have focused on using the pluripotent stem cells to generate DAergic neurons. This study is aimed first to establish a mouse embryonic stem (mES) cell line that can stably co-express Nurr1 (Nuclear receptor subfamily 4, group A, member 2) transcription factor in order to efficiently generate DAergic neurons, and glutathione peroxidase-1 (GPX-1) to protect the differentiated DAergic-like cells against oxidative stress. In addition to genetic engineering of ES cells, the effect of Beta-boswellic acid (BBA) on DAergic differentiation course of mES cells was sought in the present study
Methods: we established a CGR8 cell lineco- overexpressing transcription factor Nurr1 (Nurr1-ES), which is involved in development of DAergic neurons, as well as Gpx1 as a antioxidant gene. Then the ES-differentiating cells as EBs were embedded by Matrigel, a natural extra cellular matrix, and plated on Matrigel coated plates. The BBA used in the step three of gold standard protocol for DAergic differentiation (Ron D. McKay et al., 2002). Then, Real-time RT-PCR, Immunocytochemistry (ICC) for expression of neuroectermal, mesencephalic or DAergic neuron-related markers such as Nestin, Pax2, Pax5,Tau , Map2, Nurr1 and TH, were performed. The synthesis and secretion of dopamine was analyzed by reverse HPLC in supernatant and lysates of Nurr/Gpx1-ES-differentiated cells.
Results: The result showed that the programmed cells can express the marker of DAergic and intermediated cells in DAergic neurons development in the level of mRNA (RT-PCR) and protein (ICC). The differentiated cells could selectively synthesis and secrete dopamine and this effect was synergized by treatment of Nurr1/Gpx1-ES cells by BBA, whereas the cells did not express the Serotonin and Adrenalin, indicating the specificity of DAergic differentiation protocol.
Conclusion: overexpression of Nurr1 can promote DAergic differentiation of mES cells and the effect can be synergized by adding of BBA. The result of this study may have impact on future cell therapy of PD by ES or induced pluripotent stem cells (iPS).
Keywords: Mouse Embryonic Stem Cells, Nurr1, GPX-1, Dopaminergic, Lentiviruses, Beta-Boswellic Acid

Ps-004: The Role of Graphene Oxide Coating on Polymeric Wet Spun Microribbons in Neural Tissue Regeneration

Negar Abbasi Aval[1,2,3*], Rahmatollah Emadi[1], Mahshid Kharaziha[1], Mohammad Karimipour[3], Ali Valiani[2]

1. Biomaterials Research group, Department of Materials Engineering, Isfahan University of Technology, Isfahan, Iran
2. School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Stem Cells Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Email: abbasinegar@ymail.com

Background and Aim: Recovery of damaged nerve functions is a principal clinical challenge. It is known that orientated growth of neural cells and neurite plays vital roles in recovering nerve functions, since these behaviors can ensure neurite across the injured nerve gaps to target tissue, realizing nerve regeneration and functional recovery. Recently, electrospun nanofibers with natural and/or synthetic polymers similar to natural

extracellular matrix (ECMs) are extensively used for nerve regeneration due to their capacity of providing suitable microenvironment for cell attachment, proliferation and migration. Moreover, the aligned scaffolds provide contact-guidance for directional cell migration and proliferation as well as neurite growth. In particular, the aligned polymeric fibers are most widely used for nerve regeneration because of their tunable degradation rate, excellent mechanical property and nonimmunogenicity. It is presented that the chemical stability of graphene-based materials improves the integration with neural tissues. Therefore, the surface modification of polymeric fibers using Graphene Oxide (GO) nanosheets as a coating should greatly promote neural stem cell growth and differentiation.
Methods: Wet-spinning is a simple and controllable method for fabricating fibrous scaffolds with random or oriented fibers made from different materials and used to make a variety of fiber based tissue analogs. Here we develop microribbon-like fibers with wet spinning procedure for fabricating macroporous and stiff tissue engineering scaffolds and investigate their potential for supporting neural cell culture in 3D. The microribbons are made from Polycaprolactone, which is widely used for tissue engineering applications. We examined the effects of GO coating on the physical, mechanical and in vitro cell culturing properties of aligned polymeric microribbons.
Results: The morphology of ribbons are characterized with scanning electron microscopy. The mechanical properties before and after GO coating are examined with Tensile tests and the characteristics of polymer is examined by FTIIR.
Conclusion: In conclusion, here we report the development of a novel, microribbon-based scaffold with high mechanical properties as a scaffold in nerve tissue regeneration applications. The macroporosity of the microribbons scaffolds support cells adhesion, spreading and alignment which are so important in neural tissue engineering. Mechanical and physical properties of the microribbon-based scaffold can be tuned easily by varying the amount of GO coating.
Keywords: Microribbon, Wet Spinning, GO Coating, Neural Regeneration

Ps-005: Isolation and Characterization of Umbilical Cord-Derived Mesenchymal Stromal Cells by a Simple, Versatile, and Low Cost Method

Zahra Abbasi Malati[1], Mehryar Habibi Roudkenar[2*], Reza Sabzevari[2]

1. Department of Biotechnology, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
2. Department of Medical Biotechnology, Paramedicine Faculty, Guilan University of Medical Sciences, Rasht, Iran
Email: z.abbasi1369@yahoo.com

Background and Aim: One of the appropriate sources for MSC isolation is umbilical cord mucous tissue. As a source of MSCs, UC has recently focused researchers' attention due to their easy accessibility and no ethical concerns. UC-derived MSCs (UC-MSCs) show faster population doubling, and exhibit higher proliferation capacity than bone marrow-derived MSCs. It also seems that they are more primitive and low-immunogenic in comparison with other tissue-derived MSCs including bone marrow-derived ones. However, to isolate them they requires enzymatic digestion that could effect on the efficiency of isolated cells as well as tedious lab work. This study aims to employ a simple and low cost method to isolate UC-MSC.
Methods: Umbilical cords were obtained from patients who delivered full-term infants by Caesarean section after written consent was obtained. These patients faced no complications throughout pregnancy. All samples immersed in phosphate buffered saline (PBS) , at 4°C, supplemented with penicillin (5000 units/ml), streptomycin (5000 µg/ml) and immediately transferred to the laboratory. They were washed times in PBS before further processing. Blood vessels were removed from each piece after incising the cord lengthwise and the Wharton's jelly was carefully separated from the amniotic membrane. The WJ was cut into small fragments with sharp scissors and scalpels. Explant cultures were obtained from each region, minced into small pieces and grown in media containing: Dulbecco's Modified Eagle's Medium (DMEM F12), fetal bovine serum (FBS) (25%), and penicillin and streptomycin (P/S) (1 %). Tissue explants were removed after 7 days in culture. Adherent cells were passaged upon reaching 70% confluence and reseeded at 5 × 103/cm2 in either 25 cm2 or

75 cm2 tissue culture flasks. Flow cytometry was used to assess the MSC immune-profile of UC cells. Cells (P3) were harvested. One million cells of each population were used for flow cytometry. Cells were stained with directly conjugated antibodies against CD44, CD73, CD31, CD34, CD45, CD90, HLA-DR, CD105. An appropriate isotype-matched control antibody was used in all analyses. Differentiation capacity of isolated cells was evaluated in the presence of differentiation-inducing media.
Results: Isolated UC-MSCs displayed fibroblast-like morphology and express MSC-specific Surface markers. Flow cytometric analysis indicated that UC-MSCs highly expressed MSC markers including CD105, CD90, and CD73. However, no expression of hematopoietic stem cell markers such as CD34 and CD45 was reported indicating UC-MSCs developed their population homogeneously.
Conclusion: In this study we introduce a simple method to isolate and expand UC-MSCs homogeneously. The isolated UC-MSCs showed the ability to express specific markers, self-renewality, and retaining their differentiation capacities. In other words, we isolate UC-MSC by a simple, versatile, and low cost method.
Keywords: Umbilical Cord, MSC, Cell Banking

Ps-006: Changes in the Survival of NB4 Cells after Exposure to Microvesicles derived from Mesenchymal Stem Cells

Mahnoosh Abbaszade Dibavar, Masoud Soleimani*

Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: mahnooshdiba@modares.ac.ir

Background and Aim: Nowadays, one of the most prevalent forms of AML is APL (AML-M3) that has been addressed by arsenic trioxide (ATO) and/or all-trans retinoic acid (ATRA). Although, ATRA and ATO are broadly accessible and administrated, have dreadful side effects. Furthermore, long exposure to ATRA not only raised in chemo resistance, also lead to recurrence of the disease within 3 months. Altogether, there is a need for effective treatments to address chemo resistance in the APL. Stem cell-based therapy as a novel approach is propounded for treatment of autoimmune and other heamatological diseases and also used in regenerative medicine. There has been a significant growing interest in stem cells, especially mesenchymal stromal cells (MSCs) in combination with tissue engineering in recent years. The use of MSCs as a therapeutic option shows a promising future due to their immunoprivileged status and immunomodulatory properties. Recent studies considered micro-vesicles as a potential therapeutic agent. Microvesicles are small membrane-bound particles released by different cells including healthy and tumor types. Microvesicles can transfer their contents, proteins and RNAs, to target cells and thereby transform them. This may induce apoptosis or survival depending on cell origin and the target cell. As point of view, micro-environment of bone marrow, normal and leukemic cells have interchangeable interaction through micro-vesicles, so micro-vesicles derived from human bone marrow mesenchymal stem cells might affect leukemic cells. In this study, we investigate the effect of microvesicles derived from human bone marrow mesenchymal stem cells on NB4 cells to seek evidence of apoptosis or cell survival.
Methods: Mesenchymal stem cells were cultured in culture medium. Microvesicles were isolated from Mesenchymal stem cells by ultra-centrifugation and were added to NB4 cell line. Also, NB4 cells without microvesicles were cultured as control group. After 7 days, cell count, cell viability By MTT assay and qPCR for KI67 gene expression were performed.
Results: Results showed lower cell number, lower cell viability rate and lower KI67 gene expression in leukemia group in comparison with control group. In conclusion, this study showed apoptotic effect of microvesicles derived from Mesenchymal stem cells on NB4 cell line.
Conclusion: Altogether, this study showed that microvesicles derived from human bone marrow mesenchymal stem cells could have therapeutical potential, so using these microvesicles for treatment of APL should be considered. But there is a need for more studies to undrestand the micro-vesicles-mediated cell death mechanisms and path.
Keywords: Mesenchymal Stem Cells, Micro-Vesicles, Survival, NB4

Ps-007: OCT4B1 Promotes Metastasis by Induction of Cancer Stem Cell in Epithelial Cell Lines

Shahrokh Abdolahi Targhi[1*], Maryam Kabir Salmani[2], Seyed Javad Molw[3], Maryam Shahali[4]

1. Applied Cell Science Department, Tehran University Of Medical Sciences, School Of Advanced Technologies In Medicine, Tehran, Iran
2. Medical Biotechnology Department, National Institute Of Genetic Engineering And Biotechnology, Tehran, Iran
3. Genetic Department, Tarbiat Modares University, Tehran, Iran
4. Pasteur Institute Of Iran, The Production And Research Complex, Tehran, Iran
Email: shahrokh.abdolahi@gmail.com

Background and Aim: The human OCT4 gene, located on chromosome 6, is a key transcription factor involved in survival pluripotency and self-renewal of embryonic stem cells. Some different variants of these factors have been identified, such as OCT4A, OCT4B and OCT4B1. OCT4B1 like OCT4A widely expressed in pluripotent stem cells. However according to recent reports the expression of OCT4B1 decreased in differentiated cells. In addition, it has been shown that OCT4B1 considerably expressed in tumors and its anti-apoptotic effects have been demonstrated. Aim: In this study morphological and molecular changes in epithelial cells transfected with OCT4B1 were evaluated. The transfected epithelial cells transfer to mesenchymal cells. Hence, with over-expression of OCT4B1 in epithelial cells, the expression of genes involved in Cancer Stem Cells, the significant factors in cell invasion and metastasis, were examined.
Methods: MCF-7 cell line was cultured and transfected by pCMV-Poct4b1cDNA construct and cells expressing OCT4B1 was selected and stable cell line MCF7-OCT4B1 was created. The molecular behavior changes investigated through quantitative assays Real-Time RT-PCR. Moreover, the study on cell line (5637-OCT4B1) that previously had been transfected by the structural contains (oct4b1cDNA) was done.
Results: The molecular analysis performed on these cell lines (MCF7-OCT4B1, 5637-OCT4B1) showed the over-expression of biomarkers (CD44 and CD133) and no changes in the expression of CD24 (biomarkers of cancer stem cells).
Conclusion: According to the survey results, OCT4B1 has an impact on biomarkers of cancer stem cells markers and these cells in sight of molecular mechanism acquired mesenchymal features. Consequently the gene has the significant effect of inducing metastasis and invasion in cancer.
Keywords: Cancer, CSCs, OCT4B1, Oct4

Ps-008: Effect of Heart Valve Decellularization on Xenograft Rejection

Hamed Abdolghafoorian[1], Poopak Farnia[2], Raheleh Sadat Sajadi Nia[1], Afshin Bahrami[3], Atosa Dorudinia[4], Jalaledin Ghanavi[2*]

1. Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Mycobacteriology Research Center and the Experimental Medicine and Tissue Engineering Center at Masih Daneshvari Hospital
3. Experimental Medicine and Tissue Engineering Center at Masih Daneshvari Hospital
4. National Research Institute of Tuberculosis and Lung Disease, Shahid Beheshti University of Medical Sciences
Email: hamed.ghafoori@gmail.com

Background and Aim: Endothelial cells harbor many antigenic determinants that may be targets for the immune system. The aim of this study was to determine the immunologic effects of decellularization, using 3 different methods, on xenograft rejection.
Methods: In a sterile plate containing phosphate-buffered saline, fresh sheep aortic heart valves were decellularized using 3 different enzymatic methods: with 900 µg/mL of collagenase at 40°C (method A), with 450 µg/mL of collagenase at 4°C (method B), and with 900 µg/mL of collagenase at 4°C (method C). Intact and decellularized valves were implanted subdermally into inbred male albino rabbits and extracted after 21 days (extra valve pieces were also extracted after 60 days, as control samples, for assessing chronic rejection). Valves were histologically analyzed for inflammatory cell infiltration. Subendothelial structure integrity was determined using surface electron microscope.

Results: No inflammatory cell infiltration was seen around the decellularized valve with method A, and no subendothelial structure change was observed by surface electron microscope. Infiltration of immune cells involved in rejection was not seen around valves decellularized with method B, although the subendothelial structure was relatively preserved and valve stiffness was increased. With method C, we observed a foreign body-type reaction around the intact valve and the decellularized valve.
Conclusion: Method A is considered the optimal method of decellularization in our study, as this method significantly reduced the immune response to xenograft tissue, while maintaining subendothelial tissue
Keywords: Collagenase, Endothelial Cells, Xenotransplant

Ps-009: The Association of MTHFR Promoter Methylation with Recurrent Abortion and Idiopathic Male Infertility

Shokoufeh Abdollahi[1], Shiva Poorang[1], Zahra Anvar[2*], Mohammad bagher Tabei[1]

1. Department of Medical Genetics, Shiraz University of Medical Sciences, Shiraz, Iran
2. Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Email: shokufe.abdollahi@yahoo.com

Background and Aim: Abnormal sperm DNA methylation has been proposed as a possible mechanism compromising spermatogenesis of some men currently diagnosed with idiopathic infertility. The connection between abnormal sperm methylation and the history of recurrent abortion is not well diagnosed so far. The aim of the present study is to evaluate the association between sperm aberrant methylation of non-imprinted MTHFR gene and recurrent abortion during the pregnancy. Moreover, the role of sperm aberrant methylation in infertility of the male partner is studied.
Methods: Sperm DNA from male partner of women with Recurrent Abortion (RA), and fertile men (non-RA) were analyzed by methylation-specific PCR amplification using primers which anneal to the methylated or unmethylated cytosine within the promoter region of MTHFR. The presence of methylated or un-methylated products was confirmed by agarose gel electrophoresis.
Results: Among 25 male partners of RA women, 2 individuals (8%) showed M/U (Methylated/Un-methylated) epigenotypes. In non-RA group 7 out of 25 individuals have shown positive result for M/U epigenotypes (28%). This difference was not statistically significant (P= 0.06). The methylated allele was amplified in 4 out of 17 men with 1 or more sperm parameter deficiency (23%) and 5 out of 33 healthy men (15%). This difference in frequency was not statistically significant (P= 0.7).
Conclusion: Our results did not show any correlation between MTHFR promoter methylation aberration and the frequency of RA. However according to our results the frequency of MTHFR M/U haplotype is higher in idiopathic infertile men in comparison with fertile men.
Keywords: Epigenetic Modification Methylation Infertility Sperm Recurrent Abortion

Ps-010: Breast Milk Stem Cell Isolation and Expansion in 3D Environment

Anneh Mohammad Gharravi[1*], Muhammadhussein Abedi[2]

1. Stem Cells and Tissue Engineering Research Center, Shahroud University of Medical Sciences, Shahroud, Iran
2. Student Research Committee, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
Email: annehgh@yahoo.com

Background and Aim: Breast milk stem cells develop into a range of different tissues in the offspring, including the brain, liver and kidneys and are hypothesized to be involved in growth and development of the infant. The aim of present study was isolation and expansion of breast milk stem cell in 3D environment inside perfusion chamber
Methods: Breast milk was collected, and exosomes and stem cells were isolated using centrifuging methods. Harvested cell pellete, seeded on hydrogel scaffold of alginate and cultured under direct perfusion flow. Flow cytometry was performed and specific antibody staining data were visualized using FlowJo software. Data were compared using one-way ANOVA.

Results: Under invert microscopy, milk cell counts and viability by hemocytometer revealed 100,000-300,000 per millilitre. Under direct perfusion flow, stem cell seeded in hydrogel expanded and proliferated efficiently but, ibroblast like cell appearedin 2D culture system. There was significant correlation between count cells and breast milk type.
Conclusion: The stage of lactation is associated with major changes in milk stem cells count. Fresh post term breast milk is efficient in stem cell. 3D scaffold provides optimum environment for isolation and expansion of milk stem cells.
Keywords: Breast Milk, Stem Cell, Isolation, 3D Environment, Scaffold

Ps-011: Dendritic Cell-Based Vaccine and B Cell Lymphoma

Saeid Abediankenari[1*], Yousef Yousefzadeh[1], Ghasem Janbabaei[2]

1. Immunogenetics Research Center, Department of immunology, Faculty of Medicine Mazandaran University of Medical Sciences, Sari, Mazandaran, Iran
2. Interrnal Medicine Department, Gasterointestinal Cancer Research Center, Mazandaran University of Medical Sciences, Sari, Iran
Email: abedianlab@yahoo.co.uk

Background and Aim: Dendritic cells (DCs) are professional antigen presenting cells that have a potential role in the initiating of immune responses. The cell vaccination is a new strategy in treatment of infectious diseases and cancers. In this study, we have generated monocyte-derived dendritic cells of lymphoma patient's peripheral blood mononuclear cells, then, these cells were used as vaccine in lymphoma patients.
Methods: We generated dendritic cell vaccine from lymphoma patient's blood monocytes with human interleukin-4, granulocyte monocyte colony stimulating factor and then, antigen-primed Dcs were administrated subcutaneously close to the inguinal lymph nodes after maturation of dendritic cells. After 7 days, we analyzed immune response in lymphoma patients with determining of LDH, Beta 2 Microglobulin, CD4+T cell percent, CD8+ Tcell percent and Tumor size before and after vaccination. Furthermore, phenotypic and functional analysis of dendritic cells was performed using anti CD83-FITC monoclonal antibodies.
Results: Before vaccination, the mean ± SD of LDH was 530.62±140.65 but after vaccination it was 459±109.45 that significantly different between experimental groups (P=0.002). In addition, the CD8+ T cells percentage significantly different between two groups (P=0.002).
Conclusion: We concluded that the use of dendritic cell probably is one of the suitable noninvasive treatments for lymphoma patients that they have not response to chemical drugs.
Keywords: Dendritic Cell, Vaccine, Monocyte

Ps-012: Bone Marrow Derived Mesenchymal Stem Cells Transfected with Lentivirus, a Potential System for Diagnosis and Targeted Therapy of Tumours in BALB/C Mouse Model

Roya Abedizadeh[1*], Hossein Rezvan[1], Sara Soudi[2], Mohamadreza Baghaban Eslaminejad[3]

1. Department of Laboratory sciences, Faculty of Paraveterinary Science, Bu-Ali Sina University, Hamedan, Iran
2. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: r.abedizadeh@gmail.com

Background and Aim: Mesenchymal stem cells (MSCs) are being developed as a new vehicle for delivering diagnostic and therapeutic elements to a large number of degenerative diseases such as cancers, asthma and injuries. Recently, a number of studies have focused on the potency of MSCs for targeted therapy relying on the capacity of MSCs in accepting and delivering Lentiviruses as vehicles and their tropism to the site of infection. Lentiviral vector system is also shown to be a promising tool for incorporating into genomic DNA with high efficiency as the expression of Lentiviral vector-mediated transgenic genes are maintained in the cells for long time.
Methods: A second generation of lentiviral vector including pTRH plasmid (pTRH1-mCMV-dscGFP) was

used together with two packaging plasmids pSPAX2 (encoding rev, tat, gag/pol) and pMD2G (encoding env proteins) co-transfected into 293 T (HEK) cells using lipofectamine 2000. The virus was harvested by collecting the cell culture supernatant after 24, 48 and 72 h. After filtering the cell supernatant through 0.45 µm filters, the virus was concentrated by centrifuging at 9000 g for 15 min fallowed by a second spin (6000 g, 5 min). The concentrated virus was stored at -20ᵒ C. Bone marrow derived MSCs were cultured in growth medium and passage 3 was performed in 24-well plates (1×104cells per well). When the MSCs reached to 60 to 80% confluent, they were incubated with the lentivirus for 72 h. After collecting the MSCs with tripsinizing (Tripsin-EDTA 0.25% for 5-6 min), whole genome of transfected MSCs were purified with genomic DNA culture cell purification kit. The PCR reaction was done to detect GFP (Green Flourcent Protein) gene located in the vector. PCR product was assessed with electrophoresis with 1.5% agarose gel.

Results: It was showed that MSCs can efficiently be transfected by lentiviral vectors. Since the PCR was performed on the whole genome of MSCs, the virus was inserted in the genome properly.

Conclusion: Our results provide a potential and new system in engineered MSCs for detecting injuries such as metastasis using tracking ability of GFP gene in lentiviral vectors, which can be performed by PCR as a reliable and easier technique. Indeed, this system has therapeutic potential in targeted therapy by inserting therapeutic genes such as cytokines, immunologic agents or drug metabolizing enzymes.

Keywords: Mesenchymal stem cell, Lentivirus, Vector

Ps-013: Effects of Human Embryonic Stem Cell on Cell Cycle in HL60 Cells in Vitro

Fatemeh Adeli[1], Mohammad Jafar Rezaie[1*], Ayoob Rostamzadeh[2], Sona Zare[3]

1. Department of Anatomical sciences, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
2. Department of Anatomical Sciences, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
3. Skin and Stem Cell Research Center, Tehran University of Medical Sciences, Tehran, Iran

Email: Rezaiemjafar@gmail.com

Background and Aim: Acute myeloid leukemia (AML) is a malignancy hematopoietic cells and severe threat to human health. AML that is recognized as the ordinary form of acute leukemia among adults with higher outbreak in older patients. At present, the basic therapy for leukemia includes allogeneic hematopoietic stem cell transplantation, chemotherapy and immunotherapy. The aim of this study was having provided evidence for anti-proliferative function of human embryonic stem cells- conditioned medium (CM) (hESC-CM) on acute myeloid leukemia cells progression.

Methods: The HL-60 cell line obtained from the cell bank Pasteur Institute. We measured proliferation of myeloid leukemia cell by XTT assay. Cell growth and Cell viability was assessed using the Colorimetric Cell Viability Kit based on the reduction of tetrazolium salt to soluble formazan compounds by mitochondrial enzymes. Also cell cycle and apoptosis was assay by flow cytometry after the cells stained.

Results: Our experiment indicated that the human embryonic stem cells conditioned medium has anti-proliferative effects on progression HL60 cell. Incubation of the HL60 cell with one day hESC-CM significantly suppressed cell proliferation after 48 h of culture. Utilization of 2 day CM resulted stronger suppression was compared to one day CM. HL60 cells treated with conditioned medium, the results of the flow cytometry demonstrate that hESC CM was capable of increasing apoptosis of HL60 cells and inhibit the cell cycle progression. To demonstrate the therapeutic effect, we investigated ability of CM to suppress cell proliferation of normal Mononuclear Cell (MNC). Cell proliferation was evaluated by XTT assay. The results showed 1, 2 days hESC CM did not change significantly on MNC proliferation than the control groups. The findings confirmed that was not inhibitory effect CM on normal cells and is safer clinical application.

Conclusion: The results of Current study confirmed that both concentrations of conditioned medium produced significant reductions cell proliferation and similar to other studies demonstrated that evacuation of nutrients in hES cell-conditioned medium not cause to inhibit of cancer cell proliferation. Our Result show that Human embryonic stem cell conditioned medium is containing factors that are able to inhibit the growth

and inducing apoptosis of HL60 cells and this can be a novel therapeutic strategy for leukemia.
Keywords: Embryonic Stem Cell, Cell cycle, Apoptosis, Leukemia.

Ps-014: Effects of Different Synthesis Parameters on the Controlled Growth Factor Delivery from the Smart Temperature Sensitive Polymer for Regenerative Medicine Application

Afsaneh Adibfar[1], Ghassem Amoabediny[1*], Mohamadreza Baghaban Eslaminejad[2], Javad Mohamadi1, Behrouz Zandieh Doulabi[3], Fatemeh Bagheri[4]

1. Department Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University, MOVE Research Institute, Amsterdam, The Netherlands
4. Biotechnology Group, Chemical Engineering Department, Tarbiat Modares University, Tehran, Iran
Email: afsaneh_adibfar@yahoo.com

Background and Aim: The aim of this research was to synthesis a smart temperature sensitive polymer and to investigate its capability to deliver the bioactive growth factors. Different formulation of Poly (N-isopropylacrylamide) (PNIPAM) was successfully synthesized by free radical polymerization technique. The formation of PNIPAM nanoparticles and its optimal formulation was confirmed by different analytical methods. The presence of particles with spherical grains in the range of 50–100 nm was observed in all samples. Optimized polymeric nanoparticles had particle sizes less than 100 nm and LCST of 30-32°C. Temperature dependent release kinetic of the basic fibroblast growth factor (bFGF) was closely studied. The cumulative release profile study revealed that around 37 °C the synthesized smart nanoparticles maintain sustained release of bFGF over the first 96 hr. The biological evaluation revealed that the released growth factor was bioactive proteins and the particles up to a concentration of 3 mg/ml did not show any toxicity on MC3T3-E1 mouse osteoblast cell lines. Smart polymeric particles are a kind of hydrogels and different kind of these compounds have been proposed. PNIPAM is the most prominent candidate thermally responsive polymer to deliver the drug due to its sharp transition temperature which is around physiological temperature. In this study, controlled release of GF from PNIPAM was assessed. According to the results, it seems that these nano systems represent promising candidates for efficient growth factor and drug delivery for tissue engineering applications.
Methods: To study the effects of operating parameters, PNIPAM nanoparticles with different feed compositions were synthesized by the free radical polymerization method.
Results: According to the given data, the presence of SDS led to the formation of tiny and more homogenous polymeric particles. Though, no stable polymeric compound was made in the presences of high concentrations of SDS. It has been found that the monomer/solvent ratio is a determinative synthesis parameter as the polymerization efficiency decreased with increasing solvent. Furthermore, bisacrylamide had determinative effects on the formation of polymeric hydrogel and also on the drug encapsulation efficiency as well as release kinetic profile. Release kinetic profile of this particles changed regarding to the phase transition temperature. Low critical solution temperature (LCST) of the synthesized polymeric nano particles, altered with respect to the concentration of mentioned parameters. LSCT of the prepared polymers measured by DSC, zeta sizer and zeta potentioal analysis, revealed that the temperature was varied from 29-33 °C. High content of crosslinker resulted to the formation of PNIPAM nano particles with higher LCST and more condense hydrogel, which caused slow conformational changes due to the temperature and gradual release.
Conclusion: According to the result of this study, the PNIPAM nanoparticles could serve as a smart carrier for drug and GF delivery. By controlling the synthesis parameters, the optimum drug release profile could achieve. The best release kinetic for growth factors and bioactive drugs should exactly choose according to the biological cell signaling and cell niche.
Keywords: Smart Polymer- PNIPAM- Drug Delivery- Regenerative Medicine

Ps-015: Evaluating the Metformin Effect on BCSC Subpopulation of MCF7 by Examining CD44 Expression

Farzaneh Afzali, Mossa Gardaneh*

Department of Stem Cells and Regenerative Medicine, Faculty of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: farzaneafzali@gmail.com

Background and Aim: The cancer stem cell hypothesis proposes that, unlike most cancer cells within a tumor, cancer stem cells (CSCs) resist chemotherapeutic drugs and cause relapse. Epidemiologic studies demonstrate that diabetes is related with increased risk of breast and other cancers. Metformin a biguanide drug which is most commonly used drug in Type-2 diabetes and has anticancer effects. Metformin inhibits the growth of breast cancer cell lines and also has impact on breast Cancer Stem Cells. The breast cancer stem cell markers such as CD44, CD24 have been recognized.
Methods: We treated breast cancer cell line MCF7 with increased concentrations of Metformin in search of its lethal dose 50 on the cells measured by the MTT viability assay. We then examined changes in expression levels of breast CSC markers and others by RT-PCR.
Results: Our preliminary data indicates the potential of metformin in inducing death in our cancer cell line. We have also detected gene expression changes in the cells upon metformin treatment.
Conclusion: In the present study Metformin has shown to inhibit breast cancer cell growth and induce cell death.
Keywords: BCSC, Metforrmin, MCF7, CSC

Ps-016: Appraising the Metformin Effect on Cancer Stem Cell Markers in Breast Cancer Cell Line SKBR3

Farzaneh Afzali[1], Mossa Gardaneh[1*], Zahra Nayeri[2]

1. Department of Stem Cells and Regenerative Medicine, Faculty of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Molecular Medicine Department, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: farzaneafzali@gmail.com

Background and Aim: Cancer chemotherapy can proportionally diminish tumor mass, however the disease frequently relapses. The cancer stem cell hypothesis advocates that in the turmeric population there is a small fraction of cells called cancer stem cells (CSC) and are responsible for tumorigenesis and initiation of secondary tumor formation. Metformin is a biguanide drug most commonly used in Type-2 diabetes and has anticancer effects. Metformin has been shown to eliminate stem cell populations in HER2-amplified breast carcinoma cells like SKBR3. It inhibits cancer cells proliferation and additionally affects Breast CSCs (BCSCs). BCSC markers such as CD44, CD24 have been recognized.
Methods: The breast cancer cell line skbr3 had been treated with serial concentrations of Metformin to hunt its lethal dose 50. For measurement MTT viability assay used. Then expression alterations of breast CSC markers measured by RT-PCR.
Results: Our introductory data reveals the cell death inducing capacity of metformin in our cancer cell line. Besides we have also perceive gene expression alterations in the cells upon metformin treatment.
Conclusion: In the current research Metformin has shown to inhibit breast cancer cell growth and induce cell death.
Keywords: BCSC, Metforrmin, SKBR3, CSC

Ps-017: The Study of Gene Expression Pattern in Osteocyte Differentiation of Mesenchymal Stem Cells on Polymer Nanofiber

Mojtaba Salouti[1*], Nasrin Aghapur[1], Samad Nadri[2]

1. Biology Reserch Center,Zanjan Branch, Islamic Azad, Zanjan, Iran
2. Zanjan University of medical Sciences, Zanjan, Iran
Email: aghapur_n@yahoo.com

Background and Aim: Tissue engineering approaches have recently been devised to repair large bone losses. Tissue engineering takes advantages of the combined

use of cultured living cells and 3D scaffolds to deliver vital cells to the damaged site of the patient. To observe the expression profiles of osteoblast-related genes in mesenchymal stem cells (MSCs) derived from bone marrow during osteogenic differentiation on polycaprolactone and poly L-lactide (PLLA/ PCL) nanofibres scaffoled.
Methods: MSCs were cultured on PCL and PLLA scaffoled for 21 days. Expression of osteoblast-related genes, including, Runt-related transcription factor 2 (Runx2), osteonectin and collagen type1, was assessed by real time-PCR.
Results: The real time PCR showed that the osteogenic gene expression including osteonectin, collagen type 1 and runx2 seeded in the nanfiber culture has higher expression than cells differentiated in control culture.
Conclusion: It can be concluded that PLLA and PCL are suitable substrates to support the proliferation and osteogenic differentiation of MSCs and holds promising potential for bone tissue engineering and regenerative medicine applications.
Keywords: Tissue Engineering, Mesenchymal Stem Cells, Poly L-Lactide, Polycaprolactone, Osteogenic, Gene Expression

Ps-018: Wound Healing Dual-support by Adipose Derived Stem Cell Conditioned Medium (ADSC-CM) Impregnated Scaffold Made from Amniotic Membrane

Aftab Ahmad Chattha[1*], Khuzema Hammad[2], Sana Saleem[2], Shahzad Anwar[1], Muhammad Shahbaz Aslam[2]

1. Iffat Anwar Medical Complex, Lahore, Punjab, Pakistan
2. Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan
Email: aftab.nays@gmail.com

Background and Aim: Wound infection has become a major problem in Pakistan and in rest of the world. Nearly 61% of people suffer from the deadliest infections after they encounter wound through accidents and burning etc. Current solutions to this problem includes scaffolds like autografts, Integra, cell-free substrates and topical synthetic chemicals but these have limitations as either they are from animal origin or not cost effective. A scaffold which is made from amniotic membrane has the ability to undergo efficient wound healing. Adipose derived stem cells (ADSC) are pluripotent in nature and they secrete variety of chemokines, cytokines and growth factors which improves the healing process. These cells can be easily isolated during liposuction and their secreted proteins play an essential role in both acute and chronic wounds healing. The objective of this study is to provide a cost-effective and beneficial solution to the patients with wounds.
Methods: Two membranes were separated from placenta; amnion and chorion. These layers were de-cellularized by the use of specific detergent and stored at 4°C in glycerol and antibiotic. Later it was sterilized by gamma-rays in order to make it contamination free. ADSC were isolated after liposuction procedure from healthy human. The cells were cultured in DMEM/F12 media until they reached confluency. Conditioned medium (CM) was prepared after growing the stem cells. Both CM and de-cellularized membrane were applied on wounds to observe their healing effects.
Results: Soaking of scaffolds in CM enhances its power of wound healing. When wounds were treated only with CM, there was improvement compared to control but there was significant improvement in wound healing in the presence of de-cellularized membrane and CM. The material developed is cost-effective and provide ready to use treatment at wound site which will reduce the mortality related to wound injuries.
Conclusion: The de-cellularization was done by detergent, rather than DNases, RNases and enzymatic cocktail, makes it more economical. In addition to the physical support, addition of growth factors quickly heals the wounds and could be better for diabetic patients which had hard time in healing wounds.
Keywords: Tissue Engineering, Healing, Regenerative Medicine, Wounds

Ps-019: Induced Pluripotent Stem Cell-Derived Podocyte Cells Transplantation Improves Mouse Model of Focal Segmental Glomerulosclerosis

Amin Ahmadi[1], Reza Moghadasali[1,2], Zeinab Taghizadeh[1], Reza Salman Yazdi[2], Seyed Mahdi Nasiri[3], Vahid Ezzatizadeh[1,4], Nasser Aghdami[1*]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Bioloy and Technology, ACECR, Tehran, Iran
2. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
4. Medical Genetics Department, Medical Laboratory Center, Royesh Medical Group, Tehran, Iran
Email: amin.ahmadi@yahoo.com

Background and Aim: Focal segmental glomerulosclerosis (FSGS) is one of the most important causes of acquired chronic kidney disease (CKD) causing end-stage renal disorder at any age. FSGS is a podocytopathy resulting in sever proteinuria and renal malfunction. Podocytes are terminally differentiated cells which involve in glomerular barrier formation. Adriamycin induced nephropathy (AN) is a rodent CKD model representing human FSGS symptoms. AN is characterized by podocyte depletion resulting in glomerulosclerosis and fibrosis. The aim of this study was to evaluate of injured podocytes replacement in this disease, using transplantation of induced pluripotent stem cell (iPSCs)-derived podocyte cells.
Methods: AN was induced by intravenous injection of adriamycin in female C57BL/6J mice, resulted in abrupt decrease in podocyte number by day 3, proteinuria and glomerulosclerosis and fibrosis development which were detectable by day 60. We efficiently differentiated mouse tail tip fibroblast derived iPSC into podocyte cells employing Activin A, BMP7, Retinoic acid and GDNF. Then, we transplanted the iPSC-derived podocytes into kidney parenchyma of injured mice on day 7.
Results: We have found that iPSC derived podocytes transplantation significantly decreases proteinuria level (not to control level) and attenuates renal tissues sclerosis and fibrosis.
Conclusion: We herein suggest that transplanted iPSC-derived podocytes can improve CKD model-associated proteinuria and glomerular function by homing in glomeruli and replacing damaged podocytes. On the other hand, several glomerular filtration barrier components are being altered upon AN, like glomerular endothelium, and thus, podocyte transplantation alone does not seem to be a final therapeutic order to fight the devastating disorder and further considerations such as transplantation of both podocytes and endothelial cells together are required to translate the knowledge into clinic.
Keywords: Focal Segmental Glomerulosclerosis, Proteinuria, Induced Pluripotent Stem Cells, Podocytes

Ps-020: Induced Pluripotent Stem Cell-Derived Podocyte Cells Transplantation Improves Mouse Model of Membranous Nephropathy

Amin Ahmadi[1], Reza Moghadasali[1], Zeinab Taghizadeh[1], Seyed Mahdi Nasiri[2], Reza Salman Yazdi[3], Koorosh Shahpasand[1], Mehdi Alikhani[1], Vahid Ezzatizadeh[1,4], Nasser Aghdami[1*]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Bioloy and Technology, ACECR, Tehran, Iran
2. Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Department of Andrology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Medical Genetics Department, Medical Laboratory Center, Royesh Medical Group, Tehran, Iran
Email: amin.ahmadi@yahoo.com

Background and Aim: Podocytes as part of the glomerular filtration barrier play a major role in the initiation and progression of both immune and non-immune mediated glomerular disease. Subepithelial immune deposition in glomerular disease causes nephrotic syndrome (NS) by podocyte disruption. Membranous nephropathy (MN) is an immune mediated and one of the most common causes of NS in adults leading to end stage renal disease. MN is thought to be caused by specific binding of Igs to podocyte-associated targets. The aim of this study was to evaluate of injured podocytes replacement in this disease, using transplantation of induced pluripotent stem cell (iPSCs)-derived podocyte cells.
Methods: In this study, we efficiently differentiated mouse tail tip fibroblast derived iPSC into podocyte cells and characterized them. Then we stablished the mouse model of MN employing a generated purified

rabbit anti-podocyte polyclonal antibody by intravenous injection 5 days after pre-immunization of female C57BL/6J mice. The urine, serum and kidney samples were collected for 90 days. Then, we transplanted the iPSC-derived podocytes into kidney parenchyma of injured mice on day 10.
Results: We showed that differentiation efficiency of iPSCs into podocytes increased when in the first step, iPSCs were differentiated into cells of the intermediate mesoderm following subsequent differentiation into podocytes. Here for the first time, we showed that iPSC derived podocytes transplantation significantly decreases proteinuria close to control level. Histological studies also showed that evaluated pathological parameters improved significantly.
Conclusion: We herein suggest that transplanted iPSC-derived podocytes can improve CKD model-associated proteinuria and glomerular function by homing in glomeruli and replacing damaged podocytes.
Keywords: Membranous Nephropathy, Proteinuria, Induced Pluripotent Stem Cells, Podocytes, Anti-Podocyte Antibody

Ps-021: Graphene Oxide Nanoparticle Incorporated Gelatin/Polycaprolactone/Chitosan Nano-Fibrous Scaffolds: Biological, Mechanical and Structural Properties

Amir Aidun[1*], Abolfazl Nazbar[2], Seyedeh Aida Razavi[3], Nooshin Haghighipour[2]

1. Tissues and Biomaterials group, Universal Scientific Education and Research Network (USERN), Tehran, Iran
2. National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
3. Department of Biomedical Engineering, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran
Email: amir.aidunn@gmail.com

Background and Aim: In bone tissue engineering, it is crucial to provide a biocompatible and mechanically strong scaffold. In the present study, nanofibrous tissue engineering-scaffolds consisting of Gelatin (G), polycaprolactone (PCL) and a chitosan (CS) were fabricated by electrospinning method, using a new cost-effective solvent mixture: acetic acid and formic acid. The aim of this study was designing a novel scaffold improved by GO for bone tissue engineering.
Methods: The G (5%), PCL (10%) and CS (3%) are added separately to the solvent mixture. The final homogenous mixture is made with the weight ratio of PCL/G/CS sufficiently high (2: 1: 2). Then in order to evaluate the physico-chemical and biological properties improvement of the nanofibers, graphen oxide (0.1%) is incorporated in the solvent mixture. Four different samples with four different amount of GO were made (first sample with 0% GO, the second one with 0.5%, the third one with 3% and the fourth one with 6% GO). The microstructure, morphology, and mechanical properties of the scaffolds were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM). We also performed some biological tests including MTT to observe the effects of GO on biological properties and cell adhesion and proliferation. For biological test, we used human osteosarcoma cells (MG68).
Results: The morphology of the scaffold was observed using a scanning electron microscope (SEM). The nanofibers have smooth surface without the incidence of bead defects. The nanofiber diameter was from 90 to 110 nm. The presence of graphene oxide in the nanofibers was established by transmission electron microscopy (TEM). Mean of biodegradation percentage in 7, 14, 21, 28 and 35 day periods have been evaluated and the result demonstrate that Maximum and minimum amount of bio-degradation in 35 days period was related to sample 1 with average of 85.80% and sample 4 with 45.50% respectively. Analysis of variance for mean of biodegradation percentage showed a significant difference between all samples. Amount of cell viability for each sample and during four time periods, 1, 3, 7 and 14 days. The highest growth rate related to Sample 3 (GO 3%) and in the 14 days after culture. Also the lowest cell growth rate related to Sample 4 (GO 6%) and in the 14 day after cell culture. The results of the two-way ANOVA test showed that all differences between the percentage of cell growth in the first and 14 days were significant ($P = 0.05$). The results indicate that the graphene oxide incorporated scaffold nanofibers support the MG68 cell adhesion and proliferation.
Conclusion: In summary, the prepared scaffolds are able to support cell attachment and proliferation. This research also demonstrated that the synthesized scaf-

folds are suitable for ongoing bone tissue engineering studies.
Keywords: Tissue Engineering Scaffold Bone

Ps-022: Autologous Mesenchymal Stromal Cell Transplantation for Spinal Cord Injury: A Phase I Pilot Study

Humayoon Shafique Satti[1*], Akhtar Waheed[2], Parvez Ahmed[3], Khalil Ahmed[2], Zaineb Akram[1], Tariq Aziz[2], Tariq Mehmood Satti[1], Nighat Shahbaz[1], Mehreen Ali Khan[1], Salman Akbar Malik[4]

1. Armed Forces, Bone Marrow Transplant Center, CMH, Rawalpindi, Pakistan
2. Armed Forces, Institute of Rehabilitation Medicine, Rawalpindi, Pakistan
3. Armed Forces, Institute of Pathology, Rawalpindi, Pakistan
4. Quaid i Azam University, Islamabad, Pakistan
Email: xanab.akram@gmail.com

Background and Aim: Mesenchymal stromal cell (MSC) transplantation has immerged as promising therapeutic approach to treat spinal cord injury (SCI). In this pilot study, we investigated the safety of intrathecal injection of autologous bone marrow– derived MSCs in nine patients with SCI.
Methods: Patients with complete SCI at the thoracic level were divided into two groups: chronic (>6 months, group 1) and sub-acute SCI (<6 months, group 2), according to time elapsed since injury. MSCs were isolated by density gradient separation of autologous bone marrow harvested from the iliac crest. Cells were cultured in a Good Manufacturing Practice–compliant facility to produce clinical scale dose. After quality control testing, MSCs were injected back to patients by intrathecal injection. Safety was defined as absence of adverse event and side effects after 1 month after receiving the injection.
Results: Six patients had chronic SCI with a median duration of 33 months since date of injury (range: 10–55 months), and three patients were in sub-acute phase of disease. Each patient received two or three injections with a median of 1.2 × 106 MSCs/kg body weight. No treatment-related adverse event was observed during median follow-up of 720 days (range: 630–826 days) in group 1 and 366 days (range: 269–367 days) in group 2, respectively.
Conclusion: This pilot study demonstrated that autologous MSCs can be safely administered through intrathecal injection in spinal cord injury patients. Further investigation through randomized, placebo-controlled trials is needed.
Keywords: Clinical Trial, Mesenchymal Stromal Cells, Spinal Cord Injury, Transplantation

Ps-023: Safety of Treatment with Autologous Mesenchymal Stem Cells in Ckd Patients; a 12 Month Follow-up

Sudabeh Alatab [1*], Gholamreza Pourmand[1], Iraj Najafi[2], Mandana Moheyedin Bonab[3], Behrooz Nikbin Moghaddam[4,5]

1. Urology Research Center, Sina hospital, Tehran University of Medical sciences, Tehran, Iran
2. Nephrology Research Center, Shariati hospital, Tehran University of Medical sciences, Tehran, Iran
3. Immunogenetics Laboratory, Department of Immunology, Tehran University of Medical Sciences, Tehran, Iran
4. Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran
5. Immunogenetic Laboratory, Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: sudabehalatab@yahoo.com

Background and Aim: Chronic kidney disease (CKD) is a prevalent condition (8 to 16%) that might lead to End Stage Renal Disease in which patients need renal replacement therapy in order to survive. Recently cell-based therapy has been regarded as a promising strategy for improving CKD and is currently the focus of many preclinical and clinical studies. In this study we aimed to evaluate the safety of treatment with autologous bone marrow derived mesenchymal stem cells in CKD patients in a single arm, prospective, single-center, phase I clinical trial) IRCT201204248349N1(.
Methods: 14 patients who had documented CKD-phase III-IV, were selected after reviewing the medical records of 100 patients attending a private nephrology clinic and informed written consent form was obtained. Autologous MSCs were isolated from bone marrow of

CKD patients and culture expanded. Patients received one IV injection of MSCs under sterile condition and were then monitored at baseline and over a course of 12 months (every three months). The primary outcome was safety and secondary outcome was renal function.
Results: of 14 patients who entered study, only in 9 patients MSCs were expanded and therefore MSCs injection was performed only in 9 patients. 55% of subjects were male. The mean age of patients was 47.2±10.6 (32-61 years), with a CKD duration of 8.4±2.2 (4-11 years). Patients received 650116±4 cells/kg (9090-1192307). MSCs injection was safe. No acute or chronic adverse events were occurred during this period. Evaluation of hematologic markers, liver function and lipid profile showed no change over time. Over 12 month period, MSCs injection stabilized the serum creatinine and rate of decline in GFR. Change in serum creatinine and Glomerular filtration rate (GFR, based on MDRD calculation) from 12 months before intervention to 12 months after were as follow respectively: 2.4 ± 0.8, 30.2 ±9 (12 month before); 2.5±0.6, 27.5 ±0.7 (9 month before); 2.5±0.6, 26.6 ±5 (6 month before); 2.4 ±0.6, 29.3 ±6 (3 month before); 2.5±0.5, 27.1±0.7 (baseline); 2.6 ±0.8, 27.4±9 (3 month after); 2.6 ±0.9, 26.4 ±7 (6 month after); 2.5 ± 0.8, 27.7 ±9 (9 month after); 2.5 ± 0.8, 27.7 ±8 (12 month after). Change in serum creatinine and GFR after intervention compared to baseline was non-significant (p=0.1).
Conclusion: intravenous injection of autologous MSCs was safe and feasible in CKD patients. Moreover MSCs might be able to stabilize the rate of decline in GFR. Further clinical trials with larger sample size and having placebo group are needed to confirm these findings.
Keywords: Chronic Kidney Disease, Mesenchymal Stem Cell, GFR

Ps-024: Multi Spectroscopic Revealing Stability of KLF4 Extracted from Mouse Stem Cells: A Disease Diagnostic Model

Negin Alavi[1*], Jamshidkhan Chamani[2], Mohammadreza Saberi[3]

1. Department of Biology, Faculty of sciences, Science and research university, Tehran, Iran
2. Department of Biochemistry and Biophysics, Faculty of Sciences, Mashhad Branch, Islamic Azad University, Mashhad, Iran
3. Medical Chemistry Department, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
Email: neginalavi@yahoo.com

Background and Aim: KLF4 is a zinc-finger transcriptional regulator that plays a crucial role in the reprogramming of somatic cells into induced pluripotent stem cells. During cancer progression KLF4 could act either as a tumor promoter or as a suppressor, depending on the cancer type and the cellular context. The opposing functions of KLF4 may be related to an interaction between it and the tumor suppressor p53. Down regulation of KLF4 in colon adenomas, gastric cancer, intestinal adenomasand lung cancer may contribute to cellular hyper proliferation and malignant transformation, which is consistent with its role in cell cycle arrest and growth inhibition. However, high levels of KLF4 expression are also reported in primary breast ducal carcinoma and oral squamous carcinoma. So our findings have indeed pointed out the importance of the evaluation of conditions which can cause the instability of this protein due to prevent the progression of cancers related to KLF4.
Methods: The investigation of stability of KLF4 protein was done by usingCircular dichroism (CD), and Zeta potential spectroscopy. 2ml of %0.01 KLF4 in KH2PO4 buffer in various amounts of pH and ionic strengths was added and the ranges were measured in each experiment
Results: In order to evaluate the secondary structure of KLF4, the CD spectroscopy in far UV circular area simultaneously by changes of pH amounts was studied. As sigmoidal diagrams show, major parts of protein structure is consisted of α-helix content. By increasing ionic strength ranges from 25mM to 150mM, the amounts of θ become more positive and then the reduction of α-helix content is done. That is a proof of decrement of hydrogenic bonds and electrostatic forces which can cause the structural instability. By reduction of pH ranges from pH=7.4 to pH=5, the decrement of contents of α-helix, β-sheets and increment of random coil values were seen, that is done because of secondary structural changes of the protein which can lead to reduction of biological function of protein and unfolding

of KLF4. The Zeta potential can determine the ranges of polarity and polarization of proteins. High levels of zeta potential is due to electrostatic repulsion between particles and rising hydrophobicity values that causes restructuring and unfolding of the protein. In pH=5, Zeta Potential values decrease by increasing of ionic strentghs due to enhancement of hudrophobicity and denaturing of the protein. In other words, in various ranges of pH, chenges of surface charges have been done that cause increasing of Zeta Potential values. The highest values of Zeta Potential are related to to pH=7.4 and the lowest ranges are anent Ethanol-Distilled water solutions.
Conclusion: The secondary structural changes of protein in different environments show the dependence of protein to pH, which at pH=7.4 protein maintains it's secondary structure better and from pH=7.4 to the higher and lower pH, secondary structure will experience many changes.Also. These findings show the relativity between increasing of pH values and decreasing of Zeta Potential ranges that cause reduction of surface charges of the protein and decline of electrostatic interactions and finally increment of hydrophobicity and unfolding of KLF4 protein.
Keywords: KLF4, Stem Clell, Tumor Suppressor, Disease Diognostic, Cancer Prevention

Ps-025: Influence of Strontium on the Structure and Biological Properties of Mechanical Activation Sr-Doped Flourapatite Nanopowder for Bone Replacement

Morteza Alehosseini*, Hossein Edris, Mohammadhossein Fathi

Department of Materials Science, Isfahan University of Technology, Isfahan, Iran
Email: alehosseini.m@gmail.com

Background and Aim: Bone mineral consists mainly of a poorly crystalline fraction made of apatite and other crystalline calcium phosphate complexes. Strontium has a great affinity for bone and enhances stimulated bone formation and prevent from bone resorption. Also, Fluorapatite constitute the inorganic component of the human bone tissue and can be incorporated in hard tissue from dissolution of soluble compounds placed in the close vicinity of bone and result in the release of fluoride ions in the physiological body fluids. Fluoride may also be ingested in food along with water and is transported as dilute hydrofluoric acid in the intestinal cavity which is eventually stored in bone when exposed to apatite. The continuous daily exposure to the fluoride including therapeutics results in the formation of fluoride rich apatite in bones. Strontium flouroapatite nanopowder by different amount of Sr was prepared by mechanical alloying using a high-energy ball mill. It is well known that strontium promotes bone formation, reduces bone resorption and it is used to treat osteoporotic diseases as stated previously by researchers.
Methods: The aim of this study was to investigate the effect of biological properties of strontium (Sr) doping into Fluorapatite (Ca10-xSrx (PO4)6F2) powders. To preparing this nanopowder, specific amount of CaF2, P2O5, CaF2 and SrCO3 powders were milling for 10hrs by high energy ball mill to obtain Ca10-xSrx-(PO4)6(OH)2 with X= 0, 0.5, 1, 1.5, 2, 4, 9.
Results: The XRD patterns and Rietveld refinement analysis presented that with increasing amount of Sr, the crystallite size of SrFAp decreased firstly up to 20% Sr/(Ca+Sr) to 55 nm, and then increased for 40 and 90% Sr/(Ca+Sr) to 66 nm and 67 nm, respectively. The effect of Sr on the biocompatibility of nanopowder was evaluated in Simulated Body Fluid (SBF) after 7 and 21 days. It showed that the addition of Sr resulted increasing the biocompatibility of powder and after 21days and indicated that the release of Ca in SBF solution and phosphate groups resulted in formation of apatite (Ca10-xSrx)(PO4)6-x(CO3)x(OH)2 on the surface of nanopowder. Moreover, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay results confirmed that Sr released from SrFAp nanopowder not only did not show cytotoxic effect but also improved the viability of osteoblast cells (MG63) compared to Sr-free samples (FAp). For instant, the cell viability of SrFAp containing 40% and 90% Sr/(Ca+Sr) revealed 2.3 and 1.5 times greater than FA after 3 days of culture, respectively.
Conclusion: The merits of SrFAp such as good cytocompatibility, excellent biocompatibility make them a promising platform for bone applications.
Keywords: Strontium Fluorapatite, Mechanical Activation, Ball Mill, MG63, Osteoblast Cells, Rietveld

Ps-026: Lectin Profile Variation in Mesenchymal Stem Cells Derived from Different Sources

Elham Aliabadi*, Fatemeh Aleahmad, Tahereh Talaei-Khozani, Zahra Vojdani

Laboratory for Stem Cell Research, Anatomy Department, Shiraz University of Medical Sciences, Shiraz, Iran
Email: aliabade@sums.ac.ir

Background and Aim: Human mesenchymal stem cells (hMSCs), as a good source of stem cells in regenerative medicine, have different morphological and functional features. Carbohydrate moieties on the cell surface have important roles including cell-cell interaction and cell recognition. The objective of this study was to find the variation in glycoconjugate distribution pattern of MSCs derived from various sources.
Methods: MSCs were isolated from the adipose tissue, bone marrow, Wharton's jelly, and cord blood. Then, they were stained with FITC-conjugated Wheat germ agglutinin (WGA), Peanut agglutinin (PNA), Concanavalin A (Con A), Ulex Europaeus (UEA), Dolichos Biflorus (DBA), Phytolacca Americana (PHY) lectins. The intensity of the reactions was scored by an arbitrary scoring system.
Results: MSCs from various sources had different distribution patterns of lectin reactivity. All MSCs showed the highest intensity of reaction with WGA. They were also stained with PNA "weakly", except for the bone marrow-derived MSCs that reacted with PNA "strongly". Bone marrow-derived MSCs also failed to react with UEA, DBA and Con A. Wharton's jelly derived MSCs also could not be stained with Con A. Both adipose and cord blood-derived MSCs contained subpopulations with different lectin reactivates.
Conclusion: It seems that the MSCs derived from various sources had different lectin reactivity. Different glycoconjugate content may explain the variety in the functional aspects of the cells.
Keywords: Mesenchymal Stem Cells, Wharton' Jelly, Adipose Tissue, Bone Marrow, Umbilical Cord Blood

Ps-027: In Vitro Evaluation of Therapy Targeting CD133 Aptamers for Colorectal Cancer

Mona Alibolandi[1*], Sajjad Anvari[1], Seyed Mohammad Taghdisi[2], Khalil Abnous[1], Mohammad Ramezani[1]

1. Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
2. Targeted Drug Delivery Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
Email: alibolandim@mums.ac.ir

Background and Aim: Targeted, disease-specific delivery of therapeutic nanoparticles shows wonderful promise for transmitting highly cytotoxic anti-cancer agents.
Methods: Using the reaction of colorectal cancer (HT29 and caco-2 cell lines) as representative of other cancer types', the present study examines the effects of CD133-fluoropyrimidine RNA aptamer-decorated carboxymethyl cellulose conjugated SN38, the prepared self-assembled NPs that bond specifically to the extracellular domain of CD133 marker.
Results: Results demonstrate that CD133 aptamer-conjugated SN38-carboxymethyl cellulose significantly enhance cellular nanoparticle uptake in HT29 and caco-2 cell lines and increase the cytotoxicity of the SN38 as compared with non-targeted SN38- carboxymethyl cellulose conjugate ($P < 0.05$).
Conclusion: This study proves the potential utility of CD133 aptamer-conjugated SN38-carboxymethyl cellulose for therapeutic application in colon adenocarcinoma. In the future, CD133-targeted therapies might play a key role in treating colon adenocarcinoma.
Keywords: Aptamer; SN38; Colon Adenocarcinoma; HT29; Caco-2

Ps-028: In Vitro and in Vivo Evaluation of Therapy Targeting Epithelial-Cell Adhesion-Molecule Aptamers for Non-Small Cell Lung Cancer

Mona Alibolandi*, Khalil Abnous, Mohammad Ramezani, Fatemeh Sadeghi, Farzin Hadizadeh

Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
Email: alibolandim@mums.ac.ir

Background and Aim: Targeted, disease-specific delivery of therapeutic nanoparticles shows wonderful promise for transmitting highly cytotoxic anti-cancer agents.
Methods: Using the reaction of non-small cell lung cancer (SK-MES-1 and A549 cell lines) as representative of other cancer types', the present study examines the effects of EpCAM-fluoropyrimidine RNA aptamer-decorated, DOX-loaded, PLGA-b-PEG nanopolymersomes that bond specifically to the extracellular domain of epithelial-cell adhesion molecules.
Results: Results demonstrate that EpCAM aptamer-conjugated DOX-NPs (Apt-DOX-NP) significantly enhance cellular nanoparticle uptake in SK-MES-1 and A549 cell lines and increase the cytotoxicity of the DOX payload as compared with non-targeted DOX-NP ($P < 0.05$). Additionally, Apt-DOX-NP exhibits greater tumor inhibition in nude mice bearing SK-MES-1 non-small cell lung-cancer xenografts and reduces toxicity, as determined by loss of body weight, cardiac histopathology and animal survival rate in vivo. After a single intravenous injection of Apt-DOX-NP and DOX-NPs, tumor volume decreased 60.9% and 31.4%, respectively, in SK-MES-1-xenograft nude mice compared with members of a saline-injected control group.
Conclusion: This study proves the potential utility of Apt-DOX-NP for therapeutic application in non-small cell lung cancer. In the future, EpCAM-targeted therapies might play a key role in treating non-small cell lung cancer, the most common type of lung cancer.
Keywords: Epithelial Cell Adhesion Molecule, Doxorubicin, Nanopolymersome, PEG-PLGA, Non-Small Cell Lung Cancer, SK-MES-1

Ps-029: Analyzing Stem Cell Dimensions Using Confocal and Inverted Microscopy: A Postilion Approach in Cell Engineering

Zakieh Alihemmati[1*], Bahman Vahidi[1], Nooshin Haghighipour[2]

1. Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran
2. National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
Email: z.hemati@ut.ac.ir

Background and Aim: Body cells, including mesenchymal stem cells are subject to a lot of mechanical forces. The type and magnitude of these forces are different in different physiological and pathological conditions. A way for analyzing cell stimulations is preparation of three-dimensional images of cultured undifferentiated cells, modeling of the cell geometry in specialized software and numerical analysis using finite element method. Regarding to a crucial need for accurate stem cell dimensions, confocal and invert microscopy were used and then obtained dimensions were compared.
Methods: Suitable cover slip has been prepared for plate and 12000 cells were cultured before painting. Based on the used confocal microscope in the Pasteur laboratory (National cell bank of Iran, Pasteur Institute of Iran, Tehran, Iran), Z axis laser is applied on the surface of the cell cover and the light scans the surface area of cells. Using Image J software, pictures and dimensions, features and morphological properties of cell was determined. For comparison of obtained cell dimensions from confocal microscopy, inverted microscope was also used. The cells were cultured in the same way as before and then by using of inverted microscopy, mesenchymal stem cells, two-dimensional images were produced. Then, using ts view software, length and width of 12 cells were measured. All experiments were conducted for three times. Data were reported as the mean ± standard deviation (SD). Degrees of freedom was 12 and accordance with the critical values of t-test ($P=0.01$) was significant.
Results: several images of the stem cells produced by confocal microscopy and inverted microscopy and their dimensions were determined and compared. Consequently, dependent T of the cells length is measured in the present study and P value is Smaller than 0.01. Therefore, there is no significant difference between determined dimensions by the confocal microscope and inverted microscope and the dimensions are close and meaningful. In our study confocal microscopy provide a new way to obtain exact, 3-D image from stem cells.
Conclusion: In conclusion, we have spercified the cell-to-cell variation associated with geometrical differences across cells from the same type and culture, in terms of the localized characteristics. Better understanding of variations in cells morphology using of high resolution confocal microscopy seems attainable to get the exact dimensions.

Keywords: 3-D Imaging, Confocal Microscopy, Inverted Microscopy, Stem Cell

Ps-030: Stabilizing Primitive Properties of Mesenchymal Stem Cells in-Vitro by Histon Deacetylase Inhibitor Trapoxin A

Leila Mousazadeh[1], Effat Alizadeh[2*], Nosratollah Zarghami[3], Sedigheh Fekri Aval[4], Leili Hasanifard[5], Shahryar Hashemzadh[6], Roya Herizchi[7]

1. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran & The Umbilical Cord Stem Cell Research Center (UCSRC), Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
4. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
5. Department of Clinical Biochemistry, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
6. Department of General Surgery, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
7. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: alizadehe@tbzmed.ac.ir

Background and Aim: Adipose tissue mesenchymal stem cells (ASCs) have a great importance in regenerative medicine and cell therapy. Their frequency is low in adipose tissue upon isolation, therefore ex-vivo cultivation of them is needed. But through in vitro expansion, they lose their stemness potential and become senescent. Recent studies indicated that the ASCs quality lose in ex-vivo conditions is a possible result of histone deacetylation. In the present study, we investigate the effect of using an exogenous histone deacetylase inhibitor (HDACi) Trapoxin A(TPX A) for stabilizing the primitive features of ASCs through ex-vivo culturing.
Methods: We isolated ASCs from subcutaneous adipose tissue, evaluated their CD markers according to standards of International Society for Cell Therapy. Different doses of TPX A (0.1-0.001 μg/ml) were administered in the medium of ASCs and the viability of the cells were checked using MTT assay. DAPI staining was used for study of the distribution of cells in TPX A treated cell in comparison with control. The expression levels of stemness marker genes (Oct-4, Sox-2, Nanog, Rex-1, and TERT) were evaluated using qRT-PCR after TPX A treatment. The effect of TPX A on cell cycle also studied by PI staining using flowcytometry.
Results: MTT assay results indicated that ASCs are viable and actively proliferating after TPX A treatment. DAPI showed viable cells comparable to or in higher number than control. Our results clearly showed significant up-regulation of stemness marker genes ($p<0.05$) by TPX A. Also, the number of cells in S-phase were significantly ($p<0.01$) increased after TPX A treatment.
Conclusion: TPX A treatment could be recommended for stabilizing stemness properties of ASCs.
Keywords: Trapoxin A, Mesenchymal Stem Cells, Stemness

Ps-031: Bio-Fabrication and Accelerated Detachment of Adipose Derived Mesenchymal Stem Cells Sheet by Thermo-Sensitive Hydrogel- Copolymer Mediated Substrate

Behnaz Banimohammad-Shotorbani1, Effat Alizadeh[2*], Roya Salehi[3], Abolfazl Barzegar[1], Nosratollah Zarghami[2], Shahryar Hashemzadh[5]

1. Research Institute for Fundamental Sciences (RIFS), University of Tabriz, Tabriz, Iran
2. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Medical Nanotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
4. Research Institute for Fundamental Sciences (RIFS), University of Tabriz, Tabriz, Iran
5. Department of General Surgery, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Email: alizadehe@tbzmed.ac.ir

Background and Aim: Recently, cell sheet engineering emerged as promising approach in tissue engineering. Cell sheet is a technology which directs replacement of artificial extracellular matrix (ECM) with natural cell derived matrix. Maintaining the viability, integrity and preventing functional damage to released cell sheets is pivotal for therapeutic applications. Adipose tissue mesenchymal stem cells (AT-MSCs) have the ability for ECM secretion and showed promising outcomes in regenerative practice. The aims of this study were construction and evaluation of AT-MSCs sheet formation, detachment, viability and functionality on a thermosensitive hydrogel based on poly N-isopropyl acryl amide-Methacrylic acid P (NIPAAm-MAA) copolymer.
Methods: Thermo/pH sensitive P (NIPAAm-MAA) copolymer was synthesized and characterized by HNMR, FTIR experiments. The cell sheet fabrication was performed, subsequently the viability and functionality of released sheet were studied.
Results: We found that AT-MSCs have the potential for sheet production on thermo-sensitive P(NIPAAm-MAA) copolymer, also using MAA increased LCST point of polymer, therefore detachment of sheet didn't require more than 2ºC thermal reduction from 37 ºC (LCST=35) and consequently guaranteed the viability of cells in sheet. SEM and H&E staining showed the well-formed properties of a tissue layer with a proper ECM. The fabricated sheets exhibited osto/adipogenic differentiation potential. The cells of sheet showed less β-galactosidase associated senescence.
Conclusion: We concluded that, using P (NIPAAm-MAA) could facilitate the detachment and prevented functional damage to the engineered AT-MSCs sheet by a sol to gel transition at a temperature near to physiologic conditions.
Keywords: Cell Sheet, AT-MSCs, P (NIPAAm-MAA), Sol to Gel Transition

Ps-032: Evaluation of In Vitro and In Vivo Wound Healing Activity of Copper Nanoparticles

Sanaz Alizadeh[1], Bagher Seyed Alipour[2], Parvaneh Mohammadi[1], Nasser Aghdami[1*]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology , ACECR, Tehran, Iran
2. Department of Cellular and Molecular Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Iran
Email: alizadeh.01@gmail.com

Background and Aim: Wound healing is a complex biological process where multiple parallel and interrelated pathways are activated and synchronised to induce wound repair. Impaired wound healing, evidenced by increased closure time, decreased collagen deposition and granulation tissue formation. Current treatments based on the use of autografts, allografts, xenografts and bioengineered skin substitutes suffer from limitations such as, quantity of donor skin available, donor-site infection, potential risk of disease transmission and rejection of the graft. Given these problems, copper nanoparticles due to their unique properties such as high surface area to volume ratio, high stability, antibacterial effective and effect on the collagen deposition to be used in wound treatment. In this study we explore the potential therapeutic effects of copper nanoparticles (cu-NP) in vitro and in vivo model of wound healing.
Methods: fibroblast cells were exposed to copper nanoparticles of concentration (1-10-100 uM) and sizes (40-80nm) for 24-48-72h. MTS assay for cell viability, Real time PCR for collagen deposition were performed. Animal modeling was studied with hematoxylin and eosin (H&E) staining samplings of skin were conducted on days 3, 7, 14, 21 with copper nanoparticles of concentration (1-10-100 uM) and sizes (40-80nm).
Results: The results from cell viability (MTS) showed cu-NP of concentration (1-10-100uM) and sizes (40-80nm) was not toxic to fibroblasts in vitro and Real time PCR revealed cu-NP (80nm/1uM) significantly increased collagen expression. Also when cu-NP used to treat full thickness skin defects in rats, H&E results demonstrated that cu-NP (80nm/1uM) improved wound treatment after 21 days.
Conclusion: The results showed that the copper nanoparticles (cu-NP 80nm (1uM)) significantly accelerated wound healing
Keywords: Copper Nanoparticle, Collagen Deposition, Wound Healing

Ps-033: Study the Effects of Phenylalanine Levels on Fibroblast Cell Migration, and Proliferation

Niyaf AlKadhem[1], Melville B. Vaughan[2], Rami Mohanad Mahdi[3], Saad Salih Aldujaily[1]

1. Biotechnology Research Center, Al-Nahrain University, Baghdad, Iraq
2. Biology Department, University of Central Oklahoma, Edmond, Oklahoma, USA
3. Analytic Pathological Techniques, Al kut University, Kut, Iraq
Email: Biotechno.new.era@gmail.com

In this study the targeted cell is a fibroblast for its importance in healing wounds through cell migration and the phenylalanine applied in vitro on those celles type tissue will be grown in the absence or presence of Phe (10 μM, 1 μM, and 0.1 μM). Human fibroblast acquired from Dr. Melville Vaughan. From UCO/biology department, then sub culturing these cells and Conduct scratch assay photographing before and after migration has occurred "The scratch wound assay is a method to monitor cell migration. It mimics cell migration during wound healing in vivo and are compatible with imaging of live cells during migration to monitor intracellular events then conducted the proliferation assay with cells on cover slips using a purchased kit. Cells were plated on glass cover slips and cultured in the presence or absence of phenylalanine for 48 hours. A labeled nucleotide was added in the final hour of culture to determine proliferation. Cover slips were then fixed, stained, and photographed using an Olympus IX71 microscope .The results shows that after taking the measurement of the healing area after scratching and in presence or absence of Phenylalanine in 24 hr. by comparing the results with control the faster healing and the largest area of healing in 24 hr. 0.1m M Phe. (n.:40) 0.99 mm2/24hr whereas the low healing rate and less healing area for 1mM Phe mM Phe. 0.68 mm2/24hr compared with control group in absence of Phe 0.89 mm2/24hr the healing area while the 10 mM Phe. Concentration shows 0.75 mm2/24hr all data is an average mean of 40 sample of fibroblast scratched tissue (n.:40) the conclusions of this study are for Migration The process of wound healing have 2 sides positive and negative. If Phe speeds up healing, it would be good for diabetic patients who really face a problem healing wounds as observed in 0.1 mM Phe treatment. In the case that Phe slows down or inhibits the healing process it is a good compound for localizing cancer cells as in 1mM Phe. treatment. For Proliferation the Phe concentrations we used resulted in non-significant differences when compared to control group

Ps-034: Differentiation of Human Mesenchymal Stem Cells into Insulin Producing Cells by Using A Lentiviral Vector Carrying PDX1

Amir Allahverdi, Saied Abroun*

Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: amir_6122@yahoo.com

Background and Aim: Type I diabetes is an immunologically-mediated devastation of insulin producing cells (IPCs) in the pancreatic islet. Stem cells that produce β-cells are a new promising tool. Adult stem cells such as mesenchymal stem cells (MSCs) are self renewing multi potent cells showing capabilities to differentiate into ectodermal, mesodermal and endodermal tissues. Pancreatic and duodenal homeobox factor 1 (PDX1) is a master regulator gene required for embryonic development of the pancreas and is crucial for normal pancreatic islets activities in adults.
Methods: We induced the over-expression of the PDX1 gene in human bone marrow MSCs (BM-MSCs) by Lenti-PDX1 in order to generate IPCs. Next, we examine the ability of the cells by measuring insulin/c-peptide production and INSULIN and PDX1 gene expressions.
Results: After transduction, MSCs changed their morphology at day 5 and gradually differentiated into IPCs. INSULIN and PDX1 expressions were confirmed by real time polymerase chain reaction (RT-PCR) and immunostaining. IPC secreted insulin and C-peptide in the media that contained different glucose concentrations.
Conclusion: MSCs differentiated into IPCs by genetic manipulation. Our result showed that lentiviral vectors could deliver PDX1 gene to MSCs and induce pancreatic differentiation.
Keywords: PDX1, Diabetes Type I, Meneschymal Stem Cells

Ps-035: Treatment of Incurable Corneal Injury by Using Platelet Product a Case Report

Zahra Ameri, Seyede Mehrnaz Khuhbanani Nejad, Alireza Farsinejad*

Department of Hematology, Faculty of Allied Medicine, Kerman University of Medical Sciences, Kerman, Iran
Email: farsinezhad239@yahoo.com

Background and Aim: Platelet derived products have demonstrated their capacity to enhance healing and stimulate the regeneration of different tissues and this enhancing effect is attributed to the growth factors. Efficacy of autologous platelet-rich plasma eye drops is comparable with standard medical treatment in acute ocular chemical injury. This report describes a 47 year old woman with a 7 milimeter corneal injury and fungal infection in her left eye. Any kind of eye drop was prescribed to cure her corneal injury for mounts but none of them was efficient and making it worse. After that we use autologous platelet growth factor plus white blood cells eye drop to cure her injury.
Methods: 10 mL of patient's whole blood on ACD was collected once every three day. Leukocyte Rich Platelet rich plasma (L-PRP) was obtained by centrifuging the blood at 300g for 10 minutes. To separate components of L-PRP leukocytes were precipitated by centrifugation of L-PRP at 300 g for 5 minutes of supernatant , 90% PRP was removed, and the leukocytes were resuspended. 12.5 mM calcium chloride was added to the PRP and serum rich growth factor (SRGF) was obtained and then separated leukocytes was added to it. One drop of SRGF plus leukocytes was dropped to eye every 8 hour till one mount.
Results: The eye was examined by fluorescein eye stain every week. Fungal infection was cured and the injury was getting better and smaller and it closed in one mount.
Conclusion: PRP eye drop is a reliable and effective treatment that promotes corneal wound healing in severe corneal ulcers and corneal perforations. The PRP showed to be more effective than conventional treatments for the regeneration of the extensive and deep corneal ulcers.
Keywords: Corneal Injury, Platelet Rich Plasma (PRP), Serum Rich Growth Factor (SRGF)

Ps-036: Study of the Effects of Satureja Hortensis Extracts on Induction of Differentiation in NB4 Cell Line

Zahra Amirghofran*, Morvarid Asadipour

Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran
Email: amirghz@sums.ac.ir

Background and Aim: Induction of differentiation of cells toward mature cells is an important way for removing tumor cells or stopping their proliferation especially that numerous tumors have small groups of stem-resembling cells with the ability to renew the tumor. Plants have a long history in the treatment of cancer. The plant Satureja hortensis, belonging to Lamiaceae family has shown growth inhibitory effect on various tumor cell lines. In this study, the effect of different extracts of S. hortensis on induction of cell differentiation in NB4 cell line was investigated.
Methods: The NB4 promyelocytic cell line was used for studying the effects of the extracts on cell differentiation. We used retinoic acid as a differentiation-inducing compound as the control and examined cells for the induction of cell differentiation by studying cell morphological changes, reduction of nitroblue-tetrazolium dye and the expression of CD11b differentiation marker.
Results: The differentiation tests using retinoic acid was set up. The growth inhibitory effects of hexane and dichloromethane extracts from S. hortensis were determined by MTT assay on NB4 cells and data showed that the hexane extract of the plant was more effective than dichloromethane extract. According to Annexin V/PI staining, hexane extract of S. hortensis was able to significantly induce apoptosis at concentrations of 25-50?g/mL ($p<0.05$) 72 h after treatment. Despite of this finding, the extract was unable to induce cell differentiation in NB4 cell line in comparison to the retinoic acid differentiation effects.
Conclusion: It was concluded that the observed growth inhibitory effects of S. hortensis extract was due to induction of apoptosis and not differentiation of NB4 cells toward mature cells.
Keywords: Differentiation, NB4 Cell Line, Medicinal Plants, Satureja

Ps-037: Numerical Simulation of a Microfluidic Platform for Controlled Double Concentration Gradient Generation of Biochemical Cues to Differentiate Neural Stem Cells

Leyla Amirifar*, Amir Shamloo

Mechanical Engineering Department, Sharif University of Technology, Tehran, Iran
Email: l.amirifar@gmail.com

Background and Aim: Several biochemical cues exist within the microenvironment of living cells that control cellular behavior. Cells are induced by absolute concentrations and concentration gradients of the biochemical factors. Adequate absolute concentration initiates cell stimulation and concentration gradient maintains the process. Microfluidic devices have offered precise control in generating desired concentration fields of multiple biochemical factors for cells. The importance of achieving an adjusted concentration field is highlighted when dealing with stem cells. It is ideal to achieve a variety of target cells from differentiating stem cells so that the resulting combination is a better representative of living tissue. This requires generating more than one concentration field within the microenvironment. In this study, a microfluidic device is presented and evaluated numerically for creating double overlapping concentration gradients based on diffusion mechanism to differentiate neural stem cells into astrocytes and neurons using fetal bovine serum (FBS) and nerve growth factor (NGF), respectively.
Methods: The presented microfluidic device consists of five microchannels and a reservoir, connected through micropillars. The reservoir is intended for cell seeding, while the other four side microchannels generate concentration gradients. Navier-Stokes equations of continuity and momentum for incompressible fluid and also convection-diffusion equation were solved, leading to the calculation of concentration fields. The properties of cell culture media was considered for the fluid and diffusion coefficients of FBS and NGF were set to be 7.8e-11 m2/s and 6e-11 m2/s, respectively. Due to small values of concentration coefficients, a small value of injection flow rate must be applied to the inlet of each side microchannel.
Results: Concentration distributions of FBS and NGF were determined using numerical simulations for the introduced microfluidic device. The results showed that after 15000 second, concentration profiles of both FBS and NGF became parallel along the reservoir, demonstrating stability of the created concentration fields. By choosing the right pattern for inlets and outlets of the side microchannels, two overlapping descending and ascending concentration fields were generated within the reservoir. Noticing the trend of FBS and NGF concentration profiles along the reservoir was significant in this matter. The maximum concentration of FBS was observed in the far left side of the reservoir with gradual decrease by advancing to the right. NGF concentration increased from left to right with its maximum in the far right side. In other words, the population of astrocytes differentiated under the influence of FBS reduces, while neuron population resulted from NGF effect enhances by moving from left to right. Somewhere in the middle of the reservoir, these two concentration fields meet and create an area in which neural stem cells can be induced by both biochemical cues.
Conclusion: We have numerically studied a microfluidic device for generating double overlapping concentration gradients of biochemical factors. Diffusion was the dominant mechanism, leading to more stability and less cell destruction. Differentiating neural stem cells into two different types of target cells can be performed experimentally in our design. This implies the applicability of our microfluidic device to examine the behavior of different types of cells under biochemical stimulations.
Keywords: Double Concentration Field, Microfluidic Device, Numerical Simulation, Neural Stem Cells

Ps-038: Efficacy Assessment of Mesenchymal Stem Cell Therapy for Wrinkle Skin Care

Mohammad Amir Amirkhani, Mohammad Ali Nilforoushzadeh*, Alireza Shoae-Hassani, Somayeh Hejazi, Mahsa Mollapour

Skin and Stem Cell Research Center, Tehran University of medical Sciences, Tehran, Iran
Email: Amirkhani2000@yahoo.com

Background and Aim: The rejuvenation characteristics of fat tissue grafting have been established for many years. Recently it has been shown that stromal vascular fraction (SVF) of fat contributes to its rejuvenation properties. As the preparation of SVF is a minimally handling process based of FDA guidance's make it a suitable cell therapy for treatment of aged skin. This work is aimed to clinical evaluate the ultrastructural improvement of aged skin at the facial nasolabial region after transplantation of autologous SVF.
Methods: Our study was conducted in 16 patients aged between 38 and 56 years old that were interested for face lifting at first. All of the cases went under liposuction from the abdomen for sampling fat tissue. Quickly the SVF was harvested from 100 mL of abdominal adipose tissue and then transplanted at dose of 2.5×107 nucleated cells in each nasolabial folds. The changes in the skin was evaluated using Visioface scanner, skin-scanner DUB, Visioline and Cutometer with multi probe adopter
Results: By administration of autologous SVF the elasticity, plastisity, thickness and density of skin was improved significantly. There was no changes at the epidermis density in Scanner results but we noticed a significant increase in the dermis density and thickness with enrichment in vascular bed of hypodermis. The score of Visioface scanner showed slight changes in wrinkle scores. It seems that endothelial cells and mesenchymal progenitors from the SVF changed the architecture of skin but there was not obvious phenotypic changes in nasolabial grooves.
Conclusion: The current clinical trial showed the modification of dermis region and its microvascular bed but no changes in the density of epidermis. It represents rejuvenation process of facial skin after SVF administration.
Keywords: Cell Therapy, Dermatology, Facial Skin, Nasolabial fold, Regenerative Medicine, Rejuvenation, SVF

Ps-039: Induction of Apoptosis by Pro-Apoptotic Micrornas in Glioblastoma Multiforme and Analyzing Their Targets

Sara Amirpour Amraii[1*], Ehsan Arefian[2], Rezvan Rezazadeh[2]

1. Department of Microbiology, Faculty of biology, University College of Sciences, University of Tehran, Tehran, Iran
2. Stem Cell Biology Department, Stem Cell Technology Research Center, Tehran, Iran
Email: sara.amirpour20@gmail.com

Background and Aim: Glioblastoma multiforme (GBM) is an incurable form of brain cancer with a very poor prognosis. Because of its highly invasive nature, it is impossible to remove all tumor cells during surgical resection, making relapse inevitable. Further research into the regulatory mechanism underpinning GBM pathogenesis is therefore warranted.Research in the past decade has brought microRNAs (miRNAs) to the forefront of cancer molecular biology. Experimental and clinical scenarios have widely shown that miRNAs play pivotal roles in human cancer development and progression. Abnormalities in miRNA expression or function in glioma are usually associated with the development of representative biological hallmarks that distinguish malignant cells from normal ones, including increased cell proliferation, abrogated apoptosis, enhanced invasiveness and cell motility, and promotion of angiogenesis.
Methods: we applied analytical and bioinformatics approaches to identify a set of pro-apoptotic microRNAs that it's over expression correlates with apoptosis in glial tumors. We analyzed the impact of this microRNAs with bioinformatics tools such as GEO. Three miRs (miR-125a, miR181b, miR34a) were selected. These microRNAs were cloned in the appropriate plasmid vectors and then transfected to HEK-293 cell line by lipofection method. Glioblastoma cell lines (U-87 & U-251) were transfected with microRNA-containing viruses and subsequently apoptosis was analyzed.
Results: It has been reported that our selected microRNAs inhibits expression levels of genes like BCL2 and BAX and increase expression levels of genes such as p53 and caspase 3. p53 is a gene that codes for a protein that regulates the cell cycle and hence functions as a tumor suppression and Caspases are crucial mediators of programmed cell death (apoptosis). Among them, caspase-3 is a frequently activated death protease.
Conclusion: Apoptosis is going to be measured using Annexin V and Flow Cytometry. And the luciferase assay will be used to investigate how efficient our selected microRNAs bind to mentained targets.

Keywords: MicroRNA, miR, Glioblastoma, Apoptosis

Ps-040: Autologous Platelet Rich Plasma Effect on Wound Healing: A Systematic Review and Meta-Analysis

M. H. Amirzade-Iranaq*, S. M. R. Masoumi

Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Email: h.amirzade@gmail.com

Background and Aim: Autologous platelet rich plasma is a novel technique for wound therapy which is still a "hot-topic" and matter for discussion. For exact determining the use and clinical outcomes of the therapy, this systematic review was performed.
Methods: With a proper search strategy which include MeSH and non-MeSh words, an electronic and also hand searches for randomized controlled trials were performed. Included studies most have comparative group studies using platelet rich plasma therapy in wounds and publish time limit set for recent 10 years. Eligible studies were assessed for validity, quality, and bias using CASP appraisal tool with separated operators. The primary outcomes were effect of platelet rich plasma and control wound care on wound healing and related healing measurements. Secondary outcomes related to healing such as infection, pain, exudate, adverse events, and quality of life were also considered. The meta-analysis were operated using CMA software version 2.
Results: Among all included studies after removing duplicates and screening for protocol eligibility, a total number of 31 articles finally included. The meta-analysis of chronic wound studies revealed platelet rich plasma therapy is significantly favored for complete healing. The meta-analysis of acute wounds with primary closure studies demonstrated that presence of infection was reduced in platelet rich plasma treated wounds.
Conclusion: This meta-analysis of effect of platelet rich plasma therapy in cutaneous wounds healing determined usefulness and significant better primary and second outcomes compared to control wound care.
Keywords: Platelet Rich Plasma, Autologous, Wound healing, Therapy, Systematic Review

Ps-041: Establishment and Banking Human Mesenchymal Stem Cells

Masoumeh Asadi*, Neda Sadat Gohari, Meysam Ganjy Bakhsh, Parvaneh Farzaneh, Seyed Abolhassan Shahzadeh Fazeli

Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR and Tehran, Iran
Email: masome.asadi@gmail.com

Background and Aim: In recent years, human mesenchymal stem cells (hMSCs) considered as a proper cell source for regenerative medicine. Therefore, establishment and banking of mesenchymal stem cells with appropriate differentiation ability and expression of specific molecular markers and higher safety is needed for continuous research. Iranian biological resource center established a human mesenchymal stem cell bank from different tissues with varying potencies including adipose tissue, amnion membrane, placenta and dental pulp.
Methods: Mesenchymal stem cells from different tissues were isolated by two isolation methods including enzymatic digestion and explants culture. These cells were characterized and quality control tests were performed to bank them in proper condition.
Results: MSCs were successfully isolated. Quality control tests revealed that isolated cells had no fungal, bacterial, mycoplasma contamination. In addition no cross contamination with other species was observed in these cells. The cells differentiated to osteogenic and adipogenic lineages. Isolated MSCs expressed MSC surface markers including CD29, CD90 and CD105 and lack the expression of CD34 and CD45.
Conclusion: Our results show that an appropriate processing for mesenchymal cell isolation is very time consuming process and costs high. Also these cells contaminate easily with mycoplasma and other cell culture contaminants. We suggest that researcher obtain these cells from cell bank instead of isolation and cell establishment.
Keywords: Human mesenchymal stem cells, Establishment, Cell bank

Ps-042: Study of Hypoxemic Effect on the Cancer Stem Cells Subpopulation in Breast

MCF7 Cell Line and Inhibitory Effect of Curcumin

Mehrnaz Asadi, Naser Ostad*, Mohammad Hosein Qhahremani

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
Email: m_asadi93@yahoo.com

Background and Aim: Hypoxia develops more in solid tumors because the rate of proliferation of tumor cells is more rapidly than the vessel formation. In response to the hypoxic condition, the hypoxia-inducible factor (HIF) mediates transcriptional responses in solid tumors so it can lead to tumor progression. HIF1 level was found to be positively related to metastasis, and resistance to chemotherapy. Curcumin has been shown anti-inflammatory and anti-cancer activities.In the present study, we want to examine the possibility that curcumin inhibits tumor progression by reduction in HIF-1 and HIF-2 expression in the cancer stem cells subpopulation in breast MCF7 cell line.
Methods: MCF7 and CSC of MCF7 mammary carcinoma cells were grown in a humidified 5% CO2 atmosphere and 20% O2 (normoxic conditions) , 1% O2 (hypoxic conditions) at 37°C in an incubator.The effects of curcumin on cell viability was determined by MTT Assay and cells treated with 5-160 μM curcumin for 24 h under normoxic and hypoxic conditions. We use flow cytometry analysis for the identification of cancer stem cells (CD44+, CD24-) from MCF7. The effects of curcumin on HIF expression was detected by Western blot method.
Results: We examined the effect of curcumin on cell viability using MTT assays. We use flow cytometry analysis for the identification of cancer stem cells (CD44+, CD24-) from MCF7. The inhibitory effect of curcumin were investigated on HIF-1, ARNT and HIF-2α protein levels in MCF-7 and CSC of mcf7 cells after incubation for 24 h under normoxic and hypoxic conditions by Western blot method.
Conclusion: In summary, HIF1α has an important role in tumor progression and its expression was increased in hypoxic condition. In many reports Curcumin has been shown an anticancer and anti-inflammatory effects with relatively low toxicity.We hypothesize that curcumin can suppress HIF levels in CSC of MCF7 by degrading ARNT in hypoxic condition. However, further studies required to establish the effects of curcumin on HIFs expression.
Keywords: Hypoxia-Inducible Factor (HIF), Curcumin, Cancer Stem Cell (CSC)

Ps-043: High Expression of Long Noncoding RNA H19 in Induced Pluripotent Stem Cells

Marjan Asadii[1], Masoud Soleimani[2], Shaban Alizadeh1*

1. Hematology Department, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran
2. Hematology Department, School of Medicine, Tarbiat Modares University, Tehran, Iran
Email: marjan_asadi69@yahoo.com

Background and Aim: Long noncoding RNAs (lncRNAs) comprise a different class of transcripts that structurally resemble mRNAs, but they are not functionally encoding any proteins. As a documented substantial player of embryonic development and tumorigenesis. we could mention the oncofetal lncRNA gene H19. Moreover, it is also studied that the overexpression of this IncRNAs has the capability to enhance both the motility and invasiveness of tumor cells. Induced pluripotent stem cells (iPSCs) are a new types of stem cells which are obtained by reprogramming differentiated cells into stem cells. Recent studies also mentioned that IncRNAs such as Linc-ROR has potency to same role in the maintenance of stemness.The aim of this study is to examine the expression of H19 in comparison between iPS and CD34+cells by quantitative real-time RT-PCR (qRT-PCR) . For this purpose, CD34+ cells were isolated from human cord blood(CB)by MACS CD34 isolation kit , then were resuspended in StemPro medium which was enriched by essential growth factors such as TPO, SCF and Flt-3 Ligand, after that as hematopoietic stem cell (HPSc) were confirmed by flowcytometery to express CD34 marker, next cells were extracted for total RNA and cDNA Synthesis .Our results revealed that The expression of H19 in iPS significantly increase of 116.6-fold ($p< 0.0001$) compared to HPSc . In this

study we demonstrated, in addition linc-ROR, another lncRNAs such H19 have expressed in iPS.
Methods: CD34+ cells were sorted by Miltenyi Biotec (Bergisch Gladbach, Germany) and iPS cells were gifted by Stem Cell Technology Research Center (Bon Yakhteh research center). Then CD34+ cells were cultured in serum-free conditioned erythrocyte culture medium with 50 ng/mL SCF, 20ng/ml TPO and 50 ng/mL FLT-3. Isolation of total RNA from cells was carried out using RNeasy Mini Kit (Qiagen, Hilden, Germany). cDNA was obtained by RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) with random primers according to the manufacturer's protocol.qRT-PCR were performed using specific primers with SYBR® Green PCR Master Mix SYBR ® Green RT-PCR reagent kit (Applied Biosystems) and run on Applied Biosystems StepOne™ instrument (Applied Biosystems, Foster City, USA).
Results: AS Yue Wang etal.reported that linc-RoR is expressed in embryonic stem cells (ESCs) and iPS.In addition we observed that H19 is expressed in iPS as well. LncRNA H19 expression was examined by qRT-PCR and normalized to β-actin expression.The expression of H19 higher than CD34+cells (116.6-fold, $P < 0.001$). Agarose gel electrophoresis of the q-RT PCR-products displayed a specific band with the expected size (180bp). Furthermore, melthing curve analysis of real-time PCR revealed that the single product was amplified without primer dimers.Comparison between the CD34+ cell and iPS was performed using T-tests. P values of <0.05 were considered as statically significant.
Conclusion: These results showed that H19 is expressed in iPS. The expression of H19 was higher than CD34+cells ($P (< 0.001$).It seems that different lncRNA expressed in iPS cells and play pivotal role.
Keywords: RNA, Long Noncoding H19 Long Non-Coding RNA iPS

Ps-044: Tumor Exosome-Mediated Delivery of miRNAs

Ali Asadirad[1], Davar Amani[1]*, Seyed Mahmoud Hashemi[1], Hossein Ghanbarian[2], Esmaeil Mortaz[3], Kaveh Baghaei[4], Mohammadreza Zali[4]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Biotechnology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Chronic Respiratory Disease Research Center, National Research Institute of Tuberculosis and Lung Disease (NRITLD), Department of Clinical Immunology, Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: ali.asadirad@gmail.com

Background and Aim: Many types of cells release phospholipid membrane vesicles that are thought to play key roles in cell-cell communication, antigen presentation, and the spread of infectious agents. These membrane vesicles, derived from the late endosomes, are called exosomes. Various proteins, messenger RNAs (mRNAs), and microRNAs (miRNAs) are carried by exosomes to cells in remote locations, like a message in a bottle. Because they can protect encapsulated small RNAs from Ribonucleases (RNases) in body fluid, exosomes represent ideal carriers for nucleic acid drugs. We showed that tumor cell-derived exosomes can function as vehicles to deliver exogenous miRNA.
Methods: CT26 cell line were cultured in DMEM medium plus 1% penicillin/streptomycin. After 72 hours the culture media were harvested and exosomes were isolated. The size, concentration, morphology, of isolated exosomes were identified by Nano drop and scanning electron microscopy. For loading mirna into exosome with electroporation, exosomes were diluted in Electroporation buffer in 1:1 ratio then mixture of miRNA and exosome transferred into cold 0.2 cm electroporation cuvettes and electroporated at 0.200 kV and 100μF. The relative amount of encapsulated miRNA-155 was determined using Real time PCR.
Results: We used electroporation to transfer miRNA into exosome. The Real Time PCR result showed that electroporation can elevate the level of miRNA into exosome significantly
Conclusion: the result showed that electroporation can transfer miRNA into exosome with high quality. Exosomes could be used as efficient vehicles in RNA-based therapeutic strategies and provide proof of concept for using exosomes as efficient delivery Na-

no-vehicles with minimal cytotoxicity. This identifies exosomes as novel frontiers in expanding Nano medicine applications.
Keywords: Exosome, miRNA, Electroporation

Ps-045: Aspirin-Releasing Composite PLA/ AML Scaffolds Seeded with HMSC for Vascular Tissue Engineering

Saba Aslani[1*], Mahboubeh Kabiri[1], Hana Hanaee-Ahvaz[2], Masoud Soleimani[2]

1. Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
2. Stem Cell Technology Research Center, Tehran, Iran
Email: saba.aslani@gmail.com

Background and Aim: Cardiovascular disease and in particular coronary artery occlusion remains the leading killer around the world. Although the gold standard for small diameter blood vessels replacement in the clinic is an autograft, the need for off the shelf blood vessel substitutes is unquestionable. Few of the main challenges to overcome regarding Tissue-Engineered Vascular-Grafts (TEVGs) are the ability for long-term patency and their mimicry mechanical properties that are yet to be resolved. Of the main approaches in vascular tissue engineering "scaffold guided approach" can be mentioned. Biodegradable polymers work as a temporary scaffold to support tissue growth. Scaffolds from the decellularized tissue skeletons to biopolymers and biodegradable synthetic polymers have been used for fabricating TEVGs. Human amniotic membrane (AM) is largely composed of collagen, laminin, and fibronectin which are all sought proteins for best cell-scaffold interactions. The low immunogenicity and high healing power of AM make it attractive for use in regenerative medicine.
Methods: Our work is based on fabricating aspirin-loaded electrospun nanofibrous polylactic acid grafts coated with amniotic basement membrane lysate. The surface of the scaffolds was plasma treated in order to increase the hydrophilicity of the composite sheet. Morphological and surface characteristics of the scaffold were investigated using SEM and FTIR. Human mesenchymal stem cells (HMSC) were isolated from adipose tissue. MTT Cell Proliferation assay was performed to assess the biocompatibility of the composite scaffold.
Results: According to the SEM images, the size of fibers ranged from 250 to 500 nm. The images also showed that drug particles did not impose unfavorable effects on fiber diameter. The presence of amide bands representing proteins in the scaffolds was proved using FTIR. MTT assay results suggested appropriate HMSC proliferation rate on aspirin-loaded AML-coated PLLA scaffolds.
Conclusion: The results showed that AML can be easily and safely applied to PLLA nanofiber surface in order to make biocomposite scaffolds with favorable properties for cell attachment and growth, which would potentially better support tissue remodeling and forming of new vascular conduits.
Keywords: Vascular Tissue Engineering, Electrospinning, PLLA, Amniotic Membrane Lysate

Ps-046: Cytocompatibility of Graphene Oxide Incorporated Electrospun Nanofibrous Scaffolds

Maryam Ayazi[1], Mahboubeh Kabiri[*], Maryam Karimzadeh, Reza Mosavikia[2], Mohammad Hossein Karami

1. Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
2. Department of organic chemistry, Faculty of Chemistry, College of Science, University of Tehran, Tehran, Iran
Email: mkabiri@ut.ac.ir

Background and Aim: of cells and synthetic scaffolds is the core part of tissue engineering in order to control differentiation towards specific lineages. Graphene oxide (GO) is a biocompatible and implantable platform and due to its high stiffness can serve as a suitable additive to give conductivity and stiffness to conventional synthetic polymers. However, the potent role of GO in guiding uncommitted stem cells towards osteogenic phenotypes is not known.
Methods: Herein, we used electrospinning to make nanofibrous polyethersulfone (PES) polymers and doped it with graphene oxide in order to increase the conductivity of the substratum of the stem cells and enhance the resultant osteogenesis. To do that we first separately

dissolved the GO and PES in DMF and after 1 hour of sonication of GO, the solutions were then mixed together and electrospun on a rotatory drum. Scaffolds were characterized using SEM and hydrophilicity test. Stem cells were isolated from adipose tissue and characterized by immuphenotyping. Stem cells were then grown on the scaffolds and the cytocompatibility of the scaffolds were studied using MTT assay.
Results: We observed that fibers had formed a uniform mesh like structure. The diameter of the fibers were in the range of nanometer and GO seemed to spread uniformly in the fibers. The inclusion of GO would not result in any cytotoxicity or inhibitory effects on stem cell growth. We will further assay the osteoconductivity of the scaffolds.
Conclusion: Conductive nanofibers obtained via electrospinning of PES and GO are appropriate substrates for tissue engineering of bone or other tissues such as neural or muscular ones. Our study showed the manufacture and cytocompatibility of GO endowed PES nanofibrous sheets.
Keywords: Graphene Oxide, Polyethersulfone, Electrospinning, Tissue Engineering, Stem Cells

Ps-047: Scaffolds Based Gelatin - Chondroitin Sulfate and Poly- Caprolactone (PCL) Promote Chondrogenic Differentiation of Human Bone Marrow Mesenchymal Stromal Cells for Tissue Engineering

Golrokh Azadegan[1], Shiva Irani[1], Mohamad Pezeshki-Modarres[2*], Mojgan Zandi[2]

1. Department of Biology, Science and Research Branch Islamic Azad University Tehran Iran
2. Department of Biomaterial, Iran Polymer and Petrochemical Institute, Tehran, Iran
Email: golrokh.azadegan@gmail.com

Background and Aim: Management of Cartilage Defects Is One of the Most Challenging Problems for Public and Medical Communities. Since Articular Cartilage Is Characterized By A Poor Vascularization, Which Limits The Capacity To Repair Itself. Tissue Engineering Field Focuses On Restoring And Regeneration Of The Damaged Tissues By Means Of Cells , Biological Cues And Scaffolds Made Of Biodegradable Synthetic And Natural Biopolymers To Help Cell Attachment, Proliferation And Migration.The Scope Of This Chapter Is To Provide An Overview Of Mechanical, Biological And differentiation of Mesenchymal stem cells derivate from human bone marrow stem cells to Chondrocytes on 3D scaffolds in vitro.
Methods: Blending of Gelatin (Gt) with chondroitin-sulfate and Poly-Caprolactone (Pcl), all had been used separately as control and also in Ratio (1:1,) As Scaffolding Materials in order To Enhance Cell Attachment, Expansion and Proliferation. The Scaffolds Fabricated By Electrospining Method And Morphology Of The scaffolds after electors pining And Also morphology of Human bone-marrow Mesenchymal Stem Cells in both proliferation and differentiation Was Evaluated By Scanning Electron Microscope (Sem).Cellular Activities in proliferation and differentiation of stem cells to chondrocytes Were Also Studied By MTT Test .
Results: We hypothesised that a combination of the biological composition and physical properties of the scaffolds would direct hB MMSCs differentiation .we evaluated the ability of hBMMSCs to adhere, proliferate, and differentiate on scaffolds with MTT Test and All scaffolds allowed for cell attachment and viability throughout 21 days. Scanning electron microscopy (SEM) images contained a microstructure of pores that visually interconnected, with round or elongated pores and affected cellular attachment, morphology and differentiation.
Conclusion: demonstrated that customizable 3D scaffolds are excellent candidates for promoting chondrogenic differentiation of hMSCs and are therefore candidates for future cartilage regenerative applications.
Keywords: Tissue Engineering, Scaffolds, HMSC, Differentiation

Ps-048: Animal Models of Ischemic Stroke and Their Application in Cell Therapy and Clinical Research

Fereshteh Azedi Tehrani[1], Mohammad Taghi Joghataei[1*], Kazem Mousavizadeh[2], Amir Hassan Zarnani[3], Somaieh Kazemnejad[4], Masoud Mehrpour[5]

1. Department of Neuroscience, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran

2. Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Nanobiothecnology Research Centre, Avicenna Research Institute, ACECR, Tehran, Iran
4. Reproductive Biotechnology Research Centre, Avicenna Research Institute, ACECR, Tehran, Iran
5. Department of Neurology AND Stroke Center, Firoozgar General Hospital, Iran University of Medical Sciences, Tehran, Iran
Email: joghataei@iums.ac.ir

Background and Aim: Stroke has been identified as the second leading cause of death worldwide. Stroke is a focal neurological deficit caused by a change in cerebral circulation. The use of animal models in stem cell research in recent years has improved our understanding of the physiopathology and role of cell therapy in this disease. Rats and mice are the most commonly used stroke models, but the demand for larger models, such as rabbits and even nonhuman primates, is increasing so as to better understand the disease and its treatment, however mimicking all aspects of human stroke in one animal model is not feasible because ischemic stroke in humans is a heterogeneous disorder with a complex pathophysiology. Therefore, choosing the most appropriate stroke model and optimizing the study design of preclinical trials might increase the translational potential of animal stroke models.
Methods: The transient or permanent middle cerebral artery occlusion (MCAO) model is one of the models that most closely simulate human ischemic stroke. Furthermore, this model is characterized by reliable and well-reproducible infarcts. Therefore, the MCAO model has been involved in the majority of studies that address pathophysiological processes or neuroprotective agents. Another model uses thromboembolic clots and thus is more convenient for investigating thrombolytic agents and pathophysiological processes after thrombolysis. However, for many reasons, preclinical stroke research has a low translational success rate.
Results: One factor might be the choice of stroke model. Whereas the therapeutic responsiveness of permanent focal stroke in humans to cell transplantation declines significantly within 3 hours after stroke onset, the therapeutic window in animal models with prompt reperfusion is up to 12 hours, resulting in a much longer action time of the investigated agent. Another major problem of animal stroke models is that studies are mostly conducted in young animals without any comorbidity. These models differ from human stroke, which particularly affects elderly people who have various cerebrovascular risk factors. A stroke model with prompt reperfusion (ie, a long therapeutic window) might be critical when attempting to translate these results achieved through this method to clinical studies.
Conclusion: The ideal model for stroke research incorporates several factors. The ideal model should have a sufficient number of features that are similar to those in humans to allow the study of the biologic, behavioral, and physiologic factors of the pathology so that, after the induction of the pathologic process, the outcomes can be investigated and treated with minimal limitations. The most applicable animal models for research related to stroke are rodents and lagomorphs. These models satisfy all of the basic requirements needed to induce, manipulate, and treat diseases that affect humans. However, other models should still be explored through similar studies.
Keywords: Ischemic Stroke, Animal Model, Cell therapy, Clinical Research

Ps-049: Candidate Micrornas Regulated Self-Renewal and Resistance to Drug in Gastric Cancer

Mahnaz Azimi[1], Marzieh Ebrahimi[2*], Ali Sharifi Zarchi[2]

1. Department of Genetics, University of Science and Culture, ACECR, Tehran, Iran
2. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: mahnaz.azimi90@gmail.com

Background and Aim: Cancer Stem Cells (CSCs) are tumor cells with self-renewal ability and play a key role in clonal tumor initiation, metastasis and resistance to drugs. They are known as novel targets treatment of aggressive tumors. Recent studies has emphasize the important role of miRNAs that influence cancer cell activities such as stemness, metastasis and chemoresistance

Therefore, the aim of this study is to find these multi functional miRNAs in gastric cancer.
Methods: First of all, we used published studies from PubMed online database through search strategy. After searching, 138 studies were conducted through search strategy and after removing duplicates, non-full-text and non-related topics and abstracts, 21 studies were selected. In 21 studies, a total of 180 miRNAs were observed which regulate stemness feauture. For extracting miRNAs that regulate drug resistance feature, we used CORMINE search engine and then the results were analysed using R programming language that 14 miRNAs were detected. Finally we found out that 10 miRNAs including miR-30a, miR-34a, miR-20a, miR-181a, miR-140, miR-197, miR-23b, miR-21, miR-223 and miR-27a are common in these 2 features. Based on previous studies we then chose 3 miRNAs among them which regulated stemness target genes in different cancers and evaluated the expression of them in MKN-45 gastric cell line using qRT-PCR.
Results: Based on different sets of data, 10 miRNAs play a significant role in resistance to conventional therapy and stemness in gastric cancer. The expression of 3 miRNAs were downregulated in MKN-45 gastric cell line.
Conclusion: These data suggest that three mentioned properties in gastric cancer can regulated by common miRNA therefore, targeting these miRNAs or their target genes may be useful as as diagnostic and prognostic biomarkers to inhibit tumor growth.
Keywords: Gastric Cancer, Bioinformatics, miRNA, Stemness, Drug Resistance

Ps-050: Maintenance of Spermatogonial Stem Cell line in Mouse Testicular Culture

Hossein Azizi[1], Thomas Skutella[2*]

1. Faculty of Biotechnology, Amol University of Special Modern Technologies, Amol, Iran
2. Institute for Anatomy and Cell Biology, Medical Faculty, University of Heidelberg, Heidelberg, Germany
Email: hosseinazizi1358@gmail.com

Background and Aim: Spermatogonial stem cells (SSCs) are able to differentiate to sperm cells in order to transfer genetic information to the next generation. There are different methods to keep SSCs for long term culture. For example, SSCs can be cultured on laminin or different feeder layers such as mouse embryonic feeder (MEFs), SIM mouse embryo-derived thioguanine and ouabain-resistant (STO), Sertoli cells or testicular stroma cells (TSCs). Our aim was to use the SNL cell line, which is an immortalized subclone of the STO line, as a feeder layer to support the maintenance of SSCs. The SNL cells are able to express continuously leukaemia inhibitory factor (LIF) genes, which are important in self-renewal and maintenance of pluripotent stem cells in culture.
Methods: The testis of Oct4-GFP transgenic mice were used for isolation of testicular cells. Mouse testis were decapsulated and treated by a one-step enzymatic digestion protocol. Suspension of testicular cells was plated onto 0.2% gelatine-coated culture dishes.
Results: Here we established a new cell line of small round cells, isolated from neonate and old mice, which unlike undifferentiated SSCs have a smaller nuclear/cytoplasm ratio. This new cell line emerges on primary TSCs feeder and it was isolated by morphology based selection. Afterwards it was possible to expand these cells on SNL feeders for more than a year. Fluidigm analysis for pluripotency and germ cell gene profile showed that the isolated SSCs on TSCs feeder have nearly the same profile as those cells expanded in long term culture using the SNL feeder. Immunocytochemistry results showed that SSCs in long term culture using the SNL feeder express OCT4, KLF4, SOX2 and N-MYC, and flow cytometry (FACS) analysis showed expression of CD49, CD29, CD9, GFRa1 and E-Cadherin.
Conclusion: These data demonstrate that SNL feeder cells can support proliferation, self-renewal and maintenance of SSCs during long term culture.
Keywords: Spermatogonial Stem Cells, Proliferation, Maintenance

Ps-051: Spontaneous Establishment of Pluripotency in the Neonate, but neither in Real Adult Mouse Spermatogonial Stem Cells (Sscs) nor from Continuous SSC Culture

Hossein Azizi[1], Thomas Skutella[2*]

1. Faculty of Biotechnology, Amol University of Special Modern Technologies, Amol, Iran
2. Institute for Anatomy and Cell Biology, Medical Faculty, University of Heidelberg, Heidelberg, Germany
Email: hosseinazizi1358@gmail.com

Background and Aim: Although testis-derived embryonic stem cell-like (ES-like) cells have been obtained in several studies, up to now it is unclear up to which age of the animal it is really possible to generate ES-like cells and if it is possible to generate ES-like cells continuously from a long-term SSC culture. Also the time window for the shift to pluripotency during cell culture has not been investigated in more detail.
Methods: The testis of Oct4-GFP transgenic mice were used for isolation of testicular cells. Mouse testis were decapsulated and treated by a one-step enzymatic digestion protocol. Suspension of testicular cells was plated onto 0.2% gelatine-coated culture dishes.
Results: Here we show that it possible to generate ES-like cells from neonate and nearly adult up to 7 weeks old promoter-reporter Oct4-GFP transgenic mouse testis during short term and long term cultivation of germ line stem cell (GSCs) cultures, but not from older mice. We also describe that spontaneous appearance of germline-derived pluripotent stem (gPS) cells from both neonate and adult GSCs occurred only during a special time window (46 until 143 days) after initiation of GSCs cultures. The isolated and long-term cultured (more than one year) GSCs which were isolated by a morphology based selection procedure expressed germ cells markers and exhibited a similar morphology with a high nucleus/cytoplasm ratio in comparison to undifferentiated SSCs (spermatogonial stem cells) in vivo. A more detailed RT-PCR of analysis showed that the expression of some germ cells markers were different in the short term and long term GSCs culture. The generated gPS cells expressed pluripotency markers, in-vitro differentiated into all three germ lineages, formed complex teratoma after transplantation in SCID mice and produced chimeric mice.
Conclusion: Although the exact mechanism of the development of gPS cells from GSCs is still unclear, this new information provides more insights for scheduling natural conversion mechanisms of ES-like cells from mouse testis.

Keywords: Spermatogonial Stem Cells, Proliferation, Pluripotency

Ps-052: Controlled Release Drug Delivery to Hepatocellular Carcinoma by EpCAM-Targeted Gold -Capped Mesoporous Silica as a Theranostic Platform

Maryam Babaei[1,2], Mona Alibolandi[2*]

1. Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
Email: mbabaie78151@yahoo.com

Background and Aim: Hepatocellular carcinoma (HCC) is one of the most pernicious malignancies with rising morbidity and mortality worldwide that mainly is due to the lack of diagnostics methods. Thus, innovation of efficient medical diagnostics accompanying with therapeutics agents with fewer side effects can present an elegant solution to the aforementioned problems. Here, we developed EpCAM-targeted pegylated mesoporous silica nanoparticles for controlled-release of 5-FU. In this system, gold NPs are hybridized onto the exterior surface of mesoporous silica as gatekeeper for intelligent release of drug meanwhile its feasibility to effectively induce contrast enhancement in X-ray based computed tomography (CT) was examined.
Methods: The prepared targeted and non-targeted formulations consists of pegylated gold-capped mesoporous silica was evaluated in vitro in terms of their cellular internalization, toxicity and controlled release efficiency. The prepared theranostic hybrid system was also implemented to compute tomography of HepG2 tumor bearing nude mice in vivo.
Results: Fluorescence microscopy and MTT assay demonstrated that the developed EpCAM-PEG-Au@Si-5FU had higher cytotoxicity than non-targeted PEG-Au@Si-5FU in 2D and 3D HepG2 cell cultures. Moreover, the targeted hybrid system preferentially accumulated in HepG2 tumor cells in vitro and in vivo.
Conclusion: This work introduces a novel strategy for developing multimodal NPs via nanoparticulate hybrid materials

Keywords: Gold Nanoparticle, Mesoporous Silica, HCC, Polyethylene Glycol, Theranostic

Ps-053: Study of Senescence of Chondrocyte in Different Cell Culture Conditions

Hajar Shafaei[1], Zahra Baghaie Shah Abad[2*]

1. Department of Anatomical Sciences, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Basic Science Genetic Islamic Azad University, Iran
Email: baghaie2003@gmail.com

Background and Aim: Cartilage tissue engineering is defined the concept for repair of articular cartilage defects. In last decades, many of studies attempt to investigate the best source for articular cartilage regeneration. Chondrocytes are logical cell source and also in vivo derived materials for culture of them. In clinic, chondrocyte have been broadly applied. Autologous blood products such as serum or platelet lysate are containing growth factors for proliferation of chondrocyte. However Fetal Bovine Serum (FBS) is routinely used in cell culture for proliferation of chondrocyte. The effect of FBS and umbilical cord blood serum was investigated on morphology of chondrocyte and on age related genes IGF1 and p16INK4 expression.
Methods: Isolated chondrocytes in FBS and human umbilical cord blood serum were cultured up to passage 6 and assessed morphologically. Also total mRNA of ASCs was extracted and relative expression of IGF1 and p16 genes were assessed by quantitative real-time RT PCR.
Results: Proliferation of chondrocytes is markedly decreased in FBS groups in comparison with human serum groups. Phenotypes of cells were spindle at passage one and after six passage changed to flat appearance. Chondrocytes in medium containing human umbilical cord blood serum were fast growing than FBS ones. Morphologic results were in agreement with Real-time RT PCR results. The expression IGF1 and p16INK4a genes significantly increased in chondrocytes grown in FBS containing medium.
Conclusion: Human umbilical cord blood serum prevents senescence in Chondrocytes; therefore our results indicate human serum for human chondrocytes is good choice in cartilage tissue engineering
Keywords: Cartilage Tissue Engineering, Chondrocytes, FBS, Human Umbilical Blood Serum, Senescence

Ps- 054: Isolation and Identification of Sphere-Forming Cells in Tumor Tissues Derived From Gastric Carcinoma Patients

Vahid Bagheri[1], Mohammad Reza Abbaszadegan[2], Mehran Gholamin[3*]

1. Department of Immunology, Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Medical Genetics, Medical Genetics Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Laboratory Sciences, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran
Email: eftekhar2006@gmail.com

Background and Aim: Gastric cancer (GC) is the third and fifth cause of cancer-associated mortality for men and women throughout the world, respectively. Despite the use of surgery and chemotherapy for GC therapy, there are no efficient therapeutic protocols for it to date. Cancer stem cells (CSCs) due to their pivotal role in tumor initiation, growth, progression, invasion, distant metastasis, recurrence and resistance to anticancer drugs are very appealing targets for cancer therapies.
Methods: Tumor samples from patients with GC obtained directly after surgical removal were mechanically and enzymatically dissociated into single-cell suspensions. 1×105 cells/ml were cultivated in T-75 low attachment flask in serum-free DMEM/F12 media containing EGF, bFGF, LIF, heparin, B-27 supplement and penicillin/streptomycin at 37°C for 1-2 months in a humidified 5% CO2 incubator to form primary spheres. After dissociating spheres, sphere-forming cells were incubated with FITC, PE and APC-conjugated monoclonal antibodies against CD44, CD54 and DLL4. The fluorescence intensity was measured by a flow cytometry and data was processed by the FlowJo software. The mRNA levels of stemness-related genes

(OCT4, SOX2, SALL4 and Cripto-1) in sphere-forming cells were quantitated by comparative real-time PCR. Sphere-forming cells were utilized in animal experiments. 1×106 of viable single cells were subcutaneously inoculated into male nude mice and kept for 4 weeks. Tumor growth was inspected every week and tumor size was measured with a caliper.
Results: Small subpopulation of dissociated cells obtained from four tumor specimens formed floating three-dimensional spheroid clus¬ters, called tumor spheres in serum-free media under the non-adherent condition. The frequency of CD44+ cells, CD44+CD54+ cells and CD44+CD54+DLL4+ cells dissociated from spheres were 50-70%, 20-30% and 6-10%, respectively. Comparative RT-PCR results indicated that stemness factors OCT4, SOX2, SALL4 and Cripto-1 were upregulated in sphere-forming cells in comparison with gastric normal tissue. Subcutaneous injections of sphere-forming cells generated tumors in nude mice after 4 weeks.
Conclusion: Spheroid body formation as a functional approach is useful to isolate, enrich and identify CSCs. CSCs as tumor-initiating cells play critical role in tumor generation, invasion, metastasis and chemotherapy resistance. Thus, targeting CSCs observed in GC patients can provide effective therapeutic strategies for curing cancer.
Keywords: Cancer stem cells, Spheres, Sphere-forming cells

Ps-055: The Assesment of Expression of Ectoderm, Mesoderm and Endoderm Markers in Embyoid Body-Like Cell Aggregates Formed from Wharton's Jelly Mesenchymal Stem Cells Using in Regenerative Medicine

Soghra Bahmanpour*, Rezaei F, Tahereh Talaei-khozani

Shiraz University of Medical Sciences, Shiraz, Iran
Email: bahmans@sums.ac.ir

Background and Aim: Regenerative medicine is an area of medicine with the potential to heal damaged tissues and organs. Stem cells have the ability of differentiation into many different types of cells and are a key component of regenerative medicine, as a new clinical application. Mesenchymal stem cells (MSC) isolated from Human umbilical cord Wharton's jelly (HUCWJ) have been shown to be able to differentiate into various cell types. As they are readily available, do not raise any ethical issues and showed higher differentiation potential compared to adult stem cells. Therefore, HUCWJ is a potential source of material that can be used in regeneration medicine. The objective of this study was to find if these cells could form cell aggregates similar to that formed by ESCs (embroid body-like and form three germ layers.
Methods: The Umbilical Cords were cut into small pieces and the explants were cultured in the presence of α-MEM containing 10% fetal bovine serum (FBS), 1% L-glutamine, 100 g/mL penicillin/ streptomycin. At passage 3rd, 1000, 5000 and 10.000 cells/ 20µL were cultured in hanging drops for 3 days. Then, they were incubated for additional 3 days in non-adhesive dishes. The cell aggregates were fixed by 4% paraformaldehyde and were incubated with human three germ layer, 3 color antibodies and the flowcytometry was done.
Results: The data showed that the embryoid-body-like aggregates had little expression for ectoderm and endoderm markers and much expression for mesoderm markers.
Conclusion: These aggregates stay at the mesenchymal cell mass manner and showed a poor differentiation potential toward the ectoderm and endoderm.
Keywords: Wharton's Jelly, Embyoid Body-Like Cell, Ectoderm, Mesoderm and Endoderm

Ps- 056: Role of Indoleamine 2, 3-Dioxygenase (IDO) in Immunosuppressive Properties of Mscs Mesenchymal Stem Cells (MSCs)

Ahmadreza Bahrami[1,2*], Maryam Moghadam Matin[1,2,3], Moein Farshchian[2]

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Cell and Molecular Biotechnology Research Group, Institute of Biotecnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Stem Cell and Regenerative Medicine Research Group, Iranian Academic Center for Education, Culture and Reserch (ACECR) khorasan Razavi, Mashhad Branch, Iran
Email: molood.asadi93@gmail.com

Background and Aim: Indoleamine 2,3-dioxygenase (IDO) have a immunosuppressive function. It has been described in both mice and humans. IDO is the rate-limiting enzyme in the catabolism of tryptophan. IDO may result in tryptophan depletion together with the accumulation of its metabolites may result in the suppression of T cell activation and proliferation. Mesenchymal Stem Cells (MSCs) are capable to different cell lineages such as: adipose, bone, cartilage and myelosupportive stroma. MSCs have been successfully used in the treatment of steroid-resistant acute GvHD (aGvHD). IDO may play a pivotal role in MSC-dependent immunosuppression. Blastema cells can compare with marrow mesenchymal stem cells. Blastema from rabbit ear much similar in characteristic to bone marrow mesenchymal stem cells.
Methods: Reverse transcription polymerase chain reaction (RT-PCR)
Results: according to similar blastema with MSC in some of properties, there is hypothesiz that IDO may express in blastema.
Conclusion: The researchers have been shown IDO activity might alter the immunosuppressive properties of MSCs. according to similar blastema with MSC in some of properties, there is hypothesiz that IDO may express in blastema.
Keywords: Indoleamine 2,3-dioxygenase (IDO), MSCs, Blastema, GVHD

Ps-057: Neural Differentiation of the Wisdom Tooth Follicle Stem Cells on the Nano-Hydrogel Scaffold Containing Salvia Chloroleuca

Naghmeh Bahrami[1,2*], Sahar Shojaei[3], Zeinab Manafi[3], Abdol Reza Mohamadnia[4,5]

1. Craniomaxillofacial Research center, Tehran University of Medical Sciences, Tehran, Iran
2. Oral and Maxillofacial Surgery Department, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran
3. Cell Therapy Center, Gandhi Hospital, Tehran, Iran
4. Virology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRI TLD) Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: n-bahrami@sina.tums.ac.ir

Background and Aim: Repair of neural damages is one of important and complex treatment in medicine and dentistry. On one side possession of adult stem cells from bone marrow considering the limited source of these cells, is restricted. Finding the reachable source of stem cells with high differentiating potential is important. One of these sources is human dental follicle. The differentiation of these cells is induced by growth factors which are costly. The results of multiple studies indicate that Salvia Chlorolouеca has neuroprotective properties. This study aimed to determine the effect of alcoholic extract of Salvia chloroleuca on neural differentiation of stem cells derived from dental follicle (DFSCs) of impacted third molar.
Methods: In this experimental study, 3 samples of impacted 3rd molar follicle were used to isolate stem cells. Cultivation of digested tissue pieces was used. Differentiation to osteoblast and adipocyte was used to prove the existence of stem cells. Also, to prove the mesenchymal origin of stem cells, expression of mesenchymal cell surface markers CD44, CD73, CD90, CD105 and hematopoietic cell surface markers CD34 and CD45 were examined with flow cytometry. The nanohyaluronic acid scaffold was synthesized by sol-gel method and extract of salvia chloroleuca entered in the porosity of scaffolds as a differentiation factor. Before implanting the cells, scaffold was analyzed for mechanical properties, drug releasing and cell toxicity. After that, we seed the cells onto the scaffold by immersion method. Five groups were studied: 1. Nano hyaluronic acid scaffold without salivia chlorolueca. 2. Nano hyaluronic acid scaffold containing salivia chlorolueca. 3. Nano hyaluronic acid scaffold containing salivia chlorolueca + 10% RA 4.DMEM+FBS 10% (Negative control). 4. Standard neural inductive medium (Dexamethasone 10ng + Ascorbic acid 50 µgr/ml + RA phosphate 10mMol). After passing of 21 days, neural differentiation was investigated by morphology, expression of MAP2 and b-Tubulin genes and their protein products by RT-PCR and Western blot technique.
Results: Stem cells were successfully isolated from dental follicle tissues. All cells were successfully differ-

entiated to adipocyte and osteoblast after passing of 14 days in adipogenic and osteoblastic inductive mediums. Flowcytometry revealed that these cells are positive for CD44, CD73, CD90 and CD105 and negative for CD34 and CD45. Morphology of neuronal cells was observed in nano hyaluronic acid scaffold containing Salivia Chlorolueca, nano hyaluronic acid scaffold containing salivia chlorolueca + 10% RA and standard neural inductive medium. Expression of MAP2 and b-Tubulin were confirmed by RT-PCR and western blot tests.
Conclusion: Dental follicle of impacted third molar containing stem cells with high potential for differentiation, which is available source of stem cells to be used in cellular treatment and tissue engineering especially in repair of neural damages. Also the Salvia Chlorolueca extract alone and without presence of any nerve growth factor, induces the neural differentiation to the stem cells.
Keywords: Dental Follicle Stem Cell, Wisdom Teeth, Salvia Chlorolueca, Neural Differentiation

Ps-058: Growth Kinetics, Characterization, and Plasticity of Human Menstrual Blood Stem Cells

Roshanak BahramiNazarabadi*

Department of Biology, Science and Research Branch, Islamic Azad University, Shiraz, Iran
Email: roshanakbahrami53@yahoo.com

Background and Aim: During spring 2014 in the southern Iranian city of Shiraz, menstrual blood (5 mL) was obtained from 10 women on their third day of menstruation in 2 age groups of 30 to 40 and 40 to 50 years old. Ficoll was used to separate the mononuclear cell fraction. After the Men-SCs were cultured, they were sub cultured up to passage 4. Growth behavior and population doubling time were evaluated by seeding 5×104 cells into 12- and 24-well culture plates, and the colonies were enumerated. The expression of CD44, CD90, and CD34 was evaluated. The osteogenic potential was assessed by alizarin red staining. The Men-SCs were shown to be plastic adherent and spindle-shaped. Regarding the growth curves in the 12- and 24-well culture plates, it was demonstrated that in the women aged between 30 and 40 years, population doubling time was 55.5 and 62 hours, respectively, while these values in the women aged between 40 and 50 years were 70.4 and 72.4 hours, correspondingly.
Methods: After centrifugation and removal of the supernatant, the cell pellet was suspended in 6 mL of fresh DMEM-F12 media, supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% L-glutamine, and was then transferred into T25 flasks. The flasks were kept in a CO2 incubator (Memmert, Germany) at 37°C, 5% CO2, and saturated humidity. The media were replaced twice a week
Results: Positive expression of CD44 and CD90 and negative expression of CD34 were noted. In the osteogenic differentiation medium, the cells differentiated toward osteoblasts. As human Men-SCs are easily collectable without any invasive procedure and are a safe and rapid source of MSCs, they can be a good candidate for stem cell banking and cell transplantation in women.
Conclusion: The stem-cell phenotypic markers of Men-SCs were confirmed in our study, demonstrating not only the great potential of these cells for plasticity, self-renewal, and proliferation for long periods of time but also their differentiation properties. Human Men-SCs are a readily available and inexpensive source of stem cells and are collected noninvasively, rapidly, and safely; they can, therefore, be considered a good candidate for stem-cell banking and cell transplantation in women needing a cell therapy measure.
Keywords: Menstrual Blood, Mesenchymal Stem Cells, Plasticity, Reverse Transcriptase Polymerase Chain Reaction

Ps-059: Fabrication of a Three Dimentional Spongy Scaffold Using Human Wharton's Jelly Derived Extra Cellular Matrix for Tissue Engineering

Bahareh Beiki[1], Bahman Zeynali[1*], Ehsan Seyedjafari [2]

1. Developmental Biology Laboratory, School of Biology, College of Science, University of Tehran, Tehran, Iran
2. Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
Email: bahar_beigy58715@yahoo.com

Background and Aim: The umbilical cord connective tissue, called Wharton's jelly (WJ), contains significant amounts of extra cellular matrix (ECM) components and rich source of endogenous growth factors that is good candidate for using as a natural material in tissue engineering and regenerative medicine applications. In this study, we designed a new spongy scaffold from decellularized WJ derived ECM and used it as a skin substitute.
Methods: A biomimetic scaffold with highly degree of porosity and interconnectivity was fabricated from human WJ by applying frizzed- dried technology. The ECM components were determined before and after decellularization processor and the structural properties of scaffolds such as porosity, mechanical strength and biodegradability were also examined.
Results: Our results showed that the decellularization method had no effects on the reduction of ECM components and the scaffolds had a heteroporous structure with high degree of interconnectivity for cellular infiltration and proliferation. The potential of WJ derived scaffolds in the regeneration of full thickness wound healing was assessed through in vivo experiments. The scaffolds were well integrated into the mouse tissue and absorbed the exudates at first week. In addition to complete wound closing and the disappearance of scab within 12 days, complete re-epithelialization, newly generated epidermal layers and appendages were seen in WJ scaffold group compared to control group.
Conclusion: Altogether, the biological composition and physical structure of WJ derived scaffolds were able to improve attachment, penetration and growth of fibroblast cells and accelerate wound healing process. These findings suggest that human WJ derived scaffolds have enough properties to provide an acceptable skin graft for wound healing and different types of tissue substitutes.
Keywords: Wharton's Jelly, Scaffold, Tissue Engineering, Wound Healing

Ps-060: Evaluation of Alginate-Based Hydrogel Incorporating Sustained-Release Platelet-Rich Plasma Cartilage Tissue Engineering Applications

Behzad Bolandi[1], Rana Imani[1*], Shahin Bonakdar[2], Hossein Fakhrzadeh[3]

1. Tissue Engineering group, Biomedical engineering Faculty, Amirkabir University of Technology (Polytechnic of Tehran), Tehran, Iran
2. National cell Bank Department, Pasteur Institute of Iran, Tehran, Iran
3. Endocrinology & Metabolism Research Institute, Tehran University of Medical Sciences, Doctor Shariati University Hospital, Tehran, Iran
Email: behzad.bolandi@gmail.com

Background and Aim: Nowadays tissue engineering provide a new promising strategy by using cell, scaffold and growth factor all together. Aim of cartilage tissue engineering is preparing a scaffold that in addition to mimic the same structure of native cartilage, embedding cell and growth factors that are effective on cartilage healing, to inject in defect for accelerating regeneration process. Hydrogels as a new form of tissue engineering scaffolds, are one of the most interesting and suitable options to meet our goal due to the similar structure to the natural cartilage. The purpose of this study is to fabricate a sustained release PRP, as a source of growth factors, embedded in an alginate / polyvinyl alcohol composite hydrogel for the articular cartilage regeneration.
Methods: PRP preparation protocol was set up successfully. Hydrogel composition was optimized based on compressive modulus, microstructure, swelling ratio and biodegradation properties. Cytotoxicity and cell proliferation of the optimized hydrogel with and without PRP were evaluated by MTT assay of human adipose-derived mesenchymal stem cell (hADMSC).
Results: The total concentration of hydrogel was optimized on 4% (w/v) and the hydrogel components ratio was optimized around 3:1 Alg: PVA according to the mechanical tests results. Adding PVA to Alg resulted to a mils increase of swelling ratio. The proliferation of hADMSC cells was significantly increased by PRP releasing from the hydrogel.
Conclusion: Regarding the articular cartilage, the Alg: PVA composite hydrogel with ratio of 3:1 showed the optimized mechanical properties. Incorporation of PRP as a growth factors depot, could increase the regeneration of the hADMSC cells; so accelerate articular cartilage defects regeneration.
Keywords: PRP, Hydrogel, Alginate, hADMSC, Cartilage Tissue Engineering

Ps-061: Overexpression of Immunosupressive Micrornas in Human Adipose-Derived Mesenchymal Stem Cells (Had-Mscs) Exosomes

Zohreh Bolandi[1], Seyed Mahmoud Hashemi[2], Sara Soudi[3], Hossein Ghanbarian[1*]

1. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Immunology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: Z.bolandi@sbmu.ac.ir

Background and Aim: Mesenchymal stem cells (MSCs) are multipotent cells, with immunosuppressive and anti-inflammatory effects, which can be used for treatment of numerous autoimmune disorders. Recent studies have suggested that immunomodulatory effects of MSCs contribute to their paracrine factors such as exosomes and micovesicles. Exosomes are 30-150 nm vesicles secreted by most cells and contain proteins and RNAs such as microRNAs (miRNAs). Previously, immunosuppressive effects of miR-10a and miR-29b have been reported; regulating of naive T cells differentiation into regulatory T cells and Th1, respectively. We hypothesized whether overexpression of these miRNAs in MSCs might improve immunosuppressive effects of hAD-MSCs derived exosomes.
Methods: MSCs were isolated from human adipose tissue and characterized by flow cytometry. Moreover, MSCs were differentiated into osteoblasts and adipocytes. Initially, miR-10a and miR-29b were cloned in pLenti-III-mir-GFP vector. Mesenchymal stem cells were transduced by the miRNA-expressing lentiviral vectors after viral packaging. Overexpression of miR-10a and miR-29b microRNAs was determined by quantitative real time PCR assay. Exsomes were then isolated using ExoSpin Exosome Purification Kit and characterized by scanning electron microscopy and dynamic light scattering (DLS).
Results: To overexpress of immunosuppressive microRNAs in MSCs derived exosomes, miR-10a and miR-29b expressing lentiviral base vectores were transduced into MSCs. First, fluorescent microscopy confirmed high efficiency transduction of MSCs. Then, quntitative real time PCR determination showed that miR-10a and miR-29b were overexpressed significantly in MSCs. MSCs derived exosomes were scanned by electron microscopy. Furthermore, DLS number distribution measurement of transduced hAD-MSC-exosomes had a single peak (~117nm) diameter.
Conclusion: Here, we suggest that miR-10a and miR-29b can be enriched in hAD-MSCs-exosomes after overexpression in MSCs. Enriched exosomes might be considered as a new cell free method for treatment of immune disorders.
Keywords: Mesenchymal stem cells, Exosomes, miRNA

Ps-062: Investigating Anticancer Effects of Parthenolide and Melphalan on Human Hepatocellular Carcinoma Cells

Arad Boustan[1], Mahdi Moussavi[1], Fatemeh B. Rassouli2[*], Mehrdad Iranshahi[3]

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Pharmacognosy and Biotechnology, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
Email: arad1234@live.com

Background and Aim: Hepatocellular carcinoma (HCC) is one of the main causes of cancer-related death worldwide, with high rate of incidence in our country Iran. Although a lot of progress has been achieved in therapy and management of this malignancy, survival of HCC patients is still low. Therefore, it is necessary to introduce new and more effective anticancer agents for clinical application. The natural sesquiterpene lactone parthenolide and nitrogen mustard alkylating agent melphalan are two chemical structures that have been used for their antineoplastic purposes
Methods: In this study, we examined cytotoxic effects of both agents on human a hepatocellular carcinoma cell line. To do so, HepG-2 cells were treated with increasing concentrations of parthenolide and melphalan

for 3 consecutive days. Then, cell viability was evaluated using colorimetric MTT assay.
Results: Obtained results indicated that 20 µg/ml parthenolide and 80 µg/ml melphalan caused 50% cell death in comparison with relevant control treatment (0.8% DMSO) upon 48 h treatment, and more cytotoxicity were observed after 72 h.
Conclusion: According to current findings, parthenolide and melphalan could be considered as potent anticancer agents for future in vitro and in vivo studies.
Keywords: Hepatocellular Carcinoma, Parthenolide, Melphalan, Cytotoxicity

Ps-063: Viability of Colon Adenocarcinoma Cells Decreased by a Sesquiterpene Derivative and an Alkylating Agent in Vitro

Arad Boustan[1], Mahdi Moussavi[1], Fatemeh B. Rassouli[2*], Mehrdad Iranshahi[3]

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Pharmacognosy and Biotechnology, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
Email: arad1234@live.com

Background and Aim: Colon adenocarcinoma is a serious health concern around the world, with high incidence and rate of mortality specifically in developing countries. Several anticancer agents have been investigated in order to eradicate or at least lessen cancerous cells while most of them have been inefficient. In this regard, designation of novel and more efficient therapeutic approaches is necessary for adenocarcinoma treatment.
Methods: In present attempt, we aimed to investigate cytotoxic effects of sesquiterpene derivative parthenolide and nitrogen mustard alkylating agent melphalan on human colon adenocarcinoma cells. Accordingly, HT-29 cells were treated with increasing concentrations of both agents for 24, 48 and 72 hours. Then, viability of cells was evaluated using MTT detection kit, to determine half maximum inhibitory concentration (IC50) in comparison with relevant control treatment (0.8% DMSO).
Results: Results of current study revealed that IC50 values of parthenolide and melphalan were about 10 and 80 µg/ml after 72 h, respectively. Since considerable cytotoxic effects of parthenolide and melphalan were observed in present study, it seems that these agents are good candidates for further anticancer studies.
Conclusion: Since considerable cytotoxic effects of parthenolide and melphalan were observed in present study, it seems that these agents are good candidates for further anticancer studies.
Keywords: Colon Adenocarcinoma, Cell Viability, Anticancer Effects, Parthenolide, Melphalan

Ps-064: SigMiR: Novel Software to Find Novel MicroRNAs for Conserved Target Sites in Genes That Implicate Single Cellular Function in Whole Genome Sequence

Hamed Dabiri*, Majid Sadeghizadeh

Molecular Genetics Department, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
Email: hdabiri@hotmail.com

Background and Aim: Nowadays, given that increasing in biological data using computer is Inevitable. Moreover, computational soft wares illustrate the ways to discovery functional sequences in genome. MicroRNAs are ~22 nucleotide non-coding RNAs that post transcriptionally regulate gene expression and consequently cellular function especially differentiation. About 2500 microRNAs of estimated 55000 microRNAs have been discovered in human genome till now. Existing soft wares predict microRNAs based on genome sequence segments. In this study we created and introduced the novel software that find microRNAs in whole genome sequence base on target genes that are important in stemness states of stem cells.
Methods: For this aim we used python programing language. In the first step, we found common microRNA target sequences in 5 stemness marker genes (Oct4, Nanog, Sox2, Klf4, C-myc). Conserved target sites were chosen following comparison of 100 species homolog sequences. Reverse complement of target sequences were investigated in human genome sequence. Stem-

loop formation potential of obtained sequences with flanking region was evaluated. Candidate stem-loops were filtered in multiple steps to reach real microRNA producing stem-loops. We analyzed RNA-Seq data of embryonic stem cell differentiation to confirm existing of MicroRNAs mature forms.
Results: Our data shown there are two common target sites in 3'UTR of stemness genes that are conserved in mammals. We found overall 320 stem-loops in human genome sequence may produce microRNAs that target 5 important stemness target genes. RNA-Seq Analyzing data revealed 5 stem-loops transcribe and produce mature microRNAs in early differentiation of embryonic stem cells. Tow precursor of mature microRNAs are located in genes that involved in embryonic development and stem cell differentiation.
Conclusion: In this study we created and introduced a novel software that find novel microRNAs that target genes implicate to single cellular function. Based on our data there are 5 novel microRNAs that can target all 5 important stemness genes. This finding is interesting and important for clear differentiation mechanisms that can use for elevate the differentiation efficiency or blocking Undesirable differentiation in iPS producing process.
Keywords: Differentiation, Bioinformatics Software, MicroRNA

Ps-065: Proliferation and Stemness Preservation of Mesenchymal Stem Cells on Nasturtium Officinale Incorporated PCL/PEG Electrospun Fibers

Mehdi Dadashpour, Akram Firouzi-Amandi, Mohammad Nouri*

Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: mehdi.dadashpor@yahoo.com

Background and Aim: Background: In the field of stem cells research, design of biomaterials that can control the stem cell proliferation without the loss of its pluripotency is a crucial component for the fabrication of scaffolds. Nasturtium officinale R. Br. is one of the greatest important medical plant with a wide range of biological functions such as anti- cancer, anti-inflammatory and anti-oxidant properties. The present study describes the influence of N. officinale -loaded PCL/PEG nanofibrous mats on the regulation of proliferation and stemness preservation of Mesenchymal Stem Cells (MSCs).
Methods: Methods: N. officinale -loaded nanofibrous mats were synthesized and characterized by SEM and FTIR. MSCs were seeded on two types of PCL/PEG nanofibrous mats (N. officinale -loaded PCL/PEG nanofibrous mats and PCL/PEG nanofibrous as a control), and their morphology, proliferation, and stemness expression were analyzed using FESEM, MTT assay, and quantitative real-time polymerase chain reaction (qRT-PCR) after 2 weeks of incubation, respectively
Results: In this study, we found that N. officinale-loaded nanofibrous mats had great antioxidant potential and exhibited higher cytoprotection, better adhesion, and significantly increased proliferation of MSCs. More importantly, N. officinale-loaded nanofibrous mats significantly unregulated the expressions of stemness markers Rex-1, Sox-2, Oct4 and Nanog3.
Conclusion: Conclusions: These results demonstrate that N. officinale -loaded PCL-PEG electrospun nanofibrous mats appear suitable to support MSCs adhesion and proliferation while concurrently preserving the cell stemness, therefore representing a hopeful approach for applying in stem cell based regenerative medicine.
Keywords: Nasturtium Officinale, Electrospun Nanofibers, Mesenchymal Stem Cells, Stemness

Ps-066: In Vitro Gene Editing by CRISPR-Cas9 System

Mahintaj Dara*

Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
Email: dara.mp.pd@gmail.com

Background and Aim: CRISPR-Cas9 is an acquired immune system in prokaryotic cells that help them for resistance against foreign genetic element such as phages. Today researchers are using CRISPR-Cas9 for in vivo and in vitro gene editing. The aim of our study was dts gene editing in HEK-293 cell line.

Methods: In our work, dts gene was selected and gRNAs were designed for it. This gRNAs were inserted in a vector that carrying Cas9 and GFP. After cloning, this vector transected to the HEK-293 cell line. According to the gRNAs that we designed, we expected a 700 bp deletion in dts gene.
Results: Cells that were transfected with the vector were emitting florescent light. After DNA extraction and PCR, we demonstrated 700 bp band in edited cells and 1400 bp band in non edited cells.
Conclusion: In conclusion, CRISPR-Cas9 can be use as a powerful tool for gene editing and helpful for genetic disorder and cancer treatment.
Keywords: CRISPR-Cas9, Gene Editing, dts

Ps-067: Effect of Hepatic Differentiation on Fatty Acid Composition of Induced Pluripotent Stem Cells Derived from Human Dermal Fibroblasts

Masoud Darabi[1*], Nasim Parsafam[2], Yaghob Rahimi[2], Amir Mehdizadeh[3], Hojjatollah Nozad Charoudeh[1], Mohammad Nouri[4], Maghsod Shaaker[2]

1. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
3. Liver and Gastrointestinal Endocrine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
4. Stem Cell and Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran
Email: darabim@tbzmed.ac.ir

Background and Aim: Induced pluripotent stem (iPS) cells have been recognized as key progenitor cells for liver regeneration that could potentially repair liver injury. Differentiation of stem cells involves specific changes in lipid metabolism to form competent hepatic cells. However, the relationship between the hepatic differentiation of iPS cells and change in cellular lipids has not been well characterized. The purpose of this research was to determine the fatty acid pattern during hepatic differentiation of hiPS cells derived from human dermal fibroblasts.
Methods: Hepatocyte differentiation was induced in three stages using Wnt-3a, HGF and oncostatin M, and was impaired by a chemical ERK inhibitor PD98059. Hepatocytes-specific metabolic markers including gamma-glutamyl transferase and aminotransferases were measured using kinetic chromogenic assay kits. During the hepatic differentiation of hiPS cells, changes in fatty acids were determined at multiple time points using gas-liquid chromatography.
Results: Significant increases were observed in the hepatic enzyme markers during the hepatic differentiation in hiPS cells. Endodermal induction produced a transient increase in saturated fatty acids (+33%, $p<0.01$) and decrease in monousaturated fatty acids (-15%, $p<0.01$) following endodermal induction. Total n-6 polyunsaturated fatty acids was high in undifferentiated hiPS cells and a gradual downward trend was observed after endodermal induction (-10%, $p=0.08$) and hepatic lineage commitment (-19%, $p<0.01$). All of these changes in metabolic differentiation markers and fatty acid pattern suppressed by impaired induced hepatic differentiation.
Conclusion: Our findings indicate that the pattern of cellular fatty acids is dynamic and changes with the progress of hepatic differentiation, which includes both transient fluctuations and linear trends.
Keywords: Aminotransferases, Fatty Acids, Hepatic Differentiation, Human iPS Cells

Ps-068: Bisphenol an Induces Autophagy and cell death in Adipose stem Cells

Shahram Darabi[*], Yasaman Ebrahimikia, Farzad Rajaei

Cellular and Molecular Research Center, Qazvin University of Medical Science, Qazvin, Iran
Email: shahram2005d@yahoo.com

Background and Aim: The food contaminants bisphenol A (BPA) is an endocrine disrupting compound used in the production of polycarbonate plastics and epoxy resins. Previous studies have demonstrated that BPA induced cell toxicity with the generation of oxidative stress, and there is a potent genotoxic agent and affects the normal physiological functions. However, the molecular mechanisms of the effects of BPA on autophagy

and association with cell death are still unknown. The aim of this study was to investigate the effects of BPA in Adipose stem Cells (ASCs). In the current study, BPA increased cell death in ASCs in a dose- and time-dependent manner.
Methods: In this study, after ASCs were indubated with the BPA, autophagy genes (LC3-II and SQSTM1) were analyzed by RT-PCR and immunofluorescence and ASCs were counted using Trypan blue dye.
Results: We observed that BPA exposure during ASCs culture, enhanced the expression and the levels of autophagy LC3-II and SQSTM1 genes. BPA significantly decreased the number of ASCs cells as compared to those of the control.
Conclusion: These results suggest implication of autophagy against BPA-mediated ASCs degeneration.
Keywords: Bisphenol A, Adipose stem Cells, Autophagy

Ps-069: In Vitro Stable Non-Viral Murine Pro-Neurotrophin 3 Gene Transfer Into Rat Bone Marrow Stromal Cells

Shahram Darabi[1*], Taki Tiraihi[2], AliReza Delshad[3]

1. Cellular and Molecular Research Center, Qazvin University of Medical Science, Qazvin, Iran
2. Department of Anatomical Sciences, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3. Department of Anatomy, Shahed University, Tehran, Iran
Email: shahram2005d@yahoo.com

Background and Aim: Neurotrophin 3 (NT-3) is an important factor for promoting prenatal neural development, as well as regeneration, axogenesis and plasticity in postnatal life. Therapy with NT-3 was reported to improve the condition of patients suffering from degenerative diseases and traumatic injuries, however, the disadvantage of NT-3 protein delivery is short half live time and an alternative approach is the use of NT-3 gene therapy
Methods: In this study, the bone marrow stromal cells (BMSCs) were isolated from adult rats, cultured for 4 passages and transfected with either pEGFP-N1 or a constructed vector containing murine proNT-3 (pSecTag2/HygroB-murine proNT-3) using Lipofectamine 2000. They were permanently transfected with pSecTag2/HygroB-murine proNT-3 by Hygromycin B (200 mg/kg).
Results: A quantitative evaluation of the NT-3 expression of mRNA using real time RT-PCR shows that there was double fold increase in NT-3 gene expression, also, the culture supernatant shows that there was double fold increase in its excretion using ELISA technique and the data supported by a semi-quantitative immunoblotting technique.
Conclusion: This suggests that the use of this transfection technique could be useful for gene therapy in different neurological disorders with neurodegenerative or traumatic origins.
Keywords: NT-3, Transfection, BMSCs, Secretory Vector, Non-Viral Transfection

Ps-070: Low Level Laser Irradiation Effects on Proliferation and Apoptosis in Bone Marrow Mesenchymal Stem Cells

Somayeh Delavarifar[1], Zahra Razi[1], Seyed Mojtaba Hosseini[1,2,3*]

1. Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
2. Cell and Molecular Medicine Student Research Group, Medical school, Shiraz University of Medical Sciences, Shiraz, Iran
3. Stem Cell laboratory, Department of Anatomy, Shiraz University of Medical Sciences, Shiraz, Iran
Email: somayeh.delavari@gmail.com

Background and Aim: Low level laser irradiation is an effective method in treating some diseases and it could improve regeneration and wound healing. Some studies have reported that low level laser irradiation could enhance proliferation by increasing ATP level in the cells mitochondria. Mesenchymal stem cells (MSCs) are a kind of stem cells which are capable of differentiating to other cells. Nowadays, MSCs are an appropriate option for regenerative medicine in this study we want to investigate the optimum protocol for using low level laser irradiation to improve proliferation and make less apoptosis in Mesenchymal stem cells.
Methods: The Mesenchymal stem cells were isolated from femur and tibia of a male rat and cultured in culture media. The isolated cells were differentiated to

adipocyte and osteocyte to confirm their multi potency. The cells were irradiated with different exposure protocols of 808 nm diode laser. After exposure the stem cells markers (CD90, CD44 and CD45), doubling time, colony forming frequency and Caspase 3 activity (for apoptosis evaluation) were assessed.
Results: Both 640 mW (8th group) and 830 mW (14th group) have best result in colony forming and doubling time; however the 8th group had the least Caspase 3 activity. The patterns of stem cells markers expression have not changed after laser irradiation. The protocol used in 8th group could improve Mesenchymal stem cells proliferation more than the rest protocols and also it might make the apoptosis less than the other group.
Conclusion: This irradiation protocol could be useful in regenerative medicine due to its effects on Mesenchymal stem cells.
Keywords: Laser, Mesenchymal Stromal Cells, Proliferation, Apoptosis

Ps-071: A New Strategy for the Ca-Alginate Microbeads Synthesis with the Similar Physical Characteristics to the Microcarriers in Cell Culture

Sorour Derakhti[1], Ghassem Amoabediny[1*], Mozhgan Sheinkpour[2], Amir Esmaeili[1], Reza Nahavandi[1]

1. School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
2. Department of Microbial Biotechnology, Faculty of Biology, College of Sience, University of Tehran, Tehran, Iran
Email: Sderakhti@gmail.com

Background and Aim: Ca-alginate hydrogels have been widely applied in cell culture due to advantages they have such as biocompatibility, low cost and simplicity of preparation procedures. Small size gel beads (<300μm) are favored for anchorage dependent cell culture, as they undergo a lower level of shear stress and provide a higher surface area to volume ratio. However, production of the small microbeads has been failed by conventional techniques. In this study, we aimed to develop a strategy in the traditional electro spray method to produce the Ca-alginate microbeads with the sizes similar to the commercial microcarriers.
Methods: We employed electro spray method to create micro droplets of high viscous sodium alginate solution by applying constant DC voltage. The droplets fell into the calcium chloride solution and formed the hydrogels. The beads sizes were measured under various conditions of the flow rate and concentration of alginate solution and also the DC voltage to meet the conditions, which led us to obtain the microbeads with the desired sizes.
Results: Among the tested variables, the flow rate of alginate solution was found to be the predominant factor, since the sizes of the beads significantly decreased by reducing this parameter. The reduction of the alginate solution concentration also led to decrement in the microbeads sizes but in lesser extent compared to flow rate. In the voltage range we worked (7-12 KV), the beads sizes were approximately independent of the voltage. Finally, we could produce spherical microbeads with the proximate mean size of 210μm by incorporation of alginate solution 1.25 % (w/v) with the flow rate of 0.5 ml/h under the voltage of 8kV in calcium chloride solution.
Conclusion: Our findings suggested that it is possible to synthesis Ca-alginate hydrogels with the sizes similar to the commercial microcarriers by electro spray method. Further decrease in the beads sizes would be reachable by application of apparatus which could provide lower flow rates.
Keywords: Ca-Alginate, Microcarrier, Size, Electro Spray Method

Ps-072: The Effect of Peppermint Extract on Proliferation and Senescence of Rat Derived Mesenchymal Stem Cells

Mahla Deylam[1], Effat Alizadeh[2*], Masomeh Firuzamandi[1], Marzie Hejazy[1], Manizeh Sarikhani[1], Roya Herizchi[3]

1. Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
2. The Umbilical Cord Stem Cell Research Center (UCSRC), Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: mahla.deylam1992@gmail.com

Background and Aim: Peppermint is an aromatic plant, which have been used in the pharmaceutical, food, cosmetic industries. Recent studies showed that Peppermint have some antioxidant properties by trapping free radicals. The aim of this study is to evaluate the effect of Peppermint extract on proliferation and aging of rat derived adipose or bone marrow mesenchymal stem cells.
Methods: After isolation of mesenchymal stem cells from either adipose (rADSCs) or bone (rBMSCs), the characterization of them were performed by FACS. The differentiation capacity of rADSCs or rBSCs were analyzed by osteogenic differentiation. The rASCs or rBMSCs were treated with Peppermint extract in different concentrations (1 to 300µg/ml) in 96-well plates. Then, MTT assay was performed after 48 and 72hours. The aging of rADSCs or rBMSCs after Peppermint extract treatment was studied by senescence associated beta-galactosidase staining.
Results: It has been shown that peppermint extract significantly enhanced the viability and proliferation of both rADSCs and rBMSCs. Additionally, the beta galactosidase activity of peppermint extract was significantly decreased as compared to controls ($p<0.05$).
Conclusion: Our results of highlighted the possible proliferative and anti-senescent activity of peppermint extract. The findings of this study could be useful for applying herbal extract in cell therapy.
Keywords: Peppermint, rat MSCs, Cytotoxicity

Ps-073: Investigation of the Expression Pattern of Hsa-Mir-11181 during the Course of Human Cardiac Progenitor Cells Differentiation

Sadat Dokanehiifard[1], Bahram Mohammad Soltani[1*], Samaneh Ekhteraeei Tousi[1], Saman Hosseinkhani[2]

1. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
Email: dsadat870@gmail.com

Background and Aim: Cardiovascular diseases (CVDs) are globally the number 1 cause of death. Despite improvement in treatment strategies, heart disorders are strongly increasing. Therefore, identification of new regulatory factors involved in the cardiac differentiation is very important. TrkC receptor, part of the large family of receptor tyrosine kinases, is involved in development of the heart and central nervous system. There are many contradictory functions related to the TrkC gene which might be attributed to the non-coding RNAs located in it. Recently, a novel miRNA, hsa-miR-11181 located in TrkC gene, has been reported which is involved in nervous differentiation. MiRNAs are small non-coding RNAs regulating their target genes via mRNA degradation or protein inhibition. The goal of the present study was to investigate the expression pattern of hsa-miR-11181 during the course of cardiosphere derived cells (CDCs) differentiation.
Methods: Human cardiac progenitor cells were prepared from Royan Stem Cell Bank (RSCB) and differentiated into cardiomyocytes. Cells were treated with 5-azacytidine for initiating differentiation. Ascorbic acid (every other day) and TGFB1 (twice per week) were then added. Successful differentiation of cardiomyocytes was confirmed through microscopy method and expression analysis of the molecular markers, 4, 8, 12, 16 and 21 days after starting differentiation. The expression pattern of hsa-miR-11181 was also analyzed through RT-qPCR during the cardiac differentiation course.
Results: Expression of hsa-miR-11181 during the course of differentiation was initially increased and after this was reduced, as its expression at 3th week after starting differentiation was lower than undifferentiated cells.
Conclusion: Significant expression alteration of hsa-miR-11181 during the course of cardiosphere derived cells revealed that this miRNA potentially has a critical role in differentiation of cardiac progenitor cells.
Keywords: hsa-miR-11181, TrkC Gene, Cardiac Differentiation

Ps-074: A Label Free Microfluidic Device for Separating Circulating Tumor Cells

Dina Dorri Giv, Manouchehr Vosoughi[*], Mina Razzagh zadeh Bidgoli

Faculty Department of Chemical Engineering, Sharif University of Technology, Tehran, Iran

Email: dorri24@gmail.com

Background and Aim: Circulating Tumor Cells (CTCs) are the starters of metastasis, the process in which cancer spreads in body and seize healthy organs. Early detection of CTCs can help a doctor to overtake the cancer. Isolation of CTCs is an essential step for many therapeutics in cancer medicine. CTC separation methods have been targeted to miniaturize conventional devices by using microfluidics. In this paper, a label-free, hydrodynamic, microfluidic device for CTC separation is introduced. The proposed device separate cells based on their size. Shorter separation process time and cost effectiveness in comparison with conventional methods are the most noteworthy advantages of the proposed device.
Methods: Design principles: The device contains a set of contraction expansions in a straight michrochannel in which particles are mainly affected by two forces. First, The Dean drag force that is a cause of sudden change of cross section in the channel. Its magnitude is calculated by FD = 3πμUDeanap, where μ, UDean, and ap are the density of the fluids, transverse velocity by Dean flow and particle diameter. Second, Inertial lift force that is a balanced form of shear-induced lift with wall-induced lift forces. The magnitude of inertial lift force is calculated by FL = ρUm2ap4CL/Dh2, where ρ, Um, and CL are the density of the fluid, x-axial maximum flow velocity, and the lift coefficient. Finally, Force balance between these two forces determines the lateral positions of the particles. Simulation: Simulation for flow characteristics was performed using COMSOL multi physics 5.1. Design and fabrication: The microchannel have two inlets. It is 350μm width, 700μm length in expansions and 50μm width, 1200μm length in contractions. The depth is 60 μm. The micro channels stamps were fabricated using soft lithography process. Then poly (dimethylsiloxane) (PDMS) was poured on the stamps, cured and formed. Finally, bonding was made between a PDMS replica and a glass slide, with oxygen plasma. Experiment: For particle fluid, we used human whole blood enriched with breast cancer cell line (MCF-7). The MCF-7 cells were stained. For focusing fluid phosphate-buffered saline (PBS) was used. Fluids were injected with a syringe pump into the device.
Results: The range in which the parameters change are chosen based on simulation results and past researches. 1-Effect of Reynolds number The Reynolds number of channel is Re=?UD/? . Effect of Reynolds number on separation efficiency was observed with changing the total inlet flow rates. 2-Effect of the ratio of particle fluid flow rate to focusing fluid flow rate as the focusing fluid dilutes the particle fluid and washes away the unwanted particles, an optimum ratio between the flow rates is determined. 3-Effect of the angle between two inlets the effect of the angle is on mixing the particle fluid and the focusing fluid.
Conclusion: We have designed and fabricated a microfluidic label-free method for separation of CTCs from whole blood. The device separates cells based on their size. The specialty of our method is the ability of working with whole blood without any pretreatment.
Keywords: Microfluidic Device Circulating Tumor Cell CTC Separation Label Free Separation

Ps-075: Anti-Cancer Effects of Atorvastatin in Human Glioblastoma Spheroids Cultured in Three-Dimensional Model

Somayeh Ebrahimi-Barough[1*], Neda Bayat[2], Jafar Ai[1]

1. Department of Tissue Engineering and Applied Cell Sciences, Faculty of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Brain and Spinal Cord Injury Research Center, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran
Email: s_ebrahimi100@yahoo.com

Background and Aim: Glioblastoma multiform (GBM) is primary malignant brain tumor with a few therapeutic targets available for it. The interaction between immune system and glioma is important factor that could lead to novel therapeutic approaches to fight glioma. In this study, we investigated in vitro anti-inflammatory and apoptotic activity of atorvastatin in different concentrations 1, 5, 10μM on glioma spheroids cells cultured in three-dimensional model in fibrin gel that indicate the complex in vivo microenvironment better than simple two-dimensional cell culture. A mechanistic insight into the role of IL-17RA, TRAF3IP2 and apoptotic genes in progression of glioma could provide important way to therapy of malignant tumors with manipulation of this inflammatory axis.

Methods: To reach for these aims, After 24 and 48 hours exposing with different concentrations of atorvastatin caspase-8, caspase-3, Bcl-2, TRAF3IP2 and IL-17RA genes expression were assayed. TUNEL assay and cell cycle assay were used for evaluating the cell apoptosis and proliferation.
Results: The results showed that atorvastatin has anti inflammatory and apoptotic effects against glioma spheroids. Atorvastatin induced the expression of caspase-3 and caspase-8, and down-regulated the expression of Bcl-2, TRAF3IP2 and IL-17RA especially at 10μM concentration. These effects are dose dependent. The most likely mechanisms are the inhibition of inflammation by IL-17RA interaction with TRAF3IP2 and NF-κB signaling pathway.
Conclusion: Finally these results suggest that atorvastatin could be used as anticancer agent for glioblastoma treatment.
Keywords: Glioblastoma spheroids, Atorvastatin, Inflammation, Apoptosis, IL-17RA

Ps-076: The Impact of Kidney Mesenchyme to Convert EB-derived Renal Progenitor like Cells into Renal Epithelial Lineage

Ehsan Ehsani[1*], Nasser Aghdami[2], Hosein Baharvand[2]

1. Department of Biology, Roudehen Branch, Islamic Azad University, Roudehen, Iran
2. Department of Stem Cell and Development Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: ee_ehsan@yahoo.com

Background and Aim: End stage renal disease (ESRD) is a major public health problem in the world. At presents, the treatment options to these patients are transplantation or dialysis, which both are imperfect and costly. Renal progenitor (RP) cells hold promise in development of cell based therapies for kidney disorders. Embryonic stem cells offer a novel opportunity to develop an unlimited source of RP cells. Recently two cell surface markers, CD133 and CD24, were used to identify and isolate RP cells. In this study, we assessed the impact of bFGF in frequency of CD133+CD24+ cells presented in embryoid bodies (EBs). Following isolation of CD133+CD24+ cells from EBs, we evaluated the effect of mouse embryonic, newborn and adult kidney mesenchyme (EKM, NKM and AKM respectively) on further differentiation of the isolated cells
Methods: undifferentiated human embryonic stem cells (hESCs) were cultured in suspension for 9 days in the presence or absence of bFGF. We determined day 7 as summit quantity of the CD133+CD24+ cells in the bFGF treated EBs. We also tested the effect of EKM, NKM and AKM on differentiation of these isolated cells into epithelial lineage (renal tubular epithelial cells) in co-culture system
Results: Results revealed down-regulation of several RP marker gene expressions in the CD133+CD24+ cells. In contrast, renal tubular epithelium marker gene expressions were up-regulated after 7 days of co-culture with a preference of EKM, NKM and AMK respectively.
Conclusion: Hence, bFGF could be used to enhance the number of RP-like cells characterized by CD133 and CD24 markers, from human EBs. We suggest EKM as a superior supplementary factor to further induce differentiation of RP-like cells into renal tubular epithelial cells.
Keywords: Embryonic Stem Cell, Embryoid Body, Differentiation, Renal Progenitor Cells

Ps-077: Preparation and Characterization of Barium Titanate Scaffold for Bone Tissue Engineering

Arian Ehterami[1], Mansure Kazemi[2], Mahmoud azami[2*]

1. Department of Mechanical and Aerospace Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Medical Technologies, Tehran University of Medical Sciences, Tehran, Iran
Email: arianehterami@yahoo.com

Background and Aim: Bone tissue engineering is an important therapeutic strategy of healing severe bone injuries by utilizing the engineered scaffolds and cells. It is well known that natural bone exhibit electrical potential in response to mechanical stress due to its inherent piezoelectric property. The piezoelectricity generated in bone is found to be applicable in sensing the biologic

forces, which promotes bone regeneration at the repair site. Based on this fact, piezoelectric ceramics such as Barium titanate (BaTiO3; BT) have been wildly used in the fabrication of scaffolds for hard tissue applications. Barium titanate exhibits excellent biocompatibility and has the ability to form strong interfacial bonds with the living bone. The aim of this study was to fabricate porous Barium titanate scaffolds coated with gelatin-calcium phosphate (CaP) and characterize its mechanical and biological properties.
Methods: Firstly, the highly porous interconnected BT scaffolds were fabricated by foam replication method. The samples were sintered at 1100, 1200, 1300 and 1400 oC for 3h. In order to improve bioactivity and mechanical properties of the prepared scaffolds, samples were coated with gelatin and CaP using soaking and precipitation methods. For this purpose, the scaffolds were immersed in 0.5 M of calcium nitrate tetrahydrate and 1% gelatin solution for 24 h. Then the scaffolds were dried at room temperature and subsequently immersed in 0.3M of Na2HPO4 and 0.25% glutaraldehyde solution in order to enhance their mechanical properties and forming a calcium phosphate layer on the scaffolds surface. The coated scaffolds were dried for 3 days in an oven at 50 oC followed by 1 day in a fume hood. The effects of composite coatings on scaffold porosity, microstructure, mechanical property and in vitro mineralization were investigated.
Results: The results showed that nanocomposite scaffolds were porous with three-dimensional interconnected microstructure and pore size ranging from 200 to 300 μm. Porosity was about 80% and nanocrystalline precipitated minerals were dispersed evenly among gelatin coating. The results showed that the scaffolds with surface coating resulted in significant improvement in both mechanical and biological properties while retaining the 3D interconnected porous structure. The bioactivity of scaffolds upon immersion in simulated body fluid (SBF) were markedly improved by the coating and the bone-like apatite crystals were well mineralized on their surfaces. The in vitro cytotoxicity (MTT assay) and cell attachment using SEM of the nanocomposite scaffolds were tested using human osteosarcoma MG-63 cell line and results demonstrated that the scaffolds were biocompatible enough to support cell attachment and proliferation.
Conclusion: Finally, this research demonstrated that highly porous Barium titanate scaffolds with in situ surface formation of CaP and gelatin coating can be a promising candidates for bone tissue engineering applications.
Keywords: Piezoelectric Barium Titanate Bone Tissue Engineering

Ps-078: Survey Effect of Pancreatic and Liver Extract to Induction Definitive Endoderm Cells

Elham Hoveizi[1*], Mahmoud Hashemitabar[2], Tayebeh Mohammadi[1]

1. Department of biology, Faculty of Science, Shahid Camran University of Ahvaz, Ahvaz, Iran
2. Center for Cellular and Molecular Research, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran
Email: elham.hoveizi@gmail.com

Background and Aim: Embryonic stem (ES) cells have a pluripotent ability to differentiate into a variety of cell lineages in vitro. Investigating the roles of the co culture of mouse embryonic stem cells (mESCs) with pancreatic islets (PL) and liver stromal cells (LSCs) in the differentiation of definitive endoderm (DE) cells was the purpose of this study.
Methods: Here by using PL derived from adult mouse and LSCs derived from mouse fetal liver, we are trying to introduce a new protocol which is devoid of growth factors. We calculated mESCs indirectly for 7 days and then we analyzed the resulting cells regarding DE genes and protein expression using qRT-PCR and immunocytochemistry.
Results: It was proved that mESCs can differentiate into DE cells and also co-culture system with PL provides a proper enviroment for differentiation of mESCs. However co-culture system with LSCs isolated from mouse fetal liver, are not able to increase DE differentiation. As the first report we showed that a PL induced microenvironment can improve DE differentiation.
Conclusion: More modifications of the PL microenvironment may present another approach to providing DE cells for differentiation into hepatocyte and beta cells.

By applying these results, production of DE from stem cells facilitates in vitro.
Keywords: Mouse Embryonic Stem Cell, Differentiation, Co-Culture, Pancreatic Islets, Definitive Endoderm

Ps-079: Study of Spermatogenesis in NMRI Azospermic Infertile Mouse after Injection of Adipose Tissue-Derived Mesenchymal Stem Cell

Masoumeh Eliyasi[1], Masoud Hemadi[1], Ghasem Saki[1*], Seyed Reza Kazemi Nezhad[2]

1. Cellular and Molecular Research Center, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Department of Genetics, Shahid chamran University of Ahvaz, Ahvaz, Iran
Email: ma.eliasi@yahoo.com

Background and Aim: Infertility is one of the most common problems among couples. In recent years, researchers differentiated stem cells into male and female germ cells in vitro. Adipose tissue derived mesenchymal stem cells are more interesting due to easier isolation method, safety, and immune-modulating properties. The important restriction to apply these cells is low number and viability of the cells. To overcome this obstacle, many growth factors were studied. The aims of this study is to investigate the evolutionary process of Adipose tissue -derived mesenchymal stem cells cultured in a medium with and without EGF, LIF and GDNF growth factors after transplantion to torsion/de torsion mice.
Methods: AT-MSCs were isolated from male NMRI mice. Nature of the cells was approved by flow cytometry. After torsion/de torsion surgery, mice were injected with AT-MSCs Brdu labeled that cultured in medium with and without growths factors. The evolutionary process of the cells has been shown by examining the expression of germ cell specific markers including C-Kit, Mvh, Scp3, Gcnf with Real-Time PCR technique and expression of GCNF and C-KIT proteins with Western-blot technique and tracking labeled cells by means of immunohistochemical staining.
Results: After 8 weeks, it is observed that a great number of transplanted AT-MSCs conducted homing in the basement membrane of seminiferous tubules. The expression of spermatogonial stem cells and spermatogonia marker, gcnf and mvh, in testes transplanted by AT-MSCs cultured in medium containing growths factor were greater than other groups ($p<0.001$ and $p<0.02$ respectively). C-kit and scp3 genes expression did not show a significant change compared to the control group.
Conclusion: AT-MSCs might be studied for human infertility in future and use of the growth factors to culture the cells for clinical applications can be very helpful.
Keywords: Mesenchymal Stem Cells Transplantation, Infertility, EGF, LIF, GDNF

Ps-080: The Effect of Boswellia Carteri on Morphology, Growth Kinetics, Differentiation and Cytogenetics of Bone Marrow-Derived Stem Cells

Tahereh Emam[1,4*], Davood Mehrabani[2], Mehdi Pasalar[3], Safora Sameni[4], Shahrokh zare[2], Iman Jamhiri[2]

1. Department of Biochemistry, Fars Science and Research branch, Islamic Azad University, fars, Iran
2. Stem Cells and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Research Center for Traditional Persian Medicine and History of Medicine Shiraz University of Medical Sciences Shiraz, Iran
4. Department of Biochemistry, Shiraz Branch, Islamic Azad University, Shiraz, Iran
Email: Tahereh1011@gmail.com

Background and Aim: Boswellia carteri (frankincense) is a gum resin of Burseraceae family with many therapeutic applications. Stem cells can differentiate into specialized cell and play an important role in treating a number of diseases. This study was undertaken to investigate the effect of Boswellia Carteri on morphology, growth kinetics, differentiation and cytogenetics of bone marrow stem cells (BMSCs).
Methods: BMSCs were provided from femoral bone of 6-8 weeks old Guinea pigs. BMSCs at passage 4 were seeded in a 24-well plate and different doses of essential oils of Boswellia Carteri were added. Cells were

collected from each well 1–8 days after seeding and population doubling time and cell growth curves were determined. BMSCs were characterized morphologically and by RT-PCR for mesenchymal markers and for osteogenic differentiation property. They were assessed by karyotyping analysis for chromosomal stability.
Results: BMSCs were attached to the culture flask and displayed spindle-shaped morphology. An increasing growth and mitotic division were shown for all doses of frankincense, while the greatest growth was at a dose of 1:100,000. All cells were positive for mesenchymal markers and osteogenic induction after exposure to Frankincense.
Conclusion: Boswellia carteri was demonstrated to increase the growth and proliferation of BMSCs without any change in chromosomal stability and may be safely added to the media if higher proliferation is needed in a shorter time interval.
Keywords: Boswellia Carteri, Growth, Mesenchymal Stem Cells, Bone Marrow

Ps-081: Generation of Insulin-Producing Cells from Human Induced Pluripotent Stem Cells Using a Stepwise Differentiation Protocol Optimized with Platelet-Rich Plasma

Seyed Ehsan Enderami[1], Yousof Mortazavi[2], Masoud Soleimani[3*], Rehaneh Nassiri mansour[4], Samad Nadri[2], Alireza Biglari[2]

1. Stem Cell Technology Research Center, Tehran, Iran
2. Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
3. Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
4. Department of Clinical Biochemistry, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
Email: ehsan.enderami@gmail.com

Background and Aim: Pancreatic islet transplantation is considered as an efficient therapy for type 1 diabetes mellitus (TIDM). Limited access to human islet donors and long term need for an immunosuppressant has launched a search for an alternative source of beta cells for cell-replacement therapy purpose in T1DM. Human-induced pluripotent stem cells (hiPSCs) offer a potential source for obtaining a patient-specific insulin producing cells (IPCs) for transplantation and clinical applications. Studies on patient-specific hiPSCs as well as a series of autologous growth factors presumably will revealed their many benefits for cell base replacement therapy in T1DM patient in the future. For this purpose, in this study, we established a multistep protocol by adding platelet-rich plasma (PRP) that induce the hiPSCs into in IPCs.
Methods: We present here a differentiation protocol consisted of 5 stages for 22 days in two groups including: protocol with PRP and without PRP. Characteristics of derived IPCs in both groups were evaluated at the mRNA and protein levels, cell cycle and viability in the end stage of cell differentiation. . In response to glucose stimulation medium, insulin and C-peptide release was detected by ELISA.
Results: The in vitro studies indicated the treatment of hiPSCs in the protocol with PRP compared with protocol without PRP resulted in differentiated cells with strong characteristics of IPCs including islet-like cells, expression of mature and functional pancreatic beta cell specific marker genes, including Pdx1, Glucagon, Insulin, Ngn3 and Glut2. In addition to this qRT-PCR results, Pdx1, Glucagon, insulin and C-peptide protein was detected by immunochemistry and western blot. Our differentiated cells in two groups secreted insulin and C-peptide in a glucose stimulation test by ELISA showing in vitro functional.
Conclusion: We demonstrated for the first time that PRP might be ideal additive in the culture medium to induce pancreatic differentiation in the hiPSCs. This study provides a new approach to investigate the role of PRP in pancreatic differentiation protocols and increase the feasibility of using patient-specific iPSCs and autologous PRP for future beta cells replacement therapies for T1DM.
Keywords: Induced Pluripotent Stem Cells, Insulin-Producing Cells, Platelet-rich plasma

Ps-082: Improved Stem Cell Therapy of Spinal Cord Injury Using GDNF-Overexpressed Bone Marrow Stem Cells in a Rat SCI Model

Seyed Ehsan Enderami[1], Reyhaneh Nassiri Mansour[1], Masoud Soleimani[2*]

1. Stem Cell Technology Research Center, Tehran, Iran
2. Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: ehsan.enderami@gmail.com

Background and Aim: The spinal cord injury due to various problems, which lack of neuronal growth factors and the neuronal death are the most important of them that prevent its recovery. In this study, we improved the therapeutic outcome with utilize both of N-neurotrophic factor derived Gelial cells (GDNP) gene and the differentiation of bone marrow mesenchymal stem cells (BMSC) into the neural-like cells.
Methods: Rats were divided into four groups of six randomly. Spinal cord injury was then performed under general anesthesia using the weight dropping method. The cells were injected on the 3th day of post-spinal cord injury. Groups included rats receiving normal saline as a control, BMSC, BMSC infected with lentivirus without the GDNF gene (BMSC-vector) and BMSC infected with lentivirus encoding the GDNF gene (BMSC-GDNF). A Basso, Beattie and Bresnahan (BBB) score test were performed for four weeks. Two weeks before the end of BBB, biotin dextranamin was injected intracrebrally and at the end of the fourth week, the tissue staining was performed.
Results: There was a significant difference in BBB scores between animals received BMSC-GDNF and normal saline but there was no significant difference between other groups. A significant difference in axon counting was observed in BMSC-GDNF treated animals compared with other groups.
Conclusion: This study provides a new strategy to investigate the role of simultaneous in stem cell and gene therapy for future neural-like cells transplantation base therapies for SCI.
Keywords: Spinal Cord Injury, Differentiation Medium, BMSC, GDNF

Ps-083: Investigation of hsa-miR-19 on the Neuronal Differentiation Pathway on NPSC

Fatemeh Esfandiari[1], Fatemeh koohkan[2*], Mehrdad Hashemi[3]

1. Department of Molecular Genetic, Islamic Azad University of Tehran Medical Science, Tehran, Iran
2. Department of Molecular Genetic Engineering, Stem Cell Reaserch Center, Tehran, Iran
3. Department of Molecular Genetics, Islamic Azad University of Tehran, Medical Science Branch Tehran, Iran
Email: mahlaesfandiary@yahoo.com

Background and Aim: Introduction Oligodendrocytes, the myelinating glia in the central nervous system (CNS), are differentiated chiefly from neuroepithelial (NE) cells in the ventral neural tube. Using iPSC-derived oligodendrocyte progenitor cells (OPCs) which were shown to successfully to oligodendrocyte. Because of the low efficiency and time consuming of using hNPCs, scientists become interested in molecular ways such as microRNAs. miRNAs are a group of small noncoding RNAs which control many biological processes, including cell proliferation, differentiation, apoptosis, stress response and inflammation. Moreover, previous reports have shown that miRNAs are key regulators of neuronal differentiation. Also, miRNAs play an important role in induced differentiation to Oligodendrocyte and myelin production was proved. Therefore, miR-19 is chosen in this research for differentiating human neural progenitor stem cells (hNPSCs) to oligodendrocytes.
Methods: Maintenance and oligodendrocyte differentiation of hNPSCs. Has-miR-19 was cloned in a suitable lenti-viral vector then were concentrated with ultracentrifuge and titrated. We expanded hNPSCs in their specific media and transduced them by lentiviral viruses which contains miR-19. QRT-PCR and ICC were done to investigate expression changes of target genes.
Results: QRT-PCR and ICC results showed the down regulation of targeted genes in path of differentiation to neuron through blocking genes of neuronal differentiation pathway and also up regulation of oligodendrocytic genes such as Olig2 and MBP.
Conclusion: In this study, we provided evidence that miR-19 is involved to to blocking genes of neuronal differentiation pathways on NPSC and induction to oligodendrocyte differentiation .
Keywords: miRNA, Oligodendrocyte, OPC, h NPSC.

Ps-084: Analysis of Dignity Human Embryo in Islam and Legal System of Iran

Hasan Eskandarian*

Stem Cell Department, National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

Background and Aim: Shiite jurists don't know dignity for embryos before four months in field of fetus, its atonement, and abortion. The aim of this study is proving dignity for embryo in the time of fertilization or combination of gametes. The proving of dignity for embryo leads to important results in practical. Is it justified to derive stem cell from embryo when dignity has been proved for embryo? How to determine the embryo's atonement? What is the status of Iran legal system about the embryo? Is there a codified legal system of the embryo in Iran?
Methods: This analytic -descriptive study is based on sources of jurisprudence and Quran verses and traditions in the field of fetus and abortion.
Results: In none of the verses of the Quran embryo dignity has not been halted to elapse four months of embryo life. In all the verses concerning the creation of human creation is mentioned. Traditions entered in this field are very diverse sorts. Despite these important, dignity of human embryos in view of jurists is ascribed to four months of age or shortly after it than that. The author with analyzing Quran verses and Hadiths comparative study concludes that human dignity embryo stage will be in the time of combination of gametes. These results will challenge interpretation of Shi'a jurists about dignity human embryo. This study has provided a solution to the ethical challenge. In the view of author, the conflict rule can justify extracting stem cells. The author with search of the Iranian legal system has announced the legislator has never purpose to create a comprehensive legal framework on the embryo.
Conclusion: The findings of this study imply the codification of a legal system in the embryo. Iran currently lacks a codified legal system of the embryo. What legislators and commentators conventional understand is that the traditional conception of the embryo with the interpretation of this study does not coincide. Iranian legislator knows human dignity embryo in Fourth month while this study has demonstrated human dignity embryo in the stage of composition of gametes.
Keywords: Stem Cell Assay Embryo Aborted Islamic

Ps-085: Osteogenic Differentiation by Beta-Carotene/ PLGA Nano-Fibrous for Bone Tissue Engineering

Setareh Esmailian[1], Shiva Irani[1], Mojgan Zandi[2*], Hadi Bakhshi[3]

1. Department of Biology, School of Basic Sciences, Sciences and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Biomaterial, Iranian Polymer and Petrochemical Institute, Tehran, Iran
3. Macromolecular Chemistry II, University of Bayreuth, Universitätsstraße 30, 95440 Bayreuth, Germany
Email: setareh.esm@gmail.com

Background and Aim: Nowadays using Tissue engineering (TE) science can make a leap in this field by combining the Molecular Cell Biology science, Polymer Engineering science. According to previous studies, beta-carotene has been reported as an important factor for maintaining bone health and also it has been reported as an effective factor in pre-osteoblast differentiation. The aim of this study is to fabricate an Electrospinning scaffold containing Beta-carotene natural conductive electrical pigment, in order to differentiate Mesenchymal stem cells towards the osteoblast.
Method: To achieve this, first, a biopolymer scaffold containing Poly (lactic-co-glycolic) Acid (PLGA)/beta-carotene will be prepared using electrospinning technique. To examine pore size and porosity and morphology of scaffold SEM technique have been used. Human mesenchymal stem cells isolated from bone marrow (hBMSC). Cell viability and differentiation were evaluated respectively by MTT and calcium content assays.
Results: SEM analysis showed good porosity of scaffold. The result of MTT assay indicated that cells had good attachment and viability on this three-dimensional scaffold containing PLGA and a certain amount of beta-carotene. Calcium content approved differentiation of mesenchymal cells.
Conclusion: According to the result of this study, PLGA/beta-carotene scaffold can be a well-nominated matrix for using in bone tissue engineering (BTE) in order to differentiate mesenchymal stem cells to osteoblast.

Keywords: Bone Tissue Engineering, Mesenchymal Stem Cell, Beta-Carotene, Electrospinning

Ps-086: Proteomics Basis of Human Cancer

Milad Esmatparast*

Department of Biotechnology, Islamic Azad University Urmia, Iran
Email: Miladesmatparast@yahoo.com

Background and Aim: Cancer is a complexes disease that is caused by various genetic and environmental changes. Understanding the fundamental biological concepts and treatment of cancer has always been one of the concerns of scientists and physicians. More effort is needed to determine the molecular genetics behind the cancer development. Furthermore the early detection of cancer has crucial role in effective treatment.
Methods: Proteomics studies are performed to increase the chance of early detection, better diagnostics, and improved treatment. These new techniques have been used to discover protein biomarkers of cancers. Proteomics analysis by exploring some of the fundamental differences between proteins in normal cells and cancer cells, play a major role in the detection of cancer and its treatment.
Results: In this research proteins of normal and cancerous cells were extracted and separated from non-protein compounds. Proteins were separated base on their own isoelectic point and their mass. Separated proteins were detected by autoradiography, staining, or immunodetection after blotting onto a membrane. Markers can be easily found by comparing protein maps.
Conclusion: In the present study novel biomarkers and proteins for early detection of cancer were identified. Unlike genomics, protein biomarkers in a variety of tissues or fluids will give different results. This reflects the high sensitivity of proteomics methods.
Keywords: Proteomics, Cancer, Biomarker, Early Detection

Ps-087: Effect of Low Level Laser Therapy on Osteoporotic Bone Marrow-Derived Mesenchymal Stem Cells from Ovariectomy-Induced Osteoporotic Rats in Vitro

Somaye Fallahnezhad[1*], Abbas Piryaei, Mohammad Bayat

Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: sfallahnejad@gmail.com

Background and Aim: Postmenopausal osteoporosis (OP) is a major concern to public health. Osteoporotic bone marrow mesenchymal stem cells (BMMSCs) are critically involved in the pathogenesis of OP. Low -level laser therapy (LLLT) has positive effects on healthy BMMSCs. The purpose of this study was to evaluate the influences of LLLT on viability of ovariectomized induced osteoporosis (OVX) BMMSC in osteogenic culture at 24, 48, 72 hours after one time laser radiation.
Methods: Twelve female rats were randomized into two groups to undergo either a sham surgery (sham BMMSCs group) or ovariectomy induced OP (OVX-osteoporotic-BMMSCs group). MSCs harvested from the BM of sham and OVX rats went through culture expansion. There were three groups on main part of study: In groups one and two, sham -BMMSC and OVX-osteoporotic-BMMSC were hold respectively in osteogenic condition medium without any intervention. In group 3, laser-treated -OVX- osteoporotic -BMMSCs, were treated with optimum protocol of LLLT (one time laser radiation, 1.2 J/cm2). At 24, 48, 72 hours after one time laser radiation, surviving cells of all studied groups were counted by the MTT assay.
Results: The biostimulatory effect of LLLT is demonstrated by a significant increase in viability of OVX-osteoporotic BMMSCs compared to control -OVX- osteoporotic BMMSCs and or sham group in osteogenic medium in vitro. MTT assay showed that there were significant increase in optical density of 24 hours post-treatment holding time, compared to other time holdings.
Conclusion: Based on this study we concluded that LLLT with a He-Ne laser (1.2 J/cm2, one time radiation) significantly improved cell viability of the OVX-osteoporotic-BMMSCs.
Keywords: Low-level laser Irradiation (LLLI), Bone Marrow Mesenchymal Stem Cells (BMMSCs), Ovariectomy (OVX), Rat

Ps-088: Telomere Length Maintenance as a Key Step in Senescent Tendency of Mscs upon Expansion in the Presence of Zinc Ion

Raheleh Farahzadi[1,2,3*], Ezzatollah Fathi[4], Seyed Alireza Mesbah-Namin[5], Nosratollah Zarghami[6]

1. Cardiovascular Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Stem Cell And Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
4. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
5. Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
6. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: r.farahzadi@iaut.ac.ir

Background and Aim: The use of mesenchymal stem cells (MSCs) for cell therapy and regenerative medicine has received widespread attention over the past few years, but their application can be complicated by the factors such as reduction in proliferation potential, senescent tendency of MSCs upon expansion, and age-dependent decline in their number and function. It was shown that all mentioned features were accompanied by reduction in telomerase activity and telomere shortening. Telomeres are composed of long hexamer (TTAGGG) repeats at the end of eukaryotic chromosomes which prevent to chromosomes instability, replicative senescence and accelerated aging. During the process of cell division, as a result of the imperfect replication of linear chromosomes, telomeres are shortened that called 'end-replication problem'. Although the detailed molecular mechanisms of aging is not fully understood, but the progressive telomere shortening is one of the molecular mechanisms underlying ageing, as critically short telomeres trigger chromosome senescence and loss of cell viability. Despite the advantages of MSCs, specified properties of MSCs are so much affected by aging. As a result, the use of MSCs from older donors is lower than younger donors, which restricts the clinical applications such as regenerative medicine or cell therapy. It seems aging is the result of the accumulation of oxidative damage caused by free radicals generated as by-products during normal metabolism. So it is crucial to maintain the proliferation and differentiation capacity of MSCs. The use of antioxidants to prevent cellular aging is important. The role of zinc element as an antioxidant and anti-inflammatory agent for aging process was showed. Therefore, this study was carried out to investigate the effect of ZnSO4 on the telomere length maintenance in hADSCs.
Methods: In this study, MSCs were isolated from adipose tissue with enzymatic digestion. Flow cytometric analysis and immunocytochemical staining were performed to investigate the cell surface markers. Also, alizarin red-S, sudan III, toluidine blue and cresyl violet staining were done to evaluate the multi-lineage differentiation of hADSCs. In order to improve the effective application of MSCs, these cells were treated with 1.5×10-8 and 2.99×10-10 M ZnSO4 for 48 h. Absolute telomere length measurement with quantitative real-time PCR.
Results: The results of the present study showed that telomere length increased in the presence of 1.5×10-8 M (6.53 Kbp) and 2.99×10-10 M ZnSO4 (4.27 Kbp) compared to the control group (3.89 Kbp). This increase just significant at concentration of 1.5×10-8 M ZnSO4 ($p<0.001$).
Conclusion: It seems that ZnSO4 as an proper antioxidant could improve the aging-related features due to lengthening of the telomeres and would be of potential benefit for enhancing the application of aged MSCs.
Keywords: Zinc Sulphate (ZnSO4); Telomere Length; Human Adipose Tissue-Derived Mesenchymal Stem Cells (hADSCs)

Ps-089: Electromagnetic Field in the Presence of Zinc Ion as Fundamental and Stimulating Factor for Bone Mineralization and Development

Ezzatollah Fathi1, Raheleh Farahzadi[2,3*]

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
2. Cardiovascular Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

3. Stem Cell And Regenerative Medicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran
Email: r.farahzadi@iaut.ac.ir

Background and Aim: Osteoporosis is the most common metabolic bone disorder that frequently afflicts many women after menopause and some men in older age. It is characterized by low bone mass and structural deterioration of bone tissue. A great deal of evidence suggests that osteoporosis results from the interaction of genetic susceptibility and environmental factors. Among which, zinc ion (Zn+2) as an essential trace element, was reported to be involved in the pathogenesis of osteoporosis and has direct effect on bone mineralization. In addition to the use of Zn2+ in promoting bone formation; the use of electromagnetic fields (EMFs) exposure is another method in the treatment of osteoporosis. The practical clinical use of EMFs in the treatment of osteoporosis has been restricted due to parameters such as the uncertainty of frequencies, intensities and duration of exposure. Many researchers have tried to investigate the mechanism of physical–chemical interactions between biological tissues and EMF. The aim of this study was to evaluate the effects of EMF in the presence of ZnSO4, on the osteogenesis of ADSCs in order to further elucidate the simultaneous effects of ZnSO4 and EMF as a strategy in osteoporosis therapy.
Methods: In the present study, 0.432 µg/ml was used as the final concentration of ZnSO4 with MTT assay. The exposure condition is 50 Hz, 20 mT EMF. ALP activity measurement, calcium assay and expression of several osteoblastic marker genes such as ALP, OCN, BMP2 and Runx2, were examined to assess the effect of ZnSO4 on the osteogenesis of ADSCs under EMF. The expression of cAMP and PKA was evaluated by ELISA. To further demonstrate the correlation between the signaling pathways and the osteogenic markers induced by the magnetic field in the presence of Zn+2, the inhibitors of PKA, and ERK1/2 pathways were used to determine whether inhibiting them could also reduce the osteogenic markers of the ADSCs induced by the magnetic fields and Zn+2.
Results: The results of the present study showed that 0.432 µg/ml ZnSO4 and 50 Hz, 20 mT EMF activated pathways through increase in ALP and PKA activities, calcium and cAMP levels and expression of ALP, OCN, Runx2 and BMP2 as osteogenic differentiation genes, as well as expression of β-catenin, Wnt1, Wnt3a and LRP5 as Wnt/β-catenin pathway-related genes. Furthermore, the inhibition of the PKA and ERK1/2 signaling pathways reduced the expression of ADSCs osteogenic markers. However, the findings of this study are consistent with the hypothesis that EMF, in the presence of ZnSO4, can promote ADSCs osteogenesis via the cAMP–PKA and MAPK pathways.
Conclusion: This research indicated that EMF, in the presence of ZnSO4, promoted bone mineralization and formation of ADSCs, and that it correlated with the PKA, ERK1/2 and Wnt/β-catenin signaling pathways.
Keywords: Electromagnetic Field (EMF); Osteogenic Differentiation; Zinc Sulphate (ZnSO4); ERK1/2; Protein Kinase A (PKA); Wnt/β-Catenin

Ps-090: Senescence Enhancing Effect of Fetal Bovine Serum on Adipose Derived Stem Cells

Hajar shafaei[1], Azam Farani[2*]

1. Department of Anatomical Sciences, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Basic Science, Islamic Azad University of Tabriz, Tabriz, Iran
Email: Farani1990azam13@gmail.com

Background and Aim: Mesechymal Stem cells (MSCs) is used for treatment of several disease. However it is important to preserve of the stemness property MSCs for clinical applications Fetal Bovine Serum (FBS) is routinely used in cell culture for proliferation of MSCs. However, in vitro expansion of them resulted in their senescence. This study amid to culture human Adipose derived Stem Cells (ASCs) in culture media containing FBS and umbilical cord blood serum. The effect of these two types of serum was investigated on age related genes IGF1 and p16INK4a expression.
Methods: ASCs were cultured in two different medium up to passage 6 and assessed morphologically. Also total mRNA of ASCs was extracted and relative expression of IGF1 and p16 genes were assessed by quantitative real-time RT PCR.
Results: We observed that cell proliferation in FBS group prominently decreased and phenotypes of cells were changed to flat appearance. Human umbilical cord blood serum prevented these aging phenomenons

which demonstrate better culturing condition for human ASCs. Real-time RT PCR results confirmed morphologic results by significant increase in the expression IGF1 and p16INK4a genes in cells grown in FBS containing medium.
Conclusion: FBS promotes senescence in ASCs, therefore for cell therapy and tissue engineering purposes, human MSCs have to culture in human serum to preserve their stemness properties.
Keywords: ASCs, FBS, Senescence, Human Umbilical blood serum

Ps-091: Adipose-Derived Stem Cells as a Feeder Layer Increase C-Myc Oncogene Expression of Human Expanded Hematopoietic Stem Cells Derived from Cord Blood

Aisan Farhadi*, Tahereh Foroutan

Department of Animal Science, Faculty of Biological Science, Kharazmi University, Tehran, Iran
Email: a.farhadi69@yahoo.com

Background and Aim: The number of hematopoietic stem cells (HSCs) per cord blood unit is limited, and this can result in delayed engraftment or graft failure. Therefore, Scientists suggest the use of cytokines to enhance proliferation of HSCs. Along with cytokines; feeder layers are used to maximize the proliferation and survival of these cells. A Feeder layer and cytokines can affect the expression of genes involved in proliferation and differentiation such as c-Myc .Since c-Myc is required for the correct balance between self-renewal and differentiation of HSCs, in the present study we used cytokines such as stem cell factor (SCF), FLt3-Ligand (FL), Thrombopoietin (TPO), and adipose-derived stem cells (ADSCs) as a feeder layer to examine the expression of c-Myc in CD34+ cells expanded after co-culture with feeder layer.
Methods: CD34+ cells were cultured for7 days in three groups with mentioned cytokines including: (a) directly in contact with ADSCs feeder layer (b) separated by a transwell insert membrane (c) without a feeder layer. Gene expression was evaluated by real-time reverse transcriptase-PCR.
Results: Expression level of c-Myc in co-culture system with cytokines was higher than the other groups. Also our data showed that direct culture of CD34+cells on feeder layer was important for HSCs expansion.
Conclusion: The high expression of c-Myc indicates to increase in self-renewal of HSCs. Therefore, the proliferation of HSCs in co-culture system was higher than the other groups. Also, the results showed that direct culture of CD34+cells on feeder layer was important for HSCs expansion. Several studies have demonstrated the different inductive capacities of feeder layers to support and maintain HSCs proliferation. It has been reported that HSCs in the presence of feeder layer expand more than the other groups.
Keywords: Adipose-Derived Stem Cells, C- Myc, Feeder Layer, Hematopoietic Stem Cell

Ps-092: The Potential of Human Amniotic Membrane Proteins in Cardiac Regeneration; the Role of Extraction Methods

Yousef Faridvand[1], Samira Nozari[2], Mohammad Nouri[1*]

1. Stem Cell and Regenerative Medicine Institute, Tabriz, Iran
2. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Email: y.faridvand@yahoo.com

Background and Aim: The strategy to induce proliferation and cardio-protection against cardiac damage is one of the main approaches for inhibition of cardiac complication in heart disease. In this current study, we aimed to assess the proliferative activity of human amniotic membrane (HAM) extracted protein by three different extraction methods from HAM derived from human placenta in H9C2, a cardiomyocyte cell line
Methods: The rat cardiomyocyte cell line, H9c2, was cultured with DMEM/HG medium, supplemented with 10% FBS and antibiotics. Placenta was obtained from healthy women after normal or caesarean delivery. After removing HAM, three methods were used for protein extraction. First, HAM was chopped, sonicated and homogenized. Then, proteins were collected after 10,000g centrifuge in PBS buffer. In the second method, HAM was homogenized at the RIPA buffer and after being dialyzed against 10 mM PBS pH 7.4, the proteins were collected after 10,000g for 30min at 4°C. In

the third method, HAM was minced and grinding into powder using liquid nitrogen and homogenized into PBS buffer. After characterizing the extracted proteins by SDS-PAGE, H9c2 cells were exposed to 1 mg/ml and 0.5 mg/ml of HAM extracted proteins. The effects were evaluated by using the MTT assay test for 24, 48 and 72 h, Ki-67 staining and flow-cytometry with Annexin V/PI.
Results: The results indicated that treatment with 1 mg/ml of HAM extracted protein induced the cell proliferation but higher concentration was accompanied with cell toxicity which observed in MTT assay results. The incubation with protein extracted by PBS method showed the highest number of cell proliferation compared with the RIPA and liquid nitrogen methods. Also, increase in Ki-67 expression was observed in PBS extraction method which is in line with Annexin V/PI staining.
Conclusion: Taken together, the study results demonstrated that HAM extracted proteins exert proliferative effect in H9C2 cell line. This proliferation potency was significantly depended on methods of preparing extracted protein
Keywords: Human Amniotic Membrane, Protein Extraction, Cardiomyocyte, H9C2

Ps-093: Histone Modification of Embryonic Stem Cells Produced by Somatic Cell Nuclear Transfer and Fertilized Blastocysts

Fattaneh Farifteh[1], Mohammad Salehi[1], Elham Fazeli[2], Mojgan Bandehpour[1], Marefat Ghafari Novin[2], Mohsen Nourozian[2], Nariman Mosaffa[3], Ahmad Hosseini[1*]

1. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Science, Tehran, Iran
2. Department of Cell Biology and Anatomical Science, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Immunology, Faculty of Medicine,Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: elhamfazeli15@yahoo.com

Background and Aim: Nuclear transfer-embryonic stem cells (NT-ESCs) are genetically identical to the donor's cells; provide a renewable source of tissue for replacement, and therefore, decrease the risk of immune rejection. Trichostatin A (TSA) as a histone deacetylase in¬hibitor (HDACi) plays an important role in the reorganization of the genome and epigenetic changes. In this study, we examined whether TSA treatment after somatic cell nuclear transfer (SCNT) can improve the developmental rate of embryos and establishment rate of NT-ESCs line, as well as whether TSA treatment can improve histone modification in NT-ESCs lines.
Methods: In this experimental study, mature oocytes were recovered from BDF1 [C57BL/6×DBA/2) F 1 mice] mice and enucleated by micromanipulator. Cumulus cells were injected into enucleated oocytes as donor. Reconstructed embryos were activated in the presence or absence of TSA and cultured for 5 days. Blastocysts were transferred on inactive mouse embryonic fibroblasts (MEF), so ESCs lines were established. ESCs markers were evaluated by reverse transcription-polymerase chain reaction (RT-PCR). Histone modifications were analyzed by enzyme linked immunosorbent assay (ELISA).
Results: Result of this study showed that TSA treatment after SCNT can improve devel¬opmental rate of embryos (21.12 ± 3.56 vs. 8.08 ± 7.92), as well as establishment rate of NT-ESCs line (25 vs. 12.5). We established 6 NT-ESCs in two experimental groups, and three embryonic stem cells (ESCs) lines as control group. TSA treatment has no effect in H3K4 acetylation and H3K9 tri-methylation in ESCs.
Conclusion: TSA plays a key role in the developmental rate of embryos, establishment rate of ESC lines after SCNT, and regulation of histone modification in NT-ESCs, in a man¬ner similar to that of ESCs established from normal blastocysts
Keywords: Somatic Cell Nuclear Transfer, Trichostatin A, Epigenetics Modification

Ps-094: Down Regulation of Asncmtrna-1 and Asncmtrna-2 and Upregulation of Sncmtrna in Patients with Chronic Myeloid Leukemia

Mina Farokhian[1], Saeed Abroun[1*], Seyyed Mohammad Mozaffar[1], Amir Atashi[2], Monire Ajami[1], Masoumeh Ajami[1], Majid Niazpoor[1]

1. Hematology Department, School of Medicine, Tarbiat Modares University, Tehran, Iran
2. School of Paramedical Sciences, Shahroud University of Medical Sciences, Shahroud, Iran
Email: optima_enamel@yahoo.com

Background and Aim: Chronic myeloid leukemia is a myeloproliferative disorder which myeloid cells undergo clonal expansion, due to translocation between chromosome 9 and 22, leading to BCR-ABL fusion. One of the most important factors in cancer progression is deregulation of oncogenes and tumor suppressor genes. Long noncoding RNAs also play a prominent role in carcinogenesis and can function as oncogenes and tumor suppressor genes. Recent studies have shown that mitochondrial genome also can produce noncoding RNAs which due to known importance of mitochondria in carcinogenesis, study on mitochondrial noncoding RNAs will help in diagnosis and treatment of cancer. The aim of this study is to compare the expression of three long noncoding mitochondrial RNA; ASncmtRNA-1 and ASncmtRNA-2 and SncmtRNA in newly diagnosed patients with chronic myeloid leukemia and imatinib-treated patients.
Methods: Total RNA extraction and cDNA synthesis were performed on buffy coats of five new-case and five treated CML patients. Then, the gene expression was analyzed using Real-Time PCR for ASncmtRNA-1 and ASncmtRNA-2 and SncmtRNA.
Results: In treated patients, the gene expression of ASncmtRNA-1 and ASncmtRNA-2 were upregulated in comparison with newly diagnosed patients, respectively.and the gene expressin of SncmtRNA were down regulated in treated patients in comparison with newly diagnosed patients.
Conclusion: Recently, researchers pay attention to the role of LncRNAs in cancers. This study was the first study in investigating the noncoding mitochondrial RNAs in CML which can be a novel biomarker for diagnosing of CML and needs future studies.
Keywords: Chronic Myeloid Leukemia, LncRNA, Mitochondrial Noncoding RNA, ASncmtRNA-1, ASncmtRNA-2

Ps-095: The Effect of Sodium Selenite on Proliferation of Bone Marrow Mesenchymal Stem Cells

Akram Farzandi Esfahlan[1], Abolfazl Barzegar[1], Effat Alizadeh[2*], Roya Salehi[3]

1. Research Institute for Fundamental Sciences (RIFS), University of Tabriz, Tabriz, Iran
2. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
3. Drug Applied Research Center and Faculty of advanced Medical Science, Tabriz University of Medical Sciences, Tabriz, Iran
Email: akramfarzan9@gmail.com

Background and Aim: Bone marrow mesenchymal stem cells (BMSCs) are the best source for developing cell therapeutics but during expansions, they are exposed to various types of oxidative stresss. Oxidative stresss can cause damage to whole cell parts and their function. Studies showed that selenium is an effective antioxidant. In the present work, we aimed at the study of the effect of sodium selenite on viability and proliferation of BMSCs from rat origin.In the present study, we isolated MSCs from bone marrow (BMSCs) of rat then we characterized them using flow cytomery and osteocyte differentiation. The BMSCs were treated with sodium selenite with concentrations of 10,10 2,103,104,105,15×104,2×105nM for 48 hours and the non-treated cells were used for control then the viability of them was studied using MTT assay. Statistical analysis was performed using ANOVA method. All experiments were done in triplicate. The data were presented as mean ±SD.
Methods: In the present study, we isolated MSCs from bone marrow (BMSCs) of rat then we characterized them using flow cytomery and osteocyte differentiation. The BMSCs were treated with sodium selenite with concentrations of 10,102,103,104,105,15×104,2×105nM for 48 hours and the non-treated cells were used for control then the viability of them was studied using MTT assay. Statistical analysis was performed using ANOVA method. All experiments were done in triplicate. The data were presented as mean ±SD.
Results: Our results showed that sodium selenite has dose dependant effects. At doses higher than104nM, it showed cytotoxcity but at doses lower 104nM, it was not cytotoxic. Even sodium selenite showed best viability and significant proliferative effects at doses

10,102,103,104nM(115.21%±1.5%, 103.72%±2.6%, 107.43%±3.4%, 106.91%±0.6%, respectively) as compared to control non-treated BMSCs.
Conclusion: We concluded that sodium selenite could enhance viability and proliferation of BMSCs from rat origin. The findings of this study may be applicable for development of BMSCs source for cell therapy.
Keywords: Sodium Selenite, Bone Marrow Mesenchymal Stem Cells (BMSCs), Proliferation

Ps-097: Endothelial Differentiation of Human Amniotic Mesenchymal Stem Cells in Vitro

Farzaneh Fathi[1*], Mohammad Reza Rashidi[1], Reza Rahbarghazi[2]

1. Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, 51664- 14766, Tabriz, Iran
2. Stem Cell Research Center, Tabriz University of Medical Sciences, 51664-14766, Tabriz, Iran
Email: farzan_fathi2000@yahoo.com

Background and Aim: Mesenchymal-to-endothelial transition is an important phenomenon during tissue regeneration. Human amniotic mesenchymal SCs (hAMSCs) have been demonstrated to offer great applications in cell-based therapies, and regenerative medicine and display differentiation potential to various tissue. In biomedical science, the finding of cell differentiation could be valuable for predicting the reconstitution of target tissue. Ve-Cadherin is a transmembrane protein that cause related junction of endothelial cells and plays a specific role in the preservation and renewal of endothelium integrity. In this work, differentiation of mesenchymal stem cell to endothelial cells was investigated by increasing the ve-cadherin on the cell surface at various differentiation days.
Methods: To induce endothelial differentiation, hAMSCs were maintained in Endothelial Cell Growth media M-199 supplemented with EGM-2 cocktail (Cat No: C-22010, Promocell) and 2% fetal calf serum (FCS, Promocell) for 14 days. The medium was replenished every 2 to 3 days. Differentiation of hAMSCs into endothelial-like phenotype was studied flow cytometry analysis on days 1, 2, 3, 5, 7 and 14. The morphological changes in relation to the endothelial acquisition were monitored through the experiment.
Results: The expression of ve-cadherin increased during differentiation toward endothelial cells and reached a maximum level at the end of the experiment as compared to the first time. The flow cytometric method was able to discriminate the hAMSCs expressing ve-cadherin after 5 days of the differentiation.We observed that endothelial differentiation of hAMSCs coincided with morphological changes into epithelial-like appearance. The morphological changes along with the expression pattern are identical to the endothelial differentiation.
Conclusion: The flow cytometric method was able to detect the hAMSCs expressing endothelial cells after 5 days of the differentiation.
Keywords: Endothelial Differentiation, Mesenchymal Stem Cells, In Vitro

Ps-098: PCL/CNF Composite Scaffolds for Electrical Stimulation of Estm Cells

Samiye Fathi*, Niloofar Nazeri, Hossein Ghanbari, Reza Faridi Majidi

Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: samiyefathi@gmail.com

Background and Aim: Electrospun carbon nanofibers (CNFs) have great potential for applications in neural tissue regeneration due to their electrical conductivity and biocompatibility but they are very brittle. To overcome this, we produced electrospun CNF/PCL nanofibers. These nanofibers are morphologically similar to natural extracellular matrix and flexible and have diameter of 500±100 nm. They can use for cell stimulation and differentiation. Background: An ideal scaffold should mimic the nanofibrous structure of native ECM to help the damaged tissue to repair. Electrospining is applied to fabricate the nanofibers scaffolds. These scaffolds mimic the collagen fibrils and increased surface area to support cell attachment (). Synthetic polymers such as PCL, PLA, PLGA have been investigated as a scaffolds for nerve regeneration but they lack electrical conductivity.To overcome this, due to high electrical

conductivity of carbon nanofibers we add CNF to PCL nanofibers.
Methods: Fabrication of nanofibrous scaffolds to have CNF suspantion it was sonicated in HFIP at concenteration 5-10% (w/w) for 3 hours. PCL was dissolved in CNF supantion to obtain 10% (w/w) solution by stirrig for an hour. After stirring for 2 h solutions with different concenteration was placed into 5 ml plastic syringe and fed through a 18 G blunted stainless steel needle at a rate of 1 ml/h. distance between the tip of the needle and the collector covered with an aluminum foil was set at 15 cm while a high voltage of 20 kV was applied. The collector was rotated with speed 300 rpm for random fibers and 2000for aligned.
Results: According to the SEM images, the fiber diameters reduced by adding CNF to polymer solution. It can be due to reduction of solution viscosity by adding CNF. This reduction increase by enhancing CNF concenteration. On the other hand, electrical conductivity of the MWCNT-adsorbed PLGA samples was significantly increased by increasing CNF concenteration due to the formation of a conductive percolation network.
Conclusion: In present study, we fabricated PCL/CNF nanocomposites with blend electrospinning and measure its size, porosity and electrical conductivity. Results show that it can be good choice for stem cell stimulation to differentiate.
Keywords: PCL, Nanofiber, Electro Spun, CNF

Ps- 099: Comparative Restoration of Acute Liver Failure by Menstrual Blood Stem Cells Compared to Bone Marrow Stem Cells in Mice Model

Mina Fathi-Kazerooni[1], Sayeh Khanjani[2], Gholamreza Tavoosidana[1], Somaieh Kazemnejad[2*]

1. Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, Iran
Email: minafathi30@yahoo.com

Background and Aim: Background: Nowadays, there is an increasing attention in the application of menstrual blood stem cells (MenSCs) in regenerative medicine. The aim of this study was to investigate the therapeutic potential of MenSCs compared to bone marrow-derived stem cells (BMSCs) in an animal model of CCL4-induced acute hepatic failure.
Methods: The injured Balb/C mice were divided into multiple groups and received MenSCs, BMSCs and hepatocyte progenitor-like (HPL) cells derived from these cells.
Results: Tracking of GFP-labeled cells showed homing of cells in injured areas of the liver. In addition, the liver engraftment of MenSCs was shown by immunofluorescence staining using anti-human mitochondrial antibody. Microscopically examination, periodic acid-Schiff and Masson's trichrome staining of liver sections demonstrated the considerable liver regeneration post cell therapy in all groups. Assessment of serum parameters including aspartate aminotransferase, alanine aminotransferase, total bilirubin, urea, and cholesterol at day 7 exhibited significant reduction, such this downward trend continued significantly until day 30. The restoration of liver biochemical markers, changes in mRNA levels of hepatic markers, and the suppression of inflammatory markers were more significant in the MenSCs-treated group compared with the BMSCs-treated group. On the other hands, HPL cells in reference to undifferentiated cells had the better effectiveness in the treatment of the acute liver injury.
Conclusion: Our data showed that MenSCs could be considered as appropriate alternative stem cell population instead of BMSCs for future treatment of acute liver failure.
Keywords: Bone Marrow Stem Cells, Menstrual Blood Stem Cells, Liver Injury, Regenerative Medicine

Ps- 100: Expression Analysis of FMR1 Flanking Genes (SLITRK2, SLITRK4, MECP2 and GABRA3) in Female Full Mutation Carriers: Advances in Understanding of Molecular Basis of FXS

Zahra Fazeli[1], Sayyed Mohammad Hossein Ghaderian [2], Hossein Najmabadi[3], Mir Davood Omrani[2*]

1. Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2. Urogenital stem cell research center, Shahid Beheshti University of Medical Sciences, No 23, Shahid Labbafi Nejad Educational Hospital, Amir Ebrahimi St, Pasdaran Ave, Tehran, Iran
3. Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
Email: zahrafazeli6@gmail.com

Background and Aim: Fragile X syndrome (FXS) has been known as the most common cause of inherited intellectual disability (ID) and autism. This disease results from loss of fragile X mental retardation protein (FMRP) expression due to expansion of CGG repeats located on 5UTR of FMR1 gene. The relationship between disturbed genes associated with epigenetic silencing of FMR1 and fragile X syndrome is still not fully understood. In the present study, the expression of FMR1 flanking genes including SLITRK2, SLITRK4, MECP2 and GABRA3 were evaluated in neuronal cells differentiated from Peripheral Blood-Mesenchymal Stem Cells (PB-MSCs) of female full mutation carriers.
Methods: At first, PB-MSCs of two females with full mutation of FMR1 gene were differentiated by inhibition of BMP signaling into neuronal cells through inhibition of BMP signaling. Then, the expression of SLITRK2, SLITRK4, MECP2, and GABRA3 genes was determined in the differentiated cells by Real time PCR. The methylation pattern and expansion of CGG repeats located on 5UTR of FMR1 gene were also analyzed by MSP and TP-PCR followed by capillary electrophoresis, respectively.
Results: The obtained results indicated that the expression of the SLITRK2, SLITRK4, MECP2, and GABRA3 genes were different in the cells differentiated from PB-MSCs of females with FMR1 full mutation as compared with a normal female, being consistent with observed phenotypic differences.
Conclusion: The observed association of expression of genes located upstream of FMR1 gene with phenotypic differences in the female carriers could increase our understanding from novel therapeutic targets for patients with mild symptoms of FXS as well as the patients affected by other FMR1- related disorders.
Keywords: Fragile X Syndrome, FMR1, CGG Expansion, Molecular Mechanism, Mesenchymal Stem Cells, Differentiation

Ps- 101: In Vivo Differentiation of Mesenchymal Stem Cells to Insulin Producer Cells by a Natural Scaffold

Afsaneh Fazili[1], Soghra Gholami[1*], Mojgan Sheikhpour[2]

1. Department of Anatomy, School of Veterinary Medicine. Shiraz University, Fars, Iran
2. Department of Microbial Biotechnology, School of Biology, College of Science, University of Tehran, Iran
Email: af.fazili@ut.ac.ir

Background and Aim: Adult stem cells, including mesenchymal stem cells have the ability to differentiate into several cells such as beta cells, and can be used as a new therapeutic approach. These stem cells are capable of causing various cell types in the body and so, the cells could be affected by certain physiological conditions to be converted to some cells with special functions such as insulin producing cells. In this research we managed to in vivo differentiation of mesenchymal stem cells to insulin producer cells by natural Scaffold.
Methods: In this study, mesenchymal stem cells were isolated from adipose tissue and differentiated to insulin producing cells through transplantation by Matricaria chammomila L. oil collagen-based scaffold in healthy and diabetic animal model. Then, the Real Time PCR and immunohistochemistry techniques were used in order to investigate the differentiation and the function of differentiated cells.
Results: Both Pdx1 and insulin genes were evaluated in the differentiated cells with scaffold and oil + MSC. The expression levels of these genes increased significantly ($P<0.05$) in the differentiated cells with scaffolds and oil +MSC. In addition, the results of immunohistochemistry analysis showed that the cells on the scaffold with oil, after 21 days of exposure in the body of healthy and diabetic rabbits, were able to differentiate to insulin-like cells.
Conclusion: These results mean that the cells, in both groups, were able to express specific markers of Pdx1 and Ngn3, which are expressed in pancreatic beta cells as well. In the other hand, this in vivo differentiation of mesenchymal stem cells to insulin producer cells by natural Scaffold has no immunological and toxicity ef-

fect on the animal models, and could be used as a natural treatment technique.
Keywords: In Vivo Differentiation, Mesenchymal Stem Cells, Insulin Producer Cells, Natural Scaffold

Ps-102: Hepatogenic Condition Media Induced Adipose Derived Mesenchymal Stem Cells Affect miRNAs Expression

Sedigheh Fekri Aval[1], Nosratollah Zarghami[2*], Seyed Abolghasem Mohammadi[1], Mohammad Nouri[3], Abolfazl Barzegar[4]

1. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran.
2. Umbilical Cord Stem Cell Research Center (UCSRC), Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Clinical Biochemistry and Laboratory Medicine, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
4. Research Institute for Fundamental Sciences (RIFS), University of Tabriz, Tabriz, Iran.
Email: fekrise@tbzmed.ac.ir

Background and Aim: Adipose tissue derived mesenchymal stem cells (ADMSCs) transplantation has recently gained widespread enthusiasm, particularly in the perspective to use them as potential alternative in regenerative medicine. Although microRNAs regulatory act in various biological processes such as proliferation and differentiation was proved, the accurate role of some miRNAs in hepatogenesis process is unknown. The objective of this study was to investigate the effect of miRNAs including miR-125b, miR-15/16 family on hepatogenesis process promotion.
Methods: Subcutaneous adipose tissue specimens were obtained from normal male donors undergoing partial thoracic surgery and mesenchymal stem cells was isolated by 1% collagenase type I (Invitrogen). To characterize the isolated human ADSCs; were used flow cytometry analysis (BD, San Jose, USA). The secretion of albumin (ALB) of the differentiating MSCs was investigated using ELISA, The expression of mature microRNAs in cells was detected by real-time PCR (Exiqon).
Results: Detection results showed a significant increase of miR-16-5p expression level in first and last weeks of the hepatogenesis. MiR-16-5p compared to other miRs, except for miR-122, shows strongly positive expression in the early stage of hepatogenesis ($P < 0.01$). Probably, high expression level of miR-16 is associated with the decrease of mesenchymal signals. Additionally, significant increases in miR-195-5p were detected, suggesting that these increases could be used for monitoring at early stage of differentiation or as drivers. In addition, it was observed that there isn't functional association between miR-16 and miR-15b, whereas miR-16 and miR-15a are concordant.
Conclusion: In conclusion, our results suggest that miR-195 and miR-16 regulates important mechanisms for hepatocyte regeneration, specifically hepatogenic differentiation of MSCs, proliferation and control of signal transduction; therefore, it is a potential target for clinical regenerative therapies and using mimics or siRNAs in the hepatic differentiation of ADMSCs can improve efficiency of differentiation.
Keywords: Adipose Tissue, Transplantation, Hepatocyte like Cell, MicroRNA

Ps-103: Comparison of Two Non-Enzymatic Methods for Adipose Tissue-Derived Stem Cells Isolation

Ahad Ferdosi[1], Laila Roshangar[1*], Jafar Soleimani Rad[1], Roya Salehi[2], Morteza Rastbar[2], Raziyeh kheirjou1

1. Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. School of advanced medical science, Tabriz University of Medical Sciences, Tabriz, Iran
Email: A.ferdosi.kh@gmail.com

Background and Aim: The isolation of adipose stromal/stem cells (ASCs) currently relies on the use of the enzyme collagenase, which is an expensive reagent derived from a bacterial source. The method holds major drawbacks; it is costly, time-consuming and results in a heterogeneous cell population. Besides, digestion of extracellular matrix causes cell injury and endangers proliferation and differentiation of the cells. Also, because of over handling the samples are prone to con-

tamination but non-enzymatic methods for isolating adipose tissue-derived stem cells are efficient, safe to human cell therapy and less expensive protocols. This experiment evaluated two method of the extraction of ASCs without an enzymatic digest.
Methods: ASCs isolated with a simple method, modified physical washing non-enzymatic method and compared this method with direct washing non-enzymatic method in terms of processing time, stem cell yield, and immunophenotype. We obtained small pieces of adipose tissue from animal or human and of each sample were washed two or three times with FBS and completely homogenized. Homogenized adipose tissue was poured into conical tubes. The bottles were vigorously shaken by hand. When the tissue was separated, the aqueous infra-natant was saved and the tissue was washed for another 2-3 times, each time saving the infra-natant. the infra-natant were centrifuged at room temperature. The SVF pellet was re-suspended and plated into T-75 flasks. The flasks were maintained in a humidified incubator at 37 C with 5% CO2. After 5 days incubation, half of flasks' mediums replace with new meduim every 3 days. Finally, after 14-15 days the cells reach confluent. The cells from subconfluent cultures were then harvested and expanded through 3 passages.
Results: Based on flow cytometric analysis of surface markers (CD44+ , CD105+ , CD34? , CD45?), Dil (Carbocyanine Dyes) staining, the stromal vascular fractions isolated with modified physical washing displayed a same immunophenotype relative to the direct washing. In vitro ASCs also were evaluation by the labeling of Dil dye. The labeled cells were determined in vitro similar to fibroblast-like cells and used to in vivo studies.
Conclusion: The two methods yield a similar cell product. The MSCs isolated by non-enzymatic method were pluripotent but our method earlier and great isolation yields, homogeneity of isolated cells, brief procedure, and high economy are the advantages relative to direct washing non-enzymatic method.
Keywords: Adipose Stromal/Stem Cells, Adipose Tissue, Non-Enzymatic

Ps-104: Psychological consequences of Hematopoietic Stem Cell (HSCT) in children with Acute Lymphoblastic Leukemia (ALL) and their caregivers

Manijeh Firoozi*

University of Tehran, Farabi Pardis, Tehran, Iran
Email: mfiroozy@ut.ac.ir

Introduction: To investigate cognitive impairment, depression and sleepiness as predictors of functional outcomes in pediatric hematopoietic stem cell transplant (HSCT).
Methods: Participants included 63 children with Acute Lymphoblastic Leukemia who refer to Mahak (Children's Specialized Hospital (. The participants were undergoing HSCT. Self-report and parent-report measures of excessive daytime sleepiness (EDS), emotional and behavioral functioning and cognitive impairment (executive functioning) were completed.
Results: Patients exhibited significant depression for self-report (M=79.21±21.11) and parent-report (M=82.15±21.76). EDS was endorsed for 25–37% of children. EDS was not significant for parent-proxy outcomes, but was associated with poorer cognitive impairment and internalizing problems (p< .0013). Canonical correlation and multi-step regression demonstrated depression and sleepiness was associated with poorer functioning across all domains.
Conclusions: A substantial number of pediatric HSCT exhibit sleepiness and depression. Depression is associated with statistically and clinically greater functional difficulties, internalizing problems and cognitive impairment. Highlighting the importance of examining sleep and depression and considering interventions to improve alertness in pediatric HSCT.
Key words: Depression, Sleepiness, Cognitive Impairment, Stem Cell Transplant

Ps-105: Posttraumatic Growth and Support among Parents of Children Undergoing Stem Cell Transplantation

Manijeh Firoozi*

University of Tehran, Farabi Pardis, Tehran, Iran
Email: mfiroozy@ut.ac.ir

Bachground and Aim: To investigated the occurrence of posttraumatic growth (PTG) among parents whose children had treatment by stem cell transplantation

(SCT). Although SCT is well established, it remains stressful and demanding procedure for children and parents. Despite pain and stress, parents maybe coping well to SCT in their children.
Methods: 38 parents with a child Undergoing SCT who refer to Mahak (Children's Specialized Hospital) participated in this study. A questionnaire batteries including the Post-Traumatic Stress Disorder (PTSD) Check List—Civilian version and the Post-Traumatic Growth Inventory were sent out to a cross-sectional national sample of parents of children who had had SCT six months or more before the study.
Results: The data were analyzed in relation to parents' appraisal of the event, gender, and perceived social support. The results confirm that SCT in childhood is an event of extreme adversity for the parents. Indications of PTSD were found among an important minority of the parents. Nevertheless, a large proportion of the parents had experienced growth as a consequence of the child's illness.
Conclusion: resiliency and personal strength were the domains with the highest scores. Moreover, a higher level of PTG was correlated with a higher level of posttraumatic stress and with an experience of the trauma as more severe. In summary, the study indicates that PTG is a relevant concept for this group of parents. Future studies must determine whether interventions can bolster PTG in parents and children undergoing SCT.
Key words: Posttraumatic Growth; Parents; Children with Cancer; Stem Cell Transplantation

Ps-106: Isolation and Differentiation Exfoliated Human Deciduous Tooth (SHED) Stem Cells from Dental Pulp

Mohammad Reza Foroughi[1*], Maryam Khoroushi[2], Abbas Ali Khademi[3], Saeed Karbasi[4], Nazanin Vaziri[5]

1. Dental Materials Research Center, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran
2. Dental Materials Research Center and Department of Operative and Art, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran
3. Torabinejad Dentistry Research Center and Department of Endodonics, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran
4. Department of Biomaterials and Tissue Engineering, School of Advanced Technologies in Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
5. Faculty of Science, University of Tehran, Tehran, Iran
Email: foroughi.mr@gmail.com

Background and Aim: The exfoliated human deciduous tooth (SHED) contain multipotent stem cells that identified to be a population of highly proliferative and clonogenic. These cells are capable of differentiating into a variety of cell types including neural cells, adipocytes, and odontoblasts.
Methods: Normal exfoliated human deciduous incisors collected from six- to eleven-years-old children. The pulp was separated from the crown and digested with collagenase. Single cell solutions were cultivated in α-MEM supplemented with ES-FCS. After two to three days, the cells reached confluency and were trypsinized and cultured for further passages. The passage-5 cells were analyzed with CD119, CD14, CD90 and CD146 markers that indicated these cells had a mesenchymal stem cell (MSC) identity. We examined the cells for Alkaline Phosphatase activity to investigate the mesenchymal (stromal) nature. Finally, the cells were differentiated into the osteoblastic and adipocytic lineages in different subcultures and analyzed by RT-PCR and different staining protocols.
Results: Viable cells growing out of the explants showed elongated shapes in clusters. These cells showed alkaline phosphatase activity. Flow cytometry results revealed high expression of pluripotent stem cell markers. In some area of the osteoinductive cultures, nodule-like structures were observed that showed red mineralizing area upon staining with Alizarin Red. In adipogenic cultures, lipid vesicles appeared after five weeks of induction with Oil Red.
Conclusion: This study shows that pulp contains cells with high plasticity and proliferation capacity and can be easily isolated without any serious intervention.
Keywords: Stem Cells, Differentiation, Dental pulp, SHED

Ps-107: Establishment of Induced Pluripotent Stem Cells from Human Foreskin Fibroblast Cell Line by Retroviral Vectors

Meysam Ganjy Bakhsh[1,2], Fereshteh Mehraein[1*], Morteza Koruji[1], Aflatoonian Reza[1], Parvaneh Farzaneh[2], Ahmad Nasimian[2]

1. Department of Anatomy, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
2. Human and Animal cell Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran
Email: Meysam_ganjy@yahoo.com

Background and Aim: Induced Pluripotent Stem Cells (iPSCs) has the potential to accelerate the implementation of stem cells for cell therapy, regenerative medicine and many other cells related studies. So, providing of iPSCs with correct characterization and distinct authentication is required for subsequent studies
Methods: In this study, we used human foreskin fibroblast cell line (HU02 IBRCC10309) as target cells for reprogramming with four transcription factors OCT4, SOX2, KLF4 and c-MYC (Retrovirus vectors). After culturing infected cells on feeder cells, iPS cell colonies were Picked and expanded. Then, these cells were examined by morphological, gene expression, embryoid body formation and quality control tests.
Results: Induced pluripotent stem cells were successfully generated and results showed that these cells expressed high level of stem cell marker such as OCT4, Nanog, SOX2 and human telomerase reverse transcriptase (hTERT). Furthermore, embryoid bodies (EBs) were produced by induced pluripotent stem cells and quality control tests revealed that generated cells had no fungal, bacterial, yeast and Mycoplasma contaminations.
Conclusion: Since the production of iPSC cells is very expensive and time consuming, we suggest that researchers can provide these cells from a valid cell bank instead of spending time and money for production of these cells. The outcome of this project would facilitate and accelerate regenerative medicine researches by using these authenticated induced pluripotent stem cells.
Keywords: Induced Pluripotent Stem Cells, Foreskin Fibroblast, Retroviral Vectors

Ps-108: Standard Identified Mesenchymal Stem Cells Derived from Human Dental Pulp Tissue for Research Services

Neda Sadat Gohari*, Meysam Ganjy Bakhsh, Faezeh Vakhshiteh, Maryam Farghadan ,Fatemeh Mansouri, Parvaneh Farzaneh, Seyed Abolhassan Shahzadeh Fazeli

Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran
Email: nedagohari66@gmail.com

Background: Establishment of valuable mesenchymal cell lines from various sources including primary and wisdom teeth has demonstrated many advances in recent years. Dental pulp is the soft tissue of the tooth that contains mesenchymal stem cells (MSCs). The MSCs derived from dental pulp are capable to differentiate to other cells such as heart muscle (tissue repair damaged heart after a heart attack), neuronal cells (production of nerve and brain tissue), muscle cells for muscle repair, osteocytes (produce bone), cartilage cells, fat cells, and pancreatic cells to treat diabetes. These stem cells are adult stem cells that are found in the primary teeth (teeth in children between the ages 5 to 12) and permanent teeth. Isolation and storage of dental pulp stem cells is useful for medical applications and regenerative medicine. Therefore, we aimed to establish qualified mesenchymal stem cells at human and animal cell bank of Iranian biological resource center (IBRC) to provide services for researchers.
Methods: The stem cells were obtained from dental pulp using both enzymatic and explant techniques. After confirmation of quality controls, characterization and authentication of mesenchymal stem cells were performed by monitoring the growth, morphology, expression of specific molecular markers, and the ability of the cells to differentiate to adipocyte and osteoblast cells.
Results: The isolated mesenchymal cells obtained in this study were fibroblast-like. Flow cytometry analysis showed that isolated MSCs from dental pulp expressed MSC surface markers including CD105, CD29, and CD90 and were negative for the hematopoietic cell surface markers including CD34 and CD45. In addition, the cells were negative for mycoplasma, bacteria and fungi and viral contaminations. The isolated cells were banked at human and animal cell bank, IBRC.
Conclusion: MSCs consider as a valuable source to be applied in tissue engineering and cell-based therapy.

Therefore, the outcome of this research is storaged and maintained the standard mesenchymal cells with related identity documents for MSCs researchers.
Key words: Mesenchymal Stem Cells, Dental Pulp, Regenerative Medicine

Ps-109: Administration of Bone Marrow-Derived Mesenchymal Stem Cells Diminishes Cytolytic CD8 + T Cells and Neuroinflammation in Experimental Autoimmune Encephalomyelitis Mice Model

Amir Ghaemi[1*], Leila Alizadeh[2], Gelareh Vakilzadeh[2], Soodeh Razeghi[2]

1. Department of Virology, Institute Pasteur of Iran, Tehran, Iran
2. Shefa Neuroscience Research Center, Tehran, Iran
Email: ghaem_amir@yahoo.com

Background and Aim: Multiple sclerosis (MS) has been recognized as a common neurodegenerative disease that occurs after an Autoreactive T cells against myelin antigens. Demyelination and inflammation are the main features of this disease. The anti-inflammatory and neuroprotective roles of bone marrow-derived mesenchymal stem cells (BM-MSCs) have been considered as a suitable treatment against autoimmune diseases. Previous studies have shown that treatment with BM-MSCs may regulate immune responses and improve the symptoms in experimental autoimmune encephalomyelitis (EAE) mice, an animal model of multiple sclerosis. Therefore, the present study was designed to evaluate immunomodulatory effects of BM-MSCs in the treatment of myelin oligodendrocyte glycoprotein (MOG) 35-55-induced EAE in C57BL/6 mice.
Methods: MSCs were obtained from the bone marrow of C57BL mice, cultured with DMEM/F12, and characterized with flow cytometry for the presence of cell-surface markers for BM-MSCs. Following three passages, BM-MSCs were injected intraperitoneally into EAE mice. Immunological responses of the transplantation were evaluated.
Results: The results demonstrated that BM-MSCs transplantation in EAE mice significantly reduced inflammation infiltration and demyelination, enhanced the immunomodulatory functions, and inhibited progress of neurological impairments compared to control groups.
Conclusion: This study suggests the potential of BM-MSCs to induce immunomodulatory and anti-inflammatory roles in the treatment of neuroinflammatory disorders.
Keywords: Multiple Sclerosis, Stem Cells, Neuroinflammation, Cytokine, Neuroprotection

Ps-110: X-IAP Gene is Regulated by Micrornas in Glioblastoma Cells

Ghaemi Shokoofeh[1], Arefian Ehsan[1*], Jamshidi Adegani Fatemeh[2], Rezazadeh Rezvan[2], Gheidari Fatemeh[2]

1. Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran
2. Department of Molecular Biology and Genetic Engineering, Stem Cell Technology Research Center, Tehran, Iran
Email: Arefian@ut.ac.ir

Background and Aim: The most aggressive form of brain tumor is glioblastoma (GBM). This tumor has an extremely poor prognosis with a very low survival rate. It is introduced as a grade 4 tumor according to WHO classification. The local invasion of glioblastoma to the surrounding brain tissue makes the complete removal of all tumor cells impossible by surgery. The main cause of malignancy of GBM tumors is that they are within the CNS, leading to physical separation and delivery of the chemotherapy fails. Almost 50% of the patients die within one year and 90% within three years following diagnosis. Each miRNA, by influencing many mRNAs, can control a wide range of biological functions including organ morphology, development, cell differentiation and proliferation, apoptosis and many signaling pathways. Therefore, microRNAs can use as a predicted therapeutic approach to interfere in biological cell functions. In this study, we induced apoptosis in cancer cells by down-regulation of the inhibitor of apoptotic proteins (IAPs) via microRNAs (miRNAs). For the selection of microRNAs that target apoptotic inhibitor genes, we use Tagetscan and Mirwalk databases. Accordingly, we selected hsa-miR-3127 that targeted X-IAP gene in apoptosis pathway.
Methods: hsa-miR-3127 was cloned into a PCDH plasmid and then transfected to HEK-293 cell line via

lipofection method. Virus particles that contain miRNA were collected and used to transduction of U251 cell line of glioblastoma. Then cells' mRNA profiles were analyzed by real-time PCR and the effect of hsa-miR-3127 on treated cells was evaluated.
Results: Real-Time PCR results show a correlation between miRNA and some understudied genes and predict hsa-miR-3127 can be used for induction of apoptosis in glioblastoma cells.
Conclusion: This study considers miRNAs as a promising and novel approach for GBM treatment. But given the problems ahead, further studies are needed to achieve an efficient treatment to reduce tumor recurrence.
Keywords: microRNAs, Glioblastoma, Apoptosis, Inhibitor of Apoptosis Proteins (IAPs)

Ps-111: The Effect of Phagocytosis of Apoptotic Mesencymal Stem Celle by Mouse Peritoneal Macrophage on Nitric Oxide (NO) Production

Maryam Ghahremani Piraghaj[1*], Seyed Mahmoud Hashemi[1], Hosein Ghanbarian[2], Sara Soudi[3]

1. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Faculty of Medical Sciences, Department of Immunology, Tarbiat Modares University, Tehran, Iran
Email: masighahremani@gmail.com

Background and Aim: Apoptotic cells are swiftly engorged by neighbor tissue cells or macrophages (efferocytosis) before they can loose pro-inflammatory intracellular contents. In addition, identification of the apoptotic cells is actively anti-inflammatory process .on the other hand Mesenchymal stem cell (MSC) itself have immunosuppressive functions.
Methods: Four days after thioglycollate stimulation, macrophages were harvested from peritoneal cavity of C57BL/6 mice .Mesancymal stem cells (MSCs) were isolated by enzymatic digestion of adipose tissues that were removed from abdomen of C57BL/6 mice. Mesancymal stem cells were exposed to UV irradiation at 254 nm for 10 min. and followed by culture in DMEM with 10% fetal calf serum for 2 h at 37 °C in 5% CO2 for induction of apoptosis .Apoptotic MSC added to Macrophages at (3: 1) ratio with and without LPS and incubate for 72 hours, after that we used supernatant for NO measurement and in other groups after 24 hours incobation of apoptotic MSC by Macrophages yeast cells was aded in two groups with and without LPS.
Results: We have investigated that phagocytosis of apoptotic MSC (efferocytosis) , limit production of pro-inflammatory mediator such as NO also we find that phagocytosis of yeast by this macrophages was decreased toward to non treatment Macrophages as expectedly
Conclusion: In this study we showed that fagocytosis of apoptotic MSC by Macrophages supres production of inflammatory NO
Keywords: MCS Eferocytosis apoptptic cells

Ps-112: In Silico Analysis of a TanCAR T Cell Targeting CD123 and Folate Receptor Beta

Ali Ghamari[1], Parviz Pakzad[1], Ahmad Majd[1], Marzieh Ebrahimi[2], Amir Ali Hamidieh[3*]

1. Cellular and Molecular Biology Department, Faculty of Life Sciences, Tehran North Branch, Islamic Azad University, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Hematology-Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Iran
Email: aahamidieh@tums.ac.ir

Background and Aim: Acute myeloid leukemia (AML) is the most common leukemia affecting the myeloid lineage of hematopoietic cells. Chimeric antigen receptors (CARs) are artificial molecules that redirect the specificity of T cells to particular antigens. We constructed a CAR in which two different antigen (CD123 and FRβ) recognition domains are present in tandem on a single transgenic receptor that mediates bispecific activation and targeting of T cells. This tandem CAR (TanCAR) recognizes each target molecule exclusively

and facilitates synergistic activation and functionality when both are encountered simultaneously. CD123 is an excellent leukemia-associated antigen given its differential expression in leukemic stem cells and leukemic blasts. CD123 expression on normal cells is low to insignificant. The folate receptor beta (FRβ) is expressed on 70% of primary AML patient tumors, thus making it an attractive target for CAR T-cell therapy. In addition, FRβ expression can be enhanced on AML blasts by treatment with all-trans retinoic acid (ATRA).
Methods: We used computational modeling tools to predict the functionality of a novel single CAR molecule that can mediate bispecific activation and targeting of T cells. The extracellular domain of the TanCAR was designed to include a CD123-specific single chain antibody variable fragment (scFv) followed by a Gly-Ser linker, a FRβ-specific scFv and another Gly-Ser tandem repeat hinge. To determine if the aforesaid molecular arrangement was possible, a TanCAR structural model predicted by modeling webserver, ModWeb. Patchdock and FireDock, software tools for docking and refining two structures based on shape complementarity done and docking of FRβ- and CD123- scFv's to their respective target molecules was simulated. Other important parameters in in-silico designing of the chimeric protein such as Stability, proper energy level and etc. also determined.
Results: From the in silico analysis of this molecule, it showed that the potential interactions of TanCAR with the target molecules could accommodate the planned bispecificity, and as such, an initial model to explore the ability of TanCAR to interface with the target molecules individually predicted. The designed chimeric antigen receptor had stability, proper energy level and same docking as the original scFv's.
Conclusion: New generations of CAR T cell therapy have described recently. One of the attractive methods in this technology is using Bi-specific CAR which can bind to two different antigen separately and enhanced specificity, activity and functionality of recombinant T cells. TanCAR molcules represented both recognition domains in tandem and give T cell this opportunity to facilitate synergistic activation when both are encountered simultaneously. Previous studies have confirmed that CD123 is expressed on the majority of AML cells and leukemic stem cells. , whereas only a few cells in the normal population expressed CD123 weakly. FRβ is expressed on 70% of primary AML patient tumors, thus making it an attractive target for CAR T-cell therapy. The present study confirmed the potency of TanCAR molecule based on CD123 and FRβ as new candidate for AML treatment. Much work is needed to be performed to establish this notion which is the theme of our future research.
Keywords: AML, Chimeric Antigen Receptor, CD123, FRβ

Ps-113: Smart Scaffolds Regulate the Biological Behaviors of Stem Cell Differentiation

Hamidreza Gharibi[1*], Mohammad Mahdi Habibi[2], Amir Abdolmaleki[1,3]

1. Department of Chemistry, Isfahan University of Technology, Isfahan 84156-83111, I. R. Iran
2. Department of Chemistry, University of Tehran, Tehran 14174-66191, I. R. Iran
3. Department of Chemistry, College of Sciences, Shiraz University, Shiraz 71467-135065, I. R. Iran
Email: hamidrezagharibi70@gmail.com

Background and Aim: Efforts to develop engineered various tissues have focused on the use of a biomaterial scaffold and a stem cell source. However, the ideal scaffold microenvironment and development of organized extracellular matrix to regulate proliferation, apoptosis and differentiation of stem cells are unknown. Through thermo-chemical process, fibre and hydrogel scaffolds can be designed with tailorable architectures to mimic the intended tissue by regulating gene expression. Clearly, the development of natural biomembrane such as Eggshell membranes (ESM) and stimuli responsive hydrogels that can be used as mucoadhesives is of significant importance and no attempts have been reported to modification of natural biomembrane as tissue engineering and mucoadhesive drug delivery systems. Herein, functionalized biomembrane using ESM as a model has been synthesized for the first time.
Methods: The modified ESM was made by thermo-chemical modification. The ESM contains amines, hydroxyls, amides, carboxylic and other hydrophilic surface functional groups which anchoring the bioactive molecules to the membrane fiber surface by hydrogen bond linkage where modification can occur. As

the temperature was increased, the adjacent carboxylic acid groups dehydrated to yield a cyclic anhydride. In this study, aligned and randomly oriented scaffolds were prepared using thermo-chemical process on the inner and outer ESMs and the effects of fibre diameter and orientation were examined. Their diameters and appearance reached the standards of tissue-engineered micrometer scaffolds. The microfiber scaffolds were characterized by a high swelling ratio, high porosity and good mechanical properties. Model stem cells were cultured on the different matrix, as well as smooth spin-coated films, and the morphology, growth and expression of their genes were evaluated.
Results: The proliferation of mesenchymal stem cells on novel microfiber scaffolds was obviously enhanced. The proportions of cells in the S and G2/M phases noticeably increased. Moreover, the proliferation rate of mesenchymal stem cells on the aligned microfiber scaffolds was high. The expression levels of cyclin D1 and cyclin-dependent kinase 2 were increased. Bcl-2 expression was significantly increased, but Bax and caspase-3 gene expressions were obviously decreased. The results demonstrated that fibre diameter affects cellular behaviour more significantly than fibre alignment. Initially, cell density was greater on the small fibre diameter mats, but similar cell densities were found on all mats after an additional week in culture. After 2 weeks, gene expression of collagen 1α1 and decorin was increased on all mats compared to films. Expression of the tendon/ligament transcription factor scleraxis was suppressed on all fibre mats relative to spin-coated films, but expression on the large-diameter fibre mats was consistently greater than on the medium-diameter fibre mats. These results suggest that larger-diameter fibres may be more suitable for in vitro development of a tendon/ligament tissue.
Conclusion: There was no significant difference in the differentiation of mesenchymal stem cells into the tendon/ligament lineage on aligned and randomly oriented microfiber scaffolds. These results indicate that novel microfiber scaffolds could promote the proliferation of stem cells and inhibit apoptosis without inducing differentiation. Microfiber scaffolds regulate apoptosis and proliferation in mesenchymal stem cells by altering gene expression.
Keywords: Stem Cells, Tissue Engineering, Oriented Microfiber Scaffolds, Proliferation, Apoptosis and Differentiation, Tendon/Ligament Regeneration, Eggshell Membrane

Ps-114: Is Platelet Rich in Growth Factor (PRGF) Superior to Hyaluronic Acid (HA) in Management of Knee OA? A 6 Months Randomized Clinical Trial Follow Up

Seyed Ahmad Raeissadat[1], Azadeh Gharooee Ahangar[2*]

1. Clinical Development Research Center of Shahid Modarres Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Physical Medicine and Rehabilitation Department, School of medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Azadeh.gh67@gmail.com

Background and Aim: Knee osteoarthritis (OA) is a highly prevalent, chronic condition that causes loss of function with a significant economic cost. The purpose of this study was to compare efficacy and safety of plasma rich in growth factor (PRGF) versus hyaluronic Acid (HA, Hyalgan) in management of mild to moderate knee OA.
Methods: In this single blind clinical trial, we randomly assigned 77 patients with symptomatic knee OA [Kellgren-Lawrence grade II to III] to receive 2 intra-articular injection of PRGF (3 weeks interval) or 3 weekly infiltration of HA. The primary clinical outcome was measured by visual analogue scale (VAS), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Lequesne index prior to treatment, 2 months and 6 months after injections. Success rate has been considered as a 30% decrease in total WOMAC score. Secondary outcome included injection induced complication and patients satisfaction with therapy. This trial was registered in Iranian registry of clinical trials (www.irct.ir) [IRCT2016071513442N11].
Results: The mean age of our study was 58.2± 7.41 year. Statistically significant improvement within pre treatment and follow up values were found for all indices in both groups, although the differences between 2 groups were not significant. Success rates for total WOMAC index was 61.1% in PRGF vs 54.5% in HA group (p=0.631). Patients' satisfaction and minor com-

plication due to injection were similar in both groups, except that higher rate of pain at injection site was recorded in PRGF group.
Conclusion: Based on this study, short-term result of PRGF or HA infiltration are comparable in reducing pain and improvement of function in mild to moderate Knee OA and has not shown any superiority up to 6 months follow up.
Keywords: Osteoarthritis, Knee, Injections, Intra-Articular, Platelet-Rich Plasma, Hyaluronic Acid

Ps-115: Effects of Organic and Inorganic Zinc Ions on the Viability of Bone Marrow-Derived Mesenchymal Stem Cells

Maryam Ghasemzadeh-Hasankolaei[1], Mohammad Ghasemzadeh-Hasankolaei[2], Mohammad Taghi Ghorbanian[3], Mohamadreza Baghaban Eslaminejad[1*]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
3. Department of Cellular & Molecular Biology, School of Biology, Damghan University, Damghan, Iran
Email: maryam.ghasemzadeh@gmail.com

Background and Aim: Chemical approaches are going to be an undeniable part of regenerative medicine and stem-cell-based therapies. Zinc (Zn) is a famous trace element which is essential for many biological activities like cell proliferation and differentiation, and also for gene expression and cell division. It plays an important role in mechanisms of the major metabolic pathways that involve maintenance of physiological balance, protein synthesis and turnover, stabilization of DNA, RNA and ribosome structures. Since, there are so many unknown issues on in vitro effects of Zn on the cells' behavior and function, the present study was fabricated to investigate the effects of different concentrations (0.1, 1, 10, 100, 1000, 10000 µM) of zinc acetate (organic zinc) and zinc sulfate (inorganic zinc) on viability of bone marrow-derived mesenchymal stem cells (BM-MSCs).
Methods: Zinc sulfate and zinc acetate at different final concentrations of 0.1, 1, 10, 100, 1000, 10000 µM were added into complete culture medium. Ram passage-3 BM-MSCs were seeded in 12-well plates. After 24 hours, the medium was changed and cells were incubated with either zinc sulfate or zinc acetate at different concentrations ranging from 0.1 to 10000 µM for one week and two weeks. Control group was treated with culture medium only (DMEM+FBS 15%). The culture medium was changed every 2-3 days. At the end of each time point, MTT assay was employed to determine the impacts of different concentrations of zinc sulfate and zinc acetate.
Results: MTT results revealed that two concentrations of both zinc sulfate and zinc acetate, 1000 and 10000 µM, led to sever cell damage in BM-MSCs. Furthermore, no significant difference was observed between the impacts of zinc sulfate and zinc acetate on BM-MSCs viability. There was not any significant difference between the tested concentrations from 0.1 to 100 µM regarding BM-MSCs viability. On the other hand, the mortality rate in two-week treatment of BM-MSCs increased approximately 25%, in comparison with one-week treatment.
Conclusion: These results demonstrated that zinc at concentrations higher than 100 µM are intolerable for BM-MSCs and cannot be used for treatment. Moreover, it is obvious that the effects of zinc ions on the cells viability are also time- dependent. These results seem to be applicable to other cell types.
Keywords: Mesenchymal Stem Cells, Zinc Acetate, Zinc Sulfate, Cell viability

Ps-116: Derivation of Germ-like Cells from Mouse Amniotic Membrane-derived Mesenchymal Stem Cells

Mohammad Abdollahi[1], Mohammad Amin Rezvanfar[1], Zohreh Afsartala[2], Mahshid Hodjat[1], Shima Tanha[3], Vahideh Assadollahi[4], Khosro bijangi[5], Mohammad Ghasemzadeh-Hasankolaei[6*]

1. Department of Pharmacology and Toxicology, Faculty of Pharmacy and Pharmaceutical Sciences Research Center (PSRC), Tehran University of Medical Sciences (TUMS), Tehran, Iran

2. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
3. Department of pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
4. Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
5. Tashkhis Baft Aragene Company Ltd (TBA), Tehran, Iran
6. Stem Cell Research Lab., Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran
Email: ghasemzadeh78@gmail.com

Background and Aim: In recent years a number of studies have shown the ability of mesenchymal stem cells (MSCs) for differentiation into germline cells. These multi/pluripotent cells have raised great hope for treatment of infertility using cell-based therapies. Amniotic membrane-derived (AM-MSCs) are multipotent cells which can differentiate into various cell types. In the present study, we intended to induce AM-MSCs for differentiation into germ cells (GCs).
Methods: AM-MSCs were isolated from mouse embryonic membrane (at days 13-15 of pregnancy) by enzymatic digestion. After characterization, the cells were induced to differentiate into GCs using a creative two-step method. At first, the cells were treated with 25 ng/ml BMP4 for 5 days and then with 1 µM retinoic acid (RA) for 12 days (total treatment time was 17 days). After the treatment, real-time RT-PCR was performed to evaluate the expression of GC-specific markers- Itgb1, Dazl, Stra8, Piwil2, Mvh, Oct4 and c-Kit. Moreover, flow cytometry and immunofluorescence staining was performed to evaluate the expression of Mvh and Dazl at protein level.
Results: Real-time RT-PCR showed that, except Oct4 which downregulated slightly, all the other tested markers were upregulated in the treated cells. Furthermore, Flow cytometric and immunofluorescence analyses both revealed that a considerable part of the treated cells expressed GC-specific markers. The percentage of positive cells for Mvh was 23% and 46% of the cells expressed Dazl after 17 days of treatment with BMP4 and RA. Our results indicated that, indeed there were a number of germ-like cells in the culture of AM-MSCs which were generated by two step treatment.
Conclusion: Finally, these results create great hope for using AM-MSCs in order to generate GCs and maybe gametes in the laboratory and also their application in cell-based therapies for treatment of infertility. Functionality of the produced germ-like cells and also generation of gametes certainly needs to be further investigated.
Keywords: Amniotic Membrane, Mesenchymal Stem Cells, BMP4, Retinoic Acid, Germ Cells

Ps-117: The Role of Invasive Colorectal Cancer Stem Cell Exosomes in Regulating Tumor Cell Drug Resistance and Invasion

Elmira Gheytanchi[1], Zahra Madjd[2*], Fatemeh Atyabi[3], Roya Ghods[1], Sadegh Babashah[4]

1. Oncopathology Research Center, Iran University of medical Sciences, Tehran, Iran
2. Oncopathology Research Center, Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran
3. Department of Pharmaceutics, Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
4. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
Email: elmira.gheytanchi@gmail.com

Background and Aim: Colorectal cancer (CRC) is a second most common cancer in the world. It has been shown that CRC invasion and metastasis is associated to the communication between tumor cell and bone marrow progenitors through secreted components including chemokines, cytokines, and exosomes. These cascade of signaling events leading to creation of tumor microenvironment (TME) which are suitable for tumor cell homing, rapid progression, angiogenesis, epithelial-mesenchymal transition (EMT) and metastasis. Tumor-derived exosomes are nanometer-sized vesicles (30-100 nm) which function to communicate between cells by transferring proteins and RNA cargo (mRNAs and microRNAs). It has been revealed that exosomes from invasive metastatic cancers including colorectal carcinoma can involve in pre-metastatic niche formation through the educating and reprogramming of bone marrow progenitors to an invasive phenotype. Studies showed that distribution and migration of non-invasive colorectal cancer cells (Caco-2) can increase after treat-

ment with exosomes derived from invasive colorectal cancer (HT-29), respectively in in vivo and in vitro models. Cancer stem cells (CSCs) have been attributed to mediate chemo-resistance, recurrence, invasion, and metastasis of several cancers. In addition, CSCs release exosomes that can be taken up by cancer cells, leading to alterations to their phenotype. The role of CSCs-derived exosomes in the induction of autophagy through the mitochondrial damage has been demonstrated in prostate and breast cancer. The molecular and biological mechanisms of the interactions mediated by CSCs-derived exosomes whiten the cells remain to be further elucidated, especially in colorectal cancer. Therefore the better understanding of how exosomes mediate intercellular communication in the tumor microenvironment will likely propose new opportunities in the development of diagnostic or therapeutic strategies against cancer. Given the prevalence and importance of colorectal cancer as a major cause of patient morbidity and mortality as well as the lack of sufficient data, this study designed to evaluate exosomes released from the colorectal CSCs with the specially focus on the invasion and drug resistance of CSCs derived exosomes.

Methods: CSCs from HT29 cell line were isolated and enriched using sphere formation assay, then identified by potential CSC markers. The HT-29 CSCs, parental, and Caco-2-released exosomes were isolated using differential centrifugation and confirmed by Electron Microscopy and exosomal marekers. Effect of exosome released from HT-29 cell line on drug resistance and invasion characteristics of Caco-2 cell line was also assessed.

Results: Findings of the present study will show the role of exosomes released from CSCs in cancer invasion and drug resistance which are two main characteristics of cancer progression. Therefore, there are still some steps (in vitro/ in vivo experiments) required to be performed for confirmation of our results until these findings can be applied for colorectal cancer patients.

Conclusion: This study plans the concepts concerning the exosomes association in CRC pathogenesis including drug resistance, invasion and metastasis. Moreover, exosomal contents may be useful as promising diagnostic and therapeutic biomarkers of CRC.

Keywords: Colorectal Cancer (CRC), Cancer Stem Cells (CSCs), Exosome

Ps-118: Neural Stem Cell Carriers Loaded with Nanoparticles Containing Super Magnetic Iron Oxide and Doxorubicin for Targeted Treatment of Glioblastoma

Leila Gholami[1], Mohsen Tafaghodi[2], Fateme Moradi[3], Reza Kazemi Oskuee[4*]

1. Department of Modern Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad Iran
3. Department of Anatomy, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
4. Department of Medical Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Email: gholamil902@mums.ac.ir

Background and Aim: Glioblastoma multiforme (GBM) is one the most lethal malignancies in the world, Despite drastic therapies the median survival of patients is about 15 years. The greatest obstacle in the treatment of brain tumors is the blood brain barrier (BBB). To overcome the BBB and low blood flow within brain tumors, different biodegradable materials, polymers and nanoparticles have been developed that can slowly release therapeutics at the tumor site, but low amount of diffusion and inefficient delivery of therapeutics is still remain a major challenge.Neural stem cells (NSCs) have intrinsic glioma-tropic properties, even to distant sites, these cells can also be loaded with therapeutic cargo. In this study, we investigate if the cytotoxic effects of NSC loaded with chitosan NP containing SPION and Doxorubicin, could be used to targeted treatment of brain tumors

Methods: "Neurosphere" cell cultures were prepared from 6 male rat (4-5 week) via published methods. Cells were plated in "neurosphere growth medium" and incubated in (5% CO2, 100% humidity, 37◦C). Neurospheres were evident after 1 week. The primary antibodies including rabbit anti-Rat nestin and mouse anti-Rat SOX2 were used as specific markers for NSC.Characterizations were carried out by Immunofluorescence staining and flowcytometry analysis. For Immunofluorescence staining Nuclei were stained with DAPI, and

the slides were detected using fluorescencemicroscopy. For evaluation Np cytotoxicity, Neurosheres were seeded at a density of 5 ×105 cells/mL. Cell metabolic activity was measured with MTT method after 24 h culture. The in vitro migration of NSCs to Glioma Cells was detected using Transwel lassay. Each well of 24-well cell culture plates was separated in to two chambers by an insert membrane of 8-µm pores. NSCs (1x104 cells in 100 µl of DMEM/F12) were then seeded into the upper chamber. After 6 h of incubation, cells were fixed with 4% PFA. Migrating cells on the bottom of the membrane were stained and quantified under fluorescent microscope

Results: Neurospheres were formed after 3 weeks and charecterazation confirmed by immunofluorescence and flowcytometry.The results show that our NPs can be efficiently internalized in to NSCs while cell viability were not affected. Furthermore, these NP-loaded cells were able to migrate and produce cytotoxicity on C6 glioma cells in vitro.

Conclusion: The results of this study showed that NSCs couldserve as efficient cellular carriers for NPs in brain tumors. These promising results open up a new field of treatment in which cellular vehicles and nanoparticles can be combined to treat brain tumors.

Keywords: Neural Stem Cell Nanoparticles Glioblastoma

Ps-119: Synthesis of Injectable Natural Polymer Compound for Tissue Engineering of Intervertebral Disc

Masoud Ghorbani, Jafar Ai[1], Mohammad Reza Nourani[2], Mahmoud Azami[1], Batool Hashebmi Beni[3], Mohammad Vasei[4*]

1. Department of Tissue Engineering and applied cell sciences, Faculty of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Chemical Injury Research Center (CIRC), Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Department of Anatomical and Molecular Biology Sciences, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
4. Department of Pathology and Digestive disease research Institute Shariati Hospital Tehran University of Medical Sciences, Tehran, Iran
Email: yasin.ghorbani93@yahoo.com

Background and Aim: Intervertebral disc degeneration is recognized to be the leading cause for chronic low-back pain .Injectable hydrogel is one of the great interests for tissue engineering and cell encapsulation especially for intervertebral (IVD) affecting rate of regeneration success.The goal of this study was to prepare and assess natural compound hydrogel for regeneration of NP on IVD invitro.

Methods: Chitosan-based hydrogel was made in the ratio of 1.5%: 7%: 1%:1%:1%-1.5%-1% (Ch:β-GP:HA-CS-Col-Ge-FS). Gelation time and other rheological properties were studied using amplitude sweep and frequency sweep tests. Also, the cytotoxicity of the hydrogel invitro assessed by MTT and trypan blue tests. Morphology of the hydrogel was evaluated by SEM

Results: result showed that NP hydrogel in 4oC is an injectable transparent solution. It started gelation in 37oC after about 30 min. Gelation temperature of NP hydrogel was 37Oc. Storage modulus (G′) of this hydrogel at 37 oC was almost constant over a wide range of strain.MTT and trypan blue tests showed hydrogel was cytocompatible

Conclusion: The obtained results suggest that this hydrogel would be a natural Choice as an injectable scaffold for using in vivo study of intervertebral disc regeneration.

Keywords: Chitosan-β Glycerophosphate- Hyaluronic Acid, Collagen, Chondroitin Sulfate, Gelatin, Fibroin Silk, Intervertebral Disc, Thermoresponsive, Injectable

Ps-120: Ovarian Cancer Cell Exosomes Promote Cell Proliferation and Migration of Human Umbilical Vein Endothelial Cells

Mohammad Ghorbanian, Sadegh Babashah*

Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
Email: m.ghorbanian71@gmail.com

Background and Aim: Purpose: Ovarian carcinoma (OC) is the most lethal gynecological cancer, rank-

ing fifth in cancer-related deaths among women in the Western. Exosomes have a diameter of 30–100 nm and have protein-specific markers such as Alix, Tsg101, tetraspanins such as CD9, CD63 or CD81. Exosomes can help the cancer to progress and disseminate by manipulating the local and distant biological environment. It has been established that cancer cells-derived exosomes could regulate intracellular communications. Establish of vessels network is an important in tumor progression. Here we investigated the effects of cancer cells-derived exosomes on migration and the proliferation of endothelial cell HUVEC in vitro.

Methods: Materials and Methods: Exosomes were isolated from ovarian cancer cells conditioned media by serial centrifugation. Scanning electron microscopy (SEM) was used to characterize exosomes. The concentration of exosomes was quantified by Bradford assay. HUVECs were treated with various concentrations of OC-derived exosomes or carrier control (PBS). Viable cells were counted by trypan blue exclusion. Wound healing assay done on cells treated with exosomes to pursue migration of HUVECs

Results: Results: SEM analysis showed that exosome size was within the characteristic diameter range of 30-100 nm. Utilization of PKH labeling method proved uptake of exosomes by endothelial cells. It appears that OC-derived exosomes promote the proliferation of HUVEC cells. We monitored that the endothelial cells migrated into the wound area via proliferation, the cells moving across the wound line.

Conclusion: Discussion: These findings may clarify, in part, the role of ovarian cancer cell exosomes in endothelial cells biology. We postulated that exosomal transfer of various genetic contents to endothelial cells might be an important event for cell-cell communication within the tumor microenvironment.

Keywords: Exosome, Ovarian Cancer Cell, Human Umbilical Vein Endothelial Cell, Cell Proliferation, Migration.

Ps-121: Effect of Mesenchymal Stem Cell Therapy on TH-17 and Regulatory T-Cells in Patients with Refractory Rheumatoid Arthritis

Mohsen Ghoryani[1], Mojgan Mohammadi[2*], Zhaleh Shariati-Sarabi[3], Jalil Tavakol-Afshari[1], Ali Ghasemi[4]

1. Immunology Research Center, Avicenna Research Institute, Faculty Of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Allergy Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Internal Medicine Department, Imam Reza hospital, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Bone marrow stem cell transplantation, Faculty Of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Email: ghoryanim931@mums.ac.ir

Background and Aim: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease affecting 0.5-1% of adults. The imbalance between regulatory T cells (T-regs) and TH-17 cells has been reported in RA. The number of TH-17 cells increases in the peripheral blood and a high level of IL-17 have been reported in serum and synovial fluid of patients with RA. Different studies show that the number and function of T-regs reduces in peripheral blood of patients with RA. Conventional therapies for RA include disease-modifying anti-rheumatic drugs (DMARDs) and in severe conditions, administration of biological agents. Conventional therapies have minor and serious adverse effects and biological agents increase risk of infections. Additionally, some patients gradually develop resistance to this therapeutic method. Due to complications associated with conventional therapy, new therapeutic approaches such as stem cell therapy have been considered in the recent years. The aim of this study is to evaluate the effect of intravenous administration of autologous bone marrow derived mesenchymal stem cells on the number of T-regs and TH-17 cells in refractory RA patients.

Methods: Five refractory RA patients who received conventional therapy with no other rheumatologic disorders and inflammatory diseases, with a mean age of 47 years were included in this study. Autologous bone marrow derived mesenchymal stem cells transfused to RA patients intravenously. To assess the effects of mesenchymal stem cell therapy on T-regs and TH-17 cells, flow cytometric staining performed using monoclonal antibodies against the markers of these cells. This study was a phase 1 clinical trial and IRCT code of this study is: IRCT2015102824760N1.

Results: One month after mesenchymal stem cell transfusion, a non-significant increase was observed in the

percentage of both T-regs and TH-17 cells compared to the baseline.
Conclusion: Although our results showed insignificant change in the percentage of TH-17 and T-regs, we need to increase the sample size and monitor these cells in additional time points in order to get a better conclusion.
Keywords: Rheumatoid Arthritis, Mesenchymal Stem Cells, Regulatory T-Cells, TH-17 Cells

Ps-122: Bi-Layer Conductive ESM-PCLF/ PVA-Alg: Gr Electrospun Fibrous Conduit for Neural Tissue Engineering

Nasim Golafshan[1*], Mahshid Kharaziha[1], Mohamadhossein Fathi[2]

1. Department of Materials Science, Isfahan University of Technology, Isfahan, Iran
2. Dental Materials Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
Email: golafshan.na@gmail.com

Background and Aim: Trauma injuries often result in peripheral nervous system damages and, therefore autologous nerve graft has been introduced as a gold standard treatment technique. Nevertheless, the clinical obstacles associated with autologous nerve grafts such as the limited availability of donor nerves and extended surgeries, led to the development of alternative treatment techniques such as tissue engineering approach. Tissue engineering of nerve grafts requires synergistic combination of scaffolds and cells in order to promote and direct neurite outgrowth for effective nerve regeneration. Fibrous conduits with aligned nanofibers have been shown to promote nerve regeneration, however, current fabrication methods rely on rolling a fibrous sheet into the shape of a conduit and suture to the nerve, which results in a graft with inconsistent size and a discontinuous joint or seam due to the low mechanical properties of aligned fibers. To overcome this problem, bi-layer nerve guidance conduit were developed in this research.
Methods: Here we developed a novel bi-layer nerve guidance conduit fabricated of a double network Eggshell membrane-Polycaprolactone fumarate (ESM-PCLF) as outer layer for mechanical support and aligned electrospun Polyvinyl alcohol-Alginate: Graphene (PVA-Alg: Gr) as inner layer to promote nerve regeneration by contact guidance mechanism and electrical conductivity of Graphene. To improve the adhesion between the ESM-PCLF layer and PVA-Alg: Gr aligned fibers, the top of the woven layer was briefly brushed with Tetrahydrofuran (THF). The PCL fibrous layer was then placed on the woven component. Double network ESM-PCLF layer was then layered above and the components were maintained together at 100 C for 30 min. At that temperature, two layer adhere to each other.
Results: In vitro studies by Dapi staining and MTT assay which demonstrated that bi-layer nerve conduits were superior to aligned fibrous conduits and the number of attached cells and proliferation of PC12 cells were 1.7 and 1.5 times greater than aligned fibrous membrane after 7 days. In summary, the present research showed that the bi-layer fibrous conduit had a significant impact on neural tissue regeneration due to the superior mechanical properties and biological behavior. Furthermore, the elastic modulus and toughness of bi-layer conduit were 1.7 and 2.1 times greater than aligned PVA-Alg: Gr, respectively.
Conclusion: It is envisioned that the offered bi-layer ESM-PCLF/PVA-Alg: Gr nerve guidance conduit might have great potential to develop the devices for peripheral nerve regeneration.
Keywords: Bi-layer Nerve Guidance Conduit, Graphene, Aligned Fibers, Double Network Membrane

Ps-123: Metformin Adversely Controls Epithelial-Mesenchymal Transition-Involved Long Noncoding RNAs in Breast Cancer Cell Line

Mahsa Golshan*, Saeedeh Khaleghi, Shiva Valaee, Mehdi Shamsara

Department of Animal Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: m.golshan1393@gmail.com

Background and Aim: Breast cancer causes the most cancer-related death among women in the world. Epithelial-mesenchymal transition (EMT) plays an important role in cancer progression. It has been ruled as the

main mechanism for breast cancer metastasis. Overexpression of long noncoding RNAs (lncRNAs) has been reported previously in cancers. They have critical roles in control of EMT. H19 and HOTAIR (HOX transcript antisense RNA) are two important LncRNAs involved in EMT during tumors' development. High expression of these LncRNAs has been reported in breast cancer cell lines, like MDA-MB-231. Metformin (dimethylbiguanidine) is the most commonly used therapy in patients with type II diabetes. Emerging evidences suggest metformin as an off-labeled drug in cancer treatment partly through inhibition of EMT. In this study, therefore, we investigated the effect of metformin treatment on EMT markers and behavior of MDA-MB-231 cell line and also expression of H19 and HOTAIR.
Methods: Human breast cancer cell line MDA-MB-231 was cultured in DMEM/F12 low glucose containing 10% FBS and treated in three different times 24, 48 and 72 hours with 10 and 20 mM of metformin. Cell migration and invasion behaviors of the cells due to metformin treatment were assayed by wound-healing and trans-well tests, respectively. The changes of H19 and HOTAIR levels were quantified in metformin treated and control samples using real-time PCR. B2-microglobulin (B2M) gene was applied as the housekeeping gene for normalization of the real-time data. The changes in level of target genes were calculated by 2-ΔΔCt formula. The significance of the results was studied statistically using t-test experiment at the p-value ≥ 0.05.
Results: The results of cell migration using wound-healing test showed that the cells in metformin treated cultures migrated more slowly compared to untreated one (control). Invasion assay of MDA-MB-231 cells also gave the results in line with the cell migration in the metformin-received wells. RNA extraction from treated and control cells was followed by reverse transcription and real-time PCR. Gene expression quantification showed the reduction of H19 and HOTAIR levels after receiving metformin.
Conclusion: Recent studies indicated metformin anti-cancer activity through inhibition of EMT. Our results in consistence with these reports showed the inhibition of EMT phenotype in the metformin-exposed MDA-MB-231 cells. EMT indicates by the increased level of cell motility, which in turn facilities cancer cell metastasis. At the molecular level, EMT is along with changes in expression of coding and non-coding genes. In this regard, we measured the level of two EMT-involved lncRNAs, including H19 and HOTAIR, by real-time PCR and found their reduction in response to metformin. This finding maybe explain one of the mechanisms, by which the metformin downregulate EMT in breast cancer.
Keywords: Breast Cancer, Metformin, HOTAIR, H19, Lncrnas

Ps-124: Mir-33 Manipulation via Autophagy Induction Reduces High Glucose Induced Denovo-Lipogenesis in Hepg2 Cells

Sattar Gorgani-Firuzjaee[1*], Masomeh Gorgani-Firuzjah[2]

1. Department of Medical Laboratory Sciences, School of Allied Health Medicine, AJA University of Medical Sciences, Tehran, Iran
2. Department of Biochemistry, Faculty of Biology, Payamnoor University, Taft Branch,Yazd, Iran
Email: gorgani59@gmail.com

Background and Aim: Type two diabetes (T2D), a significant health epidemic, is currently affecting populations of the developed and developing countries worldwide. This epidemic is accompanied by a cluster of metabolic disorders including metabolic syndrome, obesity, insulin resistance, glucose intolerance, hypertension and dyslipidemia. Due to the importance of miRNAs in gene expression pastern and their role in metabolic diseases such as diabetes, effects of miR-33 manipulation by induction of exogenous miR-33 mimic sequences on high glucose-induced lipogenesis studied.
Methods: HepG2 cells were transfected with 30 nm miR-33 mimic or negative control oligonucleotides by lipofectamin as manufacturer guideline. All experimental control samples were treated with an equal concentration of a non targeting control mimic sequence to control non-sequence-specific effects in miRNA experiments. In order to induce lipogenesis the cells treated by 33mM glucose and manitol as osmotic control. Lipogenesis analyzed by oil red O staining, FAS, ACC and SREBP1C mRNA and protein expression respectively by real time PCR and western blotting. Au-

tophagy pathway assayed by LC3B, ATG5 and ATG7 protein and Beclin mRNA expression.
Results: The results show that exogenous miR-33 mimic sequence induced autophagy and reduced lipogenesis in HepG2 cells. Autophagy induction by rapamycin reduced lipogenesis and autophagy inhibition by chloroquine, enhanced lipogenesis in HepG2 cells.
Conclusion: These findings suggest that lipid lowering effects of miR-33 is autophagy dependent in hyperglycemia situation which was seen diabetes.
Keywords: Mir-33, Autophagy, Insulin Resistance, High Glucose Induced Lipogenesis

Ps-125: Autophagy Modulation in Bone Marrow-Derived Mscs (BM-Mscs) As a Novel Strategy to Accelerate Liver Restoration in Mouse Models of Acute Liver Failure

Mehryar Habibi Roudkenar[1*], Sedigheh Molaei[2]

1. Department of Medical Biotechnology, Paramedicine Faculty, Guilan University of Medical Sciences, Rasht, Iran
2. Department of Biotechnology, Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
Email: mhr376@yahoo.com

Background and Aim: Mesenchymal stem cells (MSCs) are under intensive investigation for use in cell-based therapies. Nevertheless, major impediments to their therapeutic application, such as low proliferation and survival rates remain as obstacles to broad clinical use of MSCs. Indeed, oxidative stress-mediated MSC depletion occurs due to inflammatory processes associated with chemotherapy, radiotherapy, and expression of pro-apoptotic factors, and the microenvironment of damaged tissue in patients receiving MSC therapy is typically therapeutic not favorable to their survival. For this reason, any strategies that enhance the viability and proliferative capacity of MSCs associated with their therapeutic use are of great value.
Methods: Autophagy was induced in bone marrow-derived MSCs (BM-MSCs) by rapamycin, and was inhibited via shRNA-mediated knockdown of the autophagy specific gene, ATG7. ATG7 expression in BM-MSCs was evaluated by reverse transcription polymerase chain reaction (RT-PCR), western blot, and quantitative PCR (qPCR). ALF was induced in mice by intraperitoneal injection of 1.5 ml/kg carbon tetrachloride. Mice were intravenously infused with MSCs, which were suppressed in their autophagy pathway. Blood and liver samples were collected at different intervals (24, 48 and 72 h) after the transplantation of MSCs. Both the liver enzymes and tissue necrosis levels were evaluated using biochemical and histopathological assessments. The survival rate of the transplanted mice was also recorded during one week.
Results: Biochemical and pathological results indicated that 1.5 ml/kg carbon tetrachloride induces ALF in mice. A significant reduction of liver enzymes and necrosis score were observed in autophagy-modulated MSC-transplanted mice compared to sham (with no cell therapy) after 24 h. After 72 h, liver enzymes reached their normal levels in mice transplanted with autophagy-suppressed MSCs. Interestingly, normal histology without necrosis was also observed.
Conclusion: Autophagy suppression in MSCs ameliorates their liver regeneration potentials due to paracrine effects and might be suggested as a new strategy for the improvement of cell therapy in ALF.
Keywords: MSC, Liver injury, Autophagy, Stress

Ps-126: NGAL-Engineered Mesenchymal Stromal Cells as a Novel Strategy to Ameliorate Acute Kidney Injury: An In Vivo Study

Mehryar Habibi Roudkenar*

Department of Medical Biotechnology, Paramedicine Faculty, Guilan University of Medical Sciences, Rasht, Iran
Email: mhr376@yahoo.com

Background and Aim: Acute kidney injury (AKI) is one of the most common health- threatening diseases in the world. There is still no effective medical treatment for AKI. Recently, Mesenchymal stromal cell (MSC)-based therapy has been proposed for treatment of AKI. However, the microenvironment of damaged kidney tissue is not in favorable for survival of MSCs which would be used for therapeutic intervention. Aim: It was hypothesized that MSCs overexpressing NGAL would enhance cell survival after transplantation and restore kidney function

Methods: In this study, we genetically manipulated MSCs to up-regulate lipocalin-2 (Lcn2) and investigated whether the engineered MSCs (MSC-Lcn2) could improve cisplatin-induced AKI in a rat model.
Results: Our results revealed that up-regulation of Lcn2 in MSCs efficiently enhanced renal function. The MSC- Lcn2 up-regulates expression of HGF, IGF, FGF and VEGF growth factors. In addition, they reduced molecular biomarkers of kidney injury such as KIM-1 and Cystatin C, while increased the markers of proximal tubular epithelium such as Aqp-1 and CK18 following cisplatin-induced AKI.
Conclusion: Overall, here we over-expressed Lcn2, a well-known cytoprotective factor against acute ischemic renal injury, in MSCs. This not only potentiated beneficial roles of MSCs for cell therapy purposes, but also suggested a new modality for treatment of AKI.
Keywords: Mesenchymal Stromal Cells, Lipocalin 2/ NGAL, Acute Kidney Injury, Cell Therapy

Ps-127: Development of Anti-CD47 Single-Chain Variable Fragment Targeted Magnetic Nanoparticles for Treatment of Human Bladder Cancer

Mahdi Habibi-Anbouhi*, Gashin Rezaei, Mohamad Ali Shokrgozar

National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran
Email: habibi.mha@gmail.com

Background and Aim: Bladder cancer is defined as the seventh most common cancer type in men and the 17th most common cancer in women, globally. Even though current treatments have been effective in a certain timeframe, there is usually a risk of recurrence of the bladder tumor. Recent reports have revealed a subset of the heterogeneous cell population of tumor cells with high resistance to various treatments, known as cancer stem cells (CSCs). Following CSCs isolation and characterization, function and types of their specific biomarkers have been well identified. Among known molecular profile items, CD47 as a surface biomarker is an important target in bladder CSCs and based on previous studies, its expression level is remarkably increased in bladder tumor cells, in particular, CSCs, and hence can be of great significance in identification and treatment applications. scFv with a size of about 1/6 of the whole antibody is the smallest functional VH–VL domain for binding to antigens. scFvs with a molecular weight of 26–28 kDa, joined via a flexible and hydrophilic peptide linker are usually produced by phage display technique, in which the intended scFv with desired binding property, as a genetic fusion with a bacteriophage coat protein, is selected from a library of diverse variants. Besides, advantages such as less immunogenicity, having the potential for greater tissue penetration, and more rapid clearance from non-specific tissues have introduced scFv as an attractive candidate for clinical applications. On the other hand, it can be even more impressive when being accompanied with effective materials such as magnetic nanoparticles (MNPs) for direct targeting, detecting tumor cells with high resolution and destroying them in particular circumstances such as generating local hyperthermia by MNP with safely applied magnetic fields. Previous studies have shown that MNP as a drug delivery system can execute scFv functionality in this context. This study was designed to develop a novel anti-CD47 single-chain variable fragment (scFv) functionalized magnetic nanoparticles (MNPs) for targeting bladder cell lines and its applicability in thermotherapy.
Methods: An immunized murine antibody phage display library was constructed and screened to isolate anti-CD47 binders. A scFv was selected and conjugated to MNPs which was then utilized to discriminate CD47+ bladder cells along with assessing its efficacy in thermotherapy.
Results: A scFv with high affinity to bladder cells was efficiently conjugated to MNPs. Following a hyperthermia treatment, the function of scFv–MNP conjugates led to a considerable reduction in cell viability.
Conclusion: The anti-CD47 scFv–MNP conjugate was an effective cancer cell thermotherapy tool that might pave the way for development of bionano-based targeting techniques in both early detection and treatment of cancer.
Keywords: Cancer Stem Cell, Bladder Cancer, CD47, Magnetic Nanoparticle, Phage Display, scFv, Thermotherapy

Ps-128: A Chimeric T Cell Antigen Receptor (CAR) that Target VEGFR-2 Expressing Cells

Fatemeh Hajari Taheri[1], Mahmoud Hassani[2], Mohsen Abolhassani[1*], Arash Arashkia[3], Zahra Sharifzadeh[1], Mehdi Behdani[4]

1. Hybridoma Lab, Immunology Department, Pasteur Institute of Iran, Tehran, Iran
2. Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Molecular Virology, Pasteur Institute of Iran, Tehran, Iran
4. Biotechnology Research Center, Venom & Biotherapeutics Molecules Lab., Pasteur Institute of Iran, Tehran, Iran
Email: F.h.taheri@gmail.com

Background and Aim: Designer T cells are T lymphocytes engineered toward specific antibody-type membrane antigens through chimeric antigen receptor (CAR) and are mainly used for adoptive cellular immunotherapy. The designer CAR T cell could be used as a valuable approach for inhibition of tumors by redirecting the T cells against markers of tumor angiogenesis. In this study, we describe the development and characterization of a novel specific designer CAR T cell against vascular endothelial growth factor receptor 2 (VEGFR2) as a tumor angiogenic marker.
Methods: T-cell line (Jurkat) was electroporated with the chimeric anti-VEGFR2 construct comprising antigen binding domain against VEGFR2, Fc domain of IgG1 as spacer, transmembrane and endodomain of CD28 that were linked to signaling domain of OX40 and CD3ζ. Then T cells were analyzed for CAR expression. The specific activation was analysed by co-culturing of CAR T cell with VEGFR2-expressing cells (KDR) in vitro.
Results: T cells expressing this CAR can effectively target VEGFR2-positive cells. We show that VEGFR2-specific T cells produce the large amount of immunostimulatory cytokines such as IFN-γ(308pg/ml) and IL-2(1900pg/ml) when co-cultured with VEGFR2-positive targets and proliferate more vigorously on VEGFR2-expressing cells.
Conclusion: The anti-VEGFR2 designer T cells exhibited an antibody-type specificity that could recognize VEGFR2-expressing cells in a MHC-independent manner, resulting in T-cell activation and proliferation. This redirected T cell provides a potential method for the gene therapy of a variety of solid tumors.
Keywords: Chimeric Antigen Receptor, VEGFR2, Designer T cell

Ps-129: A New Approach in Gene Therapy of Glioblastoma Multiforme

Mahmoud Reza Haji Ghasem[1,2*]

1. Brain and Spinal Cord Injury Research Center, Imam Khomeini Hospital, Tehran, Iran
2. Department of Neuroscience, School of Advanced Technologies in Medicene, Tehran University of Medical Sciences, Tehran, Iran
Email: Mhadjighassem@tums.ac.ir

Background and Aim: Gliobalstoma multiforme (GBM) is a grade 4 astrocytoma tumor in central nervous system. This tumor has ability to grow and metastasis to the surrounding healthy brain tissue. It is the most malignant form of astrocytoma tumor. The median survival rate of patients with GBM is 6–12 months.Present treatments for GBM include surgical resection, chemotherapy, radiotherapy, and combination of surgical resection-radiotherapy. In despite of these treatments of invasive, patients' survival may increase several months.in this study, we investigate whether human olfactory ensheathing cells can be used as a cell source for the first time in gene delivery to assay the tumoricidal effect of herpes simplex virus thymidine kinase gene (HSV-tk) on glioblastoma multiforme (GBM).
Methods: We obtained OECs from superior turbinate of human nasal cavity mucosa, and cell phenotype was confirmed by the expression of cell-specific antigens including low-affinity nerve growth factor receptor (p75 neurotrophin receptor), microtubuleassociated protein-2 (MAP2), and S100 calcium binding protein B (S100-beta) using immunocytochemistry. Then, these cells were transduced by lentiviral vector for transient and stable expression of the herpes simplex virus thymidine kinase gene (OEC-tk). The migratory capacity of OEC-tk, their potency to convert prodrug ganciclo-

vir to toxic form, and cytotoxic effect on astrocyte cells were assayed in vitro
Results: Our results indicated that OECs-tk were able to migrate toward primary cultured human glioblastoma multiforme and affected survival rate of tumor cells according to exposure time and concentration of ganciclovir. Also, OECs-HSV-tk was capable of inducing apoptosis in tumor cells.
Conclusion: Our findings suggest that human OECs could employ as a possible tool to transfer anticancer agent in gene therapy of brain tumor.
Keywords: Olfactoryensheathingcells, Cell Migration, Gene Therapy, Glioblastoma Multiforme

Ps-130: Targeting the Transforming Growth Factor-B Signaling During Pre-Implantation Development in Embryos of Cattle, Sheep and Goats

Mehdi Hajian, Mohammad Hossein Nasr-Esfahani*

Department of Reproduction and Development, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mehdihajian2002@gmail.com

Background and Aim: Recently, application of chemical inhibitors against differentiation signaling pathways have improved the establishment of mESCs.
Methods: In this study, we applied inhibitors of TGFβ (SB431542) and BMP4 (Noggin) from the cleavage to blastocyst stage in cattle, goat and sheep embryos.
Results: SB significantly decrease blastocyst rate and total cell number (TCN) in sheep blastocysts, whereas only TCN was significantly decreased in cattle blastocysts. In contrast to SB, Noggin significantly improved cattle blastocyst development but decreased TCN. However, Noggin treatment led to a significant increase TCN in sheep blastocysts. Regarding pluripotency triad (OCT4, NANOG, SOX2) and cell lineage commitment (REX1, CDX2, GATA4), SB led to a significant reduction in SOX2 expression in goat and cattle, while Noggin increased at least one or two of pluripotent markers in these species.
Conclusion: Taken together, this data suggests that inhibition of TGFβ by Noggin may be more favorable for derivation of stem cells in farm animals.
Keywords: Blastocyst Formation, Small Molecule, Embryonic Stem Cell, TGFβ, BMP4

Ps-131: Differentiation of Umbilical Cord Derived Mesenchymal Stem Cell in Hydrogel Scaffold for Cartilage Tissue Engineering

Anneh Mohammad Gharravi*, Mahtab Hajian, Melika Ghorbanian

1. Stem Cells and Tissue Engineering Research Center, Shahroud University of Medical Sciences, Shahroud, Iran
2. Student Research Committee,School of Medicine, Shahroud University Of Medical Sciences, Shahroud, Iran
Email: annehgh@yahoo.com

Background and Aim: Umbilical cord (UC) contains a high number of primitive progenitor cells, can differentiated into several organs and be used as a source for clinical transplantation. The aim of present study was isolation and expansion of umbilical cord in 3D environment inside perfusion chamber and differentiation into chondrocyte like cells
Methods: After removal of umbilical cord vessels, the wharton's jelly component, placed in a 37°C incubator. During subculture, they were passaged every 5 days; the medium was replaced every 3-4 days or twice a week. For flow cytometry, the cells were incubated for 30 min at 4◦C with 5 µl the monoclonal antibodies anti-human antibodies against CD105, CD90, CD34, and CD45 in dark. The culture chamber was made from Plexiglas and housed the cell/scaffold constructs. Differentiation of MSCs in 3D scaffold in the culture chamber performed in on step protocol. Then cartilage like construct were examined histologically and by immunohistochemistry methods
Results: Under direct perfusion flow, umbilical cord dived stem cell seeded in hydrogel expanded and proliferated efficiently. There was significant correlation between time of digestion and count cell expansions. Isolated Cells were positive for MSC surface biomarkers and negative for haematopoietic lineage. UC-MSCs embedded in alginate hydrogel can undergo chondrogenesis and expressed significantly collagen II. The cartilage-like tissue had metachromatic territorial matrix with lacuna housed chondrocyte like cells.

Conclusion: In conclusion, the results showed that hydrogel scaffold and perfusion bioreactor were sufficient for expansion and differentiation of UCB, promoting engraftment of UCB derived stem cell.
Keywords: Umbilical Cord, Mesenchymal Stem Cell, Hydrogel Scaffold, Cartilage Tissue Engineering

Ps-132: OCT4B1 Suppression, Down-Regulated BCL2 Gene Family in Human Tumor Cell Lines

Hasti HajJafari[1], Mohammad Reza Mirzaei[2*], Pouya Abedi[1]

1. Rafsanjan University of Medical Science, Dental School, Rafsanjan, kerman,Iran
2. Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
Email: hasti.hh.96@gmail.com

Background and Aim: The OCT4B1, a new discovered variant of OCT4 is expressed more than other variants in both human cancer cell lines and tissues. New finding showed this variant has anti-apoptotic potency in mentioned cells and tissues. BCL2 family is one of the twelve gene families involved in apoptosis pathway with negative control in apoptosis recurrence. The aim of the present study was to investigate the effects of OCT4B1 silencing on several genes of BCL2 family in human tumor cell lines.
Methods: three human tumor cell lines; AGS (gastric adenocarcinoma), 5637 (bladder tumor) and U-87MG (brain tumor) were transfected with specific OCT4B1 siRNA and a scrambled sequence as control, using Lipofectamine 2000 comerical kit. Following, The expression rate of BCL2 gene family transcripts were evaluated, using a human apoptosis panel-PCR kit.
Results: Expressional profile of the studied BCL2 transcripts in three cell lines is almost similar. Nineteen of twenty one studied genes in BCL2 family showed down-regulation, forteen gene were decreased in expression more than 3 and three genes (BAD,BCL2 and BNIP3L) more than 10 folds. BCLAF1 showed up-regulation (in U87MG and 5637 tumor cell lines) and MCL1 showed unchanged gene expression.
Conclusion: According to these results, it may be concluded that OCT4B1 suppression can lead to apoptosis in tumor cell lines via down-regulation of several BCL2 transcripts. Thus, OCT4B1 suppression effects on BCL2 may be considered as promising target genes in future studies in cancer research and therapy
Keywords: OCT4B1, BCL2 Gene Family, Tumor Cell Lines

Ps-133: Investigation Analysis the Affective Mirnas Regulating the Differentiation of Basophil

Pouria Hajmomeni, Sadat Dokanehiifard*

Department of Biology, School of Sciences, Razi University, Kermanshah, 67149-67346, Iran
Email: zm.hmomeni@yahoo.com

Background and Aim: Basophils are one of the immune cells from myeloid cell line which derived from hematopoietic stem cell. They are containing granules with enzymes that are released during the allergic reaction. Although Basophils constitute less than 1% of blood leukocytes, but the count of them can increase in infection and inflammatory diseases. Basophil's importance in host defense and allergic reaction isn't known, because their numbers in tissues aren't high. Basophil differentiation pathway from hematopoietic stem cell (HSC) include of three effective genes that expression them can regulate by three effective miRNAs.in recent research, we have analyzed the effective miRNAs that control differentiation basophil from hematopoietic stem cell. For this, a list of 3 effective genes which propagation the differentiation of basophil was provided as reported in KEGG pathway database. All possible miRNAs which target 3'UTR of these genes were predicted separately by using miRWalk and miRmap tools, and then sorted based on the miRNAs which target common sequences. Here, we predicted 3 possible miRNAs which target 3 genes in this pathway.
Methods: we have analyzed the effective miRNAs that control differentiation basophil from hematopoietic stem cell. . For this, a list of 3 effective genes which propagation the differentiation of basophil was provided as reported in KEGG pathway database
Results: All possible miRNAs which target 3'UTR of these genes were predicted separately by using miRWalk and miRmap tools, and then sorted based on the

miRNAs which target common sequences. Here, we predicted 3 possible miRNAs which target 3 genes in this pathway.
Conclusion: Here, we predicted 3 possible miRNAs which target 3 genes in this pathway.
Keywords: Basophil Differentiation, MiRNA, Bioinformatics

Ps-134: Synthesizing of Nanostructured Surface on Magnesium Alloy by Surface Mechanical Attrition Treatment for Tissue Engineering Application

Mahsa Hasani Anzabi, Mehrdad Khakbiz*

Biomedical Engineering Division, Life Science Engineering Department, Faculty of New Sciences and Technologies, Universityof Tehran, Tehran, Iran
Email: mahsa.hassani@ut.ac.ir

Background and Aim: Magnesium alloys are materials with high strength, low density, good biocompatibility in body and young modulus similar to bones. Thus, it can be an appropriate option as biodegradable metal for tissue engineering applications. In other hand, nanostructured metals are capable material for different application and recently showed specific properties in regenerative medicine as metallic implant. However, synthesizing and characterization of this material are in their infancy step. The aim of this study is synthesizing nanostructured surface on AZ31 magnesium alloy by surface mechanical attrition treatment (SMAT) in order to optimize surface properties of this alloy for tissue engineering.
Methods: AZ31 magnesium alloy samples were prepared and surface mechanical attrition treatment was accomplished in attrition machine. Microhardness test was used for measuring surface hardness. The surface structure of samples was characterized by X-Ray Diffraction method. Also, MG63 human osteoblast-like cells were cultured on the samples and in vitro cytocompatibily tests were carried out in terms of cell viability and scanning electron microscopy to observe cell attachment.
Results: XRD test results showed the nano-grained layer was created on surface. By increasing process time, the microhardness increased considerably which is attributed to the increasing lattice defects and crystallinity in samples. MTT assay and SEM showed that the cell viability and attachment of cells to the surface were increased after treatment.
Conclusion: Results showed synthesized nanosurface have good effect on mechanical and biocompatibilities of magnesium. So the nanostructure magnesium alloy could be very high performance material compared to conventional coarsed grain magnesium alloys in tissue engineering application.
Keywords: Nanostructure, Surface Mechanical Attrition Treatment, Grain Size, Magnesium Alloy, Cell Culture

Ps-135: A Novel Mutation in a Patient with Cystinosis and Stem Cell Therapy

Sahar Hasani*

Faculty of Advanced Sciences and Technology, Islamic Azad University Pharmaceutical Sciences Branch, Tehran, Iran
Email: saharhasani90@yahoo.com

Background and Aim: Cystinosis is an inherited multi systemic disease resulting from the failure of lysosomal cystine transport (cystinosin) and identified by collecting this amino acid in lysosomes entirely the body. The hereditary pattern of this disorder is autosomal recessive and its frequency is approximately one in 100000 to 200000. The responsible gene CTNS, encoding the lysosomal cystine carrier cystinosin is located on the short arm of the chromosome 17p13. Several variants in CTNS gene have been reported that consist in small intragenic deletions/insertions, missense, nonsense and splice-site mutations.
Methods: In our studies we introduced a novel mutation in an Iranian case. A 9 years old symptomatic female with renal insufficiency was diagnosed as having Cystinosis based on her clinical features and laboratory tests. After genetic counseling, blood samples were obtained from the patient and her parents. Genomic DNA was extracted from whole blood and mutation analysis was performed using PCR and sequencing methods for all exons of CTNS gene. At least 148 different pathogenic and deleterious mutations in CTNS gene have been reported up to date. Based on prominent clinical features of Cystinosis in our patient, we carried out a

targeted search for mutations in CTNS gene. This led us to identify a novel homozygous DNA variation c.256_257delCT in exon 6 of the gene. As expected, the mentioned mutation existed in both her parents in a heterozygous state. The proband described in this study has clinical manifestations as short stature, light appearance of the skin, rickets, photophobia, failure to thrive, signs of renal tubular Fanconi syndrome as dehydration, polyuria, acidosis and polydipsia, the presence of corneal crystals and vomiting.
Results: Cyesteamin is the only drug for Cystinosis that can reduce the content of cystine intracellular and can delay the progression of damages but cannot prevent all of the complications completely that it causes patients in end-stage renal failure require dialyses or transplantation. These treatments make some other problems as severe shortage of donor organs and waiting for transplantation 3 to 6 years. Thus there is a need for a new and better therapy. In many studies mentioned that transplantation of syngeneic bone marrow cell (BMC), mesenchymal stem cell (MSC) and hematopoietic stem cell (HSC) could be therapeutic. Using the CTNS -/- mice as model revealed that transplanted HSC by utilize a self-inactivating-lentivirus vector (SIN-LV) carrying a functional human CTNS gene can act as a vehicle in order to the delivery to organs that concentration of cystine is high. With due attention to studies significant decrease of cystine in tissues was led.
Conclusion: This therapy can change the insights of treatment of Cystinosis if it be successful in humans.
Keywords: Cystinosis, Hematopoietic Stem Cell, Transplantation

Ps-136: The Effect of Mummy Substance on Matrix Protein Synthesis by Human Adipose-Derived Stem Cells and Dermal Fibroblast in Separate or Co-Culture Model

Sepideh Hasanpour Khodaei[1,2], Leila Roshangar[1,2*], Jafar Soleimani Rad[1,2], Shahnaz Sebetkam[1,2]

1. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Email: safa09@yahoo.com

Background and Aim: Wound healing is a natural restorative response to tissue injury but Wound repair remains a challenging clinical problem due to increasing prevalence of non-healing wounds; therefore, efficient wound management is essential. Traditional healers may be alternative strategies for treatment of wounds. Mummy material was used as a remedy for inflammation, articular injuries, bone fractures, and wounds healing. In addition different studies have shown that adipose derived stem cells (ASCs) also play a role in the skin biology such as wound healing.
Methods: The present study is aimed at investigating the matrix protein synthesis by using mummy substance on human adipose-derived stem cells and human fetal foreskin fibroblast cell line (HFFF-2) in separate or co-culture. For this purpose, the effective concentration of mummy on fibroblasts and ASCs was determined via MTT assay. Mesenchymal stem cells were isolated from adipose tissue. Human fibroblasts were obtained from Pasteur Institute of Iran. The cells were cultured in DMEM and DMEM and Mummy as separate or in a co-cultural method. Expression of collagen type I III and fibronectin were assessed using the Real time PCR analysis.
Results: Mummy material at concentration of 1000µg/ml led to the highest proliferation rate in ASCs and HFFF-2. The results suggested that in fibroblasts the level of mRNA expression of fibronectin up-regulated in the treatment group ($P<0.0001$), but it was up-regulated Col type I ($P<0.0001$) and Col type III ($P<0.01$) in ASCs. In co-culture, mRNA expression of Col type I, III and fibronectin increased ($P<0.0001$).
Conclusion: Accordingly, mummy may possibly improve wound healing through synthesis of ECM.
Keywords: Mummy Substance, Wound Healing, Adipose-Derived Stem Cell (Ascs), Human Fetal Foreskin Fibroblast (HFFF-2), Matrix Protein

Ps-137: Induction of Osteogenic Differentiation of Mesenchymal Stem Cells Using Crocetin as One of the Major Bioactive Components of Saffron

Maryam Hashemi[1*], Fatemeh Kalalinia[2], Hengameh Ghasim[3]

1. Nanotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
2. Biotechnology Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
3. School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
Email: hashemim@mums.ac.ir

Background and Aim: Crocetin is a carotenoid dicarboxylic acid which is one of the major bioactive components of saffron. It showed excellent antioxidant, anti-atherosclerotic, anti-inflammatory, and anti-cancer activities in vitro and in vivo. In the present study, the ability of crocetin to induce cell differentiation of rat bone marrow-derived mesenchymal stem cells (rat BM-MSCs) into osteoblasts was evaluated.
Methods: Bone marrow cells were isolated from rat's femor. Cytotoxic effect of crocetin was assayed using MTT test and IC50 was calculated from the results. Osteogenic ability of crocin has been evaluated and compared with the BM-MSCs that have been treated with osteogenic standard medium after 7 and 21 days, using alizarin red staining and alkaline phosphatase (ALP) activity.
Results: The results showed a significant osteogenic activity of crocetin. Furthermore no synergistic effects are seen between crocetin alone or when co-admistrated with osteogenic standard medium.
Conclusion: According to the findings of this study, crocetin could effectively enhance osteogenic differentiation of MSCs and can be considered as safe therapeutic agents in clinical applications.
Keywords: Crocetin, Saffron, Osteoblast, Mesenchymal Stem Cells

Ps-138: "A Systematic Review on Platelet Products as a Tool for Regenerative Medicine in Iran"

Seyed A Raeissadat, Marzieh Babaee, Zahra Hashemi*

Clinical Research Development Center of Shahid Modarres Hospital and Physical Medicine and Rehabilitation Research Center of Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: hashemi.zr@gmail.com

Background and Aim: Recently there is growing trend towards regenerative medicine for management of chronic and disabling diseases. Its aim is regeneration of human cell, tissue and organ by using of stem cells, platelet, biologic proteins and etc. Platelet cells play a key role in recent researches by effects of their various growth factors and cytokines with healing properties. In this study, we review the existing published researches done by Iranian researchers on various platelet products and application of them in regenerative medicine.
Methods: Two independent researchers systematically search Persian (Iranmedex, Irandoc) and English electronic data bases (Scopus, Cochrane Central Register of Controlled Trials, PubMed, Google scholar) by key words "platelet rich*" and "Iran" till 26 May 2016. Number of published studies per year, disorder's type, type of platelet products and method of studies were analyzed. We also compare the overall results of all studies and compared by recent global systematic review and meta-analysis in each field.
Results: Within 2138 articles in primary search, finally, 133 articles were eligible. We classified articles in 8 groups (bone, cartilage, osteoarthritis, tendon & ligaments, nerve tissue, wound & fistula, dental & gingival, skin & aesthetic and nonspecific field). Bone disorders (25%), wound & fistula (16%), dental & gingival disorders (14%) and osteoarthritis (11%) have more relative frequency based on different fields. PRP with 72%, then PRGF (13.5%) and PRF (12%) were more attractive platelet products in Iranian researches.41.3% of studies designed in animal experimental method, while randomized clinical trials (17%) and non-randomized clinical trials (17%) were the next in rank.
Conclusion: Necessity of pursuing standard protocols in preparation of the platelet products, stating the precise content of platelets and growth factors and long term follow up of study subjects were the most important points in Iranian studies.
Keywords: Platelet-Rich Plasma Platelet-Rich Fibrin Platelet-Derived Growth Factor

Ps-139: Synergistic Effects of Aerobic Exercise after Bone Marrow Stem Cell Transplantation on Recovery of Dopaminergic Neurons and Angiogenesis Markers of Parkinsonian Rats

Seyed Abdollah Hashemvarzi[1*], Ali Heidarianpour[1], Zia Fallah Mohammadi[2], Mohsen Pourghasem[3]

1. Department of Exercise Physiology, Bu-Ali Sina university, Hamedan, Iran
2. Department of Exercise Physiology, Mazandaran university, Babolsar, Iran
3. Department of Anatomy, Babol University of Medical Sciences, Babol, Iran
Email: hashemvarzi_tkd@yahoo.com

Background and Aim: Parkinson is a progressive neurodegenerative disease in central nervous system. Non-pharmacologic treatment methods such as stem cell transplantation and exercise have been considered as a treatment. The purpose of this study was to evaluate the synergistic effects of aerobic exercise after bone marrow stem cells transplantation on recovery of dopaminergic neurons and promotion of angiogenesis markers in the striatum of parkinsonian rats.
Methods: 42 rats were divided into six groups: Normal (N), Sham (S), Parkinson's (P), Stem cells transplanted Parkinson's (SP), Exercised Parkinson's (EP) and Stem cells transplanted+Exercised Parkinson's (SEP). To create a model of Parkinson's, the striatum was destroyed by injection of 6-hydroxy-dopamine into the striatum through stereotaxic apparatus. Stem cells were derived from the bone marrow of femur and tibia of male rats aged 6-8 weeks. After cultivation, approximately 5×105 cells were injected into the striatum of rats through the channel. Aerobic exercise was included 8 weeks of running on treadmill with a speed of 15 meters per minute. At the end of the study, all subjects were decapitated and striatum tissues were separately isolated for measurement of vascular endothelial growth factor (VEGF), dopamine (DA) and tyrosine hydroxylase (TH) levels.
Results: VEGF, DA and TH levels in the striatum of parkinsonian rats significantly increased in treatment groups (SP, EP and SEP), especially in SEP group compared to P group after treatment ($P<0.05$).
Conclusion: The BMSCs transplantation in combination with exercise would have synergistic effects leading to functional recovery, dopaminergic neurons recovery and promotion of angiogenesis marker in the striatum of parkinsonian rats.
Keywords: Stem Cells, Aerobic Exercise, Neurotrophic Factors, Parkinson

Ps-140: Ethyl Acetate and n-Butanol Fractions of Cissus quadrangularis Enhances the Osteoblast Differentiation and Mineralization Potential of Mouse Pre-Osteoblast Cell Line MC3T3-E1 (Sub-Clone 4)

Rabail Hassan Toor, Abdul Rauf Shakoori*

School of Biological Sciences, University of the Punjab, Lahore, Pakistan
Email: rabail.toor@yahoo.com

Background and Aim: Cissus quadrangularis is a medicinal herb which is reported in Ayurvedic literature for its various pharmacological properties especially it is famous for its bone healing property. The study aims at identifying the main chemical constituents in Cissus quadrangularis responsible for its bone healing property and to develop a plant based medicine which can be used for treatment of osteoporosis and other bone related diseases.
Methods: Crude ethanolic extract of Cissus quadrangularis was prepared and fractionated into four fractions. Two purified fractions viz, Ethyl Acetate fraction (CQ-EA) and n-Butanol fraction (CQ-But) were analyzed for their effect on growth, proliferation and osteoblast differentiation of MC3T3-E1 cell line.
Results: The results indicate that both fractions of Cissus quadrangularis i.e. CQ-EA and CQ-But have no detrimental effect on the growth, proliferation and metabolic activity of the cells and also enhance the osteoblast differentiation and mineralization potential of MC3T3-E1 cell line.
Conclusion: Both purified fractions of Cissus quadrangularis i.e. CQ-EA and CQ-But have the active constituents for bone healing and they can be further purified and used as a potential medicine for treatment of osteoporosis and other bone related diseases.
Keywords: Plants, Bone, Cissus, Osteogenesis, Mesenchymal Stem Cells, Cell Line

Ps-141: Generation and Characterization of Engineered T Cells Expressing Chimeric Antigen Receptors (CAR) Against PSMA in Prostate Cancer Cell Line

Mahmoud Hassani[1], Fatemeh Hajari Taheri[2], Mohammad Hosein Modaresi[1], Zahra Sharifzadeh[2], Arash Arashkia[3], Mohsen Abolhassani[2*]

1. Molecular Medicine Department, School of Advanced Thechnologies in Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran
2. Department of Immunology, Pasteur Institute of Iran, Tehran, Iran
3. Department of Virology, Pasteur Institute of Iran,Tehran, Iran
Email: mahmoud.hassani@yahoo.com

Background and Aim: In recent years, modification of T cells with Chimeric Antigen Receptor (CAR) has been a good approach for adoptive immunotherapy of cancer, especially in cancer cells that usually fail to express major histocompatibility complex and co-stimulatory molecules.Typically CAR contains fusion of single chain variable domain fragment (scFv) with co-stimulatory and CD3zeta domains. Since scfvs most often are derived from a monoclonal antibody of murine origin it may be a trigger for host immune system that leads to T cell clearance. Nanobody is the antigen-binding fragment from heavy chain-only antibodies (HcAbs), derived from camelidae, that have great homology to human VH and low immunogenic potential. Nanobodies show attractive characteristics for tumor targeting in cancer diagnosis and therapy. For this reason we employed nanobody instead of scFv in a CAR construct.
Methods: In this study, we used an anti-prostate specific membrane antigen (anti-PSMA) nanobody as antigen binding domain, FC domain of IgG1 as a spacer domain, CD28 as co-stimulatory domain and CD3zeta as signaling domain. At the first, PBMCs were activated with anti-CD3/CD28 antibody and IL-2. Activated T cells were electroporated with the CAR construct and then anti-FcIgG1-FITC was used for detection of CAR surface expression with flow cytometry. For functional analysis, CAR T cells were co-cultured with target cells (LnCap (PSMA+) and DU145 (PSMA-)) and analyzed for IL-2 production.
Results: The flow cytometry results confirmed the surface expression of chimeric receptors on CAR T cells. T cells co-cultured with LnCap cells secreted up to 800 pg/µl IL-2 while no difference was seen in IL-2 production in T cells with DU145.
Conclusion: Here, we describe the ability of CAR-bearing T cells to recognize PSMA on the prostate cancer cell line that leads to the activation of T cells. This new anti-PSMA CAR might be used as a promising candidate for clinical applications of prostate cancer therapy.
Keywords: Chimeric Antigen Receptor, Nanobody, PSMA, Immunotherapy, Prostate Cancer

Ps-142: Simulation of Avascular Tumor Growth and Metabolism Using In Silico and In Vitro Models: Prediction and Validation of Tumor Growth Pattern in Different Metabolic Conditions

Mahshid Heidari, Mahboubeh Kabiri*, Seyed-Amir Marashi

Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
Email: mkabiri@ut.ac.ir

Background and Aim: In recent years, scientists have made many efforts for in vitro and in silico modeling of cancerous tumors. In fact, three-dimensional (3D) cultures of multicellular tumor spheroids (MCTSs) are good validators for computational results. The goal of this study is to simulate the 3D early growth of avascular tumors using MCTSs and to compare the results of a specific computational modeling framework with in vitro models. Using these two types of models, tumor growth in different culture media could be predicted and validated.
Methods: We took advantage of a previously developed computational model of tumor growth, which was constructed by integrating a previously published generic metabolic network model of cancer cells with a multiscale agent-based framework. MCTSs were grown by hanging drop. Among the computational predictions is the importance of glucose accessibility on tumor growth behavior. To study the effect of glucose concentration experimentally, MCTSs were grown in high and low glucose culture media and tumor growth pattern was analyzed by MTT assay, cell counting and propidium iodide (PI) staining.
Results: We obviously observed that the rate of necrosis increases and the rate of tumor growth and cell

activity decreases as the glucose availability reduces which is in line with computational model rediction.
Conclusion: The results emphasize on the importance of glucose availability on the growth of tumor spheroids. The consistency of the predictions of the in silico modeling framework with the near-to-reality in vitro tumor spheroids shows that the mentioned computational model may be used for other predictions. One interesting characteristic of the modeling framework is that it provides us with the ability to predict gene expression patterns through different layers of tumor, which can have important implications especially in drug target selection in the field of cancer therapy. These sort of predictions are to be examined in near future.
Keywords: Agent-Based Modeling, Constraint-Based Metabolic Network Model, Cancer Tumor Modeling, Multicellular Tumor Spheroid (MCTS)

Ps-143: Surface Modification of Poly Hydroxybutyrate (PHB) Nanofibrous Mat by Collagen Protein and Its Cellular Study

Saeed Heidari-keshel[*]

Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Saeedhey@gmail.com

Background and Aim: In this study, the effects of CO2 plasma surface modification and cross linking with collagen on the properties of poly-[3-hydroxybutyrate] (PHB) nanofibres were investigated.
Methods: The samples were evaluated by attenuated total reflectance–Fourier transform infrared spectroscopy (ATR–FTIR), scanning electron microscope (SEM), contact angle and finally cell culture. ATR–FTIR structural and SEM analyses showed the presence of functional groups and coating on the nanofibrous surfaces. The 85° difference was obtained in the contact angle analysis, obtained for the collagen-cross-linked nanofibrous mat than the un-modified nanofibrous mat.
Results: Cellular investigation showed better adhesion of collagen-cross-linked nanofibrous samples than other samples.
Conclusion: The collagen-cross-linked mats are a suitable scaffold for enhancing the cell adhesion in nerve regeneration.

Keywords: Nanofibres Cell Adhesion

Ps-144: Simple Design of an Aligned Transparent Biofilm by Magnetic Particles and Its Cellular Study: Simple Design of an Aligned Transparent Biofilm

Saeed Heidari-keshe[l*]

Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Saeedhey@gmail.com

Background and Aim: The aim of this study was to produce aligned biodegradable films.
Methods: In this study, we used magnetic microparticles and strong magnetic field for orientation of gelatin gels. The samples were evaluated by microscopic analyses and cell culture assays with Schwann cells. Results of structural analyses showed a good arrangement and orientation of films under strong magnetic field with movement of magnetite particles.
Results: Cellular experiments showed a good cell adhesion and orientation on the designed films compared with those on unmodified ones.
Conclusion: This aligned guide appears to have the right organization for testing in vivo nerve tissue engineering studies.
Keywords: Nanofibres Cell Adhesion Nerve Tissue Engineering

Ps-145: Cost Effective and Scalable Generation of Hepatocyte-Like Cells through Microparticle-Mediated Delivery of Growth Factors in 3D Suspension Culture

Zeinab Heidariyan[1], Mohammad Hossein Ghanian[2], Mohsen Ashjari[3], Zahra Farzaneh[1], Mostafa Najarasl[1], Mehran Rezaei Larijani[1], Abbas Piryaei[4,5], Massoud Vosough[1], Hossein Baharvand[1,6*]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Cell Engineering, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

3. Chemical Engineering Department, Faculty of Engineering, University of Kashan, Kashan, Iran
4. Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Department of Tissue Engineering and applied Cell Sciences, School of Advanced Technology in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
6. Department of Developmental Biology, University of Science and Culture, Tehran, Iran
Email: Baharvand@RoyanInstitute.org

Background and Aim: Application of human pluripotent stem cells (hPSCs)-derived hepatocyte-like cells (HLCs) in cell-based therapy and drug screening relies on differentiation in large scale such as three-dimensional (3D) suspension differentiation in bioreactor. However, this approach requires large amounts of growth factors (GFs) as well as overcoming the limited diffusional transport barriers posed by the inherent 3D structure of the spheroids. In this study, we hypothesized that incorporation of degradable polymeric low concentration GF-laden microparticles (MPs) within hPSC spheroids on aggregate structure would circumvent such limitations and enhance the differentiation.
Methods: Core/shell MPs of PLGA and PLLA including HGF and FGF4 were incorporated into hPSC aggregates at a 1:3 (MP: cell) ratio.
Results: Compared to conventional soluble delivery method, the gene expression results demonstrate that incorporation of MPs results in similar expression of hepatic markers, despite using 10-fold less total GFs. The differentiated HLCs in MP group, exhibited ultrastructure and functional characteristics such as glycogen storage, indocyanine green uptake, albumin secretion, urea production and low-density lipoprotein uptake and inducible cytochrome P450 activity comparable with soluble GF group.
Conclusion: This localized delivery of low concentration GF-loaded MPs may offer a novel route towards scalable hepatic differentiation technologies and to engineer the 3D microenvironment.
Keywords: Growth Factor Delivery, Microparticles, Scalable Culture, Human Pluripotent Stem Cells, Hepatocyte-Like Cells

Ps-146: Development of a Hybrid Microfluidic System for Regulation of Neural Differentiation in Induced Pluripotent Stem Cells

Zahra Hesari[1*], Massoud Soleimani[2], Fatemeh Atyabi[3], Rassoul Dinarvand[4]

1. Neuroscience Research Center, Poursina Hospital, Department of Pharmaceutics, School of Pharmacy, Guilan University of Medical Sciences, Rasht, Iran
2. Department of Hematology and Blood Banking, Faculty of Medicine, Tarbiat Modaress University, Tehran, Iran
3. Nanotechnology Research Centre, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
4. Deparmentof Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
Email: z.hesari@gmail.com

Background and Aim: Controlling cellular orientation, proliferation and differentiation is valuable in designing organ replacements and directing tissue regeneration. The advantages of biocompatible aligned nanofibrous scaffolds and microfluidic systems has been proven in previous studies while to the best of our knowledge, the probable synergistic influence on neural differentiation has not been investigated yet.
Methods: In the present study, we developed a hybrid microfluidic system to produce a dynamic microenvironment by placing aligned PDMS microgrooves on surface of biodegradable polymers as physical guidance cues for controlling the neural differentiation of human induced pluripotent stem cells (hiPSCs). The neuronal differentiation capacity of cultured hiPSCs in the microfluidic system and other control groups was investigated using quantitative real time PCR (qPCR) and immunocytochemistry. The functionally of differentiated hiPSCs inside hybrid system's scaffolds was also evaluated on the rat hemisected spinal cord in acute phase. Implanted cell's fate was examined using tissue freeze section and the functional recovery was evaluated according to the Basso, Beattie and Bresnahan (BBB) locomotor rating scale.
Results: Our results confirmed the differentiation of hiPSCs to neuronal cells on the microfluidic device where the expression of neuronal-specific genes was significantly higher compared to those cultured on the other systems such as plain tissue culture dishes and

scaffolds without fluidic channels. However, survival and integration of implanted hiPSCs did not lead to a significant functional recovery.
Conclusion: We believe that combination of fluidic channels with nanofiber scaffolds provides a great microenvironment for neural tissue engineering, and can be used as a powerful tool for in-situ monitoring of differentiation potential of various kinds of stem cells.
Keywords: Microfluidics, Human Induced Pluripotent Stem Cells (hiPSCs), Neural Differentiation, Tissue Engineering, Nanofibers

Ps-147: Intrathecal Administration of Mesenchymal Stem Cells Improves Sensory and Motor Function in the Rat Model of Spinal Cord Injury

Farshad Homayouni Moghadam[1,2*], Maryam Dehghan[2], Abolghasem Abbasi[2,] Abbas Vakili Zarch[2], Mehrdad Mansouri[2], Mojtaba Babaei Zarch[2]

1. Department of Cellular Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Neurobiomedical Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Email: f_homayounim@yahoo.com

Background and Aim: There are several reports about stem cell therapy for spinal cord injury (SCI). Recently, several clinical trials have done or are going to be done to investigate the effect of mesenchymal stem cell (MSC) therapy on SCI in human volunteers, but the precise mechanism of MSC function is not clear yet. Otherwise, still there are some doubts about the safety of these therapies such as tumor generation. In present study MSCs were injected intrathecally to the rat model of SCI to identify the fate of the transplanted cells, the host tissue immunologic responses, the risk of tumor formation, and the rate of improvements in the sensory and motor function.
Methods: After surgical induction of SCI, animals were treated with induced or noninduced bone marrow mesenchymal stem cells. MSCs were isolated from bone marrow of male rats. Induced MSCs (iMSCs) were prepared after induction of MSCs to differentiate toward neuronal cell lineage by cultivation in serum free medium containing fibroblast growth factor and epidermal growth factor.
Results: Results indicated that injected cells could migrate to the site of the injury and interact with host tissue cells. There was not any sign of tumor formation and results of staining against CD2, CD3, CD15 and CD45 showed that there were not immunologic responses in the host tissue. There was also some partial improvement in sensory and motor function in treated animals.
Conclusion: In conclusion mesenchymal stem cell therapy is safe and could be helpful for remyelination of injured nerve fibers by secretion of growth factors and immunomodulatory cytokines.
Keywords: Mesenchymal Stem Cell, Oligodendrocyte, Spinal Cord Injury

Ps-148: Evaluate of Proliferation and Attachment of Bone Marrow Mesenchymal Stem Cells on Different Percentages Gelatin/Glycoseaminoglycan Electrospun Nanofibers

Ali Honarpardaz[1], Shiva Irani[1*], Mojgan Zandi[2], Mohamad Pezeshki-Modaress[2]

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Biomaterials, Iran Polymer and Petrochemical Institute, P.O. Box: 14965/159, Tehran, Iran
Email: ali.honarpardaz@srbiau.ac.ir

Background and Aim: Cartilage disorders and damages are the most clinical concern due to cartilage structure. Cartilage is avascular and hypo cellular tissue therefore it has limited capacity to self-repair. Tissue engineering is new technique that can help treat cartilage damages by using cells and scaffolds. In this study, we tried to evaluate that novel electrospun scaffold composed of gelatin / glycosaminoglycan (G / GAG) nanofibers are biocompatible for Bone Marrow-derived Mesenchymal Stem Cells (BMMSCs) culture.
Methods: Scaffolds were synthesized and prepared by electrospinning technique scaffolds with different concentration of glycosaminoglycan (5%, 10% and 15%). Electrospun nanofibers were cross linked by glutaraldehyde, then sterilized by 70% etanol. BMMSCs were cultured and seeded on the scaffolds for biocompat-

ibility process. Cells and scaffolds were placed in to the culture media for 3 day. MTT assay was done for scaffold's biocompatibility and also cells viability. Cell attachment also examined by MTT assay during 6 hr. Scanning electron microscopy was utilized to show nanofibers morphology and cell attachment.
Results: All scaffolds with different percent of GAG were biocompatible. Scaffold with 15 % GAG showed better result for biocompatibility ($p = 0.02$). All scaffolds have increase in cell number in 72 hour after seeding cells on them ($p = 0.05$). Results for electron microscope showed that nanofibers had good porosity and diameter also showed that cells have good attachment on scaffolds and there morphology also were normal during tests.
Conclusion: Results show that electrospun gelatin/ GAG nanofibers are biocompatible and mesenchymal stem cells can highly attach and proliferate on fabricate scaffolds. There for gelatin / GAG nanofibers have potential for using as scaffold for tissue engineering.
Keywords: Mesenchymal Stem Cells, Electrospun, Glycosaminoglycan

Ps-149: Low Intensity Sonication on Proliferation of Spermatogonial Stem Cells Isolated from Neonate Mouse Testis

Zinab Hormozi Mogaddam[1], Manijhe Mokhtari Dizaji[1*], Mansoreh Movahedin[2], Mohamad Ehsan Ravari

1.Department of Medical Physics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2.Department of Anatomy, Faculty of Medical Sciences Tarbiat Modares University, Tehran, Iran
Email: mokhtarm@modares.ac.ir

Background and Aim: Although stem cell proliferation and differentiation capabilities are high, but the frequency of replication in vitro is limited, so are need techniques to increase the number of these cells in vitro. Nowdays, studies is based on the use of bio-stimulation, such as low-intensity ultrasound, in the propagation of ultrasound in liquid acoustic cavitation is the major and important phenomenon. Therefore, in the present study, the effect of mechanical index is examined that can indicate the interaction of cavitation on the proliferation of spermatogonia stem cells.
Methods: In this study because of using low intensity ultrasound, Rayleigh integral model for computation of acoustic pressure was chosen and was solved in cylindrical coordinate for estimating mechanical parameters, calculation of the sound pressure and frequency and intensity of various transducer and Mechanical Index parameter was measured at different distances from the surface of the ultrasonic transducer at 37 ° C water. In order to isolate stem cells used of 3 to 6 days neonatal male mice and characteristic of isolated cells was confirmed by immunocytochemistry with Oct4 and PLZF proteins for SSCs. Stem cells were irradiated for 5 consecutive days, then survey of proliferation and colonization of stem cell in various group. Change of one degree under hyperthermia determine during irradiation exposure.
Results: Mechanical parameters is selected in the subthreshold, threshold and the upper threshold of acoustic cavitation in water. 40 kHz ultrasonic transducer with intensities of 0.28, 0.45, 0.96 and 1.34 watts per square centimeter at a distance 0.5 cm mechanical index was chosen 0.40, 0.51, 0.75 and 0.89. Effect of mechanical indix on stem cells cultured on a culture plate with a radius of 1.8 cm shows the proliferation of groups 0.40 and 0.51 respectively 2.89 ± 0.07, 2.17 ± 0.10, and the control group 1.74 ± 0.03 and Sham 1.52 ± 0.08. The number of colonies and colony diameter of 0.4 is maximum amount of 93 ± 3.7 and 1.74 ± 1.22 compared to radiation group and the control group and sham .The survival of cells in the control and sham groups is maintained such as other groups.
Conclusion: Low intensity ultrasound waves with 0.40 mechanical index parameter have better improvement on SSCs proliferation rate and colonization compared to other intensities and duty cycles during 7 day culture in vitro.
Keywords: Acoustic Cavitation, Mechanical Index, Proliferation of Stem Cell

Ps-150: Ultrasound Mechanical Index as a Parameter Affecting the Proliferation of Spermatogonia Stem Cells

Zeinab Hormozi Moghaddam[1], M. Mokhtari-Dizaji[1*], M. Movahedin[2], M. E. Ravari[1]

1. Department of Medical Physics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Anatomy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: zhormozi69@gmail.com

Background and Aim: Considering the use of mechanical stimulation such as low-intensity ultrasound for proliferation and differentiation of stem cells, it is essential to understand the physical and acoustical mechanisms of acoustic waves in vitro. Mechanical index is used for quantifying acoustic cavitation and the relationship between acoustic pressure and the frequency. So in this study, for the first time mechanical index obtained by extracting acoustic pressure from modeling in environment with various attenuation coefficient, frequency and intensity. Modeling of the MI was applied to provide treatment protocol and to understand the effective physical processes on proliferation of spermatogonia stem cells. Spermatogonia are an undifferentiated male germ cell. Spermatogonia undergo spermatogenesis to form mature spermatozoa in the seminiferous tubules of the testis.
Methods: The acoustic pressure and MI equations are modeled and solved to estimate optimal MI for 28, 40, 150 kHz and 1 MHz frequencies. Radial and axial acoustic pressure distribution was extracted. To validate the results of the modeling, the acoustic pressure in the water and near field depth was measured by a piston hydrophone.Isolated spermatogonial stem cells from neonatal mice were cultured in DMEM culture medium with 10% FBS. Spermatogonial stem celld stimulated by Low-level intensity ultrasound for 5 days and proliferation and colonization assessed at day 7th.
Results: Based on the results of the mechanical index, regions with threshold mechanical index of 0.7 were identified for extracting of radiation arrangement to cell medium. In order to validate the results of the modeling, the acoustic pressure in the water and near field depth was measured by a piston hydrophone. Results of modeling and experiments show that the model is consistent well to experimental results with 0.91 and 0.90 correlation of coefficient ($p<0.05$) for 1 MHz and 40 kHz. Low-intensity ultrasound with 0.40 mechanical index is more effective on enhancing the proliferation index of the spermatogonia stem cells during the seven days of culture. In contrast, higher mechanical index has a harmful effect on the spermatogonial stem cells.
Conclusion: This acoustic propagation model and ultrasound mechanical index assessments can be used with acceptable accuracy, for the extraction special arrangement of acoustic exposure used in biological conditions in vitro. This model provides proper treatment planning in vitro and in vivo by estimating the cavitation phenomenon.
Keywords: Ultrasound, Mechanical Index, Modeling, Stem Cell

Ps-151: Synthesis and Antibacterial Evaluation of Tributyl Ammonium/Alginate Modified Cationic Polyurethanes

Seyyedeh Sahar Hosseini Salekdeh[1], Seyyed Mohammad Sadegh Nourbakhsh[1*], Naser Aghdami[2], Hamed Daemi[2], Sareh Rajabi Zeleti[2]

1. Biomaterial Group, Faculty of New Sciences and Technologies, Semnan University, Semnan, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: sahar_hosseini69@yahoo.com

Background and Aim: Microorganism infection is a great concern in wound healing process. Side effects associated with the use of antibiotics and small molecules such as toxicity and bacterial resistance, led scientist to introduce antimicrobial polymeric materials. Cationic compounds such as ammonium salts have emerged as promising candidates for further developments as antimicrobial agents with decreased potential for resistance development. The purpose of this study was to synthesize and antibacterial evaluation of novel cationic polyurethanes and alginates modified by tributyl ammonium compound.
Methods: At first step, the cationic polyurethanes (CPU) films were synthesized by using of methyldiethanolamine (MDEA) as a tertiary amine emulsifier. Films were surface modified with tributylammonium/alginate (CPU/TBA-Alg) by immersion method at the second step. The structure and morphology of polymeric films were studied by fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM),

respectively. Agar diffusion method was used for analyzing the antibacterial activity of both films (CPU and CPU/TBA-Alg against Escherichia coli (ATCC 25922) and Staphylococcus aureus as the gram-negative and gram-positive bacterial strains, respectively. Briefly, agar plates were seeded with 108 CFU /mL-1 of each strains and polymeric films. After incubation at 37℃ for 24 h, the inhibition zone diameter was measured.
Results: The formation of CPU and surface modification by tributylammonium/alginate were confirmed by FTIR spectra. Both films showed antibacterial activity against Escherichia coli and Staphylococcus aureus strains. The larger inhibition zone diameter around the tributyl ammonium/alginate modified cationic polyurethanes confirmed the synergistic effect of cationic polyurethanes and tributyl ammonium /alginates on their antibacterial activity.
Conclusion: Our results showed the potent anti-microbial effect of tributyl ammonium/alginate modified cationic polyurethanes against gram positive and gram negative bacterial strains and hence these film can be used for future application in wound dressing application.
Keywords: Cationic Polyurethane, Alginate, Ammonium Salts, Antibacterial Activity

Ps-152: Anti RANKL Mirna: New Approach for Osteoporosis Therapy

Vahedeh Hosseini, Arash Khojasteh, Samira Mohammadi-Yeganeh*

Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: v.hosseini@sbmu.ac.ir

Background and Aim: microRNAs (miRNAs) attracting considerable interest due to their regulatory effects on gene expression. A single miRNA may have hundreds of different mRNA targets. Experimental detection of potential targets of miRNAs is laborious. Concerning the economically unfavorable techniques of detection, computational approaches for miRNA target prediction can be used as the first step of miRNA researches. New osteoporosis therapy have special focus on positive or negative regulators of osteogenic signaling pathways such as RANKL and DKK. Hence anti-RANKL antibodies are FDA-approved, miRNAs which can target these negative regulator of osteogenesis can increase the osteogenesis.
Methods: In this study we applied several reliable and sensitive database and tools for miRNA target prediction.After cloning in non-lentiviral vector, miR-4699 was induced in HEK-293 cells and its effect on target gene was investigate using Real Time PCR and luciferase assay.
Results: Considering all bioinformatically critical criteria, we selected hsa-miR-4699-3p that had remarkable score to target RANKL. The results of Real Time PCR and luciferase assay indicated a significant decline in expression of RANKL.
Conclusion: To sum up, the result emphasizes the validity and importance of accurate in silico investiga-tion as the first step in experimental studies. We suggest hsa-miR-4699 for further investigation as an osteogenic miRNA for therapeutics purposes.
Keywords: miRNA, RANKL, miRNA Target Prediction Tools

Ps-153: The Role of Bone Marrow Niche in Drug Resistance in Acute Myeloid Leukemia

Mohammad Houshmand[1], Alireza Kazemi[2], Narjes Yazdi[3], Mahin Nikougoftar Zarif[1*]

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
2. Department Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Molecular Genetics, Tehran Medical Branch, Islamic Azad University, Tehran, Iran
Email: hooshmand8m@gmail.com

Background and Aim: Bone marrow niche is a major contributing factor in leukemia development and drug resistance in acute myeloid leukemia (AML) patients. Although mimicking leukemic bone marrow niche relies on two-dimensional (2D) culture conditions, it cannot recapitulate complex bone marrow structure that causes the introduction of different three-dimensional (3D) scaffolds. Simultaneously, microfluidic platform by perfusing medium culture mimic interstitial fluid

flow, along with 3D scaffold would help for mimicking bone marrow microenvironment.
Methods: In this study, TF-1 cells were cocultured with bone marrow mesenchymal stem cells (BMMSCs) in 2D and 3D microfluidic devices. Then, drug screening was performed by applying azacitidine and cytarabine and cytotoxicity assay and quantitative reverse transcription polymerase chain reaction (qRT-PCR) for B cell lymphoma 2 (BCL2) were done to compare drug resistance in 2D and 3D culture conditions.
Results: Cytotoxicity assay illustrated drug resistance in 3D culture condition and qRT-PCR demonstrated higher BCL2 expression in the 3D microfluidic device in contrast to the 2D microfluidic device ($p < 0.05$).
Conclusion: On balance, mimicking bone marrow niche would help the target therapy and specify the role of the niche in the development of leukemia in AML patients.
Keywords: Acute Myeloid Leukemia, Scaffold, Microfluidic

Ps-154: In vitro Expansion of Umbilical+ CD133 Cord Blood in Poly-L-lactic Acid Nanoscaffold Treated with Fibronectin

Maryam Islami[1*], Yousef Mortazavi[1], Massod Soleimani[2], Samad Nadri[1]

1. Zanjan University of Medical Sience, Zanjan, Iran
2. Tarbiat Modares University, Tehran, Iran
Email: mahtabi2234@gmail.com

Background and Aim: Due to their -renewal and potency, Umbilical cord blood (UCB) Stem Cells, have the ability to proliferate and differentiate into most kinds of blood cells and served as an attractive alternative source to bone marrow for transplantation because of low incidence of Graft Versus Host Disease (GVHD) and HLA (Human Leukocyte Antigen) mismatching. However, insufficient numbers of stem cells in UCB is still a major constraint in clinical applications. Invitro expansion of stem cells on 3D culture system is a proper way to overcome this limitation.
Methods: In this paper, fibronectin treated Poly-L-lactic acid (PLLA) scaffolds (3D) and 2D culture system are compared and are employed for cell culture. 2×106 cord blood CD133+ cells isolated by MACS system were seeded on PLLA scaffold and permitted to expand for 7 days. Following this period, total cells, CD133+ cells, CFU assay, MTT assay were evaluated.
Results: Data showed the CD 133 + cells growth on fibronectin treated PLLA scaffolds on day 1, 3, 7 of seeding increased respectively. In colony assay maximum number of colonies belonged to 3D culture system in comparison with 2D culture system and number of CFU-GEMM was rather than others. Flow cytometry data indicated that the percentage of CD 133+ marker was 93%, that's proper enough for continuing the downstream steps. MTT assay results showed the much better proliferation and viability on the PLLA.
Conclusion: Fibronectin treated PLLA scaffold can be a suitable exvivo mimicry niche by intensifying of surface/volume ratio and supporting the stem cell expansion
Keywords: Cord Blood Stem Cell, Hematopoietic Stem Cells, Tissue Engineering, 3D culture

Ps-155: Invitro Fucosylation of Umbilical Cord Blood CD133+ stem cells on Nanoscaffold

Maryam Islami[1*], Yousef Mortazavi[1], Massod Soleimani[2]

1. Zanjan University of Medical Sience, Zanjan, Iran
2. Tarbiat Modares University, Tehran, Iran
Email: mahtabi2234@gmail.com

Background and Aim: Umbilical cord blood (UCB) stem cells transplantation is the transplantation of multipotent hematopoietic stem cells, but delayed engraftment is a major limitation of cord blood transplantation (CBT), due in part to a defect in the cord blood (CB) cells' ability to home to the bone marrow (BM). Because this defect appears related to uncharacterized defect in binding to P-selectin. Selectin ligands must be alpha1-3 fucosylated to form glycan determinants such as sialyl Lewis x (sLe(x)).
Methods: we conducted a first to correct this deficiency in Invitro with a simple 30-minute incubation of UCB CD133+cells with fucosyltransferase-VI and its substrate (GDP-fucose) to increase levels of fucosylation. Exvivo-like situations are made up of a three-dimensional (3D) scaffold that mimic UCB naïve situations

in BM. Fucosylated and unfucosylated cell+ UCB133 were seeded on 3D Poly-L-lactic acid (PLLA) scaffolds treated with fibronectin in presene selectin and without it.
Results: Flow cytometry data indicated that the percentage of fucosylated CD 133+ marker was 90%, that's proper enough for continuing the downstream steps. In addition, data showed that fucosylated CD133(+) cells were responsible for attachment to the scaffold and selectin-fibronectin treated scaffold was the best scaffold for cells proliferation and attachment, and maximum number of colonies belonged to it.
Conclusion: These findings support ex vivo fucosylation of multipotent CD133(+) CB cells and expantion of them on 3D selectin fibronectin treated PLLA scaffold improve proliferation and attachment of these cells to scaffold.
Keywords: Keywords: Cord Blood Stem Cell, Fucosyltransferase, GDP-Fucose, Selectin, Fucosyltransferae

Ps-156: Arterial Blood-Gas Pressure in Rabbit Following Intrapulmonary Administration of Bone Marrow-Derived Mesenchymal Stem Cells (BM-Mscs) in Experimental Model of Acute Respiratory Distress Syndrome (ARDS)

Mohammad Reza Mokhber Dezfouli[1], Sirous Sadeghian Chaleshtori[1], Mohammad Mehdi Dehghan[2], Massoumeh Jabbari Fakhr[3*]

1. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2. Department of Surgery and Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Student of Veterinary Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Email: mjabbarifakhr@gmail.com

Background and Aim: The lung diseases are the ninth cause of mortality in Iran and one of the growing reasons among the mortality causes in the world. The current specific drug therapies for lung diseases are inefficient. Annual spending too much money on diagnosis, hospital care, emergency care, medication and home care for pulmonary patients. The other approach in the treatment lung diseases is lung transplant, but this method Engenders many problems for transplant patients such as lack of suitable donor tissue and require lifelong immunosuppressive drugs. New methods such as cell therapy are needed for this common clinical problem.
Methods: This study was included on ten healthy white Newzealand rabbits in two groups, control and treatment. Bone marrow aspiration was performed on treated group and MSCs were isolated and cultured. Then an experimental model of ARDS was induced by intratracheal delivery E. Coli-lipopolysaccharides strains O55:B5. Inflammation confirmed by radiography. 24 hours after administration of endotoxin, 10×106 BM-MSCs were autographed as intrapulmonary in the treatment group and PBS was injected as intrapulmonary in the control group. The Arterial samples were collected from the central ear artery in the 1-mL syringe before and 3,6,12,24,48,72 and 168 hours after BM-MSC transplantation or PBS injection and immediately the sample were processed with Blood Gas Analyzer (OPTI CCA-TS). Blood gas analyzers directly measure pH, partial pressure of oxygen (PO2), partial pressure of carbon dioxide (PCO2) and bicarbonate (HCO3) on arterial blood samples.
Results: arterial blood gas analysis was improved in lung injury following MSC treatment. Statistical inference in the HCO3 and Base Excess recorded no significant changes between the two groups at different times. But the survey of the PO2 showed a significant decrease in the two groups after creating an experimental model of ARDS. After BM-MSCs transplantation in the treatment group occurred increase in PO2, so that the increment was significant in comparison with baseline and the control group in times 12, 24 and 48 hours. While After transplantation in the treatment group occurred decrement in PCO2, so that the reduction was significant in compared with baseline in times 24, 48, 72 hours and also, in comparison with the control group in times 48 and 72 hours. Reduction blood PH were significant in comparison with the control group in times 24 hours ($P < 0.05$).
Conclusion: The results suggested that BM-MSCs could play a significant role in the repair of lung injury.
Keywords: BM-MSCs, ARDS, Blood Gases, Rabbit

Ps-157: Effects Administration of Bone Marrow-Derived Mesenchymal Stem Cells (BM-

Mscs) on Cytokines in Rabbit Acute Respiratory Distress Syndrome (ARDS)

Mohammad Reza Mokhber Dezfouli[1], Sirous Sadeghian Chaleshtori[1], Mohammad Mehdi Dehghan[2], Massoumeh Jabbari Fakhr[3*]

1. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2. Department of Surgery and Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3. Student of Veterinary Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
Email: mjabbarifakhr@gmail.com

Background and Aim: Lung diseases have a high incidence around the world. ARDS is a major cause of respiratory failure. The successful treatments for lung diseases are very limited and drug therapies in many respiratory diseases inefficient. Interstitial lung diseases cause shortness of breath and fibrosis and do not respond to existing treatments. Although early detection and timely Medical care and treatment may lead to improved early signs but in the long run the symptoms may reappear .So that new therapeutic approaches are essential. The use of BM-MSC in the lung injuries have become a promising the therapeutic approach.
Methods: 10 male, white Newzealand rabbit was randomly allocated into two groups, treatment and control. In the treatment group, the samples of bone marrow were collected and MSCs were isolated and cultured and the number of cells for each rabbit was increased to 10×106 cells. Then by endotracheal injection of E. Coli-lipopolysaccharides strains-O55: B5 was induced experimental model of ARDS and confirmed by registration of clinical signs and radiograph. After 24 hours of ARDS-induce, BM-MSCs were autographed as intrapulmonary in the treatment group and PBS was injected as intrapulmonary in the control group. Bronchoscopic BAL performed with 3 ml of sterile saline BAL was collected from the animals. After acquiring the BAL samples. In order to evaluate effects of cell therapy, BAL fluid supernatants cytokine includes IL10, IL6 and TNFα were measured by ELISA kit before and 12,24,48,72 and 168 hours after BM-MSCs transplantation or PBS infusion.
Results: The results obtained showed that IL6 and TNF? levels were significantly increased in both groups in response to administration of LPS, After cell therapy chemokines and proinflammatory cytokine levels was reduced. IL6 was a significant difference in 12, 24, 48 and 168 hours compared with baseline and treatment group compared with control group were significantly in times of 12,24,72 and 168 hours. TNF-? was also found that on in 12, 24 and 48 hours in treating groups were significantly lower than baseline ($P<0.05$). And the treatment group compared with control group were significantly in times of 12, 24, 72 and 168 hours. Increase levels of IL-10 correlated significantly in treatment group in hours of 24, 48,168 and compared with a control group in times of 24and 48 hours.
Conclusion: The results showed that transplantation of BM-MSCs could regulate concentration inflammatory markers (IL10, IL6 and TNFα). MSC treatment significantly reduces LPS-induced acute lung injury in rabbits.
Keywords: BM-MSCs, ARDS, IL10, IL6, TNFα, Bronchoalveolar Lavage, Rabbit

Ps-158: Heart on a Chip: A Good Device for Simulation of Heart

Farshid Jaberi Ansari[1], Parisa Ahmadi[1], Hossein Ghanbari[1*], Samaneh mashagi[2]

1. Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. School of Engineering and Applied Sciences, Harvard University, massachusetts, USA
Email: roialbiotech@gmail.com

Background and Aim: Biomimetic microsystems representing different organs can be integrated into a single microdevice and linked by a microfluidic circulatory system in a physiologically relevant manner to model a complex, dynamic process of drug absorption, distribution, metabolism and excretion, and to more reliably evaluate drug efficacy and toxicity. Several tissues have been used so far such as liver, lung, kidney, gut, bone, breast, eye brain and stem cell
Methods: we design and fabricate microfluidic system containing two channels then seed and cover the cavity

with endocardial endothelium then we developing therombosis with different urea and other materials concentration and investigation adhision of platelet to the endocardium after we studing endocardial fibrosis on chip for this aims we generate simple model of endocardial fibrosis and study the dynamics of clot formation as a function of the severity of fibrosis
Results: result show that as urea concentration increases the endothelium cell was more damaged and platelet adhision was more. Also fluorescent imaging with confocal microscope validate this phenomenon
Conclusion: microfluidic chip show that good similarity in the function with real organs in vivo therefore these tools were suitable devices for simulation and screening drugs and the effect of them on organs
Keywords: Microfluidic, Chip, Organ

Ps-159: Reinforced Polycaprolactone Bimodal Foam Nanocomposite by Pccnws as Scaffolds for Bone Tissue Engineering

Hafez Jafari[1], Mohsen Shahrousvand[2*], Babak Kaffashi[1]

1. Department of Polymer Engineering, School of Chemical Engineering, College of Engineering, University of Tehran, P.O. Box 11365-4563, Tehran, Iran
2. Department of Polymer Engineering & Color Technology, Amirkabir University of Technology, P.O. Box 15875/4413, Tehran, Iran
Email: Hafeezjafari@yahoo.com

Background and Aim: Biocompatible and biodegradable polycaprolactone (PCL) were filled by cellulose nanowhiskers (CNWs) obtained from wastepaper. Incorporated polycaprolactone nanocomposites were used for preparation of foamed scaffolds with bimodal cell sizes by solvent casting/particulate leaching method. Sodium chloride and sugar porogens were prepared to fabricate the scaffolds. The mechanical and thermal properties' of PCL/CNW nanocomposites were investigated. The advantage of incorporating various CNWs is to have tunable mechanical properties and biodegradability due to variety in their structure. All the bimodal foam nanocomposites were observed to be biodegradable as well as non-cytotoxic as revealed by MTT assay in fibroblast cell line SNLs. The PCL/CNW foam scaffolds were used for osteogenic differentiation of human mesenchymal stem cells (hMSCs). Our results suggest that these PCL/CNW nanocomposites, in conjunction with hMSCs proliferation, could also support osteogeneses them in three-dimensional synthetic extracellular matrix (ECM).
Methods: To fabricate traditional salt-leached scaffolds, PCL (2 g) in various percentage of filled CNWs (0 %, 0.1 %, 0.5 % and 1 % wt. related to polymer) and sugar (5 % wt. related to polymer) was dissolved in 15 mL DMF at 70 °C. To preparation of bimodal foam scaffolds with improved interconnectivity, prepared dispersion of PCL/CNW/sugar was mixed with equal amounts of NaCl salt (average crystal size of 100 – 250 μm) in combination with NaCl salt in a 2:10 PCL/CNW/sugar-to-combined salt ratio by weight. The viscous mixed suspension was placed in a Teflon mold, placed in a laminar flow hood for at least 2 days to evaporate the solvent. Next, the salt and sugar were leached out by immersion in deionized water for 72 h with water changes every 8 h. Finally, PCL/CNW foams were immersed in PBS solution before cell seeding for overnight.
Results: For determining the viability of cells on the nanocomposites, cells were cultured for 2 days and rinsed with PBS and stained with 0.01% (w/v) of acridine orange for 5 min. The nucleuses of living cells were green under a fluorescent light. Results are shown in Fig. 5A-F; cells seeded and have spread into bimodal foam scaffolds. Fig. 6A shows the results of MTT assay for PCL/CNWs after 2, 4 and 6 days contact of SNLs cultures with samples. There was a reduction above 90% in the cell viability compared to control for some of the PCL/CNWs, which can be considered as a slight and none cytotoxicity for all samples, but the lower the CNWs concentration in nanocomposites, the better the non-toxicity properties. Cytotoxicity can be rated based on the cell viability relative to controls, where activity relative to controls is less than 30% (severe cytotoxicity), between 30 and 60% (moderate cytotoxicity), and between 60 and 90% (slight cytotoxicity) or greater than 90% (not cytotoxic).
Conclusion: The aim of the present study was to fabricate bimodal nanocomposite foams based on PCL reinforced by cellulose nanocrystals and to investigate its potential to support the adhesion, proliferation and osteogenic differentiation of hMSCs.

Keywords: Poycaprolactone (PCL), Cellulose Nanowhiskers (CNWs), Human Mesenchymal Stem Cells (hMSCs), Osteogenic Differentiation, Bimodal Foam Scaffold

Ps-160: Enrichment of Antiapoptotic Exosomes by Transfection of Human Fibroblasts with Pegfp-C1-Mir-29a/Anti-Mir-21 Vectors

Saeideh Jafarinejad Farsangi[1], Sedigheh Gharbi[2*], Azam Rahim Pour[3], Maryam Shahrokhi[1]

1. Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran
2. Shahid Bahonar University of Kerman, Faculty of basic sciences, Biology Department, Kerman, Iran
3. Department of Tissue Engineering, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: sajaf1166@yahoo.com

Background and Aim: Fibrosis is one of the leading causes of mortality (~45% of deaths in the developed countries) worldwide. Uncontrolled production of extra cellular matrices (ECM) by fibroblasts leads to progressive loss of tissue function and organ failure in fibrotic diseases. MicroRNAs have attracted intensive researches because of their pivotal role in regulating production of the ECM. Although miR-29a and anti-miR-21 are well known as antifibrotic factors in fibrotic diseases, there are several outstanding obstacles including effective cellular uptake delaying therapeutic application of these micoRNAs. Exosomes have recently emerged as a novel effective, safe and cell-free system in regenerative medicine. Exosomes are a group of secreted vesicles loaded with RNAs, microRNAs and proteins which mediate intercellular communication. Here, we want to evaluate the potential of transfected fibroblasts with miR-29a/anti-miR-21 expressing vector to secrete exosomes mainly bearing the two antifibrotic factors mir-29a and anti-miR-21
Methods: Human primary fibroblasts will be transfected with peGFP-c1-anti-miR-21/mir-29a expression vector. Exosomes will be isolated from conditional medium using miRCURY™ Exosome Isolation Kit according to manufacturer's protocol. Mir-29a/anti-miR-21 differential expression in isolated exosomes will be assessed by Real Time PCR.
Results: The vesicular nature of exosomes makes them suitable vehicles for delivery of artificially synthesized anti-fibrotic microRNAs. Transfection of fibroblasts with expression vectors for mir-29a/anti-miR-21 is expected to enrichment of antifibrotic exosome.
Conclusion: Fibroblast exosomes which have been artificially loaded with antifibrotic microRNAs represents suitable vehicles for target delivery of microRNAs into fibrotic fibroblasts.
Keywords: Exosomes, MIRN21 microRNA, MIRN29 microRNA, Fibroblasts, Fibrosis

Ps-161: Effect of Preconditioning of Human Umbilical Cord Mesenchymal Stem Cells on Cyclophosphamide -Induced Premature Ovarian Failure in Mice

Mohamad Jafar Rezaie[1]*, Ladan Jalalie[1], Ali Jalilie[1], Mojtaba Karimpoor[2]

1. Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
2. Cellular and Molecular Research Center, Orumieh University of Medical Sciences, Orumieh, Iran
Email: jalalyl@yahoo.com

Background and Aim: Exposure of stem cells to sublethal concentrations of hydrogen peroxide (H2O2) can eliminate subsequent oxidative stress-induced apoptosis. We assessed the effects of H2O2 preconditioning on the therapeutic potential of human umbilical cord mesenchymal stem cells (UCMSCs) in a murine model of premature ovarian failure.
Methods: Mature mice were divided in to 2 groups: group 1- control group (10 mice) 2- CP group injected intraperitoneally by cyclophosphamide for 15 days and divided to three subgroups: subgroup IIa: CP group (10 mice) received CP injection and phosphate buffered saline intravenously. Subgroup IIb: CP+ MSC group (10 mice) that injected with CP (IP) and received single dose of 1×10 ^6 UCMSCs intravenously. Subgroup IIc: CP+pre MSC (10 mice) that injected with CP (IP) and received single dose of preconditioned MSC intravenously. 7 days later the mice were euthanized .Right ovarian of all groups were removed and processed

for histological and imunohistochemical studies. Histological studies were performed by using of HE and Trichrom masson staining and immunohistochemical studies by using Bax and TUNEL assay.
The number of different type of follicles by morpheometrical technique calculated. The apoptotic index calculated .These data interpreted by statistical analysis. Ovarian function was evaluated by monitoring CM-DIL labeled UCMSCs migration toward ovaries and detection of human (Cytocrom B gen) by polymerase chain reaction (PCR).
Results: our studies showed CP caused degenerative changes in the ovary these changes including follicular loss, stromal fibrosis and blood vessels injury. Immunohistochemical studies of CP group showed the evidence of Bax protein expression and TUNEL positivity in the oocyte and granulosa cells of primordial follicle. The other types of follicles show evidence of Bax and TUNEL positivity in granulosa and theca cell layers. In the (CP-MSC) and (CP- preMSC) groups our results show a few evidence of degenerative changes in the ovary. Immunohistochemical studies of these groups showed a few evidence of Bax protein expression and TUNEL positivity comparing with CP group. The number of follicles in the CP-MSC and CP-PreMSC group higher significantly compare to the CP group. Mean number of secondary follicles in CP-preMSC group significantly higher than CP-MSC. Monitoring of the MSC and Pre-MSC migration to the ovary by CM-DIL showed that the mean number of engrafted Pre-MSC was higher than MSC and mainly found in the ovarian tissue stroma. Human CYT B gen was detected in the both MSCs groups but in the CP and wild-type group was not found.
Conclusions: Our experiment give the new information about the using the stem cell therapy for treatment of ovarian failure function failure.
Keywords: Premature Ovarian Failure, Cyclophosphamide, Umbilical Cord Mesenchymal Stem Cell, H2O2 Preconditioning

Ps-162: Effect of Hypoxia on Expansion of Umbilical Cord Blood Mononuclear Cells in Three-Dimensional Culture Condition

Fateme Javani[1], Masoud Soleimani[2*], Saied Abroun[2]

1. Hematology and Blood Banking, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran
2. Department of Hematology, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran
Email: fateme.javani93@gmail.com

Background and Aim: The main purpose of tissue engineering is constructing of functional tissues for transplantation and repairing of damaged tissues. Approaching this goal requires making suitable environment for proliferation and differentiation of stem cells that can control them as same as body. Recently, many groups have examined the effect of dissolved oxygen concentration in maintenance, proliferation and differentiation of hematopoietic stem cells (HSCs), through in vitro culture. This study aimed to determine the effect of hypoxia on expansion of umbilical cord blood mononuclear cells contains hematopoietic stem cells in three-dimensional culture condition.
Methods: Three-dimensional culture context which is made of DBM scaffolds were prepared and placed in two same culture plates. 10,000 mesenchymal stem cells were cultured in each plate for five days in two different conditions in terms of the amount of oxygen by using two types of incubator Normoxia and incubator hypoxia. Then 1 million mononuclear cells (MNC) isolated from umbilical cord blood and co-cultured with MSCs for seven days in each plate. After completion of the culture period, cells were isolated from the scaffold and the expression of the CD34 surface marker and CD38 surface marker were studied by flowcytometry techniques.
Results: In flow cytometry study, the expression of the CD34 surface marker on day 0 and 7 for cells cultured in Hypoxia on average, reported 47% and 63% respectively, and expression of the CD38 surface marker on day 0 and 7, on average, reported 29% and 21% respectively. The expression of CD34 surface marker on day 0 and 7 for cells cultured in Normoxia, on average, reported 47% and 45% respectively, and expression of the CD38 surface marker on day 0 and 7, on average, reported 29% and 58% respectively.
Conclusion: Considering that the CD34 surface marker is hematopoietic stem cell surface marker, so high expression of this marker in cells that were cultured in hypoxic conditions indicates the positive impact of hypoxia on the expansion of HSCs.

Keywords: Tissue Engineering, Hypoxia, Three-Dimensional Culture

Ps-163: The Role of Hsf1 on Growth and Drug Resistance of Prostate Cancer Cell Line

Roya Yousefi[1], Mahboubeh Kabiri[1*], Fatemeh Koohkan[2], Sedigheh Asad[1]

1. Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
2. Stem Cell Technology Research Center, Tehran, Iran
Email: mkabiri@ut.ac.ir

Background and Aim: Prostate cancer is the most prevalent non-skin cancer among men in most western populations, and it is the second cause of cancer death among U.S. men. Its risk factors include advancing age, race and family history, androgens, diet, physical activity, sexual factors, inflammation, and obesity. Environmental and genetic factors both are implicated to have roles in pathogenesis of prostate cancer. As prostate cancers are hormone-responding malignancies, drugs that reduce the circulating testosterone levels or prevent this ligand binding to the androgen receptor (AR) could be efficient in prostate cancer treatment. The heat shock protein 90 (Hsp90) has an important chaperonic role in interaction with AR that keeps AR in a high-affinity ligand-binding conformation. It is shown that inhibiting this protein has some therapeutic effects in-vitro and in-vivo. Some of these inhibitors such as 17-AAG are now in clinical trial studies for prostate cancer therapy purposes. The heat shock transcription factor 1 (Hsf1) is the inducer of genes encoding heat shock proteins and molecular chaperones such as Hsp90. Recently, it is revealed that its expression level is significantly higher in some cancers including prostate cancer. In solid tumors, hypoxic conditions in the center of the tumor changes some gene expression profiles and consequently the growth profile and tumor response to therapeutic reagents.
Methods: In the present study, we aimed to investigate the effects of Hsf1 knock-down on proliferation and drug resistance of PC-3 cell line of prostate cancer in hypoxia-mimicking conditions. To better mimic the real tumors environmental conditions, 3D spheroid cultures are invoked. We designed two different shRNA sequences for two exclusive region of Hsf1 gene. The sequences were then cloned in pCDH plasmid vectors and propagated in DH5α E.coli. After confirmation of shRNA cloned vectors by sequencing, PC3 cells were transfected and examined for Hsf1 expression level by Real Time PCR. The difference between the growth of Hsf1 knocked down/ PC-3 in 2D and 3D culture platforms is determined using cell counting and MTT assay.
Results: Hsf1 has proven effects in drug resistance in different cancer cells. As it controls the expression of key resistance factors in prostate cancer, it can be a good target for gene therapy approaches. Herein we aimed to investigate Hsf1 knocked down PC3 cell line resistance to trastuzumab as a monoclonal antibody and Taxotere (brand name for Docetaxel) as a chemical drug, both used in prostate cancer therapy, in tumor resembling hypoxic conditions.
Conclusion: We observed that Hsf1 had no effect on PC-3 cells resistance to trastuzumab but we do expect that Taxotere resistance gets affected by Hsf1 knockdown, which would likely make this gene as a target for gene therapy of prostate cancer.
Keywords: Prostate Cancer, Hsf1, knockdown, Gene therapy, Drug resistance

Ps-164: Histomorphometric Changes in Auditory Nerve Ganglion Cells in Deaf Rats after Cell Therapy

Zahra Najjari, Gholamreza Kaka[1*], Behzad Mesbahzadeh[2]

1. Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Department of Physiology and Pharmacology, Birjand University of Medical Sciences, Birjand, Iran
Email: zahra.z.najjari@gmail.com

Background and Aim: This study aimed to investigate the histomorphometric changes in auditory nerve ganglion cells after cell therapy with Bone Marrow Stem Cell (BMSCs) in deafened rats.
Methods: Thirty-two male Wistar rats were randomly divided into four equal groups as below: 1- Normal hearing group (animals with no injection and surgery). 2- Sham group (normal hearing animals undergoing

surgery without any injection). 3- Amikacin group, deaf animals with 1.5 mg/g Amikacin. 4- BMSCs group, deaf animals undergoing surgery and injection of 4×105 BMSCs. Distortion product otoacoustic emission (DPOAE) of all rats was assessed before and after injection of Amikacin. The BMSCs were obtained from bone marrow of femur rats and the cells were passaged four times. Except the normal and sham surgery groups, the other groups were subcutaneously received 1.5 mg/g of Amikacin. Forty minutes later, these rats were intrapritoneally received 0.1 mg/g furosemide. On the 20 day of post surgery, DPOAE of all rats were measured and histological changes of cochlea were studied in all groups. The area of greatest ganglion cells and numbers of ganglion nuclei were assessed in all groups.
Results: The DP-Gram in rats with normal hearing undergoing sham surgery showed that procedure has no negative impact on cochlear function. The DP-Gram in Amikacin group revealed significant decreased with the frequencies 2KHz-8KHz ($p \leq 0.05$) compared to normal group. Mean area of greatest ganglion cells in Amikacin and BMSCs groups increased compared to normal and sham groups however these differences were not significant. Mean number of ganglion nuclei in Amikacin group increased when compared to normal and sham groups, however these differences were not significant. On the other hand, mean number of ganglion nuclei in BMSCs group decreased when compared to normal and sham groups.
Conclusion: Histomorphometric changes in rats undergoing cell therapy indicated that implanted BMSCs into the cochlea have no rehabilitation effects on deaf rats receiving 1.5mg/g Amikacin.
Keywords: BMSCs, Rat, Deaf, Auditory Nerve Ganglion

Ps-165: Evaluation of Effect Autologous Intra Spinal Transplanting of Bone Marrow Stromal Cells into Contusion Spinal Cord Injury in Rat

Zahra Najjari, Gholamreza Kaka*

Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
Email: gh_kaka@yahoo.com

Background and Aim: One of therapeutic strategies of spinal cord injury (SCI) is cell therapy. In the present study bone marrow stromal cells (BMSCs) after labeling with BrdU were transplanted to rats subjected to contusion SCI and their role in motor improvement was studied.
Methods: Under sterile conditions BMSCs were obtained from femur of adult rats. Following four passages of cell culture, these cells were prepared for autologous transplanting. For determining of mesenchymal and stemness of these cells was used from CD44, CD45 AND Fibronectin markers and Oct-4 primer by immunocytochemistry and RT-PCR methods respectively. For in vivo studies 32 adult female rats were subdivided to 4 groups. In 1st group or Sham only laminectomy was performed at T13 level, whereas in other groups after laminectomy a contusion SCI was brought about by dropping a rod 10g weight on the spinal cord from a 2.5 cm height. In 2nd group or Control 1 (C1) no treatment was performed following injury. In other two groups 7 days after SCI was performed injection in three area of caudal, central and cephalic. So in 3rd group or control 2 (C2) 9 µl normal saline was injected intra spinally (IS) and in 4th group or experimental (E) group BMSCs transplantation was performed IS. In all groups one day before SCI up to 12 weeks after it, motor improvement was assessed by BBB test. At the end of 12th week, spinal cord segments T12-L1 were studied by histomorphology and immunohistochemistry to determine histologic changes and the extent of cell replacement at the injury site.
Results: Immunohistochemical and RT-PCR studies by means of fibronectin, CD44, CD45 and Oct-4 indicated that following 4th passage of BMSCs, a considerable percentage of them were stemness and mesenchymal. The findings of BBB test showed a spontaneous gradual and restricted motor improvement in control groups. In experimental cell-therapy group a significant motor improvement could be seen again control groups. This motor improvement was more obvious during 2nd to 4th weeks and got less prominent during 4th to 12th weeks. Histomorphometric findings indicated, cavity formation significantly decreased in central area of spinal cord of E group again control groups. Number of detected BrdU positive transplanted cells at cranial and caudal regions of spinal cord were significantly more than central region.

Conclusion: Intra spinal autologous transplantation of BMSCs results motor improvement in contusion spinal cord injury.
Keywords: Bone Marrow Stromal Cell, Autologous Transplantation, Spinal Cord Injury

Ps-166: Crocin, Extracted from Saffron, as a Potent Osteogenic Agent

Fatemeh Kalalinia*

Biotechnology Research Center, Mashhad university of Medical Science, Mashhad, Iran
Email: kalaliniaf@mums.ac.ir

Background and Aim: Mesenchymal stem cells (MSCs) are adult stem cells that can differentiate into various lineages of mesenchymal tissues. These features of MSCs attract a lot of attention from investigators in the context of cell-based therapies of several human diseases. In the present study, the ability of crocin, extracted from saffron, to induce cell differentiation of rat bone marrow-derived mesenchymal stem cells (rat BM-MSCs) into osteoblasts was studied.
Methods: Bone marrow cells were isolated from rat's femor. Cytotoxic effect of crocin was evaluated using MTT assay and IC50 was calculated from the results. Osteogenic ability of crocin has been evaluated and compared with the BM-MSCs that have been treated with osteogenic standard medium after 7 and 21 days, using alizarin red staining and alkaline phosphatase (ALP) activity. Furthermore, ALP mRNA expression have been evaluated by Real time RT-PCR.
Results: The results showed a significant osteogenic activity of crocin. Crocin and osteogenic standard medium could increase ALP mRNA expression up to 19 and 3 fold increase in compare with negative control, respectively. Also, crocin and osteogenic standard medium could increase ALP activity up to 61 and 1.98 fold increase in compare with negative control. The similar results has been showed in alizarin red staining.
Conclusion: According to the findings of this study, crocin could effectively enhance osteogenic differentiation of MSCs and can be considered as safe therapeutic agents in clinical applications.
Keywords: Crocin, Osteoblast, Mesenchymal Stem Cells

Ps-167: In Silico Analysis of a Chimeric Protein Containing CD96, A Leukemic Stem Cell-Specific Marker, and Alpha-Crystallin B Chain

Shima Kalantar[1*], Ali Ghamari[2], Parviz Pakzad[3]

1. Microbiology Department, Faculty of Life Sciences, Damghan Branch, Islamic Azad University, Damghan, Iran
2. Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran
3. Immunology Department, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Shima.kalantar67@yahoo.com

Background and Aim: treatment of acute myeloid leukemia (AML) by chemotherapy alone remains evasive for most patients because of the inability to effectively annihilate leukemic stem cells (LSCs), the self-renewing component of the leukemia. CD96, which is a member of the Ig gene superfamily, is a promising candidate as an LSC-specific antigen. Studies have confirmed that vaccination based on CD96 enhances the cellular and humoral immune responses and inhibits the growth of CD96-expressing tumors. So, CD96 may be a potential target for AML immunotherapy. Alpha-crystallin B chain is part of the small heat shock protein family and functions as molecular chaperone that primarily binds misfolded proteins to prevent protein aggregation, as well as inhibit apoptosis and contribute to intracellular architecture. Previous studies have demonstrated that vaccination with Alpha-crystallin B chain elicit specific antitumor responses. These findings suggest that Alpha-crystallin B chain is involved in the process of antigen presentation and has potential as an immune-adjuvant chaperone for specific antigens in vaccines.
Methods: In the present study, we successfully constructed recombinant gene producing chimeric protein based on CD96 and Alpha-crystallin B chain which lays the foundation for the development of a vaccine for AML. We have designed an immunogen complex consist of CD96 with overall length genes of Alpha-crystallin B chain that represents a three-dimensional epitope of chimeric multitope protein. The construct were analyzed by bioinformatic's softwares. Stability, proper energy level, linear and discontinuous B-cell epitopes,

MHC class I and II binding peptides of chimeric protein were predicted.
Results: The designed chimeric moltitope had stability, proper energy level and same immunogenicity as the original protein's epitopes. The chimeric gene can clone in prokaryotic system. Our data indicates that epitopes of the synthetic chimeric protein could induce both B-cell and T-cell mediated immune responses which are important for a protective vaccine against AML.
Conclusion: Studies have confirmed that CD96 is expressed on the majority of AML cells in many cases, whereas only a few cells in the normal population expressed CD96 weakly. Many studies have confirmed that vaccination with Alpha-crystallin B chain -antigen fusion proteins elicit antitumor immune responses. The present study confirmed the potency of human Alpha-crystallin B chain as a molecular chaperone to use as immune-adjuvant for this recombinant protein. Our data may also suggest this synthetic chimeric protein as a vaccine candidate subunit against acute myeloid leukemia.
Keywords: CD96, Alpha-Crystallin B Chain, AML, Chimeric Protein, Vaccine

Ps-168: Expression and Purification of a Recombinant Protein Containing Cd96, a Leukemic Stem Cell-Specific Marker, and Alpha-Crystallin B Chain

Shima Kalantar[1*], Ali Ghamari[2], Parviz Pakzad[3]

1.Microbiology Department, Faculty of Life Sciences, Damghan Branch, Islamic Azad University, Damghan, Iran
2.Immunology Research Center, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran
3.Immunology Department, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Shima.kalantar67@yahoo.com

Background and Aim: Acute myeloid leukemia (AML) is a hematic disorder characterized by immature myeloid cell proliferation and bone marrow failure. CD96 as a cell surface molecule is differentially expressed in leukemic stem cells (LSCs), and plays critical roles in the maintenance, survival and functions of LSCs. Studies have confirmed that vaccination based on CD96 enhances the cellular and humoral immune responses and inhibits the growth of CD96-expressing tumors. So, CD96 may be a potential target for AML immunotherapy. Alpha-crystallin B chain is part of the small heat shock protein family and functions as molecular chaperone, which assists in transport, assembly and folding of proteins in the cytoplasm transmembrane. Previous studies have demonstrated that vaccina¬tion with Alpha-crystallin B chain complexes elicit specific antitumor responses. These findings suggest that Alpha-crystallin B chain is involved in the process of antigen presentation and has potential as an immune-adjuvant chaperone for specific antigens in vaccines.
Methods: The desire recombinant gene based on CD96 and Alpha-crystallin B chain which designed and analyzed by bioinformatics software was chemically synthesized. pET28a was used as an expression vector for transformation of competent BL21(DE3) Escherichia coli. The expression of chimeric multitope in recombinant bacteria induced by Isopropyl β-D-1-thiogalactopyranoside (IPTG). Nickel affinity chromatography was used for purification of chimeric protein. The purified chimeric protein identified and analyzed by SDS-PAGE and Western blotting.
Results: The chimeric gene can clone in prokaryotic system. The expression of the protein corresponding to the predicted size was induced in the presence of IPTG. Recombinant fusion protein was purified by Nickel affinity chromatography. Identification of recombinant fusion protein was performed by SDS-PAGE and Western blotting that confirmed the presence of the chimeric protein.
Conclusion: In this study, we presented evidence that human Alpha-crystallin B chain enhances the solubility of CD96. The chimeric multitope was successfully expressed to a high level in E.coli in soluble form and it is convenient for purification. Following three steps of purification, a purity of greater than 93% of the recombinant fusion protein was obtained. Western blotting revealed that the recombinant fusion protein obtained via purification had the same immunological characteristics. In conclusion, the present study confirmed the potency of human Alpha-crystallin B chain as a molecular immune-adjuvant chaperone and CD96 for a recombinant protein vaccine, which lays the foundation for the development of vaccines for AML and further clinical research.

Keywords: CD96, Alpha-Crystallin B Chain, AML, Chimeric Protein, Vaccine

Ps-169: Comparison of Different Concentrations of Triton X-100 Detergent in Lung Decellularization

Ali Karami[1], Hamid Tebyanian[1], Ebrahim Motevalian[2], Jafar Aslani[2], Mohammad Reza Nourani[3*]

1. Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Department of General Surgery, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
Email: ali.karami@gmail.com

Background and Aim: Pulmonary disease is a worldwide public health problem that reduces the life quality and increases the need for hospital admissions as well as the risk of premature death. In the event that lung diseases turn to severe complications, the patient might end up with transplantation as the only chance for survival. In this regard, a common problem is the significant shortage of lungs for transplantation. Tissue engineering techniques have offered a promising alternative for transplantation from donors: engineered lung tissue. In this study, we want to find the best decellularization approach for maintaining three-dimensional lung architecture and extracellular matrix (ECM) proteins composition which has significant roles in differentiation and migration of cells.
Methods: In this study, PBS, Heparin 5000u/ml, and 1% penicillin and streptomycin perfused via heart into the organ and whole body to remove blood. Animals sacrificed with ketamine and xylazine (100 mg/kg and 10 mg/kg). They were identified and fixed with catheters before harvesting lung to use in decellularization process. Different concentrations (Table 1) were used for decellularizing rat lungs for maintaining three-dimensional lung architecture and ECM protein composition which have significant roles in differentiation and migration of cells. All procedures performed in a sterile condition. Finally, decellularized lungs were evaluated by Hematoxylin and Eosin staining, Trichrome-Masson staining, and Elastin staining. Table 1: Triton X-100 detergent approaches for rat lung decellularization Time Methods Triton X-100 approaches
Results: Removing of nuclei was not completely observed by increasing the concentration of Triton X-100 (0.05% to 0.2%) at 24 h. Method 1 could relatively maintain the 3D of decellularized lung but it could not completely remove nuclei from tissue compare to other methods in Triton X-100 approaches. Methods 6 and 10 were observed as best methods decellularization in maintaining of structures and removing of nuclei in Triton X-100 approaches at 48 and 72 h. In Triton X-100 approaches, method 1 could maintain the 3D but it was weak in removing of nuclei from lung tissue at 24 h. Methods 6 and 10 were the best decellularization approaches in preserving 3D and ECM collagen and elastin. Destroying of collagen and elastin were increased by increasing of the concentration of detergent and times. Methods H& E Trichrome-Masson Elastin Control 1 6 10 Figure1: Trichrome-Masson staining; it shows collagen in Blue color, Elastin staining; it shows elastin in Blue to black color (40X).
Conclusion: We conclude that these approaches can help to achieve three-dimensional architecture and ECM protein composition of the lung with minimum destruction for next steps such as recellularization and in-vivo study.
Keywords: Lung, Decellularization, Triton X-100

Ps-170: The Effect of Bioactive Glass on Adipose-Derived MSC Osteogenesis in 3D Cultures

Mohammad Hossein Karami[1], Mahboubeh Kabiri[2*], Mahshid Heidari[2], Hana Hanaee-Ahvaz[3], Ali Farazmand[1]

1. Cellular and molecular Biology, Department of Biology, College of Science, University of Tehran, Tehran, Iran
2. Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
3. Department of Stem Cell Biology, Stem Cell Technology Research center, Tehran, Iran
Email: mhkarami.ut@gmail.com

Background and Aim: Regenerative medicine is an emerging field of biotechnology that combines various aspects of medicine, cell and molecular biology, mate-

rials science and bioengineering in order to regenerate, repair or replace tissues. Bone regeneration is one of the focus points in the field of regenerative medicine because of huge number of patients needing bone surgery every year. Adipose tissue (AD) represents a hot topic in regenerative medicine because of the tissue source abundance, the relatively easy retrieval, and the inherent biological properties of mesenchymal stem cells residing in its stroma .Capturing the full potential of MSC will likely require the development of novel in vitro culture techniques. Here, we describe a novel and efficient system to culture MSCs in 3D microaggregates composed of cells and bone mimicking inorganic nano-sized compounds and investigate their effects on MSC osteogenesis.
Methods: MSCs were cultured in microwells (custom made from poly dimethylsiloxane (PDMS)) to form 3D microaggregates in osteogenic induction medium. Spheroids were generated using PDMS-based concave micromolds developed using thin PDMS membranes (figure1). In addition we addded ~0.42 µg bioactive glass into single multicellular aggregates formed from 80 mesenchymal stem. The concave microwells were coated with 5% pluronic acid to prevent cell attachment. Cell aggregation and spheroid formation were observed daily under a microscope. Calcium content assay was performed to evaluate osteogenesis. Classic 2D cultures served as control group.
Results: We successfully used concave microwell arrays to form uniform-sized AD derived mesenchymal stem cell spheroids. MSC on cylindrical PDMS microwells modified with pluronic acid formed homogenous 3D microaggregates. However some spheroids formed were joining together making larger spheroids also not stable in cylindrical microwells (figure2). Our results showed that at day 7 and 14 post induction there was significantly greater calcium deposition in the microaggregates, relative to the 2D cell monolayer culture. In addition the resultant bio-glass endowed microaggregates cultured for two weeks under osteogenic induction medium showed prolonged formidability and rigidity. We would expect higher mechanical strength in composite endowed micro aggregates in comparison without bio glass or 2D controls
Conclusion: We developed concave microwells as a substrate for the formation of uniform-sized. In addition, we demonstrated that the concave microstructure offers a substantial advantage for the formation and harvesting of spheroids. Bone mimicry micro tissues composed of both cells and Bioactive glass can pave the way for macro tissue formation. We expect that our micro bone aggregates can be of so much use in the field of bone tissue engineering.
Keywords: Adipose Mesenchymal Stem Cell, Osteogenesis, 3D Micro-Aggregates, Bioactive Glass

Ps-171: Electrospun Polycaprolacton/Gelatin/Polyaniline Conducting Nanofibrous Scaffold and Neural Differentiation of Human Induced Pluripotent Stem Cells

Ali KarbalaeiMahdi[1*], Marzieh Ghollasi[2], Ali Salimi[1]

1. Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Department of Cell and Molecular Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
Email: Ali_km77@yahoo.com

Background and Aim: Tissue engineering has always been a significant tool for scientists in order to address the problems and abnormalities that take place in tissues and cells. Nanofibrous scaffolds have always been a seemingly suitable tool for cell differentiation and growth. Though, due to the huge number of polymers and solvents, it has always been a struggle to find a feasible scaffold that is superior to others. Although stem cell differentiation has always been addressed by cells such as Mesenchymal stem cells, recently due to the discovery of induced pluripotent stem cells, there has been a shift in research programs. Such cells possess multipotency and hold great promise regarding tissue engineering.
Methods: In this study, we tried to prepare a new blend in order to investigate the differentiation of human induced pluripotent stem cells to neural cells. To address this issue, we prepared a blend of polyaniline, gelatin and polycaprolactone. This blend was fabricated in order to combine the biocompatibility of PCL and GEL alongside the conductivity of PANi. Electrospinning of polymers was done to make the fibers in the finest way. After preparing a feeder layer, hIPSCs were cultured and then differentiated. Our results of 2 weeks showed

a significant difference between the capability of PCL-GEL-PANi fiber compared to other common blends.
Results: Our results were confirmed by a variety of tests. SEM imaging was done to confirm the morphological changes throughout two weeks of differentiation. In order to make certain of the genetical changes, real-time PCR was done. To investigate other aspects of differentiation, ICC was also performed.
Conclusion: Overall, our results indicated that the new blend of PCL-GEL-PANi not only supports stem cell growth, but it also helps the differentiation to take place more efficiently. We hope to make use of such blend regarding tissue engineering in order to provide a more efficient and feasible surface for stem cell differentiation.
Keywords: Human Induced Pluripotent Stem Cells, Neural, Tissue Engineering, Scaffold, Polyaniline, Polycaprolactone, Gelatin

Ps-172: Electrospunpolycaprolacton/Gelatin Nanofibrousscaffold: A Promising Composite for Neural Differentiation of Human Induced Pluripotent Stem Cells

Ali KarbalaeiMahdi[1*], Marzieh Ghollasi[2], Ali Salimi[1]

1. Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Department of Cell and Molecular Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
Email: Ali_km77@yahoo.com

Background and Aim: Recently, tissue engineering has attracted a great deal of attention due to its application in tissue and cell regeneration. To do so, in the past years there has been a great effort to improve the results regarding tissue regeneration. Recent advances in cell culture has led us to the discovery of induced pluripotent stem cells. It should be noted that such cells hold great potential compared to other types of stem cells and as a result are more feasible in terms of tissue regeneration. Nanofibrous scaffolds have been in the center of attention because of their unique structure and capabilities. Though, as great as efforts have been, it is still premature to reach a feasible scaffold to fully address tissue engineering problems. A lot of polymers have been used, both in single form and as a combination with other polymers. However, it can't be used in fibers alone since it has a rather worrying toxicity and it can't be electrospun. When used in blends, not only it has proven to be non-toxic but it also has shown to support cell growth.
Methods: In this study, for the first time, polycaprolactone (PCL) and gelatin (Gel) were used in a composite for neural differentiation of hiPSCs. The composite was fabricated by electrospinning. MTT assay was used to evaluate the toxicity on the composite. hiPSCs were seeded on the fibers and after 14 days of differentiation, to confirm the differentiation, Real-time PCR and immunocytochemistry (ICC) tests were performed. For morphological studies, Scanning electron microscopy (SEM) imaging was used.
Results: Our results indicated that hiPS cells had differentiated to neural cells completely. Our study demonstrates that PCL-GEL composite not only has the capability to support iPSC differentiation to neural cells, but it also is able to enhance and improve such process.
Conclusion: Overall, PCL-Gel seems to be a feasible, reliable and easily accessed composite for further tissue engineering experiments
Keywords: Human Induced Pluripotent Stem Cells; Neural; Tissue Engineering; Scaffold; Polycaprolactone; Gelatin

Ps-173: Effect of Simulated Weightlessness on Neurogenesis in the Dentate Gyrus and the Subventricular Zone of Adult Male Rats

Seyed Asaad Karimi[1*], Iraj Mirzaii-Dizgah[2]

1. Department of Physiology and Neurophysiology research center, Medical School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Physiology, School of Medicine, Aja University of Medical Sciences, Tehran, Iran
Email: asad_karimi_128@yahoo.com

Background and Aim: There is limited evidence that space environment, especially microgravity condition, may affect central nervous system (CNS). Exposure to microgravity results in extensive physiological changes in humans. This experiment hypothesized that there would be modifications in neurogenesis of the dentate gyrus (DG) of the hippocampus and the subventricular

zone (SVZ) of the lateral ventricle (LV) in adult male rats.
Methods: The tail-suspended, hindlimb-unweighting rat model was used to simulate the simulated weightlessness (SW). Male Wistar rats were randomly divided into control group (Con) and 2 week under (SW). Brain was fixed by infusion of phosphate buffer with 4% paraformaldehyde in the anesthetized rat and cross-sectional samples, around the dentate gyrus (DG) and SVZ, were analyzed immunohistochemically. Bromo-deoxy-uridine (BrdU) and doublecortin (DCX) were used as markers for newly generated cells and immature neuron, respectively. Six doses of BrdU (50 mg/kg body weight,) were administered i.p. for the last 6 days of SW to label newly generated cells in the DG and SVZ. DCX immunohistochemistry was performed to label immature cells in the DG and SVZ.
Results: The number of BrdU/DCX positive cells in the DG and SVZ of SW group was significantly decreased compared with control animals ($P<0.05$).
Conclusion: Our findings provide insight that a simulated weightlessness reduces the neurogenesis in the DG and SVZ region of adult's rats, which may have great effects on brain development.
Keywords: Simulated Weightlessness, Neurogenesis, Dentate Gyrus, Subventricular Zone, Rat

Ps-174: Baghdadite-Coated Electrospun Poly (L-Lactide) Nanofibers Enhance Osteogenic Differentiation of Stem Cells and Induce Ectopic Bone Formation

Zohre Karimi[2], Ehsan Seyedjafari[3], Khojasteh A[4], Hashemi SMM[5], Kazemi B[1,2], Samira Mohammadi-Yeganeh[1,2*]

1. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Biotechnology, University of Tehran, Tehran, Iran
4. Department of Tissue Engineering, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Department of Immunology, School of Medicine, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: smyeganeh@gmail.com

Background and Aim: A combination of calcium baghdadite (Ca3ZrSi2O9) with nanofibrous scaffolds holds promising potential for bone tissue engineering applications. In this study, baghdadite was coated on the plasma-treated surface of electrospun poly(L-lactide) (PLLA) nanofibers and the capacity of fabricated scaffolds for bone formation was investigated in vitro using human Adipose stem cells (hASC) under osteogenic induction and in vivo after subcutaneous implantation. PLLA and baghdadite PLLA (n-HA/PLLA) scaffolds exhibited a nanofibrous structure with interconnected pores and suitable mechanical properties.
Methods: These scaffolds were also shown to support attachment, spreading, and proliferation of hASC, as shown by their flattened normal morphology and MTT assay. During osteogenic differentiation, significantly higher values of ALP activity, biomineralization, and bone-related gene expression were observed on in baghdadite PLLA compared to PLLA scaffolds. Subsequently, these markers were measured in higher amounts in hASC on PLLA nanofibers compared to TCPS.
Results: According to the in vivo results, ossification and formation of trabeculi was observed in the baghdadte/PLLA scaffold compared to PLLA. Taking together, it was shown that nanofibrous structure enhanced osteogenic differentiation of hASC.
Conclusion: Furthermore, surface-coated baghdadi stimulated the of nanofibers on the orientation of hASC toward osteolineage. In addition, the baghdadite /PLLA electrospun scaffold showed the capacity for ectopic bone formation in the absence of exogenous cells.
Keywords: Osteogenesis hAdsc Baghdadite

Ps-175: Organoid Technology in Modeling Pancreas Development for Tissue Engineering Purposes

Maryam Kaviani*, Negar Azarpira

Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Email: mary_kaviani@yahoo.com

Background and Aim: The limitation of donor pancreas in damaged tissue replacement with healthy tissue highlights the need for other tissue sources. Organoid technology may provide appropriate functional pancreas for transplantation in regenerative medicine. Therefore, researchers intend to expand the isogenic tissue from patient biopsies and to find the process of specific tissue formation.
Methods: Researchers recruited induced pluripotent stem cells, embryonic stem cells and adult mouse/human pancreas to form pancreatic organoid structures. According to the endodermal origin of the pancreas, first, the cells were treated with Activin A to trigger TGF-β signaling that leads to the formation of definitive endoderm. After that, the differentiation of cells were achieved by the employing of specific activators and inhibitors cocktail to modulate WNT, BMP, and FGF pathways. Researchers, have done molecular and functional studies on the produced structures.
Results: In vitro studies demonstrated that embryonic pancreas progenitors can grow into 3D organoids and retain their potential to differentiate into different lineages of acinar, ductal or endocrine cells during the expansion for up to 2 weeks in culture. On the other hand, mouse pancreas and liver organoid initiating epithelial cells showed extensive similarity in transcriptome. In vivo studies showed that pancreas organoids were able to differentiate in ductal and endocrine lineages pathways after engraftment in a developing pancreas. Moreover, researchers found that pancreatic organoid cells unexpectedly reveal a hepatocyte-like cells generation capacity after transplantation in a model of animal liver damage.
Conclusion: The inability to control glucose level in diabetes mellitus and inflammation-related diseases such as pancreatitis lead to explore appropriate methods for the production of functional pancreas. It can be predicted that three- dimensional cell culture systems, organoid, will provide a physiological and functional tissue model system in vitro for biomedical applications.
Keywords: Organoids, Pancreas, Tissue Engineering

Ps-176: Characterization of Human Mesenchymal Stem Cells Derived From Outer Layer of Aorta

Maryam Kaviani, Negar Azarpira*, Mahdokht Hossein Aghdaei, Elaheh Esfandiari, Bita Geramizadeh, Alireza Shamsaeefar

Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Email: mary_kaviani@yahoo.com

Background and Aim: Cell- based therapies are one of the most promising approaches in regenerative medicine. Therefore, researchers have afforded to find appropriate cell sources for using in cell therapies. In this study, we focused on mesenchymal stem cells derived from outer layer of aorta.
Methods: After obtaining written informed consent and in accordance with the ethics committee of the institution, human aorta was transferred to cell isolation lab. The surrounding tissues were removed and after washing, aorta pieces were settled on the plate as an explant culture. The cells were cultured and characterized at 3th – 5th passages. For this purpose, surface antigens expression including; CD29, CD34, CD44, CD45, CD73, CD90, and CD105 were analyzed by flow-cytometry. Moreover, osteogenic and adipogenic differentiation potential were evaluated in appropriate condition.
Results: The cells showed fibroblast like shape and formed colony structures. These cells expressed CD29, CD44, CD73, and CD90 and did not express CD34, CD45, and CD105. On the other hand, they were able to undergo in adipogenic and osteogenic differentiation pathways. In this regard they showed intracellular lipid droplets in adipogenic induction and mineralization in osteogenic differentiation.
Conclusion: In the present study the isolated cells from outer layer of aorta showed mesenchymal stem cells features. Subsequent to more analysis of these cells, it is possible to use them in clinical purposes.
Keywords: Mesenchyme Stem Cells, Aorta, Isolation, Characterization, Regenerative Medicine

Ps-177: Characterization of Human Pancreatic Mesenchymal Stem Cells for Using In Islet Transplantation

Maryam Kaviani[1], Negar Azarpira[1*], Somayeh Keshtkar[1,2], Elaheh Esfandiari[1], Mahdokht Hossein Aghdaei[1], Bita Geramizadeh[1], Saman Nikeghbalian[1]

1. Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: mary_kaviani@yahoo.com

Background and Aim: The shortage of pancreas for organ transplantation leads to attention to alternative methods. Pancreatic islet transplantation is one of the most promising approaches for Diabetes Mellitus treatment. Recently, researchers have made some efforts to optimize islet isolation and culture methods. Co- culture of islets with mesenchymal stem cells may improve islet transplantation outcome. In the present study we characterized the mesenchymal like cells derived from human pancreas.
Methods: Human pancreas was obtained subsequent to organ donation in accordance with the ethics committee of the institution. Human pancreas was digested using enzymatic and mechanical methods. The presented mesenchymal stem cells in the digested pancreas tissue was cultured and characterized using morphology study, flow cytometry analysis, and multilineage differentiation assay.
Results: The pancreatic mesenchymal stem cells showed fibroblast-like morphology and expressed CD29, CD44, CD73 and CD90, as mesenchymal stem cells markers. However, they did not express CD45, CD34, and CD105. On the other hand, these cells reveal osteogenic differentiation subsequent to Alizarin Red staining. In adipogenic differentiation medium, a few intracellular lipid droplets were visible.
Conclusion: Pancreas is a source of mesenchymal stem cells. These cells may present appropriate condition for cultured islets and improve islet stability and function. Moreover, these cells can be considered in a variety of different cell-based therapies.
Keywords: Transplantation, Human Pancreas, Mesenchymal Stem Cells, Characterization

Ps-178: The Evaluation of Isolated Human Pancreatic Islets after Recovery Period: Viability and Gene Expression Analysis

Maryam Kaviani[1], Negar Azarpira[1*], Somayeh Keshtkar[1,2], Mahdokht Hossein Aghdaei[1], Elaheh Esfandiari[1], Bita Geramizadeh[1], Masoud Dehghan[1]

1. Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: mary_kaviani@yahoo.com

Background and Aim: Recently, researchers have focused on islet transplantation for diabetes mellitus treatment. For this purpose, they optimized their pancreatic islet isolation and culture to improve the outcome. Researchers suggested that isolated islets need an overnight culture before using in experimental or clinical purposes. In the presented study, we analyzed human pancreatic islets after this recovery period.
Methods: Human pancreas was transferred to cell isolation lab in accordance with the ethics committee of the institution. We isolated human pancreas islets using enzymatic and mechanical methods. After purification in COBE 2991 cell processor, islets were evaluated with Dithizone staining. Islets were cultured in CMRL medium overnight at 30°C in a humidified atmosphere of 5% CO 2. After that, islet viability was determined. The expression of BAX, as an apoptosis regulator, and insulin gene, as a β-cell specific gene, were analyzed using real time RT-PCR.
Results: After overnight culture islets showed about 75% viability. DTZ staining indicated that there was insulin in the cells granules. The expression of insulin was strongly confirmed by real time RT-PCR. The evaluation of BAX gene suggested that this gene was relatively expressed.
Conclusion: This study suggested that overnight culture after pancreatic islet isolation might lead to recovery of them, but it is essential to use appropriate anti-apoptotic and anti-hypoxic factors to reduce stressful conditions on the cells.
Keywords: Human Pancreatic Islets, Transplantation, Isolation, Culture, Viability, Gene Expression

Ps-179: Expression Pattern Analysis of Hsa-Mir-140-3p during the Cardiomyocytes Differentiation Process

Maryam Kay[1], Bahram Mohammad Soltani[1*], Hossein Baharvand[2]

1. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan, Tehran,Iran
Email: Maryam_kay2001@yahoo.com

Background and Aim: MiRNAs are known to be important post-transcriptional gene expression regulators of cardiogenesis process and are considered as promising candidates for therapeutic purposes. The implications of miRNAs in some pathological process of the cardiovascular system, including cardiac arrhythmia, cardiac hypertrophy, and heart failure as well as differentiation processes have been recently documented.
Methods: Here, we aimed to find novel candidate miRNA affecting Wnt and TGFB signalling pathways as a main pathways of the cardiac differentiation and analyse their expression pattern in the process of cardiogenesis. To this aim, miRNAs which are potentially target the important genes of Wnt and TGFB signalling pathway were screened using the bioinformatic analysis. RH5 cells were differentiated into the cardiomyocytes using small molecules and the expression pattern of candidate miRNA was analysed during the differentiation process (Days 0, 1, 2, 4, 6, 12) using qRT-PCR.
Results: The expression of marker genes during the differentiation process were analysed, which indicate that hESCs were differentiated to the cardiomyocytes. The expression pattern of hsa-miR-140-3p was also analysed. Our results indicate that its expression was enhanced in day1 and day4, which is correlated with the expression of Wnt and TGFB signalling pathway genes.
Conclusion: The alteration of the hsa-miR-140-3p expression pattern suggest its potential role during cardiomyocytes differentiation.
Keywords: Cardiomyocytes, miRNA, hsa-miR-140-3p, Differentiation

Ps-180: Evaluation of Autophagy Related Micrornas 204 and 30a in Acute Monocytic Leukemia

Alireza Kazemi[1], Mohammadreza Sadri[1], Narges Yazdi[2], Mohammad Houshmand[3], Amir Atashi[4*]

1. Department Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Molecular Genetics, Tehran Medical Branch, Islamic Azad University, Tehran, Iran
3. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
4. Stem Cell and Tissue Engineering Research Center, Shahroud University of Medical Sciences, Shahroud, Iran
Email: al.kazemi@yahoo.com

Background and Aim: Although the clinician has taken a different therapeutic approach for the treatment of acute myeloid leukemia, there is not a panacea for the patients and after a while, they are suffering from drug resistance and relapse. Recently, autophagy has introduced as the inevitable section of drug resistance process in AML. Autophagy is an intracellular mechanism which cellular organelles digested by own cells to conquer the stress condition within the environment, subsequently enhance their viability. Meanwhile, different studies show that microRNAs have a role in the regulation of this process and other studies confirmed the effect of mir-30a and mir-304 with drug resistance and relapse in AML patients.
Methods: In this study, we resolved to utilize Cytarabine (ara-C), as the first line of treatment in AML patients, along with Chloroquine (CQ) and 3-Methyladenine (3-MA) as autophagy inhibitors and Rapamycin as autophagy inducer on the Expression level of mir-30a and mir-204 in THP-1 cell line.
Results: Our results show that expression of these microRNAs increased while ara-C and autophagy inhibitors were used and following this, we face with a reduction in some genes related to autophagy (LC3B, Becn1), enhancement of apoptosis, reduction of proliferation rate and escalation of G1 arrest, which all of these refer the key role of autophagy in drug resistance in AML.
Conclusion: On balance, mir-30a and mir-204 would be decent prognostic markers in AML patients.
Keywords: Autophagy, Acute Myeloid Leukemia, MicroRNAs and Cytarabine

Ps-181: Stem Cell Induction and Differentiation of Photosynthetic Cells for Biosynthesis of β-Pinene in *Hyssopus Officinalis*

Elham Kazemi*

Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
Email: ekazemi2002@yahoo.com

Background and Aim: Several essential oils have been applied as therapeutic agents since ancient times, and some of them have been proven to possess medicinal properties, including anti-inflammatory, antiviral, antitumor, cytotoxic, and antimicrobial activities. Essential oils are complex mixtures of volatile, lipophilic and odiferous substances from the secondary metabolism of medicinal plants. They are mainly composed of sesquiterpenes, monoterpenes and their oxygenated derivatives (aldehydes, alcohols, esters, ketones, phenols and oxides). β-Pinene is used for the treatment, control, prevention and improvement of diseases and symptoms. The Hyssopus officinalis L. is an important medicinal plant. Antimicrobial and antifungal activities of the essential oil of hyssop have been reported. In this study the effects of hormones and elicitors on stem cell induction and cell culture condition for β-pinene production in H. officinalis were studied.
Methods: The current study included of two sections: stem cell induction and cell suspension culture. In this research, the fresh leaves of H. officinalis were used for stem cell or callus induction. In order to optimize the stem cell induction, different levels of 6-Benzylaminopurine (BAP or B) in three levels (0, 0.5, 1 mg/L), Naphthalene Acetic Acid (NAA or N) in four levels (0.0, 0.5, 1, 2 mg/L) that were evaluated in MS medium. Stem cell mass was inoculated to 200 mL liquid media with best concentrations of regulators based on the previous test (NAA: 2 mg/l & BAP: 1 mg/l). After cell growing, used five levels of yeast extract elicitor (0, 5, 10, 20 and 40 mg/L). These yeast extract was filtered to media after autoclaving. Then they were placed on incubator shaker with 100 rounds per minute in 25 °C. After seven days, cell masses were filtered by filter paper and were dried by freeze dryer and then were extracted by micro-Clevenger. Obtained extracts were analyzed by GC-MS to determine the amount of secondary metabolites in cells.
Results: Results showed that there were significant differences among levels of hormones for stem cell induction and cell growth rate. Mean comparison for effect of different hormones based on Duncan's test ($p<0.5$) on stem cell induction showed that N2B1, N0.5B1, N2B0.5 and N0.5B0.5 media had the highest induction respectively, and N2B0 and N0B0 had the lowest induction. Also the cell mass growth rate was the highest in N0.5B1 media and it was the lowest in N0B0 media It was defined that percentage of β-pinene as a secondary metabolite in yeast extract 5 mg/L (8.50%) was more than other concentrations of elicitors, and the amount of this metabolite reduced in less concentrations of yeast extract. The mean for 10, 20 and 40 mg/L were as 6.50, 4.60 and 4.80% respectively. Then there is a significant negative correlation between β-pinene induction and yeast extract concentration in cell culture of H. officinalis. The amount of β-pinene in control experiment was 8.45% and there did not showed significant differences with 5 mg/L.
Conclusion: The hormone and yeast extract can affect on stem cell induction and β-pinen production respectively in H. officinalis.
Keywords: Stem Cell Induction, β-Pinene, Hyssopus Officinalis

Ps-182: Investigation on Neural Differentiation of Adipose Derived Mesenchymal Stem Cells towards Neural Stem Cells through the Inhibition of TGFb and BMP Pathways

Azita Parvaneh Tafreshi[1,2*], Hoda Kazemi[3], Bahman Zeynali[3]

1. Department of Molecular Medicine, The National Research Institute for Genetic Engineering
2. Biotechnology, Pajohesh Boulevard, Hemmat highway, Tehran 14155-6455, Iran
3. Developmental Biology Lab, School of Biology, College of Science, University of Tehran, Tehran 14155-6455, Iran
Email: aptafreshi@yahoo.com

Background and Aim: Due to the abundance and easy accessibility of the adipose tissue, they would be an ideal source of mesenchymal stem cells. Aim: Our study

is aimed at evaluating the potential of adipose-derived mesenchymal stem cell differentiation towards neural stem cells.
Methods: We have therefore extracted mesenchymal stem cells from human adipose tissue by using collagenase, and cultured in medium DMEM/F12+10% FBS. Following subsequent passages on low attachment plates spheroid structures were formed. After the formation of the spheroids, those with the appropriate sizes were selected and transferred to matrigel coated plates by using the stereo microscope. Rossette like structures were formed following one week treatment with TGFb and BMP antagnonists.
Results: The rossette like structures were further differentiated into morphologically heterogenous population of cells consisting of neurons, oligodendrocytes and astrocytes, analysed by real time RT-PCR.
Conclusion: Altogether, adipose derived mesenchymal stem cells should be considered as a valuable source for autologous nerve cell therapy in future.
Keywords: Neural Stem Cell, Adipose Derived Mesenchymal Stem Cells, TGFB, BMP Pathways

Ps-183: Skin Regeneration in Donor Site of Burned Patients Using Laser Technology

Nooshafarin Kazemi khoo[1], Mohammad Ali Nilforoushzadeh[1], Reza Vaghardoost[2*], Mahnoush Momeni[2]

1. Skin and Stem Cell Research Center, Tehran University of Medical Sciences, Tehran, Iran
2. Burn Research center, Department of Plastic and Reconstructive Surgery, Iran University of Medical Sciences, Tehran, Iran
Email: nooshakazemi@gmail.com

Background and Aim: Background: A skin graft is standard therapeutic technique in patients with deep ulcers, but managing donor site after grafting is very important. Although several modern dressings are available to enhance the comfort of donor site, using techniques that accelerate wound healing may enhance patient satisfaction. Low Level Laser Therapy (LLLT) has been used in several medical fields, including healing of diabetic, surgical and pressure ulcers, but there is not any report of using this method for healing of donor site in burn patients.
Methods: The protocols and informed consent were reviewed according to Medical Ethics Board of Shahid Beheshti University of Medical Sciences (IR.SBMU.REC.1394.363) and Iranian Registry of Clinical Trials (IRCT2016020226069N2). 18 donor sites in 11 patients with grade 3 burn ulcer were selected. Donor areas were divided into 2 parts, for laser irradiation and control randomly. Laser area was irradiated by a red, 655 nm laser light, 150 mW, 2 J/Cm2. On day 0 (immediately after surgery), 3, 5, 7. Dressing and other therapeutic care for both sites were the same. The patients and the person who analyzed the results were blinded.
Results: The size of donor site reduced in both groups during the 7 day study period ($P<0.01$) and this reduction was significantly greater in the laser group ($P=0.01$).
Conclusion: In the present study, for the first time we evaluate the effects of LLLT on the healing process of donor site in burn patients. The results showed that local irradiation of red laser accelerates wound healing process significantly.
Keywords: Low Level Laser Therapy, Skin Graft, Skin Regeneration, Burn Ulcer

Ps-184: Skin Regeneration after Skin Graft Surgery in Burned Patients Using Low Level Laser Therapy (A Randomized Clinical Trial)

Nooshafarin Kazemi khoo[1], Mohammad Ali Nilforoushzadeh[1*], Reza Vaghardoost[2], Mostafa Dahmardehei[2], Mahnoush Momeni[2]

1. Skin and Stem Cell Research Center, Tehran University of Medical Sciences, Tehran, Iran
2. Burn Research center, Department of Plastic and Reconstructive Surgery, Iran University of Medical Sciences, Tehran, Iran
Email: nooshakazemi@gmail.com

Background and Aim: Skin graft is standard therapeutic technique in patients with deep ulcers but as every surgical procedure has complications. Although several modern dressings are available to enhance comfort of donor site, using techniques that accelerate wound healing may enhance patient's satisfaction. Low Level

Laser Therapy (LLLT) has been used in several medical fields, especially for wound healing and skin regeneration, for treating diabetic, pressure and venous ulcers but this is the first clinical trial using this technique in burned patients.
Methods: The protocols and informed consent were reviewed according to Medical Ethics Board of Shahid Beheshti University of Medical Sciences (IR.SBMU.REC.1394.363) and Iranian Registry of Clinical Trials (IRCT2016020226069N2). Nine patients with bilateral similar grade 3 burn ulcer in both hands or both feet, candidate for Split Thickness Skin Graft (STSG) were selected. One side was selected for laser irradiation and the other side as control, randomly. Laser area was irradiated by red, 655 nm laser light, 150 mW, 2 J/Cm2 for the bed of the ulcer and infrared 808 nm laser light, 200 mW for the margins, every day for 7 days.
Results: Skin regeneration was significantly better and rate of wound dehiscence after skin graft surgery was significantly lower in laser treated group in comparison to control group which received only classic dressing (P=0.019).
Conclusion: In the present study for the first time we evaluate the effects of LLLT on the healing process of skin grafted area in burn patients. The results showed LLLT is a safe effective method which improves graft survival and wound healing process and decreases the rate of wound dehiscence in patients with deep burn ulcer.
Keywords: Low Level Laser Therapy, Skin Transplantation, Wound Healing, Regenerative Medicine, Wound Dehiscence

Ps-185: 7 Years Experience in Characteristics and Therapeutic Potential of Menstrual Blood Stem Cells

Somaieh Kazemnejad*

Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, I. R. Iran
Email: kazemnejad_s@yahoo.com

Background and Aim: Nowadays, there is an increasing attention in the application of menstrual blood stem cells (MenSCs) in regenerative medicine. Here, we have reported in vivo therapeutic application of Menses in treatment of different disorders.
Methods: All donors of menstrual blood signed the informed consent approved by the medical ethics committee of Avicenna Research Institute. All animals received human care in compliance with the Guide for Care and use of Laboratory Animals published by NIH. Animal models of human diseases were divided into multiple groups and received MenSCs after immunophenotypic analysis. The regenerated tissues were examined by histo-patological, molecular and biochemical assessments.
Results: Menstrual blood includes a pool of multipotent stem cells which are characterized by the expression of pluripotency factors such as Oct-4. These highly proliferative cells can be collected by noninvasive procedures and can be expanded in vitro for prolonged periods without chromosomal abnormalities. In contrast to ES cells, no ethical controversy is associated with their use in therapeutic applications. Furthermore, animal studies suggest that no increased risk of tumor formation, in contrast to pluripotent cells, which have the inherent risk of forming teratomas in vivo. Menstrual blood stem cells (MenSCs) are immune privilege, suitable for allogeneic transplantation, and may even have an immunomodulatory effect in the recipient. MenSCs show multilineage differentiation potential and great efficiency in several animal models of human disease.
Conclusion: While MenSCs harbor the potential for autologous transplantation at least for reproductive-age female patients, the majority of potential patients would greatly benefit from MenSC cell banking. It sounds that a successful translation of the exciting recent research findings on MenSCs into clinics should be feasible in near future.
Keywords: Regenerative Medicine, Menstrual Blood Stem Cells, Animal Model

Ps-186: Combination of DAPT and XAV939 can Reduce Proliferation, Stemness Property and Migration in Metastatic Melanoma Cells

Faezeh Keighobadi[1], Maryam Mehdipour[1], Vajihe Azimian Zavareh[2], Javad Firoozi[3], Marzieh Ebrahimi[3*]

1. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell

Biology and Technology, Elm Va Farhang University, Tehran, Iran
2.Tehran University, Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology Stem Cell Department, Royan Institute, Tehran, Iran
3. Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: f.keighobadi@yahoo.com

Background and Aim: Cancer stem cells are the biggest reason of drug resistancy and not effectivable cure in melanoma patients. Notch and Wnt/βcatenin signaling pathways are over expressed in cancer stem cells relation to normal stem cells. The aim of this study was to appraise the effect of Notch and Wnt/βcatenin inhibiting using DAPT and XAV939, as small molecules, on stemness and metastasis in metastatic melanoma cells.
Methods: To find the effective dose of DAPT and XAV939 in viability of A375 cells, used MTT assay in different concentrations of these small molecules. Then the stemness potential of A375 cells was assessed with colony formation, sphere formation assay, q-RT PCR, and western blott for the expression of genes and proteins involved in the stemness and Notch & Wnt/βcatenin downstream genes in presence and absence of selected dose of DAPT and XAV939. Scratch test was utilized to obtain the effect of Notch and Wnt/βcatenin inhibition on migration of A375 cells.
Results: The results showed that the 48 hours pretreatment and 48 hours treatment with 15μM and 10μM concentrations of DAPT and XAV939 reduced migratory potential of A375 cells about 60 percents. As well significantly reduction seen about 79 percent in colony formation and and 4.78 times in sphere formation ability and also size of colonies and spheres was significantly smaller than control group. The βcatenin gene expression was significantly increased and c-Myc,cyclinD1, Notch1, Hes1, and Hes5 (Notch & Wnt downstream genes) reduced in A375 tumoric cells and all of these genes expression reduced in melanospheres. The βcatenin protein expression was reduced, and Nestin (marker of stemness) had no expression in combination treated cells.
Conclusion: These results indicate that combination of DAPT and XAV939 treated reduce the tumor initiating potential and the metastatic melanoma. It can test in animal model to preclinical exprements.
Keywords: DAPT, XAV939, Notch Pathway, Wnt/βcatenin Pathway, Melanoma, Stemness

Ps-187: Low-Level Laser Promotes Proliferation and Differentiation of Stem Cells

Hoda Keshmiri Neghab, Maryam Jahanshiri, Gholamreza Esmaeeli DJavid*

Academic Center for Education, Culture and Research (ACECR), Medical Laser Research Center, Tehran, Iran
Email: Hodakeshmiri@yahoo.com

Background and Aim: Low level laser therapy (LLLT) also known as photobiomodulation, is a treatment that uses low-level lasers or light-emitting diodes (LEDs) to change cellular function and is a clinically well accepted tool in regenerative medicine and dentistry. Low-level laser therapy (LLLT) has been used in several in vitro experiments in order to stimulate cell proliferation. Cells such as fibroblasts, keratinocytes, lymphocytes, and osteoblasts have shown increased proliferation when submitted to laser irradiation, although little is known about the effects of LLLT on stem cells.
Methods: This abstract aims to assess, through a systematic literature review, the effects of LLLT on the in vitro proliferation and differentiation of stem cells. We conducted an electronic search in all database for articles published in the last years.
Results: Most studies demonstrated an increase in the proliferation rate of the irradiated cells. Low level laser with low-energy density range appears to exert a biostimulatory effect on stemcells and enhance proliferation and differentiation on cell lines used in in vitro studies.
Conclusion: We conclude that LLLT promotes proliferation and maturation of human stem cells in vitro. These results may have clinical implications.
Keywords: Low-Level Laser Therapy, Stem Cells, Proliferation, Differentiation, Regenerative Medicine

Ps-188: Co-Culture of Wharton's Jelly-Derived Mesenchymal Stem Cells with Human Pancreatic Islets for Using in Islet Transplantation

Somayeh Keshtkar[1,2], Negar Azarpira[2*], Maryam Kaviani[2], Mohammad Hossein Ghahremani[1]

1. Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
Email: somayehkeshtkar@gmail.com

Background and Aim: Islet transplantation is an alternative treatment to daily insulin injections for patients with type 1 diabetes. The keeping of viable pancreatic islets is crucial for successful islet transplantation. In order to overcome islet quality loss during culture, it has been proposed to co-culture pancreatic islets with Mesenchymal Stem Cells.
Methods: In this study, human pancreas islets were isolated according to enzymatic and mechanical protocol. Then purified islets were co-cultured with Wharton's jelly-derived mesenchymal stem cells. The expression of CD90, CD44, CD105, and CD34 and as well as osteogenic and adipogenic differentiation of mesenchymal stem cells were identified. Also, the islets were evaluated with DTZ staining and the amount of insulin released was assessed by ELISA assay.
Results: The pancreatic Islets were viable and showed positive DTZ staining before co-culture.Wharton's jelly-derived mesenchymal stem cells expressed high levels of CD44 , CD90, and partly CD105 as mesenchymal stem cells markers. However, these cells did not express hematopoietic marker CD34. The culture of islets alone resulted in cells death and loss of function after a few days. While, viability and functionality of co-cultured Wharton's jelly-derived mesenchymal stem cells with islets was higher than islets alone after 7days.
Conclusion: Co-culture of islets with Wharton's jelly-derived mesenchymal stem cells has the potential for protecting islets from injury during culture period. Accordingly, by adjuvant co-transplantation of mesenchymal stem cells, the probability of successful outcomes of islet transplantation will increase.
Keywords: Islet transplantation, Mesenchymal stem cells, Outcome, Co-culture

Ps-189: Impact of Mesenchymal Stem Cell-Conditioned Medium on Proliferation of Anti-Inflammatory Macrophages

Aysun Khalil Nejad Sani Banaei[1], Kaveh Baghaei[2*]

1. Department Cell and Molecular Biology, College of Bio-Sciece, Islamic Azad University North Tehran Branch, Tehran, Iran.
2. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Email: aysun.sani93@gmail.com

Background and Aim: Mesenchymal Stem Cells (MSCs) have a therapeutic potential in tissue repair because of capacity for multipotent differentiation and their ability to modulate the immune response. We examined the ability of conditioned medium of MSCs (CM-MSCs) to modify the differentiation of human monocyte into macrophages and assessed the influence of CM-MSCs on important mononuclear cells function.
Methods: CM-MSC were collected after 21 days from cultured MSC. Peripheral blood mononuclear cell (PBMC) was isolated from healthy donors using ficoll. Then monocytes were co-cultured with CM-MSC for 72 hours. In the next step RNA extraction and cDNA synthesis were carried out from monocytes. The gene expression of anti-inflammatory cytokines (IL-4, Arg1, EGR2, iNOS) secreted from macrophage were evaluated by real time PCR.
Results: Increased gene expression of Arg1, iNOS, and EGR2 were observed while no distinct changes in IL-4 were detected. These results presents that, co-culture of CM-MSCs with monocytes stimulated inflammatory differentiation towards anti-inflammatory pathways.
Conclusion: MSCs are well known stem cells with immune regulation impact. Results obtained from this study showed that, the immunomodulatory effect of CM-MSCs on monocyte could result in differentiation mediated by soluble molecules such as exosomes, lipids, and peptides.
Keywords: Mesenchymal Stem Cells Conditioned Medium, Monocyte, Anti-Inflammatory Cytokines, Immunoregulatory

Ps-190: The Role of Vitamin D in Semaphorins-mediated Cancer Stem Cells Development

Fatemeh Alimirzaei, Fatemeh Khani-Habibabadi, Mehrdad Behmanesh*

Department of Molecular Genetics, Faculty of Biological Science, Tarbiat modares University, Tehran, Iran
Email: fatemeh.alimirzaie@yahoo.com

Background and Aim: There is a lot of evidence that links cancer to genes and pathways that are essential for normal embryonic development, increasing the possibility that cancer cells with stem cell properties, particularly self-renewal and multi potency, are primarily involved in tumor formation and progression. Recently identified Semaphorin signaling pathways are important in modulating the morphogenesis, proliferation, survival and growth in a variety of adult and embryonic tissues. Semaphorins are a family of cell-surface and soluble proteins involved in cell–cell interactions as well as cell differentiation, function and morphology. The effects of semaphorins are mediated by plexins, a group of nine trans¬membrane receptors. In normal tissues, semaphorin signaling is mainly active in precursor cells. This increases the possibility of tumors formation from tissue stem cells, which are unable to differentiate and/or stop proliferation. Calcitriol or 1, 25-dihydroxyvitamin D3 (1,25(OH)2D3) is an endocrine regulator of calcium homeostasis. Vitamin D Receptor (VDR) is expressed in different cell types, including stem cells, immune cells, and liver.The aim of this study was to investigate the VDR binding site on the promotors of semaphorins genes.
Methods: We used online bioinformatics data bases such as NCBI (www.ncbi.nlm.nih.gov), UCSC Genome Browser (genome.ucsc.edu) and JASPAR (http://jaspar.genereg.net/). We obtain promoters sequences and functional regulating elements from NCBI and UCSC databases and explore for VDR binding sites using JASPAR algorithms with relative profile score threshold above 80%.
Results: We found several VDR binding sites with significance score on the promotors of sema4d, sema3a, sema4a, and sema7a genes.
Conclusion: Here, we demonstrate new evidence for the regulatory role of vitamin D on semaphorins that are crucially involved in the control of tumor progression.
Keywords: Vitamin D, Semaphorin, Differentiation, Stem cells

Ps-191: Investigation of Scaffold Loaded with Mineral Pitch for Proliferation and Propagation of Mesenchymal Stem Cells

Raziyeh kheirjou[1], Leila Roshangar[1*], Jafar Soleimani Rad[1], Soodabeh Davaran[2], Ahad Ferdosi[1], Morteza Rashtbar[3]

1. Department of Histology and Anatomy, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Medical Nanotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
3. Faculty of Advanced Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran
Email: r.kheirjoo@gmail.com

Background and Aim: The Extracellular matrix (ECM) is the determining factor for regulating repair or regeneration. Scaffolds are ECM mimetic materials providing a suitable environment for cell delivery, wound support, and guidance for tissue regeneration. The ideal matrix is biocompatible and biodegradable, promotes regeneration and incorporation into the recipient tissue site. Acellular matrix, which is derived from animal tissue through the removal of cells, that originates from living tissue. This product can be safely metabolized or reabsorbed by the body. Mineral pitch or mummy is a traditional remedy, which has immunoregulatory, antioxidative and antimicrobial effects. This experiment evaluated scaffold seeded with ADSCs and mineral pitch in tissue engineering and regeneration medicine studies.
Methods: Adipose-Derived Stem Cells (ADSCs) were isolated by modified washing adipose tissue method. Then this cells with medium contained mineral pitch were seeded on small intestinal submucosal (SIS) acellular scaffold. In this study first, the existence of cells on SIS acellular scaffold were evaluated by H&E and DAPI staining and then, the Adhesion and proliferation of ADSCs evaluated using Scanning electron microscopy and MTT assay.

Results: Based on H&E and DAPI staining no intact nuclei and DNA fragments were seen in SIS acellular scaffold. Scanning electron microscopy images showed densely populated ADSCs which attached on the scaffold surfaces and MTT assay for evaluation of scaffold biocompatible revealed high cell viability.
Conclusion: The results confirm that decellularization of SIS acellular scaffold is efficient and it has no negative effects on ADSCs in vitro.
Keywords: Adipose Stromal/Stem Cells, SIS Acellular Scaffold, Mineral Pitch

Ps-192: Chemical, Biological and Mechanical Assessment of Scaffolds Produced By Fibroin Extracted From Different Silkworm Strains

Somaieh Khorasani Charghaddoz*

Avicenna Research Institute, ACECR, Tehran, Iran
Email: somaye.khorasani@yahoo.com

Background and Aim: There are variety of silkworm cocoons that could be use for this purpose. The aim of this study was to evaluate the difference between scaffolds produced by fibroin extracted from different local wild type and recombinant silkworms. Chemical, mechanical and cell response difference of these scaffolds are compared.
Methods: sericin was extracted from three wildtype strains of Bombyx mori silk cocoons (, orange shell, yellow shell, chamkhale shell) and a recombinant strain (white shell) by a high temperature technique in different times. The silk degumming was assured by SEM. In addition, the mechanical properties and biocompatibility were evaluated
Results: For all silk sources, degumming was completely done within 60 minutes. The SEM results show that sericin was successfully omitted from silk fibrers. Recombinant strain (white shell) the highest compressive modulus than other cases show the highest strength mechanical.
Conclusion: silk as a natural polymer with excellent mechanical and biological properties has been used widely by scientists for tissue engineering and regeneration applications. Chemical, biological and mechanical properties scaffolds produced by fibroin produced from different strains of silkworm were evaluated.
Keywords: Fibroin Mechanical Assesment Silkworm Strains

Ps-193: Microarray Reanalysis of Diabetic Nephropathy Data Provides Insights into the Pathogenesis of the Disease

Farnoush Kiyanpour[1], Yousof Gheisari[2*], Maryam Abedi[1]

1. Department of Genetics and Molecular Biology, Isfahan University of Medical Sciences, Isfahan, Iran
2. Regenerative Medicine Lab, Isfahan Kidney Diseases Research Center
Email: levin_no1@yahoo.com

Background and Aim: One of the important causes of death and disability in human society is chronic diseases. Despite different investigations, the molecular mechanisms are not fully discovered. Systems biology approaches can deepen our knowledge about complex diseases. Diabetic nephropathy (DN) is the most important and common secondary sign of diabetic mellitus. Although there have been many studies and researches on DN performed, a holistic view has been rarely done.
Methods: In this study, GSE30582 and GSE30529 microarray datasets were downloaded from Gene Expression Omnibus database (GEO). The gene expression profile of diabetic nephropathy (DN) patients and those from healthy individuals were compared, by using GEO2R. Differentially expressed (DE) genes were identified. In addition, using CluePedia application of Cytoscape software version 3.2.1, the networks of DE genes were constructed. The gene ontology (GO) of DE genes was collected using ClueGO application of Cytoscape.
Results: Considering p-value < 0.05, 2517 and 4958 genes were differential in GSE30582 and GSE30529 datasets, respectively. The networks of DE genes have consisted of 2517 nodes with 5163 edges and 4958 nodes with 9784 edges for GSE30582 and GSE30529 datasets, respectively. Among DE genes, which have been previously confirmed in the pathogenesis of DN, some new targets were found among COL3A1, SERPINA3, and MMP7. In addition, FGFR and WNT and MAPK cascade signaling were among GO terms.

Conclusion: In conclusion, here has shown new DE genes and key signaling pathways in the progression of DN. In addition, introduced critical genes in the pathogenesis of this complex disease that may serve as potential novel drug targets.
Keywords: Gene Ontology, Microarray Analysis, Differential Expressed Gene, Diabetic Nephropathy

Ps-194: Platelet Rich Plasma, Heparin Sulfate Embedded in Hydroxyapatite/Zirconia 3D Porous Scaffolds Accelerate in Vitro Bone Formation

Mona Latifi[1], Tahere Talaei-khozani[2], Mahsa Sani[2], Mahin Salmannejad[2], Maryam Kabir Salmani[1*]

1. Department Of Stem Cell and Regenerative Medicine, Medical Biotechnology Faculty, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Tissue Engineering Lab, Anatomy Department, Shiraz University of Medical Sciences, Shiraz, Iran
Email: latifi.mon@gmail.com

Background and Aim: Bone transplantation is the second popular approach besides blood transfusion for treatment of the injuries caused by trauma and other defects. Several investigations have been carried out to find various synthetic or natural bone substitutes and biomimetic materials for bone repair. In such a study, minimum pore size of 100 µm, osteoconductivity, mechanical strength, biocompatibility, and bioresorbability are considered as critical criteria for bone graft applications. Hydroxyapatite (HA) has been successfully coated on zirconia (ZrO2) to construct a porous scaffold with higher mechanical strength but there are still big challenges regarding the delivery of osteogenic growth factors, local reserve, and sustained release of them. Platelet Rich Plasma (PRP) can be considered as an autologous source of various growth factors, including platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), Vascular Endothelial Growth Factor (VEGF), insulin-like growth factor (IGF), and epidermal growth factor (EGF). These factors have been shown to play a pivotal role in bone repair. Bone repair is a time-consuming process that takes several weeks to accomplish. The introduction of growth factors to the engineered scaffolds can accelerate bone regeneration. Heparin sulfate (HS) is a highly sulfated glycosaminoglycan that can sequestrate the PRP growth factors by direct binding to them. Also, as a co-receptor, it can protect the PRP growth factor content from proteolytic degradation and increase the affinity to their receptors. HS also induces bone formation by accelerating the mineralization rate and osteogenic-specific marker expression
Methods: In this study, we fabricated a porous interconnected HA/ZrO2 scaffold by slurry method with 71.6 + 0.5% porosity and 310 + 150 µm pore size. Platelet rich plasma (PRP) was used as an autologous source of growth factors and heparin sulfate (HS) sequestrated the PRP growth factors. After activating PRP with 2.5% CaCl2, 5 µg/mL of HS was added and mixed well, followed by impregnation into the porous HA/ZrO2 scaffold
Results: The PRP/HS containing scaffolds showed a significantly higher mechanical strength. Culturing MG63 cell line showed that 100 percent of cells attached to the PRP/HS containing scaffolds compared with HA/ZrO2 ones. The cells also showed a significantly higher alkaline phosphatase activity and calcium mineralization in scaffolds treated with PRP/HS. MTT viability test demonstrated a significantly higher proliferation rate on the PRP/HS containing scaffolds compared to those without PRP/HS.
Conclusion: The scaffolds with PRP/HS seem to provide a superior microenvironment for osteoblast activities and can be suggested as a good vehicle for growth factor and cell delivery in bone tissue engineering applications.
Keywords: Hydroxyapatite, Platelet Rich Plasma, Heparin Sulfate, Bone Tissue Engineering

Ps-195: Effects of 1α, 25-Dihydroxyvitaminn D3 on T Regulatory Population in Co-Culture of Pbmc and Adipose-Derived Mesenchymal Stem Cells from Multiple Sclerosis Patients

Fahimeh Lavi Arab*

Immunology Center, Buali Institute, Department of Immunology Mashhad University of Medical Science Mashhad, Iran
Email: laviaf931@mums.ac.ir

Background and Aim: Multiple sclerosis (MS) is the most famous autoimmune disease attacking the central nervous system. There is enhanced-emerging treatment for multiple sclerosis but current immunomodulatory drugs are not sufficient and conventional disease modifying therapy are not divisive cure for progressive phase of multiple sclerosis. The importance of 1α, 25-Dihydroxyvitaminn D3 in immunologic processes has recently emerged. Anti-inflammatory effect of 1α, 25-Dihydroxyvitaminn D3 was evaluated for autoimmune diseases. 1α, 25-Dihydroxyvitaminn D3 is hypothesized to protect against MS by directly interacting with gene associated with immune cells. Stem cell therapy also has known to be useful for autoimmune neurodegenerative disease such MS. It has be thought 1α, 25-Dihydroxyvitaminn D3 can increase therapeutic potential of stem cell therapy for MS.
Methods: to established this hypothesis we removed abdominal fat mesenchymal stem cells (AT-MSCs) form multiple sclerosis patients, cultured in 6-wells plate and treated them with 100nM, 50 nM, 25nM and 12nM of 1α, 25-Dihydroxyvitaminn D3 for 24 and 48h after treatment. Sixth well was remained untreated. Then we assayed Treg percentage n medium with T regulatory in co-culture of adipose-derived mesenchymal stem cells and pbmc from multiple sclerosis patients by flowcytometry technique.
Results: all concentration of vitamin D in 24 and 48h post treatment increase T regulatory population in co-culture of AT-MSC and pbmc
Conclusion: vitamin D can be used as a potential treatment to increase capability of mesenchymal stem cell therapy in autoimmune diseases specially multiple sclerosis
Key words: 1α, 25-Dihydroxyvitaminn D3, Mesenchymal Stem Cells, Multiple Sclerosis

Ps-196: The Effect of Strontium Bioactive Glass Coatings for Bone Regeneration: An in Vitro Study

Nasrin Lotfibakhshaiesh[1*], Robert Hill[2]

1. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Barts and The London, Unit of Dental Physical Sciences, London, UK
Email: n.lotfiba@gmail.com

Background and Aim: Bioactive glass (BG) coatings may promote the formation of a strong bond with living bone tissue thus decreasing the likelihood of fibrous encapsulation and have the added benefit that their dissolution ions stimulate cell activity [1,2]. Strontium (Sr) ranelate used to treat and prevent osteoporosis, works via the action of Sr ions which stimulate the formation of new bone and prevent osteoclast-mediated resorption [3]. We have previously shown that Sr-substituted BGs promote osteoblast activity in vitro [4] and explored the effect of altering phosphate content on the material structure [5]. Here, we created Sr-substituted BG coatings with a range of phosphate contents, producing materials that combine the bone remodelling benefits of Sr and BG with phosphate to mediate pH changes which can affect cell viability.
Methods: Bioactive glasses in which 10% of the Ca was replaced by Sr and the P2O5 content was increased from 1.07 to 6.42 mol% were produced by a melt quench route. Sufficient cations were added to ensure charge neutrality in the PO43- complex formed. Simulated body fluid (SBF) was prepared [6]. Glass particles were immersed for up to 28 days. At indicated time points samples were filtered and dried for X-Ray Diffraction (XRD) analysis. Culture media containing ions from glasses were created by incubating glass powder in RPMI and then passed through a 0.2 micrometer filter. The human osteosarcoma cell line, Saos-2, was seeded and cultured for up to 28 days. On days 1, 14, 21 and 28 cell metabolic activity was measured using MTT assay. Glasses were coated on the surface of Ti6AL4V coupons with an enameling technique. Saos-2 were seeded on BG coatings and viability was assessed after 1, 7 and 14 days with a LIVE/DEAD stain. Some glass coatings cultures were also fixed, gold coated and viewed on SEM.
Results: BG with high P2O5 content forms more apatite after immersion in SBF for 4 weeks than BG with low P2O5 content, as examined by XRD. MTT activity in Saos-2 cells treated with dissolution ions from BG increased in all samples with time in culture. MTT activity was also significantly greater ($p<0.01$) in cells treated with dissolution ions from 4.28 and 6.24 mol%

P2O5 BGs as compared to controls at day 28. LIVE/DEAD staining indicated that all coating materials were not cytotoxic. SEM imaging demonstrated that the BG coating encouraged cell attachment and that cells spread well over the surface.
Conclusion: With increasing P2O5 content in the series of Sr-substituted BG, Bragg peaks in XRD traces associated with apatite crystallisation increase suggesting the glass becomes more bioactive. Apatite formation on the coating surface is an essential factor for bone bonding as the more apatite that forms on the glass coating the more bone bonding will be expected. Adding P2O5 to the glass composition in a controlled range prevents extreme pH rises, which can affect cell viability and proliferation.
Keywords: Bioactive Glasses, Bone Regeneration

Ps-197: Secretome from Embryonic Stem Cell-Derived Mesenchymal Stem Cell Spheroids Exhibit Enhanced In-Vitro Immunomodulation Potential

Faezeh Maghsood, Saeed Jahandideh, Mehdi Kadivar*

Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran
Email: faezemaghsoud@yahoo.com

Background and Aim: The use of mesenchymal stem cells (MSCs) has been emerged as a promising therapeutic tool in clinical and pre-clinical studies of regenerative medicine and cell-based therapies. The therapeutic mechanism of mesenchymal stem cells is related to the secretion of a broad array of angiogenic and anti-apoptotic factors, cytokines, chemokines, growth factors, and extracellular matrix proteins, referred to as secretome. Harnessing this MSC secretome for therapeutic applications requires the optimization of production of secretary molecules. It has been shown that aggregation of BM-MSCs into 3D spheroids, as a preconditioning strategy, can enhance immunomodulatory and regenerative capacity of such cells. In this study, we investigated the effect of secretome from human embryonic stem cell-derived mesenchymal stem cells (hESC-MSCs) spheroids on immunomodulatory efficiency of these cells in LPS-induced peripheral blood mononuclear cells (PBMCs).
Methods: hESC-MSCs were non-adherently grown to prepare 3D aggregates, and then conditioned medium (secretome) was collected and concentrated 15-fold. PBMCs were isolated from human peripheral blood and treated with aggregated hESC-MSCs conditioned media (aggregate-CM) and then, the secreted levels of IL-10, TNFα and IL-1β were assessed by ELISA after induction with LPS.
Results: The results showed that aggregate-CM was able to significantly decrease the secretion of IL-1 from LPS-induced PBMCs, as an indicator of inflammation, in comparison with untreated LPS-induced PBMCs. Furthermore, the secretion levels of IL-10 were significantly increased in aggregate-CM treated PBMCs, while TNF? levels remained unchanged.
Conclusion: In conclusion, our results implied that the cell aggregation can be used to promote the immunomodulatory effects of hESC-MSC secretome. It is obvious that for applying of these findings in clinical demands, the potency of different pre-conditioned MSCs secretome on immune response needs to be more clarified.
Keywords: Aggregation, Embryonic Stem Cells, Mesenchymal Stem Cells, Preconditioning; Secretome

Ps-198: Comparison of Nanoliposomal Form of Morphine with Simple Form of Morphine for BALB/C Mice

Saman Mahdavi[1*], Hamid Feyzi[1], Seyed Farzin Hosseini[2]

1. Department of Control of Materials Meals Quality, Faculty of Veterinary Medicine, University of Urmia Azad Islamic, Shahid beheshti St., Urmia, Iran
2. Department of Medicine,Faculty of Medicine, University of Tehran, Enghelab St., Tehran, Iran
Email: saman_mahdavy64@yahoo.com

Background and Aim: One of the applications of nanobiotechnology is designed nanomachines in drug delivery systems. Biological nanomachines have appropriate performance in the cell alive and today they have become to the most widely used structures in drug delivery system. Researchers have been continued their effort in the field of creation biological nanomachines for drug delivery and designed Nano-machines in the

name of polymeric nanoparticles. Bio-polymer nanoparticles such as liposomes for drug delivery are harmless and not cause an immune response. Nanoliposome, or submicron bilayer lipid vesicle, is a new technology for the encapsulation and delivery of bioactive agents. Nanoliposomes are able to enhance the performance of bioactive agents by improving their solubility and bioavailability, in vitro and in vivo stability, as well as preventing their unwanted interactions with other molecules.In this study the authors determined the duration of analgesia, toxicity, and neuraxial distribution of liposomal morphine after intrathecal administration in the mouse.
Methods: Analgesic duration was determined using the tail-flick test after intrathecal injection of 12.5,25, or 50 µg of plain or liposomal morphine (n = 6 mice/dose/formulation). Toxicity of the formulations was compared by estimating LD50. Neuraxial morphine distribution was determined after 20 pg of plain or liposomal morphine. The excised spinal cord and brain were divided into five segments at 1 min, and at 1, 4, and 8 h after injection for both formulations. In addition, for the liposomal morphine, similar sections were obtained at 24 h (n = 6 mice/formulation/time point). Segmental morphine concentration was quantified using radioimmunoassay.
Results: Liposomal encapsulation significantly prolonged duration of analgesia for the 25-pg (13.4 ± 1.64 [SE] vs. 4.1 ± 0.5 h) and 50-pg doses (16.8 + 4.0 vs. 4.6 2 1.0 h). The estimated LD50 was 200 (confidence interval 151- 257 pg) for plain morphine, but was not determinable for the liposomal formulation, since no deaths occurred at the largest dose level which could be tested (371 pg). For plain morphine, the drug was not confined to a specific neuraxial segment, and segmental levels declined rapidly. After liposomal morphine, the most morphine was concentrated and persisted in the low spinal cord segment at each time interval.
Conclusion: These results show that a single dose of liposomal morphine produces prolonged analgesia with decreased toxicity compared to the plain formulation.
Keywords: Nanoliposome, liposome, balbe/c, Morphine, Toxicology, Pathology

Ps-199: How Developmental Genes Control the Stem Cell's Fate during Postnatal Tissue Regeneration

Elahe Mahdipour*

Department of Medical Sciences, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: mahdipoure@mums.ac.ir

Background and Aim: Developmental genes paly role as the main transcription factors and signaling molecules during development. These genes are highly evolutionary conserved. After birth several key developmental regulators downregulated or switched off; however, not permanently. In fact, most of developmental genes such as homeobox, sonic hedgehog, and FGFs are reactivated during tissue repair. It is well observed that after injury, stem cells especially from bone marrow migrate to the affected site and play role in regeneration. However, the exact mechanism by which bone marrow-derived stem cells get involved in tissue regeneration is not clear. Here we present how HOX genes affect the fate of hematopoietic stem cells during cutaneous wound repair.
Methods: Bone marrow-derived hematopoietic stem cells were isolated and characterized. Hoxa3 gene was overexpressed in hematopoietic stem cells and the fate of cells were analyzed in vitro. Further the role of hematopoietic stem cell-derived cells were analyses in wound repair using animal models.
Results: we were able to show for the first time that Hoxa3 promotes the proangiogenic fate of hematopoietic stem cells during cutaneous tissue repair. This effect is impaired in chronic conditions such as diabetic wounds.
Conclusion: Developmental genes play a regulatory role in determination of the fate of cells during tissue repair.
Keywords: Tissue Repair, Wound, Hematopoietic Stem Cells, Developmental Genes

Ps-200: MicroRNAs as a Marker for Assessment of Developmental Competence in the Maturing Oocytes

Mahdi Mahdipour*

Stem Cell Research Center, Tabriz University of Medical Sciences and Department of Reproduction Biology, School

of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: mahdipourm@tbzmed.ac.ir

Background and Aim: Proper oocyte nuclear and cytoplasmic maturation is an essential factor for developmental competence of the oocyte and subsequent development of a healthy embryo. Micro-RNAs (miRNA) are known for regulating gene expressions at both transcriptional and post-transcriptional levels and are involved in various biological processes such as control of cell cycle and apoptosis. The knowledge on importance of miRNAs in the oocyte is limited.
Methods: In order to understand the functions of the miRNAs expressed during oocyte maturation, bovine oocytes at the germinal vesicle (GV) and metaphase II (MII) stages were collected and analyzed using next generation sequencing.
Results: In the total, 167 and 242 known miRNA species with more than 20 reads were identified for GV and MII oocytes respectively. The ratios of reads in MII to GV oocytes were calculated and the most differentially expressed miRNAs were miR-222, miR-199a, miR-221, miR-155, miR-214, Let-7b, miR-27a, miR-196a and miR-21. In-silico analysis revealed that target genes are involved in various cellular metabolic processes including protein modification like ubiquitination and phosphorylation as well as roles in the cell cycle and cell division. These results indicate that miRNAs play a significant role for preparation of the oocytes for the successful fertilization and subsequent embryonic development.
Conclusion: Identification and quantification of miRNAs could be used to assess the developmental competence of an oocyte. The better understanding of mechanisms involved in miRNA and messenger RNA interaction in the oocyte may help us develop approaches for specific types of subfertility and contribute in the improvement of assisted reproductive technologies.
Keywords: MicroRNA, Next Generation Sequencing, Oocyte Quality, Developmental Competence

Ps-201: Oxygen Transfer Evaluation in Bioreactor Used in Lung Tissue Engineering

Seyedhossein Mahfouzi Mousavi[1,2], Ghassem Amoabediny[1,3*], Ali Doryab[1,2], Seyed Hamid Safiabadi Tali[1,3], Mostafa Ghanei[4]

1. Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran and
2. Department of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran
3. Department of Biochemical and Pharmaceutical Engineering, School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
4. Department of Pulmonary Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran
Email: hosseinmahfouzy@gmail.com

Background and Aim: Bioreactors play a pivotal role in recellularization technology in order to obtain tissue-engineered lungs for in vivo implantation. Up to now many advances have been achieved in this field; however, some obstacles still remain to achieve a suitable bioreactor. Lack of sufficient information on mass transfer of oxygen, as a vital nutrient for cell growth, is an important issue. Accurate calculation of the volumetric mass transfer coefficient (kLa) as a significant parameter in mass transfer study is a great step for bioreactor design, operation, and scaling up [1, 2]. Several parameters such as physicochemical properties of the liquid phase, geometry of bioreactor, and aeration rate have a great impact on the kLa. In addition, in order to transfer nutrients during recellularization, due to cells sensitivity, we should apply low levels of shear stress. Therefore, in this study, we tried to apply aeration and stir rate at low levels and then investigate the impact of them on kLa.
Methods: The novel bioreactor was used in this study based on the lung tissue physiology (Figure 1) to simulate normal state. Complete medium (DMEM-F12 + 10% FBS + 1% Pen-Strep) as a culture medium for normal cells and deionized water as a control were used. In order to calculate kLa values, two strategies including surface aeration and batch sparging at different rates (10-100 ml/min) and stirrings (15-120 rpm) were applied. The temperature was constant at 37°C during whole the procedure.
Results: kLa values were determined for complete medium and deionized water at various operating con-

ditions. By batch sparging, the range of kLa values obtained 1.1- 3.5? 10-4 s-1 and 0.7- 2.4? 10-4 s-1 for complete medium and deionized water, respectively. In addition, for headspace aeration strategy, this range was calculated 0.75- 2.6? 10-4 s-1and 0.4- 1.4? 10-4 s-1 for complete medium and deionized water, respectively.
Conclusion: The results indicated that at low magnet speeds, there is a partial difference in kLa values for both of complete medium and deionized water. However, by increasing the speed up to 120 rpm in batch sparging, a significant difference was observed in kLa values. Furthermore, at any speed rate, values of kLa for the medium are over than deionized water. This result can be attributed to media components such as FBS in which has coalescence inhibitors [3]. What's more, in both strategy i.e. batch sparging and headspace aeration, the impact of stirring is higher than aeration on the value of kLa. The results indicated that stirring is generally more effective than the aeration strategy.
Keywords: Bioreactor Lung Tissue Engineering Regenerative Medicine Oxygen Transfer Rate

Ps-202: Improvement of Hepatogenic Differentiation of Ips Cells on an Aligned Polyethersulfone Compared to Random Nanofibers

Maryam Mahmoodinia Maymand[1], Hamid Reza Soleimanpour-lichaei[1], Abdolreza Ardeshirylajimi[2], Masoud Soleimani[3], Maryam Kabir Salmani[1*]

1. Stem Cell and Regenerative Medicine, Institute of Medical Biotechnology, National Institute of Genetic Engineering & Biotechnology (NIGEB), Tehran, Iran
2. Department of Tissue Engineering and Regenerative Medicine, School of Advanced Technologies In Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Hematology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: Mahmoodi.scrc@gmail.com

Background and Aim: The application of stem cells holds great promises in cell and tissue transplants. Recently, Polyethersulfone (PES) is introduced as a proper biocompatible biomaterial for liver tissue engineering and in artificial liver system. In this regard, the combination of the induced pluripotent stem cells (iPSCs) and nanofibrous scaffolds appeared as potential candidate. Considering the lack of optimal in vitro model for the terminal hepatogenic differentiation and to improve hepatocyte cells functionality in vitro, this study was designed to compare the hepatogenic differentiation of iPSCs on aligned PES/COL versus random nanofibers scaffolds.
Methods: Aligned and random PES/COL nanofibrus scaffolds were fabricated by electrospining and their surface modified through plasma treatment and collagen coating. The scaffolds were characterized using scanning electron microscopy (SEM) and ATR-FTIR. Morphology and biochemical activities of the differentiated hepatocyte-like cells were examined after 5 and 20 days of differentiation on two types of scaffolds. Then, cell activity and stable functional expression in terms of albumin secretion and urea synthesis were compared to that of the random nanofibers.
Results: Real-Time RT-PCR and ICC showed no significant difference in the mRNA and protein levels of two important definitive endoderm specific markers, including Sox17 and Foxa2 between two scaffolds after 5 days of induction. However, Real-Time RT-PCR analysis indicated an increase in the expression of Cyp7A1 gene over the period of the differentiation procedure on the aligned nanofibers but there was no difference in other genes such as Alb and CK19. Moreover, comparison of hepatogenic differentiation evaluated by Albumin and urea production in conditioned media of Hepatocyte-like Cells (HLCs) differentiated on aligned PES/COL, showed increase expression of these markers after 20 days compared to that of the random nanofibers.
Conclusion: Taken together, the results of this study may indicate that aligned PES/COL nanofibrous scaffolds can improve terminal differentiation of hepatocyte-like cells from iPSCs, however has not remarkable impact in earlier genes and marker for. Thus, PES/COL nanofibrous scaffolds might be considered as a suitable platform in hepatic tissue engineering designs.
Keywords: Stem Cell, Nanobiotechnology, Liver, Aligned Nanofiber, Cell Differentiation
Adhesion, Proliferation and Osteogenic Differentiation of hMSCs

Ps-203: Thermal Sintering Method for Preparation of Bone Tissue Engineering Scaffolds

Farid Maleki, Mohsen Shahrousvand*, Gity Mir Mohamad Sadeghi

Department of Polymer Engineering and Color Technology, Amirkabir University of Technology, Tehran, Iran
Email: farid.maleki91@yahoo.com

Background and Aim: Abstract Tissue engineering scaffolds are key components in healing damaged tissues. However, researchers sought to assess different methods for preparation of suitable scaffolds which support cell-based behaviors. In this research, we present a novel method known as thermal sintering to fabricate bone tissue engineering scaffolds. The thermal sintered scaffolds were made by using polycaprolactone (PCL), poly lactic acid (PLA) and their mixtures. The mechanical properties, biodegradability and biocompatibility of thermal sintered scaffolds were evaluated. Comparing with other current fabrication methods, the scaffolds are optimal choice for bone repairing.
Methods: 1.2. Construction of scaffolds 1.3. Structural study of scaffolds In order to study the scaffold's structure, FTIR of the samples was provided to examine the structure and chemical bonds and resulting peaks 1.4. Cytotoxicity and MTT assay all biological tests were conducted according to ISO standards 10993-5:1999 (Biological evaluation of medical devices; Part 5: tests for in vitro cytotoxicity). The samples were sterilized using ethanol (70%w/w) and UV radiation. The scaffolds were washed with PBS thrice and pre-incubated with culture media overnight. The cells were collected after trypsin/EDTA (Gibco BRL, USA) treatment and centrifuged at 1200 rpm and cultured into a 48-well plate which was loaded by punched scaffolds at a density of 10,000 cells/well and then transferred into an incubator. The proliferation of cells was determined after 2, 4 and 6 days using MTT assay.
Results: 2.1 Chemical structure investigation of scaffolds the scaffolds of PLA, PCL and PLA/PCL (50/50 % wt.) were prepared by the thermal sintering method in accordance with scheme 1. Figure 1a shows fabricated scaffolds after molding. Structures of the obtained scaffolds were characterized by FTIR (Figure 1b). The characteristic peaks of PCL and PLA are similar due to the polyester backbone. The carbonyl (C=O) characteristic peaks of PCL and PLA appear in all samples in the range of 1680–1800 cm-1. The characteristic peaks of about 1700-1770 cm−1 are due to the carboxyl group in semicrystalline PCL and PLA. The peaks of samples at 1000 and 1200 cm−1 are distributed by the backbone ester group. 2.2. Cell viability and cell attachment on sintered scaffolds Biocompatibilities of the sintered scaffolds were investigated via MTT assay, which revealed the significant increase on the proliferation rate of SNL cells (Figure 2). All these results indicated that the sintered scaffolds had an excellent biocompatibility for cell proliferation.
Conclusion: There is a growing need for improved biomedical scaffolds for bone replacement in patients due to the increasing average life-span of the human population and increased expectancy of the quality of life into old age. One of these methods can be thermal sintering method. In conclusion, the thermal sintering method could be used as an appropriate fabrication method in tissue engineering scaffold manufacturing such as the efficient regeneration of bone

Ps-204: Circulating MicroRNA-19a Is Associated with Acute Myocardial Infarction

Fatemeh Mansouri[1,2*]

1. Department of Medical Immunology and Genetics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran
2. Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran
Email: mansouri1600@hotmail.com

Background and Aim: Recent studies have shown, several microRNAs have now opened up new tools by inhibiting the translation or promoting the degradation of target mRNAs. Circulating microRNA as sensitive and novel biomarkers can provide more information that accurately reflects the severity of cardiovascular disease and cancer. Cardiovascular disease is severe disease and dysfunctions of heart, arteries and veins are caused cell death and the expression of microRNA in blood can change. Several biomarkers have been identified to be associated with increased cardiovascular events and even death. The concept of using microRNA technology in medicine derived from its ability to inhibit the expression of target genes involved in defective heart tissues. This study investigated the correla-

tion between the level of miR-19a and acute myocardial infarction (AMI).
Methods: We recruited 35 patients with acute myocardial infarction and 40 healthy individuals and whole blood were obtained from arterial blood samples at the time of cardiac infarction. Expression of miR-19a was quantified from blood using quantitative Real-Time polymerase-chain reaction system (qRT-PCR).
Results: We found that: miR-19a level was significantly higher in blood from AMI patients compared with non-AMI subjects. Increased circulating miR-19a was not associated with age, gender, blood pressure and diabetes mellitus for AMI.
Conclusions: Our results suggest that increased blood levels of miR-19a are associated with AMI. Circulating miR-19a may be an ideal independent biomarker for accelerating diagnosis of AMI and the risk of developing heart disease in emergency department patients. We can use of silenced microRNAs or anti-miRs for the direct therapeutic of heart disease.
Keywords: Acute Myocardial Infarction, MicroRNA, Biomarkers

Ps-205: Olfactory Epithelium as an Infinitive Source of Neural Stem Cells for Derivation of Inner Ear Hair Cells

Kamran Mansouri[1*], Tara Bahmani[1], Ali Mostafaie[1], Ebrahim Karimi[2]

1. Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
2. Amir Alam Hospital, Tehran University of Medical Sciences, Tehran, Iran
Email: kamranmansouri@gmail.com

Background and Aim: Surgical techniques for treatment of sensory neural hearing loss (SNHL) has unpredictable outcomes and in recent years cell therapy investigated for the treatment of SNHL. Olfactory epithelium proceeds neurogenesis life time and provides an easily accessible source of neural stem cells. So the aim of this study was isolating neural stem cells from olfactory epithelium of rat and differentiation of these cells into hair cells of the inner ear in vitro.
Methods: The epithelium tissue of olfactory mucosa of rats was removed and digested by collagenase H. The digested tissue was cultured in flasks in suspension forms to create spheres. Spheres were passaged and from passage 2 spheres selected for differentiation. At this stage cells of spheres isolated from each other and placed in the flask containing defined differentiation medium. Cells at this stage cultured in adhesive form. Immunohistochemistry and RT-PCR have used for neural stem cells and hair cells identification.
Results: Spheres formed from olfactory epithelium culture and immunohistochemistry revealed that cells of spheres from passage one and two expressed the neural stem cells markers. After culture of isolated cells in differentiation medium, the morphology of cells begun to change. The cells presented neural cells projections and after 10 days the projections elongated more and interact with each other in multi-layers. RT-PCR and immunohistochemistry revealed that differentiated cells expressed hair cells specific genes.
Conclusion: Here in, we showed that neural stem cells of olfactory epithelium can differentiate into hair cells of the inner ear and so can be used for the treatment of SNHL.
Keywords: Olfactory Mucosa, Neural Stem Cells, Hair Cells, Differentiation, Rat

Ps-206: Extracellular Vesicles Generated from Embryonic Stem Cell-Derived Mesenchymal Stromal Cells Display Potent Immunomodulatory Properties

Soura Mardpour[1,2], Seyedeh-Nafiseh Hassani[2], Amir Ali Hamidieh[3*], Hossein Baharvand[4,5]

1. Tissue Engineering and Applied Cell Sciences Department, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. Hematology, Oncology and Stem Cell Transplantation Research Center, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran
4. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
5. Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

Email: souri_mardpour@yahoo.com

Background and Aim: Mesenchymal stromal cells (MSCs) and more recently their secreted extracellular vesicles (EVs) could be involved in tissue regeneration through paracrine effect, cell empowerment mechanism and immunomodulation. Moreover, increasing rate of demand in MSC-based therapy, limited potency and individual heterogeneity lead to the raise of interest towards allogeneic sources to produce off-the-shelf products. The aim of this study is to investigate immunomodulation potential of human embryonic stem cell-derived Mesenchymal stromal cells (hES-MSCs) and their generated EVs in comparison to those from bone marrow (BM) and adipose tissue (AD).
Methods: Human ESC line (Royan H6) were differentiated to stromal cells according to the Raclure's method within four weeks. Then, morphology, immunophenotype, multipotency and immunomodulation of hES-MSCs were extensively evaluated. Differential ultracentrifugation accompanied with sucrose gradient were used for isolation and purification of EV population from serum free conditioned medium. Then, inhibitory effects of hES-MSCs and their secreted EVs were determined on peripheral blood mononuclear cells (PBMCs) proliferation.
Results: Human ES-MSCs displayed similar morphology and immunophenotype to BM and AD-derived stromal cells. They suppressed proliferation of PBMCs through the secretion of anti-inflammatory cytokines such as IL-10, TGF-B1. In addition to hES-MSCs, their secreted EVs could also elicit more potent immunosupression potential compared to other sources.
Conclusion: Results from the present study demonstrated that hES-MSC EVs revealed more immunosuppressive effect compared to other counterparts from other sources. Therefore, this study provided a new approach in which EVs could be considered as allogeneic cell-free products in autoimmune disease.
Keywords: Mesenchymal Stromal Cells, Extracellular Vesicles, Immunomodulation

Ps-207: Cold Atmospheric Plasma Surface Modification of PCL Nano-fibers for Cartilage Tissue Engineering

Marziyeh Meghdadi[1], Zahra Nourmohammadi[1], Seyed-Mohammad Atyabi[2*], Mohamad Pezeshki-Modaress[3]

1. Department of Biology, School of Basic Sciences, Sciences and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Pilot Nanobiotechnology, Pasteur Institute of Iran, Tehran, Iran
3. Tissue Engineering and Regenerative Medicine Institute, Tehran Central Branch, Islamic Azad University, Tehran, Iran
Email: marziyeh.meghdadi@gmail.com

Background and Aim: Tissue engineering (TE) is a new scientific field of research with the purpose of repairing or enhancing tissue or organ function. Biomaterial substrates have many different applications in TE. Poly Ɛ-caprolactone (PCL) is a bio-compatible polymer with potential applications for tissue engineering especial tight tissue such as cartilage. Electrospun biomaterial substrate has highly porous microstructure with interconnected pores and large surface-area-to-volume ratio for tissue regeneration. In this study, we evaluated effects of Cold Atmospheric Plasma on electrospun PCL Nano-fibrous substrates surface modification for mesenchymal stem cells proliferation.
Methods: PCL Nano-fibers were fabricated using electro-spinning technique. A cold atmospheric gas plasma system, comprised of helium 99.99 and oxygen 5% (He/O2), was used for surface modification. Modified PCL Nano-fibrous scaffold was scanned by electron microscope (SEM). The mesenchymal stem cells proliferation evaluated by MTT tests for three days.
Results: Our results showed the nano-?ber surface modi?cation with Cold Atmospheric Plasm) CAP) improved growth and proliferation of human bone marrow derived mesenchymal stem cells (MSCs) by developing three-dimensional topography.
Conclusion: The CAP modification method used in this study could be an effective step in the process of cartilage tissue engineering and favorable alteration of surface properties towards the optimization of cell conditions.
Keywords: Nano-fiber, Surface Modification, Electrospinning, Cold Atmospheric Plasma

Ps-208: Glycosylated Mesoporous Silica Nanoparticles as a Delivery System of Gadolinium for Effective Human Stem Cell Tracking

Bita Mehravi[1*], Neda Iranpour Anaraki[2], Mojdeh Mohseni[2]

1. Radiation Biology Research Center, Iran University of Medical Sciences, Tehran, Iran
2. Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran
Email: bmehravi@yahoo.com

Background and Aim: The ability to track the status of stem cells after transplantation with noninvasive imaging techniques is an important challenge for the field of regenerative medicine. To be of most use tracking methods should perfectly be non-invasive, high resolution and allow tracking in three dimensions. Magnetic resonance imaging (MRI) is one of the best methods, but needs an appropriate contrast agent to be loaded to the cells to be tracked. One of the most extensive in stem cell tracking is as magnetic nanoparticles. One of the major problems to the study in the application of stem cells in tissue regeneration is the difficulty in tracking the movement of implanted cells. There has been significant effort to develop new nanoparticle vectors that could be efficiently internalized into cells. Though, modern cellular labeling apparatus are barely satisfactory. We sought to develop a highly efficient cell labeling nanoparticle vector for stem cell tracking.
Methods: In this study, glycosylated Gd-based mesoporous silica nanoparticles (Gd @GMSN) that a MRI contrast agent are developed in order to evaluate their cell uptake and potential as effective T1 and T2-enhancing trackers for human mesenchymal stem cells (hMSCs).
Results: The hMSCs are labeled with Gd@GMSN via over expression glut transporters. Labeled hMSCs are unaffected in their proliferation, viability, and differentiation capacities into osteocytes, adipocytes, and chondrocytes, which can still be readily MRI detected. Imaging, with a 1.5-T MRI.
Conclusion: This study shows that the advantages of biocompatibility, high internalizing efficiency, and cellular uptake make GMSN a perfect vector of T1 and T2-agent for stem-cell tracking with MRI.

Keywords: Mesoporous Silica Nanoparticels, MRI, Gadolinium, Stem Cell Tracking, Mesancimal Stem Cells

Ps-209: Mesenchymal Stem Cells Enhance the Metastasis of Bladder Cell Line, 5637, In 3D Co-Culture System

Mehrshad M.[1,2], Shakibaei M.[1], Bagher pour M[1,3] Modaressi S. M.H.[2], Maryam Shahali[2*]

1. Department of Quality Control, Research and Production Complex, Pasteur Institute of Iran, Tehran, Iran
2. Department of Biology, Science and Research Branch, Islamic Azad university, Tehran, Iran
3. Department of Biology, Karaj Branch, Islamic Azad University, Karaj, Iran
Email: maryam.shahali@gmail.com

Background and Aim: The human mesenchymal stem cells (hMSCs) have low immunogenicity and paracrine effects through secretion of growth factors, cytokines, and antifibrotic or angiogenic mediators. These properties make MSCs an attractive target as a cell therapeutic agent for tissue defects as well as organ and other degenerative diseases. Also, previous studies reported that MSCs are used to treat certain cancers such as lung cancer, Kaposi's sarcoma. However, recent investigations have produced controversial results regarding whether MSCs support or suppress tumor growth and progression. Therefore, to further investigate the biological roles of MSCs in the process of tumorigenesis, we investigated the effect of hMSCs on 5637 human bladder cancer cell line using co-culture system.
Methods: hMSCs were cultured in alginate gel (ALG) beads, and then co-cultured with 5637 human bladder cancer cell line. The effect of hMSCs on cell morphological and molecular behavior of 5637 cells was investigated by microscopic observation and qRT-PCR after 24 hours.
Results: Molecular analysis showed that, compared with control groups, treatment of 5637 cells with beads containing hAD-MSC resulted in increased expression of CSC biomarkers of CD44 and CD24 ($P<0.05$). We also measured the expression of EMT-related genes. Consistent with the observed morphological changes, the levels of N-cadherin, vimentin and fibronectin was

significantly up-regulated in co-cultured group compared with that in the control group. Molecular analysis showed that, compared with control groups, treatment of 5637 cells with beads containing hAD-MSC resulted in increased expression of CSC biomarkers of CD44 and CD24 (P?0.05). We also measured the expression of EMT-related genes. Consistent with the observed morphological changes, the levels of N-cadherin, vimentin and fibronectin was significantly up-regulated in co-cultured group compared with that in the control group.
Conclusion: Therefore, these data indicated that hMSCs could significantly enhance the tumor cell initiation, progression and metastasis. However, further complementary data such as the results of tumorigenic in vitro and in vivo assays of co-cultured/co-injected 5637 cells with hMSCs and its comparison with control group should be collected to fully confirm the function of hMSC-mediated tumor cell promotion in bladder cancer cell line, 5637.
Keywords: ALG Beads, Co-Culture, Mesenchymal Stem Cells, 5637 Cell Line

Ps-210: Evaluation Micrornas Involved in Epigenetics Control in All

Fatemeh Memari*

Tabriz University of Medical Sciences, Tabriz, Iran
Email: Hani.memari@yahoo.com

Background and Aim: Acute lymphoblastic leukemia (ALL) is the most common cancer in children in the world. While transcriptional silencing of genes because of epigenetic that is one of the most significant alterations in ALL, several current studies show that DNA methylation contributes to down-regulation of miRNAs throughout tumorigenesis. The progression of DNA methylation involves the adding of a methyl group to the 5-carbon site of cytosines in CpG. Hypermethylation of cytosines in CpG islands is related with silencing of tumorsuppressor genes. MicroRNAare small, non coding RNAs of 19 to 25 nucleotides that control gene expression by targeting mRNAs in a sequence-specific manner, remind translational repression or mRNA destruction and plays an important role in proliferation, apoptosis, differentiation, or tumorigenesis. In recent years, studies has shown that epi-miRNA manage the epigenetic site of cancer by targeting epigenetic factors, for example DNMTs, HDACs or polycomb genes
Methods: In this study at first ten miRNAs were selected that involved in DNA methylation. Sample collection 50 ALL samples were obtained fromperipheral blood (PB) or bone marrow (BM) of patients in sent to specialized for diagnosis to peyvand specialized laboratories. Then Total RNA was extracted and reverse transcribed into cDNA according to manufacturer's information. Real-time quantitative PCR (qRT-PCR) was performed to analyze the status of miRNAs expression in 122 patients with de novo ALL and15normal controls. Finally MiRNAs expression profiles were analyzed.
Results: Expression profiles of miRNAs was investigated in the sample of ALL Patients and healthy controls .We could identify a number of miRNAs that were significantly differentially expressed between ALL and healthy controls.
Conclusion: Our investigation revealed that there was observed differences in expression between in patients diagnosed with ALL miRNAs and controls.According to studies MiRNA expression profiles also show that most miRNAs have lower expression levels in tumors compared with normal, then most likely some of these miRNAs can act as recognized tumor suppressor genes. accept into account the inherent reversibility of epigenetic marks, disordered epigenetic regulation poses the ability to use targeted drugs.
Keywords: Epigenetic, miRNA, Acute lymphoblastic leukemia

Ps-211: TBL1Y Knock Down Results in Malfunction of Human Embryonic Stem Cells Derived-Cardiomyocytes by Suppressing of Notch Signaling

Anna Meyfour[1], Hassan Ansari2, Sara Pahlavan[2], Mostafa Rezaei-Tavirani[3], Hossein Baharvand[2], Ghasem Hosseini Salekdeh[2*]

1. Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Science, Tehran, Iran

2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology
3. Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Science, Tehran, Iran
Email: meyfour_a@yahoo.com

Background and Aim: Despite great evidences in sex-specific cardiovascular physiology and pathophysiology, the biological basis for such dimorphism has not been identified yet. Such gender related characteristics might reside in the function of sex chromosomes during cardiac development in addition to hormonal influences. Human embryonic stem cells (hESCs) are appropriate tools to targeted gene manipulations and subsequent functional studies of Y chromosome genes during cardiac development. In this study, the proteome of Y chromosome was analyzed during cardiac differentiation of hESC into cardiomyocyte.
Methods: The expression of male-specific region of Y chromosome (MSY) genes and their X counterparts were profiled during cardiac differentiation of human embryonic stem cells (hESCs) using qRT-PCR, western blotting and immunofluorescence. The function of TBL1Y gene was further studied in differentiating cells using siRNA approach. Cell cycle analysis, contraction measurements and Notch signaling activation were assessed for functional analysis of TBL1Y knockdown.
Results: We observed alterations in gene expression of TBL1Y, PCDH11Y, ZFY, KDM5D, USP9Y, RPS4Y, DDX3Y, PRY, XKRY, BCORP1, RBMY, HSFY and UTY which influenced protein expression and localization too. Due to increase in expression of TBL1Y and totally different expression pattern to its X-homolog, TBL1X, we further studied its function by using siTBL1Y. Down regulation of TBL1Y resulted in stabilization of CtBP protein as a member of Notch corepressor complex as well as decrease in expression of Notch signaling members such as Notch1 and HES1 as a notch target gene, and some cardiac differentiation markers such as TBX5 and Gata4. Moreover, TBL1Y knock down influenced cardiac differentiation by reducing its efficacy as well as increasing the probability of impaired contractions.
Conclusion: Significant alterations of Y chromosome genes expression during cardio genesis may suggest an important role for MSY genes in cardiac development. In agreement to this hypothesis, TBL1Y which seems to be no longer a missing protein promotes cardiac differentiation by derepressing of Notch signaling corepressor complex, CtBP. This findings open a new window for investigations of sex dependent differences in cardiac development.
Keywords: Y chromosome, Human Embryonic Stem Cell, Cardiac Differentiation, TBL1Y, Notch Signaling

Ps-212: Wnt/B-Catenin Pathway Inhibitor Proteins Are Regulated by Neuron-Specific Mirnas

Elham Sadat Mirfazeli[1], Bahman Zeynali[1*], Samad Nadri[2], Ehsan Arefian[3], Mousa Kehtari[3]

1. School of Biology, College of Science, University of Tehran, Tehran, Iran
2. Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
3. School of Biology, College of Science, University of Tehran, Tehran, Iran
Email: elham.mirfazeli@ut.ac.ir

Background and Aim: Deriving dopaminergic neurons from stem cells is a promising approach for cell-based therapies in several neurodegenerative diseases. The newly discovered multipotent stem cells in the trabecular meshwork (TM) tissue of human eye are characterized as mesenchymal stem cells (MSC). Our previous studies have indicated high differentiation potential of TM-MSC into dopaminergic neurons. Several studies have demonstrated that Wnt/β-catenin signaling pathway plays an important role in different steps of neuronal differentiation. Furthermore, several reports have emphasized on the role of miRNAs in the regulation of Wnt/β-catenin signaling pathway and neural development. However these miRNAs and their targets are poorly understood. Thus, in the present study, we tried to identify key miRNA regulators of Wnt/β-catenin pathway and their targets during induced neuronal differentiation of TM-MSC.
Methods: Here, we applied miRNA-Target prediction tools, namely TargetScan and miRWalk, for identifying miRNAs targeting our candidate inhibitors of Wn-

t/β-catenin signaling pathway. The relative expression of predicted miRNAs and their targets were then evaluated by means of quantitative Real-time PCR.
Results: Our bioinformatics and analytical analysis predicted miR-9 as a key miRNA targeting many of our candidate inhibitors including DKK2, SOST. Further analysis with quantitative Real-time PCR, showed significant upregulation of the putative miRNA and significant downregulation of their targets among inhibitors of Wnt/?-catenin pathway in TM-MSC during neuronal differentiation in a time-dependent manner.(p-value <0.05)
Conclusion: Our studies therefore suggest that miR-9 might activate Wnt/β-catenin signaling pathway by targeting its key inhibitors during neuronal differentiation of TM-MSC.To further validate the results, in the next step, we are going to examine direct impact of miR-9 on their targets by means of dual luciferase reporter assay.
Keywords: Neuronal Differentiation, Wnt/β-Catenin Pathway, Mesenchymal Stem Cell, miRNA, Dopaminergic Neuron

Ps-213: Organ Printing Using Tissue Spheroids as Building Blocks

Vladimir Mironov*

3D Bioprinting Solutions, Moscow, Russia
Email: vladimir.mironov54@gmail.com

Background and Aim: Organ printing could be defined as a robotic additive biofabrication of functional human tissues and organs according to digital models from tissue spheroids as building blocks. Biological basis of organ printing technology is natural tissue fusion phenomenon.
Methods: Using original multifunctional 3D bioprinter Fabion and rounded embryonic explants of mouse thyroid gland and allantoides the functional vascularizied mouse thyroid gland have been bioprinted.
Results: Using radioactive ablation animal model it have been demonstrated functionality of bioprinted mouse thyroid gland implanted under kidney capsule. Vascular tissue spheroids derived from rounded allantoides explants and closely placed with embryonic thyroid gland explants vascularise them by sprouting angiogenesis.
Conclusion: According or best knowledge it is a first report about bioprinted vascularized and functional organ with proven functionality. Perspectives and challenges of bioprinting human thyroid gland and other human organs using multifunctional 3D bioprinter Fabion and self-assembling tissue spheroids as building blocks will be discussed.
Keywords: 3D Bioprinting, Organ Printing, Tissue Spheroids, Thyroid Gland, Tissue Fusion

Ps-214: Down-Regulation of Anti-Apoptotic Genes in Tumor Cell Lines Is Facilitated by Suppression of OCT4B1

Mohammad Reza Mirzaei*, Mehdi Mahmoodi, Gholamhossein Hassanshahi

Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
Email: mirzaeemr@rums.ac.ir

Background and Aim: The OCT4B1 as a variant of OCT4 is expressed in both cancer cells/tissues. Its anti-apoptotic potency of this variant aid cancer cells to escape apoptosis. Therefore, the aim of the present study was to determine OCT4B1 suppression effects on regulation of 25 genes involved in anti-apoptotic pathway in tumor cell lines.
Methods: AGS (gastric adenocarcinoma), 5637 (bladder tumor) and U-87MG (brain tumor) were transfected with specific OCT4B1 siRNA and a scramble siRNA by siRNA silencing gene technology, using Lipofectamine 2000 commercial kit. Real-time PCR was applied and fold changes were calculated from 2-ΔΔCT formula.
Results: Our results demonstrated that interested genes in three studied cell lines similarly expressed, 22 genes (88%) were down-regulated and three genes (CASP2, IGF1R,TNF) were up-regulated and CFLAR was down-regulated in AGS, while up-regulated in 5637 and U87MG cells.
Conclusion: It may be concluded that OCT4B1 suppression can lead to apoptosis in tumor cell lines via down-regulation of almost of anti-apoptotic genes. Thus, suppression of OCT4B1 may possibly be considered in cancer therapy/research.

Keywords: OCT4B1, Anti-Apoptosis Gene Family, Tumor Cell Lines

Ps-215: Living Status and Activity of SMF Treated Cells, Possible Biophysical Manipulation of OEC Stem Cells for Regenerative Purposes

Hamid Mobasheri[1,2*], Sajedeh Ebrahim Damavandi[1], Zahra Elyasi Goji[1,3], Parvaneh Farzaneh[3], Motahareh Aghamolaei[1]

1. Laboratory of Membrane Biophysics and Macromolecules, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
2. Biomaterials Research Center (BRC), University of Tehran, Tehran, Iran
3. Iranian Biological Resource Center (IBRC), Tehran, Iran
Email: h.mobasheri@ut.ac.ir

Background and Aim: Different structural and functional aspects of the living cells can be manipulated by means of biophysical application of magnetic fields (MF) that mimic those of chemical treatments in some instances. As the living cells are composed of various types of free and bound ions in biological medium and macromolecules, polyelectrolytes, whose native dynamics deviated by the applied field, the exposure of the cells to MF may distort different machineries, including gene expression, lipid synthesis, cytoskeleton structure and dynamics, cell motility, growth , inter and intracellular traffics and so on. The susceptibility of different cells to MF depends on their ionic and atomic compositions as well as the intensity, exposure time and direction of the applied field. Furthermore, as the dynamics of the cells varies in different phases of the exposed cell, the extent of the effects differs at different status of the cells and thus, can be used for triggering certain differentiation path particularly in stem cells.
Methods: Here, cells were exposed to static magnetic fields (SMF), with intensities of 10, 30, 50, 80, and 100 mT and different aspects of their activities, including, viability, membrane integrity, growth rate, morphology, polarization rate, orientation and migration were recorded through time-lapse technique at every 1-5 minute rate for up to 24 hours at 37C, in 5% CO2, and 95% humidity.
Results: According to our results, exposure of NIH3T3 cells to SMF caused different effects on various aspects of their life in a nonlinear manner. First of all, the applied SMF at the applied levels was not toxic to the cells and did not changed their membrane integrity. Though most of time, they migrated parallel to the field direction ir-respect of its N or S pole, it varied to some extent at 10 and 80 mT. The morphology of cells changed into more elongated shapes which were directed parallel to the field directions.The results will be compared to those identified in OEC Olfactory Ensheeting Cells.
Conclusion: The monitored changes in the activity of the SMF treated cells reveals its induction on the intracellular traffic, polymerization of certain microfibers of cytoskeleton, and orientation

Ps-216: Study Effective of Stem-Cell Mechanism on Breast Cancer Therapy

Kamelia Mobasheri[1*], Sahar Rouholah Zadegan[2], Mohammad Zaefizadeh[1]

1. Islamic Azad University Branch, Ardabil, Iran
2. Islamic Azad University Branch, Tehran, Iran
Email: mobasherikamelia@gmail.com

Background and Aim: Stem cells are fundamental to human life and offer great therapeutic potential, yet their biology remains incompletely or in cases even poorly understood. Advances in stem-cell technology have led to the identification of stem cells in normal and malignant breast tissue. The most important and useful property of stem cells is that of self-renewal. Through this property, striking parallels can be found between stem cells and cancer cells: tumours may often originate from the transformation of normal stem cells, similar signalling pathways may regulate self-renewal in stem cells and cancer cells, and cancer cells may include 'cancer stem cells' rare cells with indefinite potential for self-renewal that drive tumorigenesis. The study of these stem cells has helped to elucidate the origin of the molecular complexity of human breast cancer.
Methods: We isolated (EGFR) from breast cancer cell lines (MCF7) .Cells were analyzed for spheroid formation, morphological changes, immunofluorescence for differentiation markers, protein (Western) and RNA (RT-PCR) analysis. Effect of inhibitors on the cell vi-

ability of breast cancer cells was determined using cell titer glow assays.
Results: Utilizing Hit-Ligand interaction site with the (EGFR) hot spot residues based on 3D alignment and shape, we have identified 51 potential hits from Ligand-Based screening using a 1000 Diverse Set.Screening of these 51 potential hits using MTT based cell viability assays identified three small organic molecule inhibitors (peptidomimetics) as leads. In self-renewal assays, peptidomimetic-treated cells had decreased self-renewal capacity.
Conclusion: Collectively, our studies have discovered an essential role for (EGFR) in breast cancer stem cell. The novel small molecule inhibitors of (EGFR) could be used for therapeutic targeting of breast cancer stem cells and therapy resistance.
Keywords: Breast Cancer, Stem-Cell, Metastasis, Tumor

Ps-217: Cell Spheroid Generation from a Single Cell on a Biocompatible Hydrophobic Electrospun Scaffold

Hajar Moghadas*, Mohammad Said Saidi

School of Mechanical Engineering, Sharif University of Technology, Tehran, Iran
Email: Moghadas_stu@yahoo.com

Background and Aim: In this paper, a superhydrophobic flexible electrospun scaffold (ES) developed that used for cell spheroid generation from a single cell. The insight of single cell behavior during proliferation can discover many concepts of the growth, chemotherapy resistance, and disease recurrence of the tumor caused by cancer stem-like cells (CSCs). Uniform and homogeneous fibers with a blend of polydimethylsiloxane (PDMS) and polymethylmethacrylate (PMMA) were produced by dissolving 6% (weight/volume) PMMA in 2:1 ratio of THF: DMF. Then various weight ratios of PDMS were added to the prepared PMMA solution and electrospun successfully. The extreme wettability of the ES presented a high contact angle 138.5o. The fabricated scaffolds were characterized by a variety of tests including scanning electron microscopy (SEM), contact angle goniometer, tensile test and porosity measurement which confirms the suitability of the ES for cell culture applications. Moreover, the cell spheroids generations from a single cell on our ES have confirmed its biocompatibility. These inexpensive easily detachable scaffolds with controllable thicknesses and proper tensile strength are a good candidate for cell culture in the biological applications, especially for non-adherent mammalian cancer stem cell.
Methods: In this paper, the scaffolds are electrospun using a mixture of PDMS and PMMA from 1:1 to 6:1 for PDMS: PMMA. Our ES peeled off very easily from the collector and three was no need to additional procedures such as heat treatment or vacuum pump. The significant characteristics of the ES such as hydrophobicity, strength, flexibility, porosity, thickness and mobility were evaluated. Then its capability to cell culture confirmed by growth the cells spheroid from a single cell.
Results: the cell spheroids generations from a single cell on our ES have confirmed its biocompatibility. These inexpensive easily detachable scaffolds with controllable thicknesses and proper tensile strength are a good candidate for cell culture in the biological applications, especially for non-adherent mammalian cancer stem cell.
Conclusion: In the current paper, flexible scaffolds from the mixtures of PDMS and PMMA were electrospun. It separated from the collector very simply without any heat or vacuum treatment. This electrospun scaffold is roughly superhydrophobic with contact angle around 138.5o, which was larger than the contact angle of PDMS and PMMA sheets. The hydrophobicity of the ES limits cells adhesion to it and lead to the cells spheroid generated from a single cell which can deliver valuable insight of the various characteristics of the tumor microenvironment. As an inexpensive, simply produce, biocompatible and flexible scaffold, our substrate can be adjusted according to different parameters for CSC screening. By changing the ratio of each polymer, different microarrays and scaffolds based on the desired properties of the other polymer (PMMA) are also practicable.

Ps-218: A Novel Scaffold Composed of Platelet Rich Plasma and Hydroxy Apatite-Tricalcium Phosphate for Bone Regeneration

Hamid Reza Mohajerani[1], Mohammad Bigdeloo[2], Amir Hossein Zakerighazanii[3], Mahdieh Sadat Ghiasi[4,5*]

1. Department of microbiology, Arak Azad University, Arak, Iran
2. Department of Surgery, Qom University of Medical Sciences, Qom, Iran.
3. Department of Orthopedics, Qom University of Medical Sciences, Qom, Iran
4. Department of Pharmacology, Razi Drug Research Center. Iran University of Medical Sciences, Tehran, Iran
5. Avaye Mahd Cell Iranian Company, Health Technology Development Center of Qom University of Medical Sciences, Qom, Iran
Email: mahdieh.ghiasi@yahoo.com

Background and Aim: platelet rich plasma - hydroxy tricalcium phosphate (PRP-HATCP), a new composite scaffold, has been shown to facilitate early revascularization and speed up bone regeneration process. The objective of this study was to evaluate the effect of PRP-HATCP seeded with mesenchymal stem cells (MSCs) to provide optimal growth and development environment for stem cells.
Methods: PRP-HATCP were prepared and MSCs isolated from human adipose tissue. Cells were cultured separately on the scaffolds and 2 weeks after differentiation, osteogenic genes expersion in each scaffold were evaluated using real time-polymerase chain reaction.
Result: MSCs with PRP-HATCP shown the highest expression of osteogenic genes compared to MSCs-HATCP.
Conclusion: According to the results of the study, it can be said that biomaterial such as PRP can be used as an appropriate environment for bone differentiation
Keywords: Mesenchymal Stem Cells, PRP-HATCP, Tissue Engineering, Bone Regeneration

Ps-219: The Differentiation of Mesenchymal Stem Cells into Neural Cells by Fibrin Glue Scaffold

Hamid Reza Mohajerani[1*], Mohammad Bigdeloo[2], Amir Hossein Zakerighazanii[3]

1. Department of Microbiology, Arak Azad University, Arak, Iran
2. Department of Surgery, Qom University of Medical Sciences, Qom, Iran
3. Department of Orthopedics, Qom University of Medical Sciences, Qom, Iran
Email: mahdieh.ghiasi@yahoo.com

Background and Aim: Human adipose derived mesenchymal stem cells (hAMSCs) can differentiate into several types of mesenchymal cells, including osteocytes, chondrocytes, and adipocytes, as well as, these cells can also differentiate into non-mesenchymal cells, such as neural cells, under appropriate conditions in vitro. In this study, we induced differentiation into neural phenotype in the hAMSCs population by bio material that called fibrin glue (FG). In this treatment, hAMSCs could express neural marker.
Methods: The Human adipose derived mesenchymal stem cells were isolated by enzymatic digestion and cultured in DMEM containing 10% FBS. Cells were seeded in fibrin glue scaffold and differentiated for 7-14 days. We used Real-time PCR analys for assessment of specific nourogenic gene expersion (Nestin).
Results: Real-time PCR analys showed that after induction, cells will express neuronal marker more than control group significantly.
Conclusion: Results showed that hAMSCs treatment with FG can improve differentiation condition in vitro, depending on growth faactors.
Keywords: Mesenchymal Stem Cells, Neural Cells, Fibrin Glue, Differentiation

Ps-220: Avaluation of Morphology of Stem Cells Differentiated Into Osteoblast in Combination of Natural and Synthetic Scaffold by H & E Staining

Hamid Reza Mohajerani[1*], Mohammad Bigdeloo[2], Amir Hossein Zakerighazanii[3,4]

1. Department of Microbiology, Arak Azad University, Arak, Iran
2. Department of Surgery, Qom University of Medical Sciences, Qom, Iran.
3. Department of Pharmacology, Razi Drug Research Center. Iran University of Medical Sciences, Tehran, Iran.

4. Avaye Mahd Cell Iranian Company, Health Technology Development Center of Qom University of Medical Sciences, Qom, Iran
Email: mahdieh.ghiasi@yahoo.com

Background and Aim: Platelet rich plasma (PRP) is a new, autologous biomaterial beneficial for repairing bone and soft-tissue defects, and it has shown an excellent outcome composed in hydroxy tricalcium phosphate. In this study was evaluated the morphology of stem cells differentiated into osteoblast in platelet rich plasma - hydroxy tricalcium phosphate (PRP-HATCP) as optimal environment.
Methods: Mesenchymal stem cells (MSCs) isolated from human fat tissue. Stem cells cultured 21 days then seeded in PRP-HATCP and performed osteogenic differentiation for 2 weeks in speicific medium. Differentiated stem cells morphology were analyzed using H & E staining.
Results: Our result exibited that treatment of MSCs with PRP-HATCP was able to preserve normal morphology of Differentiated stem cells in experimental condition compared to pellet system Conclusion: it can be confirmed that natural structure such as PRP can be caused an appropriate situation for differentiation of stem cell into bone.
Keywords: Mesenchymal Stem Cells, Natural, Synthetic, Adipose, Osteoblast

Ps-221: Development of Novel Biocompatible Nano Dot-Based Multiplex Cellular Tracking Using Based Ring-Controlled Luminescence Mechanism

Nasrin Mohajeri, Mohammad Pourhassan Moghaddam, Abolfazl Akbarzadeh Nosratollah Zarghami*

Department of Medical Biotechnology, Faculty of Advanced Medical Science, Tabriz University of Medical Sciences
Email: mohajeri.nasrin@gmail.com

Background and Aim: With advent of stem cell biology and regenerative medicine, development of novel tracers of cell evolution such as division, differentiation is indispensable. On the other hands, current tracers are costly, toxic and unstable in the aqueous solution. Therefore, here in, we report the development of novel biocompatible and stable luminescent nucleic acid dots in different colors with wide potential application in tracking the cellular and molecular behavior of stem cells, in order to better understand the exact mechanism of cell growth and differentiation.
Methods: Nucleic acids of similar lengths were incubated in temperature 60° C - 80° C for 24h. In order to customize the luminescence color, a ratio of oxidizing agents were added to the above solution and incubated at temperatures higher than 80° C for another 24h and with fine tuning of the ratio of oxidizing agents, different luminescence were achieved. Finally, for evaluation of possible cytotoxicity, HGR-2 over expressing human breast cancer cells (MCF7/HGR+) were incubated with the serial concentrate of the developed nano dots using MTT assays. Also the size and morphology of particles were investigated by electron microscopy and the absorbance and photoluminescence profile of the particles were recorded using Cytation5 bio-imaging equipment. QY measurement was tested a using F-3018 quantum yield accessory.
Results: Comparing to the control nucleic acid solution, the nano dot exhibited red and green luminescence colors regarding the ratio of the oxidizing agents used. Analysis of luminescence spectra showed a direct relationship between the luminescence intensity and the degree of base ring oxidation, and particles morphology was spherical with a size of 25 nm. Moreover, the cell viability was higher than 90% that reveals the nucleic acid dots are very biocompatible. The solution of nucleic acid dot shows luminescence lifetime equals to 2.7ns with a quantum yield up to 7.5%.
Conclusion: The dramatic increase in the use of nanoparticles in stem cell research and regenerative medicine technology has enhanced studies about safe and non-toxic materials. The development next generation nano dot is described here to be used in the future investigations such as selective controlled and targeted real-time bioimaging of stem cell and cancer stem cell. We report a facile approach to the preparation of different colors of nucleic acid dot in water. Multicolor displays by red and green nucleic acid is useful strategy in biological molecules imaging and their interactions.
Keywords: Stem Cell Tracking, Nucleic Acid Dot, Nanoparticle, Luminescence Mechanism

Ps-222: Characteristics and Differentiation of Cyrtopodion Scabrum Original Tail Cells

Shiva Mohamadi Moghanjoghi*, Meysam Ganjy Bakhsh , Parvaneh Farzaneh

Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran
Email: shvmhmd@gmail.com

Background and Aim: Lizard's tail has attracted scientists' attention to discover the original tail cells as well as regenerated tail cells properties for containing putative resident stem or progenitor cells. This way the origin of regenerated tail would be discovered. These animals possess numerous stem-ness niches, long growth rate period and suitability for regenerative medicine. Production, characterization and cryopreservation of diverse source of animal cell lines are crucial approach for different aspects of research and lead to conserve genetic resources. In this study, cells derived from tail of the lizard Cyrtopodion scabrum were cultured and cryopreserved in vitro, and their biological characteristics, proliferation and differentiation features were analyzed to discover aspects like potential progenitor properties.
Methods: Tail tissue sample was washed with70% ethanol, transferred to DMEM. After removing the skin from tail tissue in DMEM, sample was cut from upper part to 1-2 mm3 pieces. Every two small tissue pieces were seeded in a 35-mm2 tissue culture dishes covering with a sterile 22-mm2 glass slip. For first growing condition, 3 ml DMEM with 20% FBS, 1% L-Glutamine, 200 U/ml penicillin and 200 mg/ml streptomycin were added in each cell culture plate. The primary cell cultures were kept in 37°C incubator with 5% CO2 for approximately two weeks. To investigate the osteogenic potential of tail cells, osteogenic medium was added including DMEM with 10% FBS, 2 mM L-Glutamine, 10 mM β-glycero phosphate, 100 nM Dexamethasone, 50 µM Ascorbic Acid. Adipose differentiation medium was prepared containing DMEM with 10% FBS, 2mM L-Glutamine, 10 µg/ml insulin, 200 µM Indomethacin, 10 µM Dexamethasone, 500 µM 3Isobutyl1-methylxanthin.
Results: Diverse incubation conditions with 18, 23 and 37˚C temperature were applied to achieve optimum growth conditions. 18˚C incubation condition was optimal for tail cell properties. The cells demonstrated fibroblast like morphology with population doubling times of approximately 24±0.5 hours. Adipose and osteoblast differentiation were vividly observed in cells. Multiplex PCR confirmed and distinguished the specie of lizard C. scabrum with no cross-contamination with other species.
Conclusion: Producing cell lines from animal tissues in non-invasive manner is considered to be advantageous in biological perspective. In this regard, cells derived from original tail of the lizard Cyrtopodion scabrum were cultured using tissue explant technique. Original tail cells were studies for their adipose and osteoblast differentiation potential. The established cell line is a valuable genetic pool and would be regarded as a source for subsequent in vitro studies and regenerative medicine.
Keywords: Primary Cell Culture, Cyrtopodion Scabrum, Differentiation, Cryopreservation

Ps-223: Umbilical Cord Derived Mesenchymal Stem Cell Expansion under Direct Perfusion Bioreactor

Anneh Mohammad Gharravi*, Ahmad Mahmodi, Mehrdad Ebrahimi

1. Stem Cells and Tissue Engineering Research Center, Shahroud University of Medical Sciences, Shahroud, Iran
2. Student Research Committee, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran
Email: annehgh@yahoo.com

Background and Aim: In comparing stem cells sources, Umbilical cord-derived mesenchymal stem cells (UC-MSCs) are more accessible to collect and expand, thus UC-MSCs are an ideal candidate for Stem cells transplantation for the treatment of several diseases. The aim of present study was isolation and expansion of UC-MSCs by a novel bioreactor technique.
Methods: The Wharton's jelly component of Umbilical cord harvested and placed in a 37°C incubator.In one group, UC-MSCs harvested by explant methods. In other group, the Wharton's jelly cut into small pieces and encapsulated in alginate hydrogel and transferred into perfusion bioreactor chamber and cultured under

direct perfusion of culture medium. Surface biomarkers of cell such as CD105, CD90, CD34, CD45 indicated by flow cytometry. Histological and morphological examination performed on tissue construct of UC-MSCs-scaffold.
Results: In convention method, UC-MSCs in 2D culture expanded 7-10 days. Cells dedifferenciated into fibroblast like cell. In perfusion bioreactor culture, UC-MSCs expanded 5-7 days. Cells appeared with round phonotype during culture. After optimum confluency, UC-MSCs safely isolated with depolymerization of scaffold. In histological examination cell appeared similar to native umbilical cord morphology.
Conclusion: Finding of the present study indicated that perfusion bioreactor is an effective protocol to isolate UC-MSCs. Since hUC-MSCs can be safely expanded without cell dedifferentiation, the novel bioreactor protocol can be recommended as alternative protocol of 2d isolation and expansion method.
Keywords: Umbilical Cord, Mesenchymal Stem Cell, Expansion, Isolation, Perfusion, Bioreactor

Ps-224: Sustained Release Liposomal Formulation of Bone Morphogenetic Peptide-2 for Bone Regeneration

Marzieh Mohammadi[1], Mahmoudreza Jaafari[1], Mohammad Ramezani[2*]

1. Nanotechnology research center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
2. Pharmaceutical Research Center, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
Email: mohammadim861@gmail.com

Background and Aim: Bone morphogenetic protein-2 (BMP-2) is an osteoinductive molecule which has opened up a new frontier in maxillofacial and orthopedic surgery. However, there are two major problems in the clinical use of BMP-2: BMPs are not retained at the site of the injury for an adequate period of time because of their systemic high clearance and the use of high doses may lead to nerve injury, cancer or bone overgrowth. So the goal of the current study was to design and develop a liposomal formulation of BMP-2 to reduce the burst release of the bioactive molecule and the cost of the final product, also to increase the safety issues.
Methods: Preparation and characterization of liposomes: eight different liposomal formulations were produced by the film rehydration method. Liposomes were filtered through polycarbonate membranes using an Avanti Polar Extruder. Then the nonencapsulated BMP-2 was removed by dialysis against PBS buffer. Hydrodynamic diameter and ζ-potential of the liposomes were determined using Malvern ZS Nano Instrument in PBS. Release assay: Liposomes were put into a dialysis bag (cut off 30KDa) in a solution of PBS supplemented with 10% FBS containing 0.02% sodium azide. The samples were withdrawn at 1day intervals and replaced with equal volume of fresh medium. The fluorescence intensity of the samples were monitored at different time points. Biological studies: To study the effect of the released BMP-2 on osteogenic differentiation, human adipose- derived mesenchymal stem cells (hADSCs) were cultured. The osteogenic potential of the cells incubated with liposomal BMP-2 was assessed and compared to the free peptide by quantifying alkaline phosphatase (ALP) activity and the amount of mineralization 21 days post-seeding.
Results: The size distribution of different liposomal formulations were 105-171nm. Loading efficiency of the formulations were 11-55% and the cumulative release were 5-80% in different formulations. The results demonstrated that ALP activity and the mineralization of the selected formulations were significantly more than the free peptide.
Conclusion: The results demonstrated that the formulation DSPC/Cholestrol/mPEG-DSPE (69%, 30%, 1%) possess a sustained release profile for up to 21 days and enhanced the ALP activity and the mineralization of the ADSCs in comparison with free peptide.
Keywords: Liposome, Bone Morphogenetic Protein, Osteogenic Differentiation, Regenerative Medicine

Ps-225: Consideration of Cardiac Glycosoids Application in Concurrence with Stem Cell Therapy? An in Vitro Study

Amaneh Mohammadi Roushandeh[*]

Anatomical Sciences Department, Medicine Faculty, Hamadan University of Medical Sciences, Hamadan, Iran

Email: dinachal@yahoo.com

Background and Aim: Mesenchymal stem cells are used recently on heart failure patients but the results are disappointed. Concurrent to stem cell therapy the patients receive the pharcological agents. As an example, cardiac glycosides such as digoxin can inhibit the cell proliferation and lead to apoptosis of cells. Present study tries to know stem cell behavior following digoxin treatment.
Methods: Mesenchymal Stem Cells were osolated from bone marrow of rat tibia and femur. Then they treated with different concentrations of digoxin for 6, 12, 24 and 48 hours. Cell viability was detected with trypan blue. Hoechst staining and tunnel assay were conducted to evaluate nuclear configuration and apoptosis in MSCs.
Results: Cell viability decreased after digoxin treatment in all groups during 6, 12, 24 and 48h significantly ($P<0.05$). After 6 hours, rate of nuclear fragmentation was significantly higher in30 and 40μM digoxinthan control group ($P< 0.001$). Treatment with 20, 30 and 40μM digoxin led to nuclear damagesignificantly compare to control groupafter 12h ($P< 0.001$). Also, after 24 and 48 hours, nuclear damagesignificantly increasedin 15, 20, 30 and 40μM of digoxin ($P< 0.001$).Digoxin induced apoptosis significantly in all groups in time and dose dependent, so that the highest rate of cell death was found after 48h.
Conclusion: It is suggested that glycosoids such as digoxin might lead to decline in cell survival and increase cell death in a dose and time dependent and interfere with stem cell therapy. Therefore, it is recommended to consider application of glycosides in concurrence with stem cell therapy.
Keywords: Cell death, Cardiac Glycosoid, Mesenchymal Stem Cell, Proliferation

Ps-226: Co-Preconditioning: A New Approach to Rescue Mesenchymal Stem Cells from Harsh Environment

Amaneh Mohammadi Roushandeh*

Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
Email: dinachal@yahoo.com

Background and Aim: During cell therapy, the scientists face to several problems. Most MSCs are going to be apoptotic after injection into the body. Because of factors such as Low Serum, Hypoxia and oxygen free radicals. A benefit strategy to increase the cells resistance against toxic stresses is cells preconditioning the present study investigated the effects of preconditioning with hydrogen peroxide (H2O2) and serum deprivation on cell viability and apoptosis.
Methods: Mesenchymal stem cells from bone marrow were cultured and preconditioned with serum deprivation and H2O2 for 6, 12, 24 and 48 hours in 6 groups (I: Control, II: 5μM H2O2, III: 10μM H2O2, IV: 5%FBS, V: 5μM H2O2+5%FBS and VI: 10μM H2O2+5%FBS). After these time periods of treatment, the MSCs were exposed to lethal dose of H2O2 (300 μM) for 24 hours. Then, trypan blue staining was conducted to evaluate the cell viability. Also, TUNEL assay was done to study the cell apoptosis.
Results: This study demonstrated that the cell viability in groupsV and VI, which were both preconditions at all times were significantly increased compared to control group. Moreover, cell apoptosis of V and VI groups was significantly lower than control group after 12, 24 and 48 hours ($P < 0.01$).
Conclusion: The present study showed that preconditioning with 5μM H2O2+5%FBS and 10μM H2O2+5%FBS can improve the cell viability as well as lead to decline in cell apoptosis.
Keywords: Mesenchymal Stem Cells, H2O2, Serum Deprivation, Preconditioning

Ps-227: Colorectal Cancer Stem Cells Secretions Induce Pro-Inflammatory Response by Alteration of Human Pbmcs Cytokine Panel

Bahareh Mohebi[1,2], Kaveh Baghaei[1*], Mehrdad Hashemi[2], Samaneh Beigi[1], Seyed Mahmoud Hashemi[3], Hamid assadzadeh[1], Mohammadreza Zali[1]

1 Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Genetic, School of Advanced Sciences, Tehran Medical Branch, Islamic Azad University, Tehran, Iran

3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: baharsp943@yahoo.com

Background and Aim: Colorectal cancer (CRC) is the third most frequent cancer worldwide. Soluble factors, chemokines cytokines and exosomes of tumor cells lead to inflammation, and macrophages are the cells recruited to the tumor microenvironment (TME) in response to these factors. Studies indicated that immune cells and soluble factors are the main key in the maintenance of the balance between tumor promoting inflammation and anti-tumor immunity. On the other hand, it is proven that secreted cytokines of CRC cell lines can have an impact on Peripheral Blood Mononuclear Cells (PBMCs) phenotype, especially monocytes and macrophages. Classically activated (M1) macrophages and Alternative activated (M2) macrophages are two main types that appear to have a central role in different kinds of tumors. So that, macrophage infiltration has been associated with good prognosis in CRC, but a poor prognosis in prostate cancer, breast cancer, and cervical cancer. In this study, the effect of colorectal cancer stem cells (Caco-2) in an immune response produced by PBMCs was evaluated.
Methods: PBMCs were isolated by ficoll gradient from peripheral blood. Only monocytes were cultured in control and test flasks. After24 hours, monocytes were treated with Caco-2 conditioned media. RNA extraction and cDNA synthesis of monocytes were performed after 72 and 96 hours. Finally, the gene expression of pro-inflammatory PBMCs phenotype (IL-6, IL-12b, IFN-ɣ, TNF-α) was examined by Real-time PCR.
Results: In comparison with controls, increasing levels of IL-6, IL-12b, IFN-ɣ and decreasing of TNF-α were observed in treated flasks. This is suggestive that secretion of pro-inflammatory cytokines by PBMCs, stimulate the immune system to anti-tumor immunity (innate, and adaptive) responses.
Conclusion: According to our results, Caco-2 conditioned media, induced the differentiation of PBMCs especially monocytes toward inflammatory phenotype by changing the gene expression. Since immunotherapy approaches are based on improvement in the immune system, enhancement of anti-tumor immunity by provoking inflammatory PBMCs would be a right therapeutic approach in CRC patients.

Keywords: Caco-2, PBMCs, IL-6, IL-12b, IFN-ɣ, TNF-α

Ps-228: Production of Functional Motor Neurons from Human Adipose Derived Mesenchymal Stem Cell Source

Rashin Mohseni, Mahmoud Reza Ashrafi, Jafar Ai, Mahin Nikougoftar Zarif, Mahmoud Mohammadi, Masoud Ghahvechi-Akbari, Anahita Majma, Alireza Shoae-Hassani, Amir Ali Hamidieh*

Applied Cell Sciences and Tissue Engineering department, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: cell.therapy@yahoo.com

Background and Aim: Cell therapy and stem cell transplantation strategies have provided potential therapeutic approaches to treat neurological disorders. Mesenchymal stem cells from adipose tissue (ADMSC) are abundant adult stem cells with low immunogenicity, which could be considered for cell replacement therapies. The differentiation of ADMSCs into acetylcholine secreted motor neuron-like cells, followed by elongation of the cell axon, is a promising treatment for motor neuron cell dysfunction in human like spinal muscular dystrophies (SMA).
Methods: Adipose derived mesenchymal stem cells were isolated by a double enzyme digestion method. The third passage of mesenchymal stem cells was induced with 2BME pretreatment and then by basic fibroblast growth factor (bFGF). Differentiation was followed by four days of induction with retinoic acid and sonic hedgehog. Motor neuron axon elongation was then induced by adding nerve growth factor (NGF) to the differentiation media.
Results: The results were evaluated using Real-time PCR, Flowcytometry and Immunocytochemistry. Our data confirmed that the cell morphology did not change after induction with basic fibroblast growth factor alone. However, neuronal morphology was visible, and Hb9, microtubule-associated protein-2 expression and acetylcholine levels increased following induction with bFGF.
Conclusion: ADMSCs are an attractive stem cell source for producing motor neurons in vitro. The meth-

ods to induce Olig2 and Hb9, have therapeutic potential for autologous cell replacement therapy to treat motor neuron disorders.
Keywords: Differentiation, Motor Neuron, Neurological Disorders, Spinal Muscular Dystrophy, Stem Cell

Ps-229: Improved Distribution and Proliferation of MC3T3-E1 Pre-Osteoblastic Cells Cultured on a Porous PLGA/B-TCP Scaffold on a Perfusion Bioreactor by Optimizing the Flow Rate

Fatemeh Mokhtari-Jafari1[2*], Ghassem Amoabediny[1,2], Mohammad Mehdi Dehghan[3], Marco N. Helder[4], Behrouz Zandieh-Doulabi[5,6], Jenneke Klein-Nulend [5,6]

1. School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
2. Department of Biomedical Engineering, Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran
3. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
4. Department of Orthopedic Surgery, VU University Medical Center, MOVE Research Institute Amsterdam, Amsterdam, The Netherlands
5. Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA)-University of Amsterdam Amsterdam, The Netherlands
6. VU University Amsterdam, MOVE Research Institute Amsterdam, Amsterdam, The Netherlands
Email: fatememokhtari@yahoo.com

Background and Aim: In recent decades, the application of bioreactors has been revolutionized the concept of regeneration of critical size bone defects. Perfusion flow rate through the scaffold appeared to play a significant role in cell growth. Therefore we aimed to evaluate the suitable perfusion flow rate for distribution and proliferation of MC3T3-E1 cells cultured on a porous PLGA/β-TCP scaffold.
Methods: MC3T3-E1 pre-osteoblastic cells were seeded onto the porous PLGA/β-TCP scaffolds (2 cm in diameter and 1 cm in thickness) by adding 200 µl of cell suspension media with 1×106 cells to each scaffold in a dropwise manner and incubated for 2 hr (200 µl of culture medium were added to each scaffold every 30 min). After 2 hr, 10 ml of culture medium was added to each scaffold and incubated in a humidified atmosphere at 37 °C and 5% CO2. After an overnight static culture, scaffolds were transferred to the perfusion bioreactors (one scaffold for each bioreactor) with different flow rates (3, 5 and 7 ml/min). After 21 days of culture, the MC3T3-E1 -seeded scaffolds were treated in Alamar Blue for 4 hours and absorbance intensity of alamarBlue® reagent was measured which is directly proportional to cell number. Assessments of cell distribution were performed using DAPI staining.
Results: It was found that the dynamic culture using 5 ml/min perfusion flow rate increased metabolic activity of MC3T3-E1 pre-osteoblastic cells which is directly proportional to cell proliferation. Flow rate of 7 ml/min caused higher cell detachment than that of 3 and 5 ml/min. In addition, DAPI staining images showed uniform cell distribution across all the scaffold sections.
Conclusion: At the flow rate of 5 ml/min, the MC3T3-E1 pre-osteoblastic cell contents of PLGA/β-TCP scaffolds cultured in the perfusion bioreactor for 21 days were increased over those cultured at the flow rate of 3 and 5 ml/min.
Keywords: Flow Rate, Proliferation, Distribution, Perfusion Bioreactor, Pre-Osteoblastic Cells

Ps-230: Conversion of Astrocytes into Oligodendrocyte-Like Cells with a Single Transcription Factor, a Strategy to Enhance Myelin Repair

Akram Mokhtarzadeh Khanghahi[1], Wenbin Deng[2], Mohammad Javan[1*]

1. Department of Stem Cells and Developmental Biology Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Institute for Pediatric Regenerative Medicine, University of California, Davis, School of Medicine, Sacramento, California, USA
Email: khanghahi@alumni.ut.ac.ir

Background and Aim: Oligodendrocytes are the myelinating cells of the central nervous system and provide the possibility of saltatory conduction. Regeneration of oligodendrocyte after demyelinating diseases by reprogramming or cell conversion strategies remains a major

challenge for myelination. Recent studies have demonstrated that astroglial cells can be directly converted into functional neurons both in vitro and in vivo.
Methods: Here, we report that single transcription factor Sox10 was capable of directly reprogramming of astrocytes into oligodendrocyte-like cells. Primary astrocytes from mouse pups were purified and transduced with Sox10 as lentiviral particles which included a GFP expressing sequence. Afterward, cells were treated with oligodendrocyte medium. The induced oligodendrocyte progenitor cells (iOPCs) expressed Olig2, endogenous Sox10 in transcript levels and Olig2 and NG2 in protein levels. After differentiation Oligodendrocyte like cells had oligodendrocytic morphology with expression of myelinating markers like MBP and PLP. For investigating the effect of application of Sox10 on in vivo reprogramming, after control transduced cell fate studies in injection site in cuprizone treated animals, specific oligodendrocyte markers were assessed by immonohistofluroscence in Sox10 receiving group.
Results: We observed that transplantation of Sox10 transduced astrocyte after three weeks could reprogram transplanted astrocytes to oligodendrocyte fate. Our findings showed the feasibility of reprogramming of astrocytes into oligodendrocyte-like cells by using a single transcription factor, Sox10 in vitro and in vivo.
Conclusion: Altogether, these results show that constitutive expression of Sox10 in astrocytes induces the transdifferentiation into oligodendrocyte fate, suggesting a master role for Sox10 in changing astrocyte gene regulatory network.
Keywords: Astrocyte, Oligodendrocyte-Like Cells, Conversion, Sox10

Ps-231: Clinical Improvement of Patients with Knee Osteoarthritis after Intra-Articular Injection of Autologous Bone Marrow-Derived Mesenchymal Stem Cells: A Case Series

Ali Ghasemi[1], Mehdi Yaghoubi[1], Zhaleh Shariati-Sarabi[1*], Bita Zargaran[1], Seyed Mostafa Monzavi[2], Jalil Tavakol Afshari[1]

1. Stem Cell Research Core, Mashhad University of Medical Sciences, Mashhad, Iran

2. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: seyedmostafamonzavi@gmail.com

Background and Aim: Bone marrow (BM)-derived Mesenchymal stem cells (MSCs) are multi-potential to form various cells. The BM-MSCs have high proliferation capacity and can be differentiated to chondrocytes, and so, they can facilitate the repair of osteoarthritis (OA)-induced damages. This study was designed to evaluate the effectiveness of intra-articular injection of BM-MSCs on clinical improvement of patients with knee OA.
Methods: Patients aged between 45 and 75 years with grade 2 and 3 knee OA according to Kellgren and Lawrence grading scale were included. After signing informed consent, the patients underwent BM aspiration under local anesthesia and the aspirate was sent to the laboratory for BM-MSCs isolation and expansion. All patients received single injection of a solution consisting of 2-3 × 10^6 BM-MSCs, 2 mL hyaluronan gel and 0.2 mL dexamethasone. The patients were evaluated prior to injection (baseline) as well as in 2 follow-up visits (3 and 6 months post-injection) according to the following parameters: Persian version of Knee injury and Osteoarthritis Outcome Score (KOOS) and numerical rating scale (NRS) for subjective measurement of pain.
Results: Fourteen patients (80% female) with mean age of 60.7 ± 7.3 years were studied. By the effect of the treatment, from baseline visit (pre-injection) to the 3rd visit (6th month post-injection), mean scores of the KOOS subscales significantly improved for symptom (34.6 ± 12.5 to 64.1 ± 8.1, P=0.001), pain (31.3 ± 14.0 to 61.4 ± 18.1, P<0.001), activity of daily living (ADL) (33.3 ± 14.3 to 64.5 ± 20.8, P=0.001), sports and recreational activities (18.6 ± 9.5 to 54.7 ± 23.0, P<0.001) and quality of life (14.0 ± 4.9 to 46.1 ± 17.5, P=0.001). Moreover, from baseline visit to the 3rd visit, joint pain based on NRS decreased significantly from 7.2 ± 0.9 to 4.2 ± 0.6 (P=0.003). Except transient local pain on the aspiration site, no notable adverse events occurred during the 6-month observation.
Conclusion: The intra-articular injection of autologous BM-MSCs was clinically effective in knee OA in terms of improvement of pain, symptoms, activity and qual-

ity of life during 6 months of follow-up. Nonetheless, long-term follow-up is needed to determine if the clinical response is durable.
Keywords: Bone Marrow, Intra-Articular Injection, Knee Osteoarthritis, Mesenchymal Stromal Cells, Quality of Life

Ps-232: No Significant Magnetic Resonance Imaging Changes after Intra-Articular Injection of Autologous Adipose-Derived Stromal Vascular Fraction to Patients with Knee Osteoarthritis

Tayebeh Asadi Sakhmaresi[1], Sara Jalali Jivan[1], Seyed Amir Jalali[2], Meysam Ganjy Bakhsh[2], Seyed Mostafa Monzavi[3], Bita Zargaran[1], Zhaleh Shariati-Sarabi[1]*, Jalil Tavakol-Afshari[1]

1. Stem Cell Research Core, Mashhad University of Medical Sciences, Mashhad, Iran
2. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: seyedmostafamonzavi@gmail.com

Background and Aim: Stromal vascular fraction (SVF) of lipoaspirates contains heterogeneous cell populations such as mesenchymal stem cells (MSCs), preadipocytes, endothelial cells, pericytes, T cells, and M2 macrophages. SVF cells have demonstrated regenerative potential in damaged tissues or organs through paracrine and differentiation mechanisms. This study was designed to evaluate the effect of intra-articular injection of adipose-derived SVF (AD-SVF) to patients with knee osteoarthritis (OA) by comparing magnetic resonance imaging (MRI) findings before and after injection.
Methods: Patients aged between 45 and 75 years with grade 2 and 3 knee OA according to Kellgren and Lawrence grading scale were included. After signing informed consent, the patients underwent lipo-aspiration from abdominal and/or femoral regions under local anesthesia. SVF cells were isolated from adipose tissue by collagenase digestion and gravity separation. SVF was filtered successively through 100 µm, 70 µm and 40 µm porous filters. Quantification of viable cells was performed. Four mL of fresh SVF cells were injected into intra-articular space of affected knee of the patients. The patients undergo MRI prior to injection (baseline) and 1 year post-injection. The effect of the treatment on the repair of articular cartilage defects was assessed using the modified version of magnetic resonance observation of cartilage repair tissue (MOCART) score.
Results: Six patients (100% female) with mean age of 56.3 ± 6.9 years were studied. The modified MOCART scores of the patients changed from 75.0 ± 11.8 before injection to 78.5 ± 18.6 in the 12th months after injection, which were not significantly different from each other (P = 0.571). Moreover, in all parameters of the MOCART scoring system including: filling of the cartilage defect, cartilage interface, cartilage surface, adhesions, cartilage structure, signal intensity, subchondral lamina, subchondral bone and effusion, no significant change was observed after the injection.
Conclusion: The intra-articular injection of autologous AD-SVF provides no significant repair of the knee articular defects according the MRI findings. SVF contains limited number of MSCs, the cells which are known to be differentiated to chondrogenic lineages capable of repairing articular cartilage. However, only 2 to 10% of SVF are MSCs which may explain why the treatment was somehow unsuccessful.
Keywords: Adipose Tissue, Knee Osteoarthritis, Lipoaspiration, Magnetic Resonance Imaging, Mesenchymal Stromal Cells, Stromal Vascular Fraction

Ps-233: Lithium Enhances Osteogenic Differentiation of Mesenchymal Stem Cells Cultured on the Ion Exchange Nano-Scaffold

Faeze Moradi[1], Bahman Zeynali[2]*, Ehsan Seyedjafari[3], Iman Shabani[4], Azita Parvane Tafreshi[5]

1. Developmental Biology Lab., School of Biology, College of Science, University of Tehran, Iran
2. Department of Biotechnology, College of Science, University of Tehran, Iran
3. Faculty of Medical Engineering, Amirkabir University of Technology, Tehran, Iran
4. Amirkabir University of Technology, Tehran, Iran

5. Department of Molecular Medicine, Faculty of Medical Biotechnology, The National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: fam_1368@yahoo.com

Background and Aim: Nowadays engineered tissue for regenerative medicine provide new approach to develop personalized medicine. In this study, we used new scaffold for investigating the effect of lithium, as a Wnt signaling activator, on the osteogenic differentiation of adipose tissue-derived mesenchymal stem cells.
Methods: Scaffolds was prepared according to a method described previously include poly (ether sulfone) (PES) and sulfonated PES (SPES) nanofibers and lithium sulfonated PES (LiSPES). Mesecchymal stem cells were cultured on the scaffolds for 7 days after which cell viability was measured via MTT assay and osteogenic differentiation was examined using alkaline phosphatase activity kit, calcium content assay and Alizarin Red Staining. Morphology of attached cells was observed using SEM. Li+ concentration in cell culture media was determined by inductively coupled plasma–atomic emission spectroscopy.
Results: Our results showed that cell proliferation was significantly increased in the stem cells cultured on Li-SPES nanofibers. Moreover, osteogenic differentiation was increased in the cells cultured on Li-SPES scaffolds compared to those cultured on PES and SPES nanofibers.
Conclusion: Taken together, it was demonstrated that the incorporation of Lithium ions into the ion-exchange nanofibrous scaffolds not only improves chemical and physical characteristic of the scaffold but also enhances osteogenic differentiation of the stem cells. These findings suggest releasing of Li+ from LiSPES may accelerate bone regeneration.
Keywords: Tissue Engineering Scaffolds Stem Cell Regenerative Medicin Personalized Medicin

Ps-234: MDM2 Gene is Regulated by Hsa-Mir-140 in Glioblastoma Cells

Atieh Moradi Motlagh[1], Ehsan Arefian[1*], Fatemeh Jamshidi Adegani[2], Rezvan Rezazadeh[2], Fatemeh Gheida[ri]2

1. Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran
2. Department of Molecular Biology and Genetic Engineering, Stem Cell Technology Research Center, Tehran, Iran
Email: Arefian@ut.ac.ir

Background and Aim: Glioblastoma multiforme (GBM) is the most malignant and aggressive type of brain tumor with an average life expectancy of fewer than 15 months. This is mostly due to the highly mutated genome of GBM, which is characterized by the deregulation of many key signaling pathways involving growth, proliferation, survival, and apoptosis. It is critical to explore novel diagnostic and therapeutic strategies that target these pathways to improve the treatment of malignant glioma in the future. Today, treatment of GBM is primarily through tumor resection and subsequent radio- and chemotherapy, typically alkylating agents, e.g., temozolomide. Molecular phenotyping of GBM is opening up the potential for molecularly targeted therapies. These can take the form of targeting specific components of oncogenic pathways, e.g., through the delivery of a therapeutic gene or microRNA (miRNA). Several miRNAs are differentially expressed in a variety of malignancies compared to corresponding healthy tissue. Some of these miRNAs have been shown to modulate oncogenes and tumor suppressors, as is the case for GBM. Therefore, miRNAs could hold a great potential for the future treatment of this disease.
Methods: In this study, we selected MDM2 gene that is involved in GBM signaling pathways and is upregulated in comparison with normal brain tissues. Subsequently, we used some bioinformatics databases like TargetScan and miRWalk to look for some specific miRNAs, which are capable of targeting 3'UTR region of desirable gene. In other words, they would degrade the mRNA of upregulated gene and disrupt proper cancer cells' signaling. Finally, hsa-miR-140 was chosen as one of the best miRNA for our studies. hsa-miR-140 was cloned into a pLenti-III-eGFP Plasmid and then transfected to HEK-293T cell line via lipofection reagent. miRNA containing virus particles, which were shed into cell culture media, were collected and used to transduce U-251 Glioblastoma cell line. Relative Real-Time PCR Will be our approach to analyze the cells' mRNA expression and evaluate the effect of hsa-miR-140 transcription on treated cells.

Conclusion: We are going to do Real-Time PCR to investigate a correlation between miRNA and understudy gene and suggest the hsa-miR-140 as an efficient modulator that can be are going to use for alteration in GBM signaling. Moreover, we used mesenchymal stem cells as a delivery vehicle because of their high ability of infiltration into blood-brain barrier and consequently more efficient gene delivery. In this study we use bioinformatics databases to find a definite a miRNA as a promising and novel tool for GBM treatment. All in all, further studies should be conducted to achieve a highly efficient treatment that diminishes consequently tumor recurrence.
Keywords: MicroRNAs, Glioblastoma, Gene Transfer Techniques, hsa-miR-140, MDM2

Ps-235: Inhibition of TGF-B Signaling Pathway Enhances the Osteogenic Differentiation of Unrestricted Somatic Stem Cells in the Initial Phase

Nahid Moradi*, Bahman Zeynali

University of Tehran, Tehran, Iran
Email: n.moradi@alumni.ut.ac.ir

Background and Aim: In recent years, extensive studies have been performed to enhance the stem cell based therapies for bone and cartilage repair. Among various sources of stem cells, the unrestricted somatic stem cells (USSCs) appear to be a more appropriate option. TGF-β is considered as an important pathway in bone formation which regulates the proliferation and differentiation of osteocytes. However, the signaling network controlling differentiation of USSCs is not well known. The purpose of this study is to investigate the role of TGF-β signaling pathway in osteogenic differentiation of USSCs.
Methods: For begining Cellular isolation was performed according to the Kogler Method 2010 and flow cytometry test was performed for cell surface markers. To address the question, the effects of TGF-β1 antagonist (SB 431542) on osteogenic differentiation of USSCs were investigated during a 21 day period. Using RT-PCR analysis and bone matrix measurement by Alizarin red for staining the osteoblasts.
Results: In this study we also evaluated the markers which corresponded to those reported by Kogler and colleagues indicating that our protocol of extraction was reliable.These antigens were mainly CD45, CD90, CD19, CD44, CD73 CD105 and CD133. Our results showed that unlike blood cells, USSCs do not express CD45, CD19 and CD133, however they express mesenchymal stem cell markers such as CD90, CD44, CD73, CD105. Also our results imply that by adding TGF-β1 antagonist (SB), the endogenous TGF-β activity is inhibited leading to a significant increase in osteogenic differentiation of USSCs.
Conclusion: We found that the inhibition of TGF-β1 in both primary and secondary phases of osteogenic differentiation, increased differentiation, although the increase in the initial phase is more than that of the secondary phase.
Keywords: TGF-β Signaling Pathway, Osteogenic Differentiation, USSC

Ps-236: Cancer Stem Cells Therapy Based on Nano Biotechnology and Targeted Drug Delivery, Anionic Amphiphilic Polymer

Puria Motamed Fath*, Bahman Ebrahimi Hosseinzadeh, Razi Sahraeian, Ashrafalsadat Hatamian

Department of New Sciences and Technologies, University of Tehran, Tehran, Iran
Email: puria.motamed@yahoo.com

Background and Aim: To overcome difficulty of cancer treatment by conventional chemotherapy methods, the targeting drug delivery based on Nano carriers has been developed, rapidly. PMBN, as an amphiphilic Nano polymer which could be solubilize hydrophobic agents, is so interested, nowadays. The most important property of the PMBN polymer, amphiphilic ter-polymer poly [2-methacryloyloxyethyl phosphorylcholine (MPC)-co-n-butyl methacrylate (BMA)-co-p-nitrophenyloxycarbonyl poly (ethylene glycol-methacrylate) (MEONP)], is solubility in water. The MEONP monomer is responsible for conjugation of the polymer to biomolecules with free –NH2 groups, due to the active ester group of the monomer. This ability makes the polymer a suitable carrier to use for targeting in drug delivery systems and nano-biosensors. In addition, this

amphiphilic polymer could directly penetrate the cell membrane of cells of interest without any toxicity. So, these polymer was selected to target Leukemia Stem Cells (LSCs) cancer to be able solve difficulties of treatment of this cancer by common methods.
Methods: For targeting LSCs overexpressing CD123 receptors, Interleukin 3 (IL-3) ligand was conjugated to the polymer, and Ganoderic Acid derivative A (GA-A) as a fungal metabolite possessing acceptable anti-cancer properties, was incorporated to the polymer. The zeta potential and size of designed system were measured, and finally toxicity of Nano system was analyzed, and compared by free agent
Results: IL-3 was conjugated to the polymer by a simple Amide Bond by active easter group of MEONP monomer of polymer. Meantime, the BMA site of polymer make hydrophobic bond by any hydrophobic drug or agents like GA-A. The efficiency of conjugation and loading were selected 72.1% and 77.5%, respectively. Analysis of conjugated polymer, indicates that it is in a rod shaped with the size about 160±30 nm and zeta potential of -43.8 mv.
Conclusion: These data demonstrate that PMBN-IL3-GA is an anionic Nanostructure material with acceptable size and structure which could be a proper remedy for cancer treatment. By cytotoxicity analysis of Nanostructure material, the great enhancement of anti-proliferation was achieved. Although, the toxicty of polymer alone was analysed and demonestrated that PMBN is biocompatible without any toxicity.
Keywords: Cancer Stem Cells, Nanobiotechnology, DDS, Anionic Nanostructure

Ps-237: The Relationship between Micro RNA 661 Secreted by the Embryonic Stem Cells, Embryos ICSI Fertilization Rates and Embryo Implantation

Bahram Farsani Eslami[1*], Masoud Hemadi[2], Qasem Saki[2], Elham Mozafari Chegeni[1]

1. Medical University of Ahvaz, Ahvaz, Iran
2. Department of Anatomy Medical University of Ahvaz, Ahvaz, Iran
Email: elham.mozafare@gmail.com

Background and Aim: Infertility as a disease, and mental problems big on family life and society affect family health. Despite much research and development in recent years in the field of assisted reproductive techniques have been successful, but fertility and embryo implantation rates have hardly increased. Studies show that biomarkers secretory microRNA levels is already the implantation of stem cells trophoblast embryonic medium to produce embryos that this microRNA an important role in the process of Successful implantation of the embryo or rather the lack of embryo implantation play on accordingly, this study aimed to investigate the relationship between biomarkers medium ICSI embryos in embryonic tissue and the implantation was successful.
Methods: 50 Examples include the medium used embryos 72 and 96 hours after ICSI is. 661 microRNA expression in embryo culture techniques by Real-time PCR after extraction and manufacture microRNA complementary strands of DNA or cDNA by the enzyme reverse transcriptase were evaluated.
Results: High-quality communication between the embryo and the secretion of microRNA exists between low expression of this microRNA but statistically significant differences can be seen by fertility rate.
Conclusion: It turns out the embryonic tissue and secretion of miR secreted in the culture medium, as well as the implantation or reject fetal cells, endometrial there, so that you can miR as a non-invasive marker for selecting an embryo suitable for implantation chance to succeed and ultimately increase the fertility rate.
Keywords: Implantation, Human Blastocyst, Micro RNA, ICSI

Ps-238: Antioxidant Effects of Microbial Carotenoids on Primary Skin Fibroblast Cells

Negar Mozahheb[1*], Mohammadali Amoozegar[2], Ehsan Arefian[1]

1. Department of Molecular Biology and Genetic Engineering, Stem Cell Technology Research Center, Tehran, Iran
2. Department of Microbiology, School of Biology,College of Science, University of Tehran, Tehran, Iran
Email: negar_mozahheb@yahoo.com

Background and Aim: In several organs Reactive Oxygen Radicals (ROS) are continuously produce and clear. An imbalance in this pathway is called Oxidative Stress.That is associated with several degerative damages in human such as cancer, heart disease, and cerebrovascular disease through multiple mechanisms. Carotenoids are ubiquitous natural antioxidant and found in high concentrations in plants, algae and microorganisms. Humans and other animals cannot synthesize them and therefore are required to source them in the external ways.So as the skin have more probability for exposure to oxidant agents, it is necessary to protect this organ against oxidative damages. For example this pigments are important components of skin's cosmetic and therapeutic agents to reduce oxidative stress that can be happen in this organ's cell caused by exogenous and endogenous complications. In this paper our aim is to evaluate antioxidant properties of our microbial carotenoids on skin fibroblast cells that treated with hydrogenperoxide in sublethal doses.
Methods: Microbial carotenoids were extracted from two bacterial and archaeal strains (Salinibacter ruber, Halovenus aranensis) by mechanical shock and use of organic solvents. Several concentration(50,25,12,6,4,2,1 microgram per milliliter) of this carotenoids were prepared, using methanol as final solvent and affected on cell to determine nontoxic concentrations of carotenoids with solvent against skin fibroblast cells and viability of cell under the effect of carotenoids were determined by MTT method. Then sublethal doses of hydogenperoxide (300 micro Molar) in the presence of different concentration of carotenoids affected on cell to find out more effective concentration of the better microbial carotenoids by determining cell viability through the MTT method.
Results: It was observed that 4 microgram per milliliter of H. aranensis and 2 microgram per milliliter of S.ruber carotenoids have no toxic effects against intended cells. Under the effect of the higher sublethal concentration of hydrogenperoxide, the cell's viability is about 20%, in comparison with control. However in the presence of maximum nontoxic concentration of carotenoids (4 and 2 microgram per milliliter for H. aranensis and S.ruber) the viability of cells under the effect of hydrogenperoxide is dramatically increased. The viabiy of cells under effects of H. aranensis carotenoid in about 78% that have about four fold enhancement compared with control cells treated with hydrogenperoxide and in the case of S.ruber cells viability reached to 55% have about 2.5 fold increase compared with control cells with hydrogenperoxide.
Conclusion: It seems that our microbial carotenoids have good antioxidant effects. This effects can caused by different mechanisms such as free radical scavenging, activation of cell antioxidant effects pathways etc. Prior to this several experiments are performed to find more effective antioxidant agents by different approachs.
Keywords: Oxidative Stress, Antioxidants, Carotenoid

Ps-239: The Study of NT3 Gene Expression Changes in Rat Sciatic Nerve under Lavender (Lavandula Officianalis) Extract

Fereshteh Naderiallaf[1], Maryam Tehranipour[2*], Khadijeh Nejadshahrokhabadi[3]

1. Cell and Developmental Biology, Biology Department, Mashhad Branch, Islamic Azad University, Mashhad, Iran
2. Physiology, Biology Department, Mashhad Branch, Islamic Azad University, Mashhad, Iran
3. Molecular Genetics, Biology Department, Mashhad Branch, Islamic Azad University, Mashhad, Iran
Email: naderifereshteh@gmail.com

Background and Aim: During an individual's life, peripheral nerves are vulnerable. Therefore, many studies have been done on prevention and treatment procedures including surgery, use the active ingredients in regeneration or special exercise programs. Retrograde transport to the cell bodies of alpha motoneurons after the nerve lesion causes spinal degeneration. The neurotrophic factor (NT3) increases the number of myelinated axons in the dorsal root during the recovery process which is necessary for corticothalamic connections. It also leads to both differentiation and survival of sensory neurons, parasympathetic neurons and motoneurons and prevents cell death. Lavender is a plant in the family Lamiaceae. While some researches have reported antioxidant, antispasmodic, diuretic, anti-asthmatic, and antipyretic effects of the plant, therefore, this study examined NT3 gene expression changes on several days after sciatic nerve compression in rats, in the presence of Lavandula officinalis extract.

Methods: Lavender Soxhlet hydroalcoholic extraction was prepared. In this study, 36 male Wistar rats (200-250 g, 3 months old) were provided, then randomly divided into 3 groups include control, compression and treatment (Compression group + hydroalcoholic extract of Lavender injections 75mg/kg) groups. In compression and treatment groups samples were taken 4 times. In controls the muscles was opened without damage to gain access to the sciatic nerve without damaging. In compression and treatment group the sciatic nerve was compressed for 60 seconds. Two occasions of the extract was injected intraperitoneally on the days 1 and 8. A biopsy was taken from the spinal cord segments L4-L6 on days 1, 7, 14, 28 after that total RNA was extracted, cDNA was synthesized and NT3 gene expression changes were studied in control, compression and treatment groups.
Results: The results showed that NT3 gene expression had a significant reduction in compression group compared to the control group. The NT3 gene expression had a significant increase in treated group (Compression group + hydroalcoholic extract 75mg/kg) compared with the compression group.
Conclusion: A significant increase in gene expression shows that Lavandula officinalis hydroalcoholic extract that has Antioxidant and anti-inflammatory properties can improve nerve regeneration via NT3 gene expression.
Keywords: NT3 gene, Sciatic Nerve Regeneration, Compression, Lavandula Officinalis

Ps-240: Exosomes Secreted by Hypoxic Cardiosphere-derived Cells Enhance Tube Formation and Increase Pro-angiogenic miRNAs

Helia Namazi[1], Sareh Rajabi Zeleti[2], Iman Namazi[3], Azam Samadian[2], Ensiyeh Hajizadeh-Saffar[2], Elham Mohit[1*], Nasser Aghdami[2*]

1. Departmen of Pharmaceutical Biotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
3. School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Email: helia.namazi2017@gmail.com

Background and Aim: Cardiosphere-derived cells (CDCs) have been shown to promote cardiac regeneration of the infarcted human heart. The delivery of exosomes as the beneficial paracrine signals generated by CDCs and other stem cells could overcome obstacles associated with cell injection-based approaches in order to repair damaged myocardium. Few studies were shown exosomes secreted by CDCs replicate the cardioprotective and regenerative effects of CDCs such as enhancing tube formation and in vivo pro-angiogenic effects in animal MI models. However, in all of these studies, CDCs exosomes were generated under normoxic conditions, which likely did not reflect the state of post-infarct tissue. Importantly, hypoxic preconditioning enhanced the benefit of cardiac progenitor cells and CPC-exosomes therapy in an animal MI model and increased pro-angiogenic effect of exosomes.
Methods: We first determined sub lethal hypoxia time for treatment of CDCs by culturing the cells in hypoxia condition (1% O2) at different time points. To assess the hypoxia induction in CDCs, Cells were immunostained with HIF-1α. We determined proliferation potential of CDCs after 48 h hypoxia precondition in comparison with normoxic condition. Next to that, CDC-exosomes were isolated from cell conditioned media after 48 h using differential ultracentrifugation. The protein content of the exosome suspension was analyzed by BCA Protein Assay kit. Exosome's size was determined by Dynamic light scattering (DLS). Exosomal markers was analyzed by flowcytometry. Uptake of exosome by human embryonic stem cell derived cardiomyocyte (hESCs) was assessed by labeling of exosomes with PKH26. Morphological characteristics of exosomes was observed by scanning electron microscopy (SEM). In vitro tube formation assay was performed using in vitro Angiogenesis Assay Kit. We quantified the expression level of miR-126, miR-130a, miR-132, miR-210 that was previously reported to be overexpressed in hypoxia and have showed cardio protective and pro-angiogenic effect.
Results: The cells viability analysis showed that 48 h, is appropriate time for hypoxia preconditioning. HIF-1a was detectable in the nucleus of hypoxia cultured CDCs at 48 h. Our data indicated no difference in the rate of proliferation between the two conditions. The

size of exosomes from hypoxic CDCs was almost similar to those of normoxic CDCs, with mean hydrodynamic diameter from 140 to 180 nm. CDCs derived exosomes possessed highly positive expression for exosome surface markers, such as CD63 and CD81. We also demonstrated the uptake of PKH26-labeled CDC-exosomes to hESC-CMs. Hypoxic exosomes secreted by human CDCs that were cultured 48 h under moderate hypoxic conditions (1% O2) increased tube formation in comparison with normoxic exosomes and the promoting activities presented at a concentration of 25 µg/ml were comparable to those induced by VEGF. Our results indicated that hypoxic exosomes promoted in vitro angiogenesis. Our finding showed that hypoxia condition significantly increased the levels of pro-angiogenic miR-126, miR-130a and miR-210 in both exosomes and CDCs.
Conclusion: Hypoxic CDCs could release exosomes that enhanced tube formation in HUVEC and containing distinct enriched pro-angiogenic miRNAs. Hypoxic exosomes contained higher levels of miR-210, miR-130a and miR-126, indicating a qualitative difference between normoxic and hypoxic exosomes and induce angiogenesis in vitro.
Keywords: Cardiosphere-Derived Cells, Exosomes, Angiogenesis, MicroRNA

Ps-241: Exosomes of Bone Marrow Derived-Mesenchymal Stem Cell as Nanocarriers for Getting Rid of Breast Cancer Stem Cells

Zahra Naseri[1*], Mahdi Forozandeh Moghadam[1], Mahmoudreza Jaafari[2]

1. Department of Medical Biotechnology, School Of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. School of pharmacy, Pharmaceutics and Pharmaceutical Nanotechnology Canter, Mashhad University of Medical Sciences, Mashhad, Iran
Email: naseryzahra@yahoo.com

Background and Aim: Exosomes are small endosomally derived vesicles that are secreted by a variety of cell types and tissues. There is increasing evidence that therapeutic effects of mesenchymal stem cells (MSCs) is mediated through exosomes, membrane enclosed vesicles (30-150 nm) containing proteins, mRNA and miRNA which can be delivered to nearby cells. The intercellular communication role of exosomes generated a lot of interest in the scientific community as exosomes could be used as natural drug delivery vehicles if they could be loaded with the desired therapeutic molecules. Cancer stem cells (CSCs) have been suggested as both the seeds of the primary cancer and the roots of chemo- and radio-therapy resistance. The ability to precisely deliver drugs to target CSCs is an urgent need for cancer therapy, with nanotechnology-based drug delivery system being one of the most promising tools to achieve this in the clinic. Exosomes are cell-derived natural nanovesicles that are widely distributed in body fluids and involved in multiple disease processes, including tumorigenesis. Exosome-based nanometric vehicles have a number of advantages: they are non-toxic, non-immunogenic, and can be engineered to have robust delivery capacity and targeting specificity. The present study aimed to isolate exosomes from bone marrow-derived MSC (BMSC) and test them to deliver a given molecule to cancer stem cells.
Methods: MSCs was derived from bone marrow of 8 weeks mice. Exosomes was isolated from cell supernatants using Exospin kit. Exosomes was characterized by western blotting, TEM and DLS. Breast cancer stem cells were derived as mammospheres from MCF-7 cell line. PKH67-labeled exosomes cocultured with breast cancer stem cells. Labeled exosomes was injected to tumor bearing mice and 24 hours after injection vital organs were fixed by cryosection method and uptake of exosomes was analyzed using fluorescence microscopy.
Results: The average exosome yield was 200 µg from 100 ml of mesenchymal stem cells supernatant. Breast cancer stem cells was observed as mammospheres using 3D imaging systems. 3 hours after coculturing, the PKH67-labeled exosomes was observed within cytoplasm of cancer stem cells and the uptake of exosomes was increased during 24 hours. Labeled exosomes was observed in tissue sections, including lung, liver, spleen and tumor tissue.
Conclusion: according to these data, MSCs are prolific producers of exosomes. They can produce high amounts of exosomes, we hypothesize that exosomes derived from MSCs could be used as an ideal nanovehicles for delivering of desired drug molecules. We

showed that MSC-derived exosomes can uptake by cancer stem cells, therefore the exosomes can be used as a controllable, manageable, and feasible approach for targeting and elimination of cancer stem cells as initiating tumor cells.
Keywords: Exosomes, Mesenchymal Stem Cells, Breast Cancer Stem Cells, Nanovesicles

Ps-242: Improving Heart Failure by Magnetized Stem Cells and External Magnet

Maryam Naseroleslami[1], Nahid Aboutaleb[2*]

1. Department of Biology, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran
2. Physiology Research Center, Iran University of Medical Sciences, Tehran, Iran
Email: naseroleslami@gmail.com

Background and Aim: Cell therapy is one of the best ways suggested to treat heart failure .the migration of stem cells after transplantation reduces their therapeutic effects .The aim of this study was to increase implantation of stem cells within the cell using iron nanoparticles utilizes a magnetic field around heart.
Methods: For this purpose, after confirmatory tests on nanoparticle entry into the cells and MTT tests to find non-toxic dosages, cells labelled with Iron oxide nanoparticles were injected into the myocardium of the rats with heart failure in both magnet-dependent and magnet-independent groups. Then the MRI was used to monitor the injected cells. After echocardiography, the iron content in various tissues was measured by ICP after a week and a month.
Results: The results indicated that the blue colour of the cytoplasm attested to the cells' ability to uptake nanoparticles without any intervention, and that there was a significant increase in ejection fraction parameters (84.6 ± 1.6) and fraction shortening (48.2 ± 1.9), as shown by echocardiography, in comparison with the heart failure group. ICP results also indicated more cell implantation in the heart (1.60 ± 0.067) ($P < 0.05$).
Conclusion: According to the findings, magnetic nanoparticles, in presence of the electromagnetic field, can be used to direct the cells to the parts of the body which require repairing or regenerating.

Keywords: Heart Failure, Super-Paramagnetic Nanoparticles, Cell Labelling, Magnetic Resonance Imaging

Ps-243: Rhesus Monkey Adipose Derived Mesenchymal Stem Cell Large Scale Expansion Using Corning Synthemax II Microcarriers in Spinner Flask

Roghayeh Navabi[1], Ensiyeh Hajizadeh-Saffar[2*]

1. Department of Medical Biotechnology, Tehran University of Medical Science, Tehran, Iran
2. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: n_rose49@yahoo.com

Background and Aim: Mesenchymal stem cells (MSCs) have recently made significant progress with multiple clinical trials targeting modulation of immune responses and tissues regeneration. The current 2-dimensional culture systems can be used only when low cell doses are needed. So, various 3-dimensional culture systems have been developed to solve this limitation. One of the best method, is microcarrier based culture systems that provide much higher surface to volume ratio for attachment and proliferation of adherent dependent cells such as mesenchymal stem cells. Here we report the expansion of Rhesus monkey adipose derived mesenchymal stem cells (rhe-AD-MSCs) on Corning Synthemax II microcarriers in spinner flask.
Methods: Abdominal adipose tissue sample was isolated from a healthy Rhesus monkey by surgery. After transferring of this tissue sample to laboratory, we isolated and cultured Mesenchymal Stem Cells in current 2-D culture flasks using Collagenase Type I digestion protocol. Also for prevention of cell attachmentto the glass spinner flask, its surface was coated by sigmacoat. 1.388 g Corning Synthemax II microcarriers (1g = 360 cm2) were weighed under laminar hood for providing 500 cm2 surface. For activating microcarrires, 30 ml complete medium (MEM-α & glutamax + 15% FBS) was added and agitated on stage for 4 hours at 37 °C. After 4 hours, Cells at passage 2 were seeded directly onto microcarriers at 1000 cells per cm2 and rested 24 hours in 37 °C without any agitation for rhe-AD-MSC attachment. Medium volume was adjusted to 50 mL.

After 24 hours, cultures were agitated at 40 rpm in 37 °C. On day 3, 50 ml of the culture medium was replenished. After that, every 3 days, 25 ml fresh medium was added to spinner flask. On day 12, cells were isolated from microcarriers by Accutase enzyme and counted. Expression of rhe-AD-MSCs markers (CD73, CD90, and CD105; CD34 and CD45) was assessed by flow cytometry. Also their differentiation potential to adipocytes and osteocyteswere checked.
Results: rhe-AD-MSCs attached to Corning synthemax II carriers and Cell numbers were increased 11 fold After 12 days. Rhe-AD-MSCs after microcarrier culture condition shows normal immunophenotype by flowcytometery. Rhe-AD-MSCs after microcarrier culture expansion shows the differentiation potential to adipocytes and osteocytes.
Conclusion: Under these microcarrier culture condition, rhe-AD-MSCs expansion increased 11 fold in 12 days. Rhe-AD-MSCs culture on Corning Synthemax II microcarriers in spinner flask maintained normal phenotypic marker expression profile and two-lineage differentiation potential.
Keywords: Mesenchymal Stem Cell, Large Scale, Microcarrier

Ps-244: Effect of Transforming Growth Factor- β1 (TGF-β1) on Bovine Nasal Cartilage against the Tissue Degradation Induced by Interleukin1α (IL-1α) in Explant Culture

Vajihe Nayebzade[1*], Mariam Yadegari[2], Morteza Anvari[2]

1. Faculty of Medicine, Shahid Sadoughi University of Medical Sciences and Health Service, Yazd, Iran
2. Shahid Sadoughi University of Medical Sciences and Health Service, Yazd, Iran
Email: vajihenayebzade16@gmail.com

Background and Aim: Osteoarthritis (OA) is the most common form of arthritis, involving cartilage, synovium and bone. An imbalance between the activities of these anabolic and catabolic factors leads to cartilage degradation resulting in OA. The aim of this study to investigate role of growth factors TGF-b1 in Repair of Bovine Nasal culture (BNC) Suffering OA and phenotype of chondrocyte. Spacial effect of TGF-b1 on cartilage chondrocyte morphology for collagen and proteoglycan production.
Methods: BNC explants were cultured in DMEM with IL-lα (10 ng/ml), TGF-β1(10 ng/ml) and culture for 14 days. Investigate At days 3, 7 and 14 the media in absent and present TGF-β1. We used Masson's Trichrome stain to visualize collagen distribution and synthesis, and Safranin O and Alician blue to highlight the proteoglycan content. The viability and number of chondrocyte was evaluated by trypan blue staining.
Results: stimulation of BNC with IL-lα show model of cartilage detraction like OA cartilage. In most chondrocytes were transformed into fibroblast-like morphology with pyknotic nuclei at day 14 and proteoglycan and collagen in extracellular matrix (ECM) was destructed. The data of the current study show that TGF-b1 could preserve cartilage from apoptotic and degenerating of chondrocyte morphology and collagen, proteoglycan in BNC suffering oA.
Conclusion: we suggest that TGF-β1 is an mportant factor for chondrocyte morphology and this way controlled ECM production.we show that A parallel relationship between chondrocyte morphology and collagen and proteoglycan production. TGF-β1 is good choice for treatment of OA.
Keywords: Chondrocytes, Interleukin-1 (IL-1), Transforming Growth Factor-β (TGF-β), Bovine

Ps-245: Ginger Anti-Inflammatory Effect in Mesenchymal Stem Cells

Afsaneh Nazari[1], Zeinab Sahraian[*2], Sanaz Mahmazi[3]

1. Department of Genetics, Faculty of Basic Sciences, Zanjan Branch, Islamic Azad University, Zanjan, Iran
2. Department of biology, Biology Research Center, Zanjan Branch, Islamic Azad University, Zanjan, Iran
3. Department of Genetics, Biology Research Center, Zanjan Branch, Islamic Azad University, Zanjan, Iran
Email: afsaneh.nazari_dr1@ yahoo.com

Background and Aim: Ginger (Zingiber officinale) belongs to the Zingiberaceae family used for the treatment of numerous ailments such as colds, nausea, arthritis, migraines and hypertension. It has anti-inflammatory and anti-oxidative properties. In inflammatory diseases increased inflammatory cytokines such as Interleukin-6

expression could be lead to additional side effects then reduction or inhibition of some inflammatory cytokines could be effective in treatment of some inflammatory diseases. IL-6 is one of important cytokines expressed in MSCs. These cells able to differentiation into Mesenchymal and nonmesenchymal cells including osteoblasts, chondrocytes, adipocytes ،muscle cells, tendon، nerve, stromal cells and myoblasts. The effects of ginger hydro alcoholic extract on the expression of IL-6 gene in rat bone marrow-derived mesenchyme stem cells were studied.
Methods: Mesenchymal stem cells aspirate from rat and cultured. Cells treated in 7 groups by 2 doses 50 and 100 µgml-1 in 3 times 2, 16 and 24 hour. After culture and treatments RNA was extracted and cDNA synthesized and IL6 gene expression level evaluated by real-time PCR. Gene expression level results were compered by 2-ΔΔCt and statistically analyzed by T-test.
Results: treatment group in dose 50 µgml-1 ginger hydro alcoholic extract after 2h significantly decreased IL6 expression level. Expression level changes after 16 and 24h were not significant but expression reduction in 100 µgml-1 treatment seen after 16 h.
Conclusion: IL-6 is a pleiotropic inflammatory cytokine that could induce transcriptional inflammatory response with wide range of biological activated in immune regulation، hematopoiesis، oncogenesis and inflammation. The function of IL-6 gene is implicated in wide range of inflammation-associated diseases states. We investigated that ginger extract reduced IL6 expression level in MSCs dose and time dependent. MSCs are one of important cells that used in cell therapy and medicine and we can use them in inflammation disease such as rheumatoid arthritis. IL-6 has important role in many diseases. It is produced by various types of cell and has multiple biological activities through its receptors system. In some autoimmune diseases declined IL6 mRNA expression level might be effective in inflammation symptoms reduction.
Keywords: Ginger, Interleukin-6, Mesenchymal Stem Cells

Ps-246: Mesenchymal Stromal Cell Conditioned Media Attenuates Intracellular Signaling Pathway Actuation and Il-1β Expression

Vida Nazemian[1*], Jalal Zaringhalam[2], Ali Mohammad Sharifi[3], Homa Manaheji[2]

1. Neurophysiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Physiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Tissue Engineering Group, Department of Orthopedic Surgery, Faculty of Medicine, University of Malaya, Kualalampur, Malaysia
Email: vida.nazemian@gmail.com

Background and Aim: Rheumatoid arthritis (RA) is an autoimmune and chronic inflammatory disease which is caused by direct stimulation of nociceptors through release of many inflammatory mediators. Despite the unknown etiology of RA, it seems that pro-inflammatory cytokines such as IL-1β elevated during the disease which has been implicated to play a noticeable role in RA pathogenesis and can cause inflammatory symptoms such as hyperalgesia and edema. Activation of intracellular signaling pathway markers have a considerable role in the development of inflammatory symptoms, in particular, in chronic phase of RA. Mesenchymal stromal cells conditioned media (MSC-CM) has anti-inflammatory factors which can adjust the immune responses without any downside on patient's health. The purpose of this study was to investigate the effect of administration of MSC-CM on serum IL-1β level and spinal NF-κB activity during acute and chronic phases of adjuvant-induced arthritis in male Wistar rats.
Methods: Complete Freund's adjuvant (CFA)-induced arthritis (AA) was caused by single subcutaneous injection of CFA into the rats' hind paw on day 0. MSC-CM was administered daily (i.p.) after CFA injection. Serum levels of IL-1β and NF-κB activity were assessed on days 0,7,14 and 21 of the study.
Results: The results of this study indicated the role of MSC-CM in declining serum levels of IL-1β and activity of NF-κB during acute (first week) and chronic (next 2 weeks) phases of RA caused by CFA adjuvant.
Conclusion: It seems that MSC-CM administration due to its direct effects on inhibition of intracellular signaling pathways and pro-inflammatory cytokines can alleviate inflammatory symptoms and pain during different phases of RA.

Keywords: Rheumatoid Arthritis, MSC-CM, IL-1β, NF-κB

Ps-247: Development of a Novel Bioactive Polymeric Scaffold for Proliferation and Stemness Maintenance of Human Adipose-derived Stem Cells: Possible Application in Regenerative Medicine

Kazem Nejati-Koshki[1], Yousef Mortazavi[1], Younes Pilehvar-Soltanahmadi[2], Effat Alizadeh[2], Nosratollah Zarghami[2*]

1. Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
2. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: nejatik@zums.ac.ir

Background and Aim: Bioactive nanofibrous scaffolds present new developments in biomedicine and recent advances in nanofiber technology reinforced their application in regenerative medicine and cell therapy. The best scaffolds in tissue engineering must have two important characteristics. One of them is the ability to stimulate stem cell proliferation and the other is stemness preservation of this cells. At the present study we developed novel composite scaffold based on polycaprolactone/collagen containing emu oil as a bioactive material to induce proliferation and stemness preservation of human Adipose-derived stem cells (ASCs).
Methods: Successful fabrication of emu oil-loaded PCL-Collagen (EO-loaded PCL/Coll) nanofibers by electrospinning technique and acetic acid, as a solvent, were characterized by FE-SEM, FTIR and tensile test. ASCs were seeded on two types NFs (PCL/collagen and EO-loaded PCL/Coll) and their proliferation, cell cycle progression and stemness gene expressions were evaluated using MTT test, propidium iodide staining and real-time quantitative polymerase chain reaction (qPCR) during 2 weeks.
Results: The results showed that ASCs exhibit better adhesion and proliferation on the emu oil loaded NFs scaffold compared to the other groups. The proliferation induction of EO-loaded PCL/Coll NFs was further confirmed by the results of cell cycle analysis. It was also found that emu oil (EO) loaded NFs significantly up-regulated the expressions of stemness markers Sox-2, Nanog, Oct4, Klf4 and c-Myc.
Conclusion: These results demonstrate that composite NFs containing bioactive materials can reinforce cell adhesion and stimulate stem cells proliferation while at the same time preserve the stemness property of ASCs, thus representing a promising method for potential application of MSCs in the field of cell therapy and regenerative medicine.
Keywords: Nanofiber, Regenerative Medicine, PCL, Collagen, Emu oil

Ps-248: Electrospinning of Polyurethane for Vascular Tissue Engineering: an Optimization Study by Using Taguchi's Methodology

Maryam Nezadi Niasar[1], Hamid Keshvari[2*], Maryam Yousefzadeh[3]

1. Department of Biomedical Engineering, (Tissue Engineering), Amirkabir University of Technology Tehran, Iran
2. Department of Biomedical Engineering (Biomaterials), Amirkabir University of Technology Tehran, Iran
3. Department of Textile Engineering, Amirkabir University of Technology Tehran, Iran
Email: Keshvari@aut.ac.irr

Background and Aim: Electrospinning of Polyurethane (PU) is a promising approach to produce scaffolds for tissue engineering of vascular grafts. Vascular grafts fabricated from PU have to match with the biomechanical properties of native vessels. This properties can be improve by producing nanofibers without beads. In this paper, the aim was finding optimal electrospinning settings in order to obtain fibers without beads by using Taguchi's orthogonal design. For this purpose, solvent concentration and process parameters (applied voltage, flow-rate, distance between the needle-tip and collector) were considered as the effective factors.
Methods: The fibers produced from electrospinning a solution of polyurethane in Dimethylformamide (DMF). Taguchi's mixed-level parameter design (L18) was employed for the experimental design and optimal electrospinning conditions were determined. Physical properties of the resulting scaffolds were evaluated by

SEM and Tensile mechanical testing was carried out on all electrospun scaffolds.
Results: Non-beaded fibers was yielded at 20 wt% polymer concentration, 14 kV of supply voltage, 0.1 ml/h feed rate and 15 cm tip-to-distance. Mechanical testing demonstrated that the Young's Modulus and ultimate tensile strength in optimal sample were 2.07 ±0.77 MPa and 4.54 ± 1.41 MPa respectively.
Conclusion: Experimental data were found as similar to the optimum conditions estimated, proving the applicability of Taguchi's method for electrospinning optimization. Mechanical properties of PU scaffolds can be suitable for vascular graft applications.
Keywords: Electrospinning, Scaffolds, Vascular Tissue Engineering, Polyurethane, Taguchi's Orthogonal Design, Optimization

Ps-249: Study the Effects of Administration of Mesenchymal Stem Cells Derived Exosomes on Improving Clinical and Pathological Symptoms and IL-17 And TGF-B Production in Mouse Model of Type I Diabetes

Shahrzad Nojehdehi[1], Ardeshir Hesampour[1*], Seyed Mahmoud Hashemi[2]

1. Department of Biology Central Tehran Branch, Islamic Azad University,Tehran, Iran
2. Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: sh.nojehdehi@gmail.com

Background and Aim: Background and objectives: Type I diabetes, is one of the most common autoimmune diseases. The autoreactive T cells activation causes destruction of beta cells. Continuous high blood sugar and metabolic disorders caused by diabetes causes secondary pathophysiologic changes in multiple organs of the body which creates a lot of trouble and expense for the person with diabetes and the healthcare community. The aim of this study was to evaluate treatment effectiveness of exosomes derived from mesenchymal stem cells in inhibition of T cell inflammatory response and reduction of clinical symptoms of the diabetic mouse.
Methods: First, by disease induction using STZ and based on disease symptoms, mice were separated into three groups: healthy group, control group and treatment group. The treatment group were treated by intraperitoneal injection (2 times per week) of exosomes extracted by centrifuge subtraction of mesenchymal stem cells derived from adipose tissue. Body weight and blood glucose of each mice were measured during 6 weeks. After 6 weeks, the mice were dissected and the pancreas were sent for pathological examination with H & E staining and also the spleen lymphocytes were examined in order to evaluate their alternation in cytokine profiles (TGF-β and IL-17) by ELISA. As well as to confirm the size and morphology of the exosomes electron microscopy (TEM and SEM) and dynamic light scattering (DLS) was used.
Results: The data showed, the mice that were not treated had a shorter life span and also exosomes derived from mesenchymal stem cells were able to reduce the symptoms by reducing the secretion of inflammatory cytokines IL-17 and increasing anti-inflammatory TGF-beta.
Conclusion: In this study, the therapeutic use of exosomes as a non-cellular therapy has a number of benefits that could properly explain the reason for replacing it with the common methods of cellular therapy.
Keywords: Diabetes, Mesencymal Stem Cells, Exosomes

Ps-250: Regulatory Effects of the Novel Synthesized Indole-3-Carbaldehyde on Expression of Cell Cycle Genes: A Study on Cyclin D and P21 Expression by Acute Promylocytic Leukemia Cell Line (NB4)

Mojgan Noroozi Karimabad, Mehdi Mahmoodi, Abdollah Jafarzadeh, Ali Darekordi, Mohammad Reza Hajizadeh, Gholamhossein Hassanshahi*

Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
Email: mojgan.noroozi@yahoo.com

Background and Aim: Aim and objectives: A large variety of heterocycles are known to date and among these, indole and pyran rings are of particular interest. The present investigation was aimed to investigate the effects of novel Indole-3-carbaldehyde derivative (NI-3-CD) analogue on growth inhibition of cells and its

regulatory effects on expression of cell cycle genes in acute promylocytic leukemia (APL) cell line.
Methods: NB4 cells were cultured in presence of RPMI1640 medium contained various concentrations of NI-3-CD and basal compound of indole (I3C) (15.12-1000 µg/mL) for 24, 48 and 72 hours. The inhibitory effects of compound on cellular proliferation were assessed by both trypan blue staining and MTT assay techniques. When was confirmed apoptosis the changes in expression of Cyclin D and P21 were determined by quantitative Real-Time PCR. Western blotting analysis was also applied for evaluating the expression of P21 at protein level. Differences were considered significant if p values less than 0.05.
Results: Our results showed a significant difference between various concentrations of NI-3-CD and I3C when cells were treated for 24, 48 and 72 h. Real Time-PCR analysis indicated that the expression of Cyclin D was down regulated while P21 upregulated in compare to untreated control cells and I3C treated cells($P<0.01$). In concert with RT-PCR, western blot analysis also showed that the P21 expression in NI-3-CD treated cells was significantly increased in compare to both untreated control cells and I3C treated cells.
Conclusion: According to these findings, the novel synthesized NI-3-CD analogue effects on the cell cycle arrest in APL cell line is possibly facilitated via modulating Cyclin D and P21 pathway mediators. NI-3-CD may introduce this compound as a promising therapeutic compound against APL.
Keywords: NI-3-CD, Cell Cycle, Cell Growth, APL, P21, Cyclin D, Antitumor

Ps-251: Evaluating Of OCT-4 and NANOG Was Differntially Regulated By a New Derivative Indole in Leukemia Cell Line

Mojgan Noroozi Karimabad*, Mehdi Mahmoodi, Abdollah Jafarzadeh, Ali Darekordi, Mohammad Reza Hajizadeh, Gholamhossein Hassanshahi

Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
Email: mojgan.noroozi@yahoo.com

Background and Aim: The potential exists to improve treatment through characterization of tumor stem cells and identification of therapeutic targets Using OCT-4 and NANOG genes. Here we have synthesized and investigated the potential of ; New Indole-3-carbaldehyde derivative (NI-3-CD) in inhibiting the expression of self-renewal regulatory factors and cancer stem cell gene in a leukemia cell line NB4.
Methods: The NB4 cells were cultured in RPMI1640 medium contained NI-3-CD and I3F (15.12-1000 µg/mL) for 24, 48 and 72 hours. Inhibition of cell proliferation was assessed by trypan blue staining technique and MTT assay. The percentage of apoptotic cells was determined by flow cytometry analysis using Annexin V/PI apoptosis detection kit. The fold changes of NANOG/OCT4 expression against β-actin were determined by real-time-PCR technique. Western blotting analysis was also applied for evaluating the expression of NANOG/OCT4 at protein level. Data were analyzed by student t and repeated measure tests. Differences were considered significant if ($P<0.01$).
Results: There was a significant difference in cell viability, when various concentrations of NI-3- were used for 24, 48 and 72 h in comparison to I3C regarding the cellular viability. Furthermore, the NI-3-CD, had markedly elevated anticancer activity than I3C (IC50 values for novel I3C in 24, 48 and 72h were 225.77, 123.13 and 63.72M respectively while for I3C were 728.05, 407.82 and 277.92M respectively). Flow cytometry results exhibited an obviously significant augmentation in apoptotic NB4 cells. Real Time- PCR analysis indicated that the expression of NANOG/OCT4 was down regulated in compare to untreated control cells and I3C treated cells($P<0.05$). In concert with RT-PCR, western blot analysis showed that the OCT4 expression in NI-3-CD treated cells was also significantly decreased in compare to both untreated control cells and I3C treated cellular populations.
Conclusion: Our results imply that NI-3-CD treatment decreases the sphere-forming ability of NB4 cells. In summary, this study provides valuable information on the presence of stem-cell genes expression in NB4 cells.
Keywords: NANOG, OCT4, Antitumor, Indole-3-Carbaldehyde, Treatment

Ps-252: Stimulatory Effects of the Novel Synthesized Indole-3-Formaldehyde on the Apoptosis of Leukemia Cell Line via Modulating Bax/Bcl-2 Ratio

Mojgan Noroozi Karimabad*, Mehdi Mahmoodi, Abdollah Jafarzadeh, Ali Darekordi, Mohammad Reza Hajizadeh, Gholamhossein Hassanshahi

Molecular Medicine Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
Email: mojgan.noroozi@yahoo.com

Background and Aim: Balancing between Bax and Bcl-2 plays critical roles in both proliferation and self-renewal activation of cancer cells. Indole-3-formaldehyde derivatives limit the growth and facilitate cell death in different cell systems. In this study, we introduced a novel indole derivative (2-AITFEI-3-F) with tendency to facilitate apoptosis in NB4 line in comparison to basal Indole-3-formaldehyde (I3F).
Methods: The NB4 cells were cultured in RPMI1640 medium contained 2-AITFEI-3-F and I3F (15.12-1000 µg/mL) for 24, 48 and 72 hours. Inhibition of cell proliferation was assessed by trypan blue staining technique and MTT assay. The fold changes of Bax/Bcl-2 expression against β-actin were determined by real-time-PCR technique. Western blotting analysis was also applied for evaluating the expression of Bax and Bcl2 at protein level. Data were analyzed by student t and repeated measure tests. Differences were considered significant if ($P<0.01$).
Results: There was a significant difference in cell viability, when various concentrations of 2-AITFEI-3-F (but similar to I3F) were used for 24, 48 and 72 h in comparison to I3F regarding the cellular viability ($P<0.05$). Real time- PCR and Western blotting analysis indicated that the gene and protein expression level of Bcl-2 down-regulated while Bax was up-regulated in compare to untreated control cells and cells treated with I3F ($P<0.01$).
Conclusion: According to these findings, the novel indole derivative 2-AITFEI-3-F probably triggered apoptosis of NB4 cells by modulating Bax/Bcl-2 ratio. Furthermore, the 2-AITFEI-3-F had markedly displayed anti-cancer activity than I3F.
Keywords: Indole-3-Formaldehyde, Apoptosis, NB4, Bax, Bcl-2

Ps-253: Comparison of Colony-Forming Efficiency between Breast milk of Mothers with Full-Term Delivery and Full-Term Delivery

Abolfazl Nosrati Tirkani[1], Nadia Boromand[1], Mohhammad Hassan Arjmand[1], Daryoush Hamidi Alamdari[2], Ahmadshah Farhat[3*]

1. Department of Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Biochemistry, Biochemistry and Nutrition Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Neonatal Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
Email: nosratia9@mums.ac.ir

Background and Aim: Stem cells have a great potency for self-renewal, proliferation and tissue regeneration. Various sources of stem cells have been developed for several therapeutic purposes, including adipose tissue, bone marrow, umbilical-cord blood and specifically breastmilk stem cells. A large body of evidence has shown that human breastmilk is a novel source of multipotent stem cells, forming during the period of pregnancy. Therefore, it can be hypothesized that mothers with preterm delivery (gestational time is 34±4 week), may not provide enough efficient stem cells in their breastmilk in compare with full-term infant mothers (gestational time is 40±2 week). Moreover, since breastmilk stem cells are thought to be involved in infant development, possible lack of efficient stem cells in preterm infants may have negative impact on their future health. In the current study, we compared the colony forming efficiency (CFE) of breastmilk-derived stem cells in preterm with full term infant mothers.
Methods: Breast milk samples obtained from neonatal research center, maternity ward, imam reza hospital. The 10CC fresh breastmilk mixed with equal amount of balance salt solution (BSS) and centrifuged in 1380 rpm for 20 minutes at clean room temperature (25°c). Then, the sediment cell pellet washed three times with BSS and cultured in 12-wells plate (Biofil). The media consist of agar-agar gel 0.8% in DMEM-F12 (Gibco). Moreover, the IMDM (Gibco), glutaMAX (Gibco), 12% FBS (), 1% antibiotic antimicotic chloral toxin A and necessary supplements add to the medium. The cells viability determined by trypan blue staining protocol, then 4×104 cells seeded per each well (1ml) and incubated at 37°c, 5% CO2, 80% humidity for 14 days.

To determine the colony forming efficiency, the colonies were counted using an inverted microscope.
Results: The cultured breastmilk-derived stem cells in preterm infant mothers presented higher colony numbers as compared with the cultured stem cells in full term infant mothers. Indeed, there are the significant relationship between the age of mothers and CFE ($P<0.05$).
Conclusion: We expected that stem cells colony numbers of breastmilk would be lower in the case of preterm birth due to the incomplete pregnancy period. Interestingly, oppose to our expectation, the results demonstrated increased numbers stem cells colony in preterm infant mothers' breast milk, which could be considered as a compensatory response in order to support the preterm infant development out of the mother's body.
Keywords: Breastmilk, Stem Cells, Preterm Delivery, Full Term Delivery

Ps-254: The Cryopreserved Technique for Preparation of Human Amniotic Membrane as an Appropriate Substrate for Limbal Epithelial Stem Cell Expansion

Abolfazl Nosrati Tirkani[1*], Nadia Boromand[2], Mohammad Jalili-Nik[2], Daryoush Hamidi Alamdari[3]

1. Department of Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Student Research Committee, Department of Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Biochemistry, Biochemistry and Nutrition Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: nosratia9@mums.ac.ir

Background and Aim: The Human Amniotic Membrane (HAM) also called amnion, is a transparent and avascular innermost layer of human placenta. HAM has been widely used for transplantation and reconstruction of damaged ocular surface. Accordingly, cultivation of adult stem cells locate in the limbus, the narrow zone between cornea and sclera named limbal epithelial stem cells on the denuded HAM is a new method for treatment of patients with limbal stem cell deficiency (LSCD). In order to localize presented procedure, production of denuded HAM was much-needed. Production of denuded HAM by non-enzymatic (biologic) procedure was the aim of current study.
Methods: The human placenta obtained from the HCV, HIV, and Syphilis seronegative cesarean part of the maternity ward of imam reza hospital. Then, the placenta washed three times in the phosphate buffer saline at the clean room to remove extra blood. Then, amnion transparent membrane separated from the placenta and chorion and washed three times with balance salt solution (BSS). The amniotic membrane sored at -80°c for one week in the cryopreservation media consist of equal volume glycerol and DMEM. After that, the amniotic membrane thawed at the clean room temperature and the epithelial layer removed and washed ten times at BSS and flatted on nitrocellulose sheet with appropriate amount of DMEM media and sored at -20°c while the denuded epithelial side was up.
Results: To confirm our decellularization procedure, paraffin block prepared. The histological results showed that there is no cells on the surface of HAM. Indeed, the HAM stained by trypan blue protocol after denuding procedure.
Conclusion: the results of the current study suggest that the cryopreserved Human amniotic membrane retains and support the proliferation of explant cultured limbal stem cells. Furthermore, the graft of cryopreserved Human amniotic membrane seems to be the appropriate biological matrix for reconstruction of other epithelialized surface.
Keywords: Human amniotic membrane, Limbal Stem Cell, Explant Culture, Cryopreservation

Ps-255: The Role of Morphine on Neuro-Angiogenesis and Neuro-Steroidgenesis Properties of Rat Neural Stem Cells

Nima Abdyazdani[1], Reza Rahbarghazi[2,3], Hojjatollah Nozad Charoudeh[2], Navid Feizy[1], Alireza Nourazarian[1*]

1. Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical sciences, Tabriz, Iran
2. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

3. Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: ali_nour65@hotmail.com

Background and Aim: A lack of comprehensive data exists on the effect of morphine on neural stem cell neuro-steroidogenesis and neuro-angiogenesis properties. We, herein, investigated the effects of morphine (100 M), naloxone (100 M) and their combination on rat neural stem cells viability.
Methods: clonogenicity and Ki-67 expression over a period of 72 h. Any alterations in the total fatty acids profile under treatment protocols were elucidated by direct transesterification method. We also monitored the expression of p53, aromatase and 5-alpha reductase by real-time PCR assay. To examine angiogenic capacity, in vitro tubulogenesis and the level of VE-cadherin transcript were investigated during neural to endothelial differentiation under the experimental procedure.
Results: Cells supplemented with morphine displayed reduced survival ($p < 0.01$) and clonogenicity ($p < 0.001$). Flow cytometric analysis showed a decrease in Ki-67 during 72 h. Naloxone potentially blunted morphineinduced all effects. The normal levels of fatty acids, including saturated and unsaturated were altered by naloxone and morphine supplements. Following 48 h, the up-regulation of p53, aromatase and 5-alpha reductase genes occurred in morphine-primed cells. Using three-dimensional culture models of angiogenesis and real time PCR assay, we showed morphine impaired the tubulogenesis properties of neural stem cells ($p < 0.001$) by the inhibition of trans-differentiation into vascular cells and led to decrease of in VE-cadherin expression.
Conclusion: morphine strongly impaired the healthy status of neural stem cells by inducing p53 and concurrent elevation of aromatase and 5-alpha reductase activities especially during early 48 h. Also, neural stem cells-being exposed to morphine lost their potency to elicit angiogenesis.
Keywords: Rat neural stem cells, Morphine, Aromatase, 5-alpha reductase, Endothelial differentiation, VE-cadherin

Ps-256: Investigation the Effects of Morphine on Insulin, IGF-1and IGF-2 Secretion with Gene Expression of Insulin Receptor in Rat Brain Neural Stem Cell

Sadegh Salarinasab[1], Alireza Nourazarian[1*], Reza Rahbarghazi[2], Nima Abdyazdani[1], Navid Feizy[1]

1. Department of Biochemistry and Clinical Laboratories, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Email: ali_nour65@hotmail.com

Background and Aim: One of the striking discoveries in the brain of patients with Alzheimer's disease is neuronal degeneration and loss of neuronal precursors in different parts of the brain, especially in the cortex and hippocampus. Neural stem cells (NSCs) are neuronal precursors and changes in the homeostasis of these cells can cause neurodegeneration. Morphine is one of the factors that can disrupt homeostasis in neuronal stem cells. However, the effects of morphine on these cells have not been clearly determined. Therefore, the aim of this study was to investigate the effects of morphine on multipotent neural precursor cells.
Methods: In order to understand the effect of morphine on multipotent neural precursors (NSCs) with the emphasis on insulin biosynthesis and its receptor as well as insulin-like growth factors (IGF-1, IGF-2), neural stem cells were extracted and their lineage potential was confirmed by specific markers. The cells were exposed to 100 μM morphine, 50 μM naloxone and combination of these two drugs for 72 hours. The neural cell growth, changes in levels of insulin and insulin-like growth factors secreted by NSCs as well as the insulin-receptor-gene expression were assessed by Flowcytometry, Elisa and real time PCR, respectively.
Results: according to the results obtained from cell cycle assay with propidium iodide (PI) the exposure of cells to morphine for 72 h increased cell apoptosis and decreased neural stem cell growth.in addition, it was proved that exposure of NSCs to morphine at the concentration of 100 μM for 24, 48 and 72 hours decreases the biosynthesis of insulin and insulin-like growth factors and also reduces the expression of insulin receptor gene. But, naloxone which acts as morphine antagonist could reverse different effects of morphine.

Conclusion: according to the obtained results, it can be concluded that morphine by reducing the secretion of insulin and insulin-like growth factors and also by downregulating insulin receptor gene expression interferes with the growth kinetics of NSCs and decreases the viability of these cells, thereby can cause neurodegeneration and possibly Alzheimer's disease.
Keywords: Morphine, Insulin, IGF-1, IGF-2, Neural stem cells

Ps-257: Evaluation of Bioactivity and Osteogenesis Activity of Electrospun Biodegradable Nanocomposite Scaffold Based on Polycaprolactone for Bone Tissue Engineering

Azar Nourian Dehkordi, Seyedeh Sara Shafiei*

Department of Stem cell and Regenerative Medicine, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: azar.noorian@yahoo.com

Background and Aim: Tissue engineering scaffolds provide biological and structural supports for cell adhesion, proliferation, and differentiation. Fibrous scaffolds properly mimic the native extra cellular matrix (ECM) fibers which play an important role in development regeneration of tissue and/or organs. One way to achieve fibrous scaffold with tailored properties is incorporating suitable nanomaterials into the polymeric matrix. In this study, Poly (ε-caprolactone) (PCL) composited with different layered double hydroxide (LDH) contents (ranging from 0.1 wt % to 10 wt %) were successfully fabricated through electrospinning process
Methods: The morphology, biomineralization, of samples were analyzed. Also, the biological effects of nanocomposites on attachment, viability, proliferation and osteogenic differentiation of MG63 osteoblast-like cells on nanocomposite scaffolds were evaluated.
Results: A decrease in the average diameter of PCL nanofibers was observed with the addition of LDH. The presence of LDH in PCL fibers promoted in vitro biomineralization, indicating bioactive features of the nanocomposite scaffolds. Compared to the pure one, nanocomposite fiber also showed better ability in protein adsorption. The in vitro cell culture studies showed that the addition of LDH did not diminish the biocompatibility of the electrospun PCL nanofiber. Furthermore, the adhesion and proliferation of MG63 cells were increased
Conclusion: Altogether, the results demonstrated that electrospun PCL-LDH nanofiber may be a suitable candidate for tissue engineering scaffold application
Keywords: Tissue Engineering, Electrospinning, Nanocomposite, Polycaprolactone, Layered Double Hydroxide

Ps-258: Homing of Epidermal Neural Crest Stem Cells after Maternal Transplantation in Animal Model of Microcephaly

Ameneh Omidi*, Iraj Ragerdi Kashani

Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: omidi_ameneh@yahoo.com

Background and Aim: Embryo has the capability of acceptance cells, which probably made it an ideal candidate for stem cell transplantation of various cerebral cortex abnormalities, such as microcephaly. The aim of this study was to determine epidermal neural crest stem cells (EPI-NCSCs) homing into the organs of fetus and mother.
Methods: Cells were obtained, analyzed for immunophenotypic features, and then labelled with CM-DiI; nestin+EPI-NCSCs or media (PBS) were intravenously delivered on day 16 of gestation in BALB/c mice which intraperitoneally received methylazoxymethanol (MAM) one day in advance, and homing was assessed at 24 hours succeeding cell injection. In the present study, involvement of SDF-1α /CXCR4 axis was also assessed by real-time PCR.
Results: Flow cytometry and immunocytochemistry manifested positive expression of nestin in EPI-NCSCs. Fetal brains were the host of cell implant, and for brain the figure was considerably higher in fetus to 4.05 ± 0.5% ($p \leq 0.05$ vs. mother). MAM-injected mice had a downward trend for SDF-1α and CXCR4 ($p \leq 0.05$ vs. control), but EPI-NCSCs group an upward for CXCR4 ($p \leq 0.05$ vs. MAM).
Conclusion: We conclude the EPI-NCSCs homing potential in experimental microcephaly which may let

these cells be a target in futures works on prenatal therapy of neurodevelopmental disorders.
Keywords: EPI-NCSCs, Homing, Methylazoxymethanol

Ps-259: The Association of Psychological Disorders with Epigenetic Modifications of FKBP5, IL-6 and IL-1β Genes

Shiva Poorang[1], Shokoufeh Abdollahi[1], Mohammad Bagher Tabei[1*], Zahra Anvar[2], Hossein Arabameri[3]

1. Department of Medical Genetics, Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran
2. Infertility Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Baghiyatallah Medical University, Tehran, Iran
Email: shiva.poorang@yahoo.com

Background and Aim: There is mounting evidence on the role of inflammatory cytokines and stress related genes in psychological health and diseases. In this study, we examined whether epigenetic modifications of IL-6, IL-1β and FKBP5 genes are associated with sleep quality, anxiety and depression in 59 young medical students who are expose to high extent of daily stress
Methods: Blood samples were collected from volunteers in one session for DNA extraction and methylation assays. The individuals were then categorized based on Beck Anxiety Inventory (BAI), Beck Depression Inventory-II (BDI-II) and Pittsburgh Sleep Quality Index (PSQI) questionnaires plus a two-week sleep diary.
Results: The results showed that all participants were hyper-methylated in the target region of FKBP5 and IL-1β genes. There was hypo-methylation in target region of IL-6 and second area of IL-1β genes. Although the results showed minor association between methylation patterns of selected genes and depression, anxiety and sleep disturbance, there was not statistical significance in methylation patterns of each gene between case and control groups (P>0.05).
Conclusion: Our data suggest that the alterations in methylation level can be associated with psychological disorders, so further studies are needed to confirm the findings of this study.

Keywords: Epigenetic Modification Methylation Immunity Cytokine

Ps-260: All-Trans Retinoic Acid Preconditioning Enhances Proliferation, Angiogenesis and Migration of Mesenchymal Stem Cell in Vitro and Enhances Wound Repair in Vivo

Mona Pourjafar[1], Rezvan Najafi[1*], Masoud Saidijam[1], Kamran Mansouri[2]

1. Research center for molecular medicine, Hamedan University of Medical Sciences, Hamedan, Iran
2. Medical Biology Research Center, Kermanshah University of Medical, Sciences, Kermanshah, Iran
Email: pourjafar_mona@yahoo.com

Background and Aim: Stem cell therapy is considered to be a suitable alternative in treatment of a number of diseases. However, there are challenges in their clinical application in cell therapy, such as to reduce survival and loss of transplanted stem cells. It seems that chemical and pharmacological preconditioning enhances their therapeutic efficacy. In this study, we investigated effects of all-trans retinoic acid (ATRA) on survival, angiogenesis and migration of mesenchymal stem cells (MSCs) in vitro and in a wound-healing model.
Methods: MSCs were treated with a variety of concentrations of ATRA, and mRNA expression of cyclo-oxygenase-2 (COX-2), hypoxia-inducible factor-1 (HIF-1), C-X-C chemokine receptor type 4 (CXCR4), C-C chemokine receptor type 2 (CCR2), vascular endothelial growth factor (VEGF), angiopoietin-2 (Ang-2) and Ang-4 were examined by qRT-PCR. Prostaglandin E2 (PGE2) levels were measured using an ELISA kit and MSC angiogenic potential was evaluated using three-dimensional tube formation assay. Finally, benefit of ATRA-treated MSCs in wound healing was determined with a rat excisional wound model.
Results: In ATRA-treated MSCs, expressions of COX-2, HIF-1, CXCR4, CCR2, VEGF, Ang-2 and Ang-4 increased compared to control groups. Overexpression of the related genes was reversed by celecoxib, a selective COX-2 inhibitor. Tube formation and in vivo wound healing of ATRA-treated MSCs were also significantly enhanced compared to untreated MSCs.

Conclusion: Pre-conditioning of MSCs with ATRA increased efficacy of cell therapy by activation of survival signalling pathways, trophic factors and release of pro-angiogenic molecules
Keywords: Stem Cell Therapy, Mesenchymal Stem Cells, All-Trans Retinoic Acid, Angiogenesis, Migration

Ps-261: All-Trans-Retinoic Acid Icreases in Vitro Mesenchymal Stem Cells Migration through Induction Matrix Metalloproteinases 2 and 9

Mona Pourjafar, Rezvan Najafi*, Massoud Saidijam

Research center for Molecular Medicine, Hamedan University of Medical Sciences, Hamedan, Iran
Email: pourjafar_mona@yahoo.com

Background and Aim: Mesenchymal stem cells (MSCs) are currently the most convenient primary source of stem cells applicable in cell therapy and regenerative medicine. Due to low number of viable MSCs after cell transplantation, novel approaches are required to improve viability, robust migration and proper homing of the cells. Herein, we investigated the effect of ATRA on caspase 3 activity, MMP-2/-9 expressions and activities, and in vitro murine bone marrow-derived MSCs cells migration.
Methods: MSCs were treated with different concentrations of ATRA for 24 h. matrix metalloproteinase-2 (MMP-2) and MMP-9 expressions and activities were assessed by Real-Time PCR and gelatin zymography. Measurement of Caspase 3 activity was carried out through an enzymatic assay. The migration potential of MSCs was observed by scratch test
Results: The expression of the MMP-2/-9 was higher at least 5 times in ATRA-treated MSCs ($P< 0.001$) and activity of the MMP-2/-9 was enhanced with increasing doses compared to the control MSCs. The caspase 3 activity was attenuated by ATRA preconditioning with significance level below 5% and 1% in different doses. Scratch test showed that ATRA could promote the migration ability of the MSCs in dose-dependent manner compared to the untreated MSCs.
Conclusion: Our data identify ATRA inhibits caspase 3 activity, stimulates the expressions and activities of MMP-2/-9 and, moreover, increases the in vitro migration capacity of MSCs.
Keywords: All-trans retinoic acid, Mesenchymal Stromal Cells, Migration, Survival, Matrix Metalloproteinase

Ps-262: Assessing Predictive Factors of Short and Long Term Clinical Response to Infliximab Treatment and Evaluation of Survival without Colectomy and Relapse in Patients with Ulcerative Colitis

Helia Pournaghshband[1], Nasser Ebrahimi Daryani[2], Ali Niksirat[3], MohammadKazem Nouri Taromlou[4], Zahra Azizi[5*]

1. Iran University of Medical Sciences, Tehran, Iran
2. Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Internal Medicine, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
4. Tehran University of Medical Sciences, Tehran, Iran
5. Southlake Regional Health Centre, Toronto, Canada
Email: Hpournaghshband@gmail.com

Background and Aim: Ulcerative colitis (UC) is a chronic, relapsing and remitting colorectal disease that is characterized by mucosalinflammation. During the past decade studies have shown the efficacy of Tumor Necrosis Factor alpha (TNF-α) blocker including Infliximab(IFX) - a recombinant chimeric IgG-1k monoclonal antibody neutralizing the activity of TNF-α (Tumor Necrosis Factor), as one of the effective therapeutic strategies in patients with moderate to severe corticosteroidrefractory UC. Despite the significant success of IFX therapy in many patients, there is still a considerable rate of failure. This is, in some cases, due to the development of antibodies against IFXthat neutralize the drug by binding to it or insufficient level of serum IFX. Therefore, we performed this study to evaluate the role of Serum IFX and IFX Ab levels besides other inflammatory markers as predictors of response to treatment in patients with corticosteroids or immunomodulator drugs refractory UC.
Methods: This is a cross-sectional study that was performed between 2015-2016 on patients with corticos-

teroids or immunomodulator drugsrefractory (UC) or patients who were unable to stop corticosteroids. Patients with latent Mycobacterium Tuberculosis infection, positive Cytomegalovirus, or Clostridium Difficile infection were excluded. Patients received a single dose of IFX (either 5 or 10 mg per kilogram of body weight) at week 0, 2, 6, 14, and then every 8 weeks. IFX and IFX Ab and C-reactive protein (CRP),Erythrocyte Sedimentation Rate (ESR) ESR, Calprotectin were assessed for all patients at weeks 0, 14 and 26(month 6).The use of Methotrexate, 6-Mercaptopurine, or Azathioprine before week 14 of IFX administration was considered as combination therapy with Immunomodulators. Disease activity and endoscopic severity were assessed using the Mayo Index to assess response to treatments and associated predicting factors. All patients signed an informed consent and the protocol was approved by ethics committee of Tehran University of Medical sciences. All data were analyzed using the SPSS version 23.0 software.

Results: Total of 24 UC patients 12(50%) male with mean age of 34.63±11.12 years were analyzed. Mean disease duration was 97±71.31 month. Overall, 4(16.7%) patients had proctitis, while 9(37.5%) and 7(29.2%) patients had Left colitis and pan colitis respectively. Mean mayo score at first injection was 10.88±1.86 (5-12) and decreased to 8.82±3.43(3-12) and 7.2±4.43(0-11) at week 14 and month 6 respectively. A simple linear regression was calculated to predict patients' response to treatment (Decrease in mayo score) 6 month after first injection, based on their IFX level at month 6. A significant regression equation was found with R2 =0.91 (F=30.42, P=0.012). Patients mean mayo score decreased -0.65 for each unit of IFX level. Patients' IFX level at week 14(p=0.29, R2=0.13) and IFX Ab level at week 14 (p=0.61, R2=0.03) and month 6(p=0.3, R2=0.34) couldn't significantly predict response to treatment (Decrease in Mayo Score). Mean difference of CRP and ESR at week 14 and month 6 in comparison to baseline couldn't significantly predict response to treatment (decrease in mayo score).

Conclusion: IFX level at month 6 later from the first injection could significantly predict patients' response to treatment by reducing Mayo score.

Keywords: Infliximab, Ulcerative Colitis, Inflammatory Bowel Disease, Colectomy, Relapse, Long Term Outcome, Short Term Outcome

Ps-263: Gene Expression Analysis of SOX2, NANOG, KLF4, OCT4 and REX1 Genes in BM Mononuclear Cells Treated with Acidic pH

Shahram Purbiyranvand*, Masoud Soleimani

Department of hematology, Faculty of Medical Sciences, Tarbiyat Modares University, Tehran, Iran
Email: sardarabadi@modares.ac.ir

Background and Aim: many studies have been done to produce induced pluripotent cells. In addition produce induced pluripotent cells going to resolve many of the problems relating to the use of embryos stem cells might serve in the gene therapy. It will be effective on gene therapy, pharmaceutical development and study on function of genes. The manufacturing process of induced pluripotent cells is kind of reprogramming, without destroying embryos. That's a controversial subject to produce embryos stem cells and this scientific discovery actually will close the scientist to dream of regenerative tissues without destroying the embryos. So far, many studies have been done to produce induced pluripotent cells. There are several methods to reprogramming somatic cells including use of small molecules and specific pluripotency proteins. In this study, without transfer of pluripotency factors and genetic manipulation, and only with pH treatment and trituration with Hamilton gage in BM cells the process of reprogramming the gene expression of pluripotency factors OCT4، SOX2، NANOG، REX1، KLF4 was observed.

Methods: In this experimental study, Bone Marrow mononuclear cells have been exposed to HCl (pH 5.7) for 30 minutes, and then have been transferred to the medium that have been supplemented with growth factor bFGF. Then RNA was extracted on day7. Quantity gene expression of OCT4، SOX2، NANOG، REX1، KLF4 was evaluated by Quantitative Real time-PCR.

Results: Gene expression of OCT4، SOX2، NANOG، REX1، KLF4 were increased after treatment with acidic pH in 7 days. In comparison with untreated cells. (0.05>P)

Conclusion: The Treatment of Bone Marrow mononuclear cells with acidic pH (5.7) lead to expression pluripotency factors in adult cells. These finding indi-

cate that adult cells may reprogram under changing environmental condition
Keywords: iPS, Bone Marrow, Reprogramming

Ps-264: Pancrease Biochemistry in Stem Cell Transplantation

Durdi Qujeq*

Department of Clinical Biochemistry and Cellular and Molecular Biology Research Center (CMBRC), Health Research Institute ,Babol University of Medical Sciences, Babol, Iran
Email: dqujeq@gmail.com

Background and Aims: After the transfer of stem cells in pancreatic tissue has been observed many problems, but mechanisms of this problem is not entirely clear for researchers. The aim of this research was to investigate the problems created in pancreatic tissue after the transfer of stem cells.
Methods: In this study, by injection of alloxan (75 mg per kg body weight of rats, n=30) diabetes was established in rats, diabetic condition confirmed by measuring blood glucose levels over 250 mg per kg. After diabetic conditioning biochemical factors such as insulin and glucose levels were measured each week until 22 weeks after the transplant. Glucose level was measured using glucose-oxidation methods: insulin concentration was measured using ELISA method according to the kit's instructions. To confirm the outcomes determined with ELISA assay, our assays were validated using other complementary methodology. The data were expressed as the mean ±SD. The endpoint level for statistical significance was set at $p<0.05$.
Results: In stem cell transplantation group, insulin levels were significantly higher than in the control group 22 week after transplantation (9.1.7 ± 0.6 vs. 6.3 ±1.2 µu/mL, $P < 0.05$). Also, decreased levels of glucose (108 ± 23.6 vs. 261.4 ±27.9 mg/dl, $P < 0.05$) were observed in rats during the 22 weeks period. Also, the relationship between pancreatic toxicity and biochemical changes were studied.
Conclusion: The findings of this research indicate that toxicity created in the pancreas was resolved, after improvement in biochemical parameters. It seems toxicity caused by imbalances in biochemical factors including glucose and insulin levels. Biochemical factors balance is essential to be successful in the cells transplantation.
Keywords: Pancreases, Insulin, Glucose, Stem Cell, Transplantation

Ps-265: Safety and Efficacy of Platelet-Rich Plasma in Treatment of Carpal Tunnel Syndrome; A Randomized Controlled Trial

Seyed Ahmad Raeissadat*, Leila Bagherzade

Physical Medicine and Rehabilitation Research Center, Shahid Modarres hospital, Shahid Beheshti University of Medical Science, Tehran, Iran

Background and Aim: Carpal tunnel syndrome (CTS) is the most common peripheral nerve entrapment, which is caused by the compression of median nerve in the carpal tunnel. The high prevalence of carpal tunnel syndrome, its effects on quality of life, and the cost that disease burden generates to health systems make it important to identify the research priorities that will be resolved in clinical trials. Considering the complication of surgery, conservative treatment is preferred for mild to moderate forms of the disease. Since the effect of PRP on peripheral nerve regeneration has been studied in recent years we designed this Randomized Clinical Trial to indicate the effects of local Platelet-Rich Plasma injection in patients with idiopathic mild and moderate Carpal Tunnel Syndrome
Methods: 42 patients with mild and moderate carpal tunnel syndrome (CTS) were randomly divided into two treatment groups: (1) Single injection of 1 ml of Platelet-Rich Plasma into carpal tunnel plus prefabricated hand wrist splint in neutral position (0-5 extension) for CTS for 8 weeks (n = 22); (2) Eight weeks of prefabricated hand wrist splint similar to group1 (n = 20). Pain visual analog scale (VAS), (BQ-SYMPT) Bostone/Levine symptom severity scale and Bostone/Levine functional status scale (BQ-FUNC) and EDX studies were assessed before intervention and after 8weeks. This research study was approved by the Ethics Committee of ShahidBeheshti University of Medical Sciences (IR.SBMU.MSP.REC.1395.382), and conducted in accordance with the principles of the Declaration of Helsinki.
Results: At 8 weeks after intervention, VAS score, symptom severity score ,functional scores and electro-

diagnosis parameters were significantly improved in both groups, compared to pre-treatment assessments (P-value<0.05). Nevertheless there was no significant difference in the cases group compared to control group after 8 weeks. Although majority of patient had no side effects, 20% reported mild pruritus and less than 10% reported tolerable pain after PRP injection

Conclusion: In short term PRP plus splint is not more effective than splint in reducing pain, symptom severity and functional status in mild and moderate carpal tunnel syndrome

Keywords: Carpal Tunnel Syndrome, Platelet-Rich Plasma , Wrist Splint

Ps-266: In Vivo Repair of Bone Defect with PGLA/HA/CS Scaffold and Mscs on Rats

Niloufar Rafiee*

Iran University of Medical Sciences, Tehran, Iran
Email: niloufar.raf@hotmail.com

Background and Aim: Human mesenchymal stem cells (hMSCs) are a very attractive option for cell engineering. In addition, as hMSCs does not cause immune response, it's nonautologous application is also possible.

Methods: This study discusses the application of hMSCs seeded in PGLA/CS/HA scaffold in femur bone defect model in rats.After separating the cells from human bone marrow sample, they were cultured on the constructed scaffold.Then some test were performed on the constructed tissue, which were: Electron microscopy studies, to determine cells's adherence to scaffold and the tissue's morphology, MTT test to determine the scaffold's biocompatibility and measuring Alkaline- phosphatase amounts to check the quantity of differentiated hMSCs to osteoblasts.After forming the bone defect model on rats, The bone defect was filled with scaffold and MSCs in one group and with scaffold alone, in another. Also, there was a third, control group for comparison.

Results: After 12 weeks of implanting the tissue in rats, CT scan radiography revealed the new bone was only formed at the two ends of femur bone in the control group and filling was much more in the other two groups, one with scaffold alone and another with scaffold and cells. Although, the difference between the amount of newly formed bone in the group with both

Ps-267: Effect of Low Level Laser Irradiation on Exosome Biogenesis in Human Endothelial Cells

Hesam Saghaei Bagheri[1], Monireh Mousavi[2], Jafar Rezaie[3], Aysa Rezabakhsh[1], Seyed Hossein Rasta[4], Reza Rahbarghazi[1*]

1. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Cellular and Molecular Biology, Islamic Azad University, Ahar Branch, Ahar, Iran
3. Department of Biology, Faculty of Science, Urmia University, Urmia, Iran
4. Department of Medical Physics & Bioengineering, Tabriz University of Medical Sciences, Tabriz, Iran
Email: rezarahbardvm@gmail.com

Background and Aim: Exosomes are nano-scale particles mirrored the dynamic of intracellular space and participate actively in cell-to-cell crosstalk especially angiogenesis. In the current experiment, we examined the potential of low level laser irradiation on the human endothelial cells 'exosome secretion exposed to low and high dose ranges.

Methods: We exposed HUVECs to various doses of low level laser irradiation from 1 to 80 J/cm2. Following cell survival screening by conventional MTT assay, we exposed the cells to selected doses of 2 and 80 J/cm2. 48 h after cell exposure to irradiation, we measured the transcripts of exosome biogenesis genes CD63, Alix, Rab-27a and b by real-time PCR assay. The protein level of CD63 was also measured by ELISA assay and immunofluorescence imaging. The release of exosomes to supernatant was monitored by detecting acetyl choline esterase activity.

Results: Our results showed that high doses of low level laser irradiation reduced cell viability 48 h after irradiation. The maximum toxic effect was recorded at 80 J/cm2 while cells under irradiation at 2 J/cm2showed an enhanced proliferation rate. Interestingly, a direct correlation was evident between the decrease of cell viability and increasing dose of irradiation. Whatever the intensity of the laser power raised a high levels of ex-

osome biogenesis transcripts and CD63 were detected. Also, the total amount of acetyl choline activity correlated with high laser intensities.
Conclusion: To our knowledge, this is the first report of direct impact of laser irradiation on exosome biogenesis in human endothelial cells. By using different dose of laser irradiation, we are able to modulate the baseline response of cell paracrine activity via exosome secretion during angiogenic response.
Keywords: Low Level Laser Irradiation, Exosome Biogenesis, Endothelial Cells

Ps-268: Investigation of Change in Expression of Some Apoptotic Proteins in KG1-a Cell Line, a Leukemic Stem Cell Model, by an Active Compound from Spiro-Quinazolinone Family

Arezoo Rahimian[1,2], Majid Mahdavi[1*], Hojjatollah Nozad Charoudeh[2], Reza Rahbarghazi[2]

1. Department of Biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran
2. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Email: a.rahimian@ymail.com

Background and Aim: Acute myeloid leukemia (AML) is a heterogeneous hematologic malignancy characterized by the clonal expansion of immature myeloid blasts. As leukemic stem cells are resistant against most of current cancer treatments, they are the main reason for treatment failure and disease relapse. KG1-a variant derived from the human acute myelogenous leukemia cell line KG1, is composed of undifferentiated blast cells and is considered as a leukemic stem cell model. Apoptosis induction is an effective strategy in cancer therapy and Spiro-quinazolinone derivatives as N-heterocyclic compounds, which have widespread bioactivities, have been considered as a potent apoptosis inducing agents. In the apoptosis pathway, the Bcl2 and IAP proteins family are the central regulators. Survivin as a member of IAP family as a suppressor of apoptosis is up regulated in the KG1-a cells. Also Bcl2 as an anti-apoptotic member of Bcl2 family, suppress apoptosis with inhibiting Bax (pro-apoptotic member of bcl2 family) function. High levels of Bcl2/Bax expression have been reported in many drug resistant cancerous cells. In this study we investigated the apoptosis inducing effect of 4-tert butyl spiroquinazoline benzene sulfonamide (4t-CHQ), compared to 4-tert butyl spiroquinazoline thiophene-2 carboxamide (4t-QTC) in the KG1-a cell line.
Methods: The cells were treated with 10 to 150 µM of both derivatives and then cell viability was determined using MTT assay. Inhibition concentration of 50% were determined using resultant formazan absorption. Apoptosis was evaluated using acridine orange/ ethidium bromide fluorescent dies and imaging with fluorescence microscopy, DNA fragmentation assay with agarose gel electrophoresis, cell cycle and AnnexinV/PI double staining assessment with flow cytometry. Furthermore, Survivin, Bcl2 and Bax expression changes were investigated using western blotting. Bcl2- 4t-CHQ and Survivin- 4t-CHQ interactions were analyzed by molecular docking simulation.
Results: According to MTT assay, 4t-CHQ derivative with IC50 value of 131.3 µM were chosen for further evaluations. Results indicated that the cells showed morphologic changes with chromatin condensation, DNA fragmentation and G0/G1 cell cycle arrest after treatment with the compound (at IC50 value). Presence of phosphatidyl serine on the outer surface of the cell membrane, confirmed the apoptosis occurrence in the KG1-a cells after treatment. Down regulation of Survivin as well as increasing in the Bax/Bcl2 ratio were resulted time dependently from western blotting. According to docking simulation data, functional BH3 domain of Bcl2 and BIR domain of Survivin were the binding sites for 4t-CHQ.
Conclusion: Our results represent induction of apoptosis by 4t-CHQ from Spiroquinazolinone derivatives in the KG1-a leukemic stem like cells.
Keywords: Apoptosis, Spiro-Quinazolinone, Survivin, Bax, Bcl2, KG1-a Cell Line

Ps-269: Stem Cell Applications in Treatment of Liver Diseases

Danial Khezri[1*], Shima Rahmati[2]

1. Student Research Committee, Kurdistan University of Medical Sciences, Sanandaj, Iran

2. Cellular & Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
Email: danialkhezri@gmail.com

Background and Aim: Liver fibrosis is a pathologic process that occurs between liver injury and liver cirrhosis, which can be triggered by viruses, alcohol abuse, drug abuse, and auto-immunity. Currently, Liver transplantation is the primary and effective treatment for various end-stage hepatic diseases, but it has many problems, including: immunological rejection, lack of donor organs and surgical complications so finding new therapeutic strategies for liver disease is essential. Stem cells have the ability to renew themselves endlessly and possess pluripotent ability to differentiate into many cell types. Two types of stem cells, embryonic stem cells (ESCs) derived from the inner cell mass of blastocysts and embryonic germ cells (EGCs) obtained post implantation embryos, have been identified.
Methods: The retrieved studies were searched through the PubMed, Google Scholar, Scopus, databases. Different studies have been demonstrated that cell therapy is an effective treatment of liver disease. Mesenchymal stem cells (MSCs) seem to be a promising cell type in of liver disease treatment. In this abstract we summarize the current uses of MSC therapies for liver fibrosis and suggest potential future applications.
Results: MSCs have multiple differentiation abilities, allowing them to migrate directly into injured tissue and differentiate into hepatocyte-like cells. Additionally, MSCs can release various growth factors and cytokines to increase hepatocyte regeneration, regress liver fibrosis and regulate inflammation and immune responses.
Conclusions: Stem cell research has offered the promise of effective cell-based therapies in treating several diseases including liver disorders. This therapeutic approaches have potential to enhance cutaneous regeneration and largely through trophic and paracrine activity. Totally Stem cell therapy may, in the future, be used as a bridge to either liver transplantation or endogenous liver regeneration, but efficient protocols must be developed and safety must be demonstrated before it can be applied to clinical practice.
Keywords: Stem Cells, Regenerative Medicine, Liver Disease

Ps-270:MCF7- Mamospheres Promote Generating Regulatory T Cells

Hajar Rajaei[1], Javad firouzi[1], Marzieh Ebrahimi[2*]

1. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
2. Department of Regenerative Medicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: hrajayi@ymail.com

Background and Aim: Cancer stem cells (CSCs) are a small population with the potential of tumor initiating, self-renewal capacity and pleuripotency ability. CSCs might be a proper target for eradication of tumor and a good candidate in dendritic cells (DCs) vaccination. But this question occurs if CSCs antigens per se are able to stimulate T cells after vaccination with DCs
Methods: To understand the impact of CSCs antigens on stimulating immune system, we generated pulsed DCs with total antigens of mamospheres derived from MCF7 cell line. Then colony formation, invasion and migration assay were performed to characterize the mamospheres. Immature DCs were differentiated from blood monocytes in medium supplemented with 50 ng/ml IL-4 and GMCSF for 5 days. Then 150 µg/ml Mamosphere antigens was added in presence of 5 ng/ml IL-1β, IL-6, 150 ng/ml TNF-α and 1 µg/ml prostaglandin E2. The profile of maturity markers on DC surface was analyzed by Flowcytometery and compared with the control group which didn`t receive any antigen. Finally, MLR test was performed between DCs and T cells. The immunophenotyping of resulted T cells was evaluated by specific antibodies with FACS machine after 14 days.
Results: In this study, we found that there was no significant difference in MHC-II, CD80, CD11c, CD14 and CD83 between DCs pulsed with mamospohere antigens and the control which didn`t receive any antigen. But flowcytometry analyses showed that the amount of regulatory T cells increased from 1.4 to 34.7% in the group which DCs pulsed with mamospohere antigens and on the flip side the number of CTLs, and T helper cells decreased from 50 to 23.1% and 18.3 to 1.19% respectively

Conclusion: In conclusion, these findings show that DC vaccination against mamospheres might play immunomodulatory effects, so that another approach along with DC application should be used to generate potent DCs against mamospheres to eradicate tumor.
Keywords: Mamosphere, DC, Regulatory T cells

Ps-271: Investigating hsa-miR-1915-3p-Mediated Suppression of Anti-Apoptotic Genes as A Tool to Rule out Apoptosis Resistance of Glioblastoma Cells

Hassan Rakhsh-Khorshid[1], Rezvan Rezazadeh[1], Ehsan Arefian[2*]

1 Department of Molecular Biology and Genetic Engineering, Stem Cell Technology Research Center, Tehran, Iran
2 Department of Microbiology, School of Biology, University of Tehran, Tehran, Iran
Email: arefian@ut.ac.ir

Background and Aim: Glioblastoma multiforme (GBM) is the most common and the deadliest primary brain tumor in adults. Growing aggressively and recurring inexorably, the disease still remains refractory to therapy, and the median survival of patients is 12-15 months since diagnosis. The high levels of inter- and intratumor heterogeneity stand out as one of the major obstacles in GBM therapeutics, resulting in the survival of resistant clones and recurrence of a tumor with distinctive new features. It seems that surviving cells have apoptosis resistance, due to inactivation of pro-apoptotic genes or activation of anti-apoptotic genes. Gene therapy is a promising tool, particularly in diseases like GBM that patients are considered as being in end stage. However, due to inter- and intratumor heterogeneity, the need for combination therapy in genetic approaches, as in chemotherapy, has been appreciated. The trend to use combination gene therapy has guided many research groups toward microRNAs. Their short length and efficacy of gene expression suppression make microRNAs interesting research topic in gene therapy. Considering apoptosis resistance of some clones in glioblastoma tumor, using microRNAs to target and suppress anti-apoptotic genes seems promising. In order to obtain the most efficient gene construct to induce apoptosis in glioblastoma cells, we aimed to find microRNAs ruling out apoptosis resistance.
Methods: We searched for microRNAs suppressing anti-apoptotic genes that render cancer cells apoptosis resistant. Using databases like TargetScan, miRWalk, and RNA22, in addition to data mining, we found hsamiR-1915-3p as a promising choice. It has been reported that hsa-miR-1915-3p inhibits expression of genes like BCL2, XIAP, and CFLAR, and its utility was previously investigated in colorectal carcinoma. In this study, we cloned hsa-miR-1915-3p in pCDH vector and banked the recombinant vector in stbl4 strain of Escherichia coli. To confirm these genes as bona fide targets of hsa-miR-1915-3p and investigate the efficiency of suppression, 3'untranslated regions (3'UTR) of BCL2 and XIAP were cloned in psiCHECK2 vector, and recombinant vectors were banked in DH5-alpha strain of E. coli. Unlike hsa-miR-1915-3p gene that was amplified with Taq DNA polymerase, 3'UTR of BCL2 and XIAP genes were amplified using Invitrogen Platinum SuperFi DNA polymerase, due to their over-5kb length.
Results: The efficacy of hsa-miR-1915-3p in suppressing anti-apoptotic mRNAs of BCL2 and XIAP has been suggested in databases and previous studies. Therefore, we cloned hsa-miR-1915-3p and banked the recombinant vector. 3'untranslated regions of XIAP and BCL2 genes were also cloned and recombinant vectors were banked.
Conclusion: We are going to do luciferase reporter assay to investigate how efficient hsa-miR-1915-3p binds to 3'UTR of BCL2 and XIAP mRNAs. Efficient binding of hsa-miR-1915-3p to aforementioned mRNAs may result in a significant decrease in expression levels of BCL2 and XIAP. This result will encourage us to use hsa-miR-1915-3p in gene constructs to rule out apoptosis resistance of glioblastoma cells and enhance the efficacy of drugs and genes inducing apoptosis in cancer cells.
Keywords: Gene Therapy, microRNAs, Glioblastoma, Apoptosis

Ps-272: Preparation and Evaluation of Conductive Biodegradable Nanocomposite Scaffolds for Nerve Regeneration

Ahmad Ramazani S.A.*

Sharif University of Technology, Tehran, Iran
Email: ramazani@sharif.edu

Background and Aim: Electroactive biomaterials are a part of a new generation of ‘‘smart’’ biomaterials that allow the direct delivery of electrical, electrochemical and electromechanical stimulation to cells. Recently, electroactive biomaterials have often been fabricated as tissue engineering scaffolds to provide electrical stimulation for neural tissue engineering as well as differentiation of stem cells. Cell growth especially nerve system cells could promoted by electrical stimulation. Electrically conductive Scaffolds could provide necessary media for electrical stimulation process. As part of our ongoing effort to develop a conductive biodegradable/ biocompatible nerve guidance conduit based on biodegradable, porous conductive scaffolds are designed and fabricated by incorporating highly conductive PANI/ graphene (PAG) nanoparticle, produced by in-situ emulsions polymerization of polyaniline in presence of graphene, in different biodegradable polymeric system. The Freeze-drying and electrospinning methods are used for single and multi-canal conduit fabrications. The effect of PAG content and other continents on the various properties of the scaffold such as mechanical properties, electrical conductivity, porosity, biodegedabilty, biocompatibility, and cell growth in presence of electrical stimulation is investigated. Mechanical investigations show highly promoted mechanical properties for scaffold prepared by simultaneously Electrospun Polycaprolactone/gelatin nanofibers. Cell growth experimental results show that electrical stimulation specially pulsated electrical stimulation could significantly increase cell growth properties.
Methods: An experimental setup has been designed and fabricated for applying ES through cultured Schwann cells on conductive scaffolds. The voltage set on 50, 100, and 150 mV/mm and investigated the effect of ES on Schwann cells growth. Before stimulation, the Schwann cells were seeded on scaffolds and incubated for 24 h. The cultured cells were stimulated with direct current with selected voltages for 4 h. Next, the stimulated cells were further incubated for 20 h. MTT assay and SEM micrographs were obtained for investigating the effect of ES on Schwann cells growth and morphology, respectively. Tissue culture plate (TCP) and non-stimulated scaffold were used as controls.
Results: The results confirmed the positive effect of ES on proliferation and morphology of cultured Schwann cells. Furthermore, it was confirmed that stimulation with pulsed current further improved cells proliferation especially for those stimulated with smaller time step (1 sec) probably due to most similarity to that happen in the body. Overall, this work supports using of conductive biodegradable scaffolds with ES for repairing nerve damages in nerve tissue

Ps-273: DNA- Neutral Lipid Self Assemblies as Nanobiocarriers

Reihaneh Ramezani*

Department of Biological Science, Women Research Center, Alzahra University, Tehran, Iran
Email: re.ramezani@alzahra.ac.ir

Background and Aim: Unique advantage of liposomes is that they are able to entrap biological macromolecules such as proteins, RNA, DNA, or small substances like drugs, nucleotides and even ions into their structures. They can even entrap these materials simultaneously. As mentioned in previous reports, it is possible to place all necessary elements for transcription, translation and protein synthesis inside the liposome, a system known as an artificial cell. This unique feature completely distinguishes these structures from other non-viral delivery systems.
Methods: DNA was encapsulated in bilayer vesicles by employing two contiguous dehydration-rehydration processes. The characteristics of obtained structure were investigated by various techniques such as electrophoresis, spectroscopy, DLS (Dynamic Light Scattering), Transmission electron microscopy.
Results: In this approach, we concluded that the neutral carriers such as dimyristoyl-phosphatidylcholine vesicles were able to encapsulate DNA with high efficiency (98%). These results also showed that lack of positive charge on the surface of liposomes and even the existence of cations does not have any important effects for the self-assembly process.
Conclusion: Due to promising results obtained in this study, it can be expected that cell membrane-based neutral liposomes would be considered in the near future as an alternative to cationic carriers in gene delivery. By

mimicking virus functions in fusion to cell membrane and transferring DNA and RNA to mammalian cells simultaneously, these neutral liposomes can be introduced as artificial viruses in gene therapy.
Keywords: Neutral Liposome, Zwitterionic Lipids-DNA Self Assemblies, Dimyristoyl-Phosphatidylcholine

Ps-274: Cytoplasmic Twist1 Expression, an EMT Related Transcription Factor Is Associated With Higher Grade Renal Cell Carcinomas and Poor Prognostic Factors in Clear Cell Renal Cell Carcinoma

Arezoo Rasti[1*], Mitra Mehrazma[1], Zahra Madjd[1], Maryam Abolhasani[1], Leila Janani[2], Leili Saeed Nejad Zanjani[1], Mojgan Asgari[1]

1. Oncopathology Research Centre, Iran University of medical Sciences,Tehran, Iran
2. Department of Biostatistics, School of Public health, Iran University of Medical Sciences (IUMS), Tehran, Iran
Email: rastirf@yahoo.com

Background and Aim: Twist is a key transcription factor which confers tumor cells with cancer stem cell (CSC)-like characteristics and has been reported to significantly enhance epithelial-mesenchymal transition (EMT) under various pathological conditions including tumor malignancy, invasion and metastasis. This study aimed to evaluate the expression patterns and clinical significance of twist1 in renal cell carcinoma (RCC).
Methods: The nuclear and cytoplasmic expression of twist1 was examined in 252 well-defined RCC samples, including 173(68.7%)clear cell renal cell carcinomas(ccRCC),45(17.9%) papillary renal cell carcinomas (pRCC) and 34 (13.5%) chromophobe renal cell carcinomas (ChRCC), by immunohistochemistry on a tissue microarray(TMA). The association between expression of this marker and clinicopathologic parameters and survival outcomes was then analyzed.
Results: Twist1 was observed mainly localized to the cytoplasm of tumor cells (98.8%). There was a significant difference in cytoplasmic expression levels of twist1 in different tumor grades (P =0.045). Cytoplasmic expression of twist1 was significantly correlated with renal vein invasion (P =0.031) and microvascular invasion (P =0.044). Higher cytoplasmic expression of Twist1 was correlated with shorter progression free survival time in patients with ccRCC (P=0.027).
Conclusion: These findings suggest that cytoplasmic expression of twist1 can be considered as a diagnostic and therapeutic marker for targeted therapy of ccRCC.
Keywords: Renal Cell Carcinoma, Twist1, Epithelial-Mesenchymal Transition, Tissue Microarray

Ps-275: Mir-204 Is Highly Decreased In Renal Cancer Spheres with Stem Cell/Mesenchymal Properties

Arezoo Rasti[1*], Mitra Mehrazma[1], Zahra Madjdi[1], Sadegh Babashah[2], Leili Saeednejad Zanjani[1]

1. Oncopathology Research Centre, Iran University of medical Sciences (IUMS), Tehran, Iran
2. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
Email: rastirf@yahoo.com

Background and Aim: Renal cell carcinoma (RCC) is the most common neoplasm of the adult kidney, with 35% chance of metastasis. In many solid tumors, cancer stem cells (CSC) represent a population with tumor-initiating, self-renewal and differentiation potentials. They are also resistant to drug and apoptosis. CSCs often exhibit EMT (Epithelial to mesenchymal) properties. This relationship between EMT and CSCs might have many implications in tumor progression. MicroRNAs (miRNAs) regulate many important cell functions and play important roles in tumor development, metastasis and progression, they regulate both normal stem cells and CSCs, and their dysregulation has an important role in metastatic RCCs.miR-204 is one of the important down regulated miRs in metastatic RCCs and inhibits RCC in xenograft models.The aim of this study was to evaluate miR-204 expression level in ACHN-derived spheres as a metastaic RCC cell line.
Methods: We isolated RCC spheres from ACHN cell line in enriched DMEM serum free medium and copmprised their characterizion with the parental cell line by sphere formation and colony assay techniques. The expression of CD44 and CD24 and a panel of surface mesenchymal and epithelial proteins was investigated by flowcytometry. Evaluation of CSC properties was

validated by quantitative real-time PCR. The expression of stem cell, mesenchymal and epithelial markers and also drug resistance and apoptotic genes were investigated in ACHN-derived spheres in comparison with parental cell line. miR-204 gene expression level was assessed in RCC spheres and their parental cells.
Results: The isolated RCC spheres from ANHN cell line showed cancer stem cell properties in sphere formation and colony assay techniques. Significant increased expression of stem cell and mesenchymal markers and decreased expression of epithetlial markers was observed in flowcytometry and quantitative real-time PCR. Most of drug resistant and apoptotic genes had elevated levels. miR-204 was highly downregulated in ACHN-derived spheres and it was differentially expressed between RCC spheres and their parental cells. ($P<0.001$)
Conclusion: Recent studies have highlighted several miRNAs to be differentially expressed in normal and cancer stem cells and established their role in targeting genes and pathways supporting cancer stemness properties. Our analysis showed that miR-204 is highly downregulated in ACHN-derived spheres. Down-regulated miR-204 represent potential diagnostic biomarker and may have therapeutic applications in targeting RCC spheres.
Keywords: Renal Cell Carcinoma (RCC), Cancer Stem Cells (CSCs), microRNA, miR-204

Ps-276: Increased Expression of SMAD4 a Key Transcription Factor in (TGF)-B Signaling Pathway in Renal Cancer Spheres with Stem Cell/Mesenchymal Properties

Arezoo Rasti[1*], Mitra Mehrazma[1], Zahra Madjd[1], Sadegh Babashah[2], Leili Saeednejad Zanjani[1]

1. Oncopathology Research Centre, Iran University of medical Sciences (IUMS), Tehran, Iran
2. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
Email: rastirf@yahoo.com

Background and Aim: RCC is the most lethal and aggressive urologic cancer.Recent evidences demonstrates that cancer stem cells (CSCs) are able to cause tumor initiation, metastasis and responsible for chemotherapy and radiotherapy failures and also apoptosis resistance. Transforming growth factor (TGF)-β participates in several biological events in cancer metastasis either under physiological or pathological conditions such as the cell cycle and apoptosis ,epithelial to mesenchymal transition (EMT) and ECM (extra cellular matrix) regulation .The (TGF)-β signaling pathway is the main pathway for epithelial to mesenchymal transition (EMT) induction in renal CSCs. The aim of this study was to evaluate SMAD4 expression level, a key transcriotoon factor in (TGF)-β signaling pathway in ACHN-derived spheres as a metastaic RCC cell line.
Methods: We isolated RCC spheres from ACHN cell line in enriched DMEM serum free medium and copmprised their characterizion with the parental cell line by sphere formation and colony assay techniques. The expression of CD44 and CD24 and a panel of surface mesenchymal and epithelial proteins was investigated by flowcytometry. Evaluation of CSC properties was validated by quantitative real-time PCR. The expression of stem cell, mesenchymal and epithelial markers and also drug resistance and apoptotic genes were investigated in ACHN-derived spheres in comparison with parental cell line. SMAD4 gene expression level was assessed in RCC spheres and their parental cells.
Results: The isolated RCC spheres from ACHN cell line showed cancer stem cell properties in sphere formation and colony assay techniques. Significant increased expression of stem cell and mesenchymal markers and decreased expression of epithetlial markers was observed in flowcytometry and quantitative real-time PCR. Most of drug resistant and apoptotic genes had elevated levels. SMAD4 was highly expressed in ACHN-derived spheres and it was differentially expressed between RCC spheres and their parental cells. ($P<0.05$)
Conclusion: TGFβ has a positive role on the CSC population promoting or sustaining stemness properties. It can contribute to the biology of cancer stem cells (CSCs) and various stromal cell types through EMT in order to facilitate cancer metastasis. Increased expression of SMAD a key transcription factor in (TGF)-β signaling pathway represent potential diagnostic biomarker and may have therapeutic applications in targeting RCC spheres.
Keywords: RCC, CSCs, EMT, TGFβ, SMAD4

Ps-277: The Controlled Release of Dexamethasone from Bioactive Electrospun PCL Nanofiber Scaffold

Fatemeh Rasti Boroojeni[1], Shohreh Mashayekhan[1*], Hojjat-Allah Abbaszadeh[2]

1. Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran
2. Department of Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: fatemeh.rasty@yahoo.com

Background and Aim: Spinal cord injury (SCI) is one of devastating disorders affecting many people. Notwithstanding the presence of neural and progenitor cells in central nervous system (CNS), the ability of CNS to regenerate is restricted due to the presence of inhibitory environmental factors in the injury site as well as the inherent limited regenerative capacity of CNS. In the site of injury, reactive astrocytes producing chondroitin sulfate proteoglycan are pivotal element in secondary injury phaseand the formation of glial scar as an inhibitory barrier to axonal regrowth. The use of anti-Inflammatory drug-loaded scaffold in an early phase of injury is recommended preventing the formation of glial scar. Dexamethasone sodium phosphate (DEXP), glucocorticoid anti-inflammatory drug, suppresses inflammation responses in the nervous system and affects astrocytes and glial cells through glucocorticoid receptors. In addition, it was showed that the use of DEXP in SCI reduced the expression of neurotrophin receptor p75, which triggered apoptosis and cell death, and therefore the use of DEXP may improve the SCI recovery. In this study, DEXP was loaded in chitosan nanoparticles, and DEXP-loaded nanoparticles were embedded in polycaprolactone (PCL) nanofibers. To improve the hydrophilicity and cell attachment, gelatin was applied.
Methods: The process of chitosan nanoparticle formation was based on ionic gelation method. 1.7 mg/ml chitosan solution containing 0.2 mg/ml DEXP was mixed with 12 ml of tripolyphosphate solution as cross-linker. To produce nanofibers, electrospinning technique was employed. The flow rate of PCL and gelatin solutions were adjusted at 0.5 ml/h. For gelatin solution, high voltage applied at syringe needle and the distance between needle and collector were 23 kV and 15 cm, respectively. The voltage of PCL solution was adjusted at 16 kV. The contact angle measurement was employed to evaluate the scaffold hydrophilicity. Drug release was studied at λ_Max=242nm by ultraviolet spectrophotometer. 100 mg of dried scaffold was immersed in 10 ml phosphate buffer solution. DEXP released from scaffold was measured at certain time. To analyze the biocompatibility of the scaffold, rat bone marrow-derived mesenchymal stem cells (BMSCs) were cultured on scaffold and cell viability was evaluated by MTT assay.
Results: To characterize the fiber diameter, the SEM micrographs of electrospun nanofibers were used. The average size of nanofibers was 320 nm ± 60 nm, which is similar to axon size and morphology. The release profile of DEXP from scaffolds containing chitosan nanoparticles showed that the presence of chitosan nanoparticles in nanofibers increased drug affinity to remain in the scaffolds. The percent of accumulated drug released from nanofiber scaffold reached to 60% after day 8. Co-electrospining of PCL and gelatin decreases the contact angle of scaffold from 112 ° to 23 °. MTT assay results indicated that PCL/gelatin nanofiber scaffold supported BMSCs proliferation, and DEXP release did not have negative effect on the cell proliferation.
Conclusion: Our composite scaffold fabricated by electrospinning technique provided sustainable release of DEXP, which may improve the SCI recovery. Moreover, this hybrid scaffold has the arranged structure of nanofibers which has similar structure axonal morphology and can be used as a bridging biomaterial.
Keywords: Spinal Cord Injury, Dexamethasone, Nanofiber Scaffold, Controlled Release, Chitosan Nanoparticle, Polycaprolactone

Ps-278: Mesenchymal Stem Cells (MSC): Identification, Proliferation and Differentiation of Mouse Omentum Stem Cells

Roudabeh Razaz, Kazem Parivar[*]

Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
Email: razzaz_r@yahoo.com

Background and Aim: Mesenchymal stromal cells (MSCs) as multipotent cells with the capacity to be differentiated into several cell lineages are promising sources for cell therapy and tissue engineering nowadays. Mesenchymal stem cells derived from omentum tissue are pluripotent cells which have been identified recently. In this study, the differentiation ability of Mesenchymal stem cells derived from NMRI mouse omentum tissue into kidney cells has been investigated with concentration on kidney genes expression profile. The inductive material was kidney tissue extracts.
Methods: For 21 days, omental Mesenchymal stem cells of NMRI mice were co-cultured with different concentrations of kidney extracts for kidney differentiation. At first to prove the mesenchyme being in cultured cells and then to show the differentiation of cells treated with kidney extracts, expressions of Mesenchymal-specific genes such as octamer-binding transcription factor-4 (Oct-4), Wilm's tumor suppressor gene-1 (WT-1) and kidney-specific genes like Pax2, Six2 and Wt1, were analyzed using reverse transcription polymerase chain reaction (RT-PCR)
Results: Mesenchymal stem cells derived from NMRI mouse omentum tissue expressed WT-1 gene as adult stem cell marker, Oct-4 as embryonic pluripotent stem cell marker and Actin Beta (ACTB) gene as housekeeping marker, of omentum tissue. OMSCs lost their spindle and fibroblastic morphology through differentiation, and changed to elliptical appearances. Messenger ribonucleic acid (mRNA) expression of kidney-specific genes include Pax2, Six2 and Wt1 in differentiated cells were detected on day 21.
Conclusion: These observations showed that omental Mesenchymal stem cells are capable to differentiate into kidney in vitro by induction of kidney tissue extract in absence of extracellular matrix.
Keywords: Mesenchymal Stem Cells, Omentum Tissue, Differentiation

Ps-279: Type 2 Diabetes Stimulated Human Mesenchymal Stem Cells Angiogenic Response by Up-Regulating Of the Autophagic Pathway

Aysa Rezabakhsh[1], Reza Rahbarghazi[2*], Alireza Garjani[1], Shahrooz Ghaderi[3], Omid Cheraghi[4], Alireza Nourazarian[5], Mehdi Hassanpour[5], Masoumeh Kazemi[4], Elaheh Nabat[s]

1. Faculty of Pharmacy, Department of Pharmacology and Toxicology, Tabriz University of Medical Sciences,Tabriz, Iran
2. Faculty of Advanced Medical Sciences, Department of Applied Cell Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
3. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
4. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
5. Faculty of Medicine, Department of Biochemistry and Clinical Laboratories, Tabriz University of Medical Sciences, Tabriz, Iran
Email: Aysapharma.rezabakhsh@gmail.com

Background and Aim: The current experiment aimed to declare the effect of type 2 diabetic serums on the angiogenic properties of human bone marrow mesenchymal stem cells (MCS) and its relationship to autophagic signaling pathway.
Methods: we exposed to isolated human MSCs with the sera from diabetic subjects over a period of 7 days. The cell survival rate was analyzed by conventional MTT and flow cytometric analysis of apoptosis. We then went on to elucidate the migration rate and in vitro tube formation of diabetic MSCs and endothelial trans-differentiation capacity, LDL lipase activity. PCR array was also exploited to monitor the autophagic status. The protein level of P62, LC3-I/II and becline-1 was also analyzed by western blotting. Angiogenic paracrine activity of diabetic MSCs was studied by in vivo Matrigel plug assay. The out data from the current experiment was subjected to Bioinformatics analysis.
Results: Compared to the control group, diabetic serum was found to induce cellular death rate ($P<0.001$) and apoptotic changes ($P<0.01$). We also showed that diabetic conditions significantly prohibited angiogenesis tube formation on Matrigel substrate, reduced cell chemotaxis ($P<0.01$) in response to SDF-1a, and inhibited endothelial cell differentiation rate ($P<0.0001$). Western blotting showed autophagic status by increase in LC3II/I ratio ($P<0.001$), beclin-1 ($P<0.0001$) and high levels of P62 ($P<0.0001$). In vivo Matrigel plug assay revealed that supernatant conditioned media pre-

pared from cells exposed to diabetic serum caused a significant reduction in the recruitment of VE-cadherin ($P<0.01$) and a-SMA-positive ($P<0.0001$) cells 7 days after subcutaneous injection. PCR array analysis confirmed the overexpression of autophagy and apoptosis genes in cultured cells in response to a diabetic condition ($P<0.05$). Using bioinformatic analysis, we clarified a crosstalk network between diabetes, angiogenesis, and autophagy signaling pathway. Type 2 diabetes could potently change angiogenesis by the interaction of IL-1b with downstream insulin receptor and upstream androgen receptor.
Conclusion: Together, diabetic serum led to abnormal regulation of P62 during the angiogenic response. These data demonstrated that diabetic serum decreased human MSC angiogenic properties directly on angiogenesis or by induction of autophagic pathway.
Keywords: hMSCs; Diabetes Type 2; Angiogenesis; Autophagy; Differentiation; Paracrine Activity

Ps-280: Separation of Mesenchymal Stem Cells from Normal and Cancerous Tissue of Pancreas and Comparison of Their Properties

Marzieh Rezaei[1,2], Saman Nikeghbalian[3], Abbas Ghaderi1[2*]

1. Cancer Immunology Group, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Immunology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
3. Shiraz Transplant Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz,
Email: bio_mrezaei@yahoo.com

Background and Aim: Pancreatic carcinoma (PC) is the fourth-leading cause of cancer death and is characterized by early invasion and metastasis. Mesenchymal stem cell (MSC)-like cells have been isolated from various types of tumor. It has previously been reported that MSCs are involved in tumorigenesis and its prognosis. The aim of the present study was to isolate and compare MSC cells from human PC and normal pancreas tissue.
Methods: MSC-like cells were isolated from 7 PC patients and 2 corresponding normal tissues by the collagenase digestion protocol. After MSC establishment in culture, between passages 3 to 5, we characterized the MSC cells by examining its morphology, culture properties and mesenchymal surface markers profiles and compared their properties with each other's.
Results: MSC- like cells were separated in both cancerous and normal pancreas tissue were similar and didn't show specific differences between their surveyed properties. In both groups we have long spindle cells that remain in the culture media without additional growth factors till passage 10-15s. The cells were positive for CD44, CD90, CD73 and CD166, negative for CD24, CD34, CD45, CD14 and expressed CD29 and CD146 in variable mode.
Conclusion: In conclusion, the present study demonstrated that MSC-like cells could be isolated from PC tissue. Moreover, Separation and culture of PC MSC-like cells provides an experimental model for investigating their role in the initiation and progression of PC and can be

Ps-281: Tissue-Specific Somatic Stem-Cell Isolation and Characterization from Human Endometriosis. Key Roles in the Initiation of Endometrial Proliferative Disorders

Mostafa Rezaei Tavirani[*]

Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Saeedhey@gmail.com

Background and Aim: The endometrial proliferation related diseases leads to endometrial hyperplasia, endometriosis. Endometrial progenitor and stem cells play key roles in the beginning of endometrial proliferative disorders. The purpose of this study are isolation of stem cells in endometriosis lesion as well as the evaluation and comparison of the stemness--related target genes in Endometriosis endometrial stem cells (EESCs), normal endometrial stem cell (ESCs), endometrial lesions stem cell (ELSCs) and bone marrow Mesenchymal stem cell (MSCs).
Methods: EESCs, ESCs, ELSCs and MSCs were isolated. Flowcytometry and Real--time PCR were utilized to detect the cell surface marker and expression pattern of sixteen stemness genes. The proliferation of all stem cells was observed by MTT assay. The differentiation

potential was evaluated by alizarin red, oil red O and RT--PCR method. The karyotyping was performed on EESCs and ELSCs at passage 20.
Results: The unique patterns of gene expression were detected although EESCs, ESCs, ELSCs and MSCs have a background expression of stemness--related genes. Spindle--like morphology, normal karyotype, adipogenic and osteogenic potential, significantly expression of Oct4, SALL4, DPPA2, Sox2, Sox17 and also specific surface markers such as CD44, CD105, CD90, CD73 and CD146 in EESCs and ELSCs was observed.
Conclusion: According to our data, stem cells in endometriosis endometrial and endometriosis are such an informative tools to study of pathogenesis of gynecological diseases. Furthermore, endometrial stem/progenitor cells which easily obtain from tissue may be valuable targets for early diagnosis of endometrial disorders in the future.
Keywords: Endometriosis Endometrial Disorders Stemness-Related Target Genes

Ps-282: The Effect of Human Mesenchymal Stem Cells Cultured in Type 2 Diabetic Serums on Cell Viability and Function of CRI-D2 Insulinoma Cell Line

Jafar Rezaie[1*], Malek Soleimani Mehranjani[1], Mohamad Ali Shariatzadeh[1], Reza Rahbarghazi[2]

1. Department of Biology, Faculty of Science, Arak University, Arak, Iran
2. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Email: J.rezaie88@gmail.com

Background and Aim: Type 2diabetes is a metabolic disorder characterized by hyperglycemia and hyperinsulinemia which can affect stem cell function. Mesenchymal stem cells (MSCs) were attracted enormous attention due to their broad therapeutic potential. These cells produce useful growth factors and cytokines with paracrine effects and play an important role in the regeneration of different tissues. Increasing evidence suggest that MSCs have significant anti-diabetic effects and interplay with pancreas β cells in animal models. We investigated the effect of conditioned media (CM) from MSCs cultured in Type 2 Diabetic Serum on cell viability and function of CRI-D2 insulinoma cell line.
Methods: Human mesenchymal stem cells (hMSCs) were divided into three groups and cultured in three different media including: 10% FBS, non-diabetic sera and diabetic sera (n=6) for a period of 7 days. After 48h of starvation, CM from hMSCs was collected and the CRI-D2 Cell line was exposed to CMs for 48 h. Cell viability, lipid accumulation and the level of insulin secretion were assessed using the MTT test, Oil Red O staining and ELISA assay respectively. Data were analyzed using one way ANOVA and Tukey's test and the means were considered significantly different at $P<0.05$.
Results: CRI-D2 cell viability significantly decreased in the diabetic group compared to the FBS ($p<0.01$) and non-diabetic ($p<0.05$) groups. The mean number of Oil Red O positive cells significantly increased in the diabetic group when compared to the FBS ($p<0.05$) and non-diabetic ($p<0.001$) groups. The level of secreted insulin did not differ significantly between non-diabetic and diabetic groups ($P>0.05$).
Conclusion: Our data revealed that treating the CRI-D2 cell line with the supernatant derived from hMSCs cultured in Type 2 Diabetic Serum reduced cell viability and disturbed lipid metabolism. It could be deduced that exposure of hMSCs to diabetic conditions intervenes with the production or function of growth factors and cytokines secreted by hMSCs
Keywords: Diabetes type 2, Mesenchymal Stem Cells, Condition Media

Ps-283: Gastric Cancer Stem-Like Cells Induce Treg Differentiation

Alaleh Rezalotfi[1,2], Ghasem Solgi[1], Marzieh Ebrahimi[2*]

1. Immunology Department, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran
2. Department of Stem Cells and Developmental Biology, Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, Tehran, Iran
Email: A.rlotfi91@gmail.com

Background and Aim: Regulatory T (Treg) cells or CD4+CD25+FOXP3+ cells, are immune suppressive

cells and prominent with tumor progress. Among tumor cells, rare populations with capacity of pluripotency mediated immune suppressive effects and are responsible for the most tumor progression. Although Treg cells has been reported in the most advanced solid tumors, but their interaction with gastric cancer stem like cells have been elusive. The object of this study is to find the effect of gastric cancer stem like cells on Treg differentiation.

Methods: Gastro-spheres were used as a model for enriching cancer stem like cell. The mixed leukocyte reaction was done peripheral blood mononuclear cells (PBMCs) from two normal individuals as responder and stimulator (inactivated with mitomycin) and in presence of MKN-45 cells and its derived gastro-spheres. Experiments were performed for 3 continues days. The expansion rate of responder T cells was evaluated by CFSE and the percentage of CD4+CD25+FOXP3+ cells was analyzed by flow cytometery.

Results: Flow cytometery analysis revealed that there was an increase in percentage of CD4+CD25+FOXP3+ Tregs in responder cells was treated with gastro-spheres compared with parental cells (7.8% vs 3.2%, P=0.04). Moreover, T cell expansion was increased about six fold in gastro-spheres treated cells compared with the control group ($P < 0.05$).

Conclusion: We concluded that gastro-spheres increases T cell expansion and also Treg population. Indeed these cells may induce CD4+CD25+FOXP3+ regulatory T cell expansion and alter CD4+ cells to another subsets.

Keywords: Regulatory T cells, Gastro-Spheres, Gastric Cancer Stem Like Cells, Tumor Microenvironment

Ps-284: Impact of Mummy Substance on Proliferation of Human Adipose-Derived Stem Cells and Fibroblasts in Separate or Co-Culture Model and Their Behavior on Plated PCL Scaffold

Roshangar Leila[1*], Hassanpour Sepideh[2], Jafar Soleimani Rad[2], Azadeh Ontaseri[2], Shahnaz Sabetkam[2]

1. Regenerative Medicine Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Stem Cell Research Center, Faculty of Medicine, Tabriz University of Medical Sciences

Email: lroshangar@yahoo.com

Background and Aim: Wound healing remains a challenging clinical problem due to increasing of the prevalence of non-healing wounds; therefore, efficient wound management is essential. At present, mesenchymal stem cells are touted as multipotent progenitors with the capacity for self-renewal and multi-lineage differentiation that could enhance cutaneous wound healing. On the other hand, traditional healers may be alternative strategies for treatment of wounds. Mummy was used as a remedy for inflammation, articular injuries, bone fractures, and wounds healing. This study is performed to evaluate the effect of mummy on proliferation and cell adhesion on Nano-fibers PCL of human adipose-derived stem cells and fibroblasts in separate or co-culture models.

Methods: For this purpose we isolated mesenchymal stem cells from human adipose tissues and human fibroblasts were procured from Pastor Institute, Iran. The cells were treated with mummy separately and co-cultured. Proliferation was assessed by Ki67 method in monolayer condition. Cell adhesion on Nano-fibers PCL scaffold was determined via SEM and cell proliferation on 3D scaffolds was evaluated based on the MTT assay.

Results: Proliferation rate was higher in treated ASCs ($p< 0.0001$) but was not different in fibroblasts ($p>0.05$). In co-cultured groups, cellular proliferation was unchanged. The cells were successfully penetrated and adhered and spread on PCL scaffolds in both control and mummy treated groups. Higher proliferation rate of fibroblasts was observed in Mummy-treated cells compared to the control group after 24h ($p<0.0001$). Increased proliferation rate in ASCs seeded on scaffolds and treated with Mummy was observed in comparison to control groups on 24 h ($p<0.01$) and 96h ($p<0.0001$).

Conclusion: Accordingly, mummy and ASCs may possibly improve wound healing through stimulating cellular proliferation and attachment and thereby help in proliferation of cells on PCL scaffold.

Keywords: Adipose-Derived Stem Cells Fibroblasts Mummy PCL Scaffold

Ps-285: Mummy Effect on Matrix Protein Synthesis of Wharton's Jelly-Derived Stem

Cells and Fibroblasts in Separate or Co-Culture

Shahnaz Sabetkam[1,2], Jafar Soleimani Rad[1,2*], Sepideh Hassan Pour[1,2], Leila Roshangar[1,2]

1. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical, Tabriz, Iran
Email: sh_sabetcom@yahoo.com

Background and Aim: In traditional medicine, mummy is used to treat inflammation, articular injuries, bone fractures, back pain and for wound repair. The healing effect of mummy on a wound in vivo has been studied and the results support its efficacy. The present study was investigated on effect of mummy on matrix protein synthesis of Wharton's jelly-derived human stem cells and human fetal foreskin fibroblast cell line (HFFF-2) separately or in co-cultures.
Methods: The effective dosage of mummy was determined by MTT assay and the human mesenchymal stem cells were isolated from Wharton's jelly. Human fibroblasts were obtained from Pasteur Institute (Tehran, Iran). The cells were cultured in DMEM and DMEM + mummy separately or in co-culture.
Results: The results suggested that mummy at concentration of 1000µg/ml led to the highest proliferation rate in WJSCs and HFFF-2. The level of mRNA expression of fibronectin in fibroblasts was up-regulated in the treatment group ($P<0.0001$), but it was up-regulated Col type III ($P<0.001$) and fibronectin ($P<0.0001$) in WJSCs. In co-culture, mRNA expression of collagene type I and fibronectin increased ($P<0.0001$).
Conclusion: The results indicate that mummy can treat wounds by synthesis of matrix protein.
Keywords: Mummy, Wound Healing, Matrix Protein, Poly-E-Caprolactone Scaffold, Human Wharton's Jelly Mesenchymal Stem Cells

Ps-286: New Developments in Three Dimensional Bio-Printing Devices

Babak Roushangar[1], Mohammadreza Shabgard[1*], Leila Roshangar[2]

1. Mechanical Engineering Department, University of Tabriz, Tabriz, Iran
2. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Email: b.roshangar@yahoo.com

Background and Aim: The most important goal of tissue engineering is production of suitable bio-scaffolds with good mechanical behavior and properties like living tissues beside its bio-compatible characteristics. Bio printers is the key to producing proper scaffolds. Bio printing devices have limitations in extrusion of bio-materials due to high viscosity. In other hand this high amounts of viscosity in bio materials is necessary for printing with higher resolutions. This viscosity must be adjusted for proper conditions which have not negative effects on cell living efficiency. So we developed advanced bio-printer with precise control in three dimensions ($0.001\geq$) and ability to extrude materials in wide range of viscosity (1000-20000 cp) in sterilized syringes and clean printing box. Also new developed temperature control systems, easy working and short set up time is some of the advantages of new designed bio-printer.
Methods: Mechanical force macro extrusion system used for precise extrusion and high quality ball-screw and rails inserted for coordinates control of device. Several air filters used for obtaining of clean box for printing in better conditions and three sensitive sensors used for controlling of room temperature, hot plate and cool bed. Mechanical properties of printed scaffolds investigated with universal axial machine.
Results: The results revealed that high resolution bio scaffolds even with living cells can be printed with new developed bio printer. High cell efficiency tested by MTT assay and scanning electron microscopy (SEM) pictures show high cell validity and good adhesion of chondrocyte cells to scaffolds structure. Also good mechanical properties obtained from tests and interconnected structure recognized in SEM observations.
Conclusion: The results indicated that this new developed bio printer has the ability of printing bio materials with living cells for using in different application of tissue engineering with high resolution in different viscosity.
Keywords: Bio-Printer, Chondrocyte, Viscosity, SEM, Scaffold

Ps-287: Study of Mummy on Proliferation of Wharton's Jelly -Derived Stem Cells and Fibroblast Cells and Their Co-Culture with and Without PCL Scaffold

Jafar Soleimani Rad[1,2*], Shahnaz Sabetkam[1,2], Leila Roshangar[1,2], Sepideh Hassan Pour[1,2]

1. Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical, Tabriz, Iran
Email: sh_sabetcom@yahoo.com

Background and Aim: Mummy was utilized as a remedy for swelling, articular wounds, bone breaks, laceration repair, and back torments in traditional medicine. The present study was aimed at investigating the proliferation by mummy on human Wharton's jelly-derived stem cells and human fetal foreskin fibroblast cell line (HFFF-2) in separate or co-culture and their behavior on plated PCL scaffold.
Methods: For this purpose, the mesenchymal stem cells were isolated from Wharton's jelly and then Human fibroblast cells were obtained from Pasteur Institute (Tehran, Iran). The cells were cultured in DMEM and DMEM + mummy separately or in co-culture. Proliferation was assessed using the Ki-67 method in monolayer condition. Cell adhesion on Nano-fibers PCL scaffold was determined via SEM and cell proliferation on 3D scaffolds was evaluated based on the MTT assay.
Results: Proliferation of WJSCs increased in the treatment group ($P < 0.0001$), but had no effect on fibroblasts and the effect of mummy in a co-culture was non-significant. The results suggested that the cells were successfully penetrated and adhered and spread on PCL scaffold in both control and mummy-treated groups. Based on the MTT assay on PCL, fibroblast cells proliferation increased after 24 h in treated group compared to control ($P \leq 0.0001$) and data indicated no marked difference between seeded WJSCs in both groups after 24 and 96 h and in co-culture on scaffold, (50-50) was significantly lower in mummy-treated constructs compared to the control on 24 h ($P \leq 0.0001$) and 96 h ($P \leq 0.001$). There was no significant difference in co-cultured cells in proportion of 70-30 in both control and treatment groups on both 24 and 96 h.
Conclusion: Based on the results, mummy may treat wound by stimulating cellular proliferation in monolayer condition and attach and proliferate cells on 3D scaffold.
Keywords: Mummy, Wound Healing, Proliferation, PCL Scaffold, Human Wharton's Jelly Mesenchymal Stem Cells

Ps-288: Acceleerating of Wound Healing by Secretion of Cytokines from Adipose Derived Stem Cells in Animol Model

Mohsen Sheykhhasan[1], Mohammad Bigdeloo[2], Mahdieh Sadat Ghiasi[3,4*]

1. Avaye Mahd Cell Iranian Company, Health Technology Development Center of Qom University of medical Sciences, Qom, Iran.
2. Department of Surgery, Qom University of Medical Sciences, Qom, Iran
3. Department of Pharmacology, Razi Drug Research Center. Iran University of Medical Sciences, Tehran, Iran.
4. Avaye Mahd Cell Iranian Company, Health Technology Development Center of Qom University of Medical Sciences, Qom, Iran
Email: mahdieh.ghiasi@yahoo.com

Background and Aim: Burns are considered the main causes of death in the world. One of the big problems in the burn was completed effective treatment to prevent damage to the skin. The variety of available methods, including the use of silver sulfadiazine 1% cream and herbs like Aloe Vera is considered researchers. Secretion of cytokine from stem cells can play a role in skin wound healing. The study of cytokine from adipose derived stromal cells to improve third-degree burn wound was used.
Methods: In this study 16 male rats Sprague Dawley 8-6 weeks 150 grams was used. The mice in the experimental and control groups were classified. In both groups, third -degree burns from boiling water 100 ° C by means of metal the size of 3 × 2 was developed for 30 seconds. After 3 weeks of the model, the burns were evaluated in both groups. In both groups burned section of each area was determined by H & E. The size of the wound on the first day and was assessed after 3 weeks. Data were analyzed using T-test.

Results: Assessment of burned area to confirm the model that led to the loss with boiling water burns and skin appendages comes epithelium. After 3 weeks in both groups were created, but speed healing of burns, repair and replacement of new skin in the experimental group than the control group.
Conclusion: Burns using secretion of cytokine from ASCs can speed up wound healing is shorter. This slowdown is likely due to secreting cytokine.
Keywords: Cytokine, Adipose Derived Stem Cell, Wound Healing, Burn

Ps-289: Bio, a Wnt Signaling Pathway Activator, Enhances the Adipogenic but Inhibits the Osteogenic Differentiation of Human Adipose Tissue-Derived Mesenchymal Stem Cells

Bahman Zeynali[1*], Forough Sadat Hosseini[1], Azita Parvaneh Tafreshi[2]

1. Developmental Biology Lab., School of Biology, College of Science, University of Tehran,Tehran, Iran
2. Department of Molecular Medicine, Faculty of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Email: aptafreshi@yahoo.com

Background and Aim: In recent years, extensive studies have been performed to enhance the stem cell based therapies for tissue repair. Although canonical Wnt signaling pathway is considered as an important regulator of proliferation and differentiation of different stem cells, its involvement especially in osteogenic and adipogenic differentiation has not been completely understood. Using human adipose tissue-derived mesenchymal stem cells (ADSCs) as a model, we sought to investigate the role of canonical Wnt signaling pathway on the osteogenic and adipogenic differentiation of ADSCs.
Methods: To activate Wnt signaling pathway, the osteogenic-or adipogenic-induced ADSCs were treated with Bio, a GSK-3β inhibitor, for 21 days and their differentiation were examined with Alizarin red and Oil red staining, respectively. The expressions of specific differentiation gene markers were evaluated with RT-PCR.
Results: Our results showed that in the ADSCs cultured in the presence of the osteogenic-inducing medium and Bio, the osteogenic differentiation was decreased whereas that of the adipogenic induced significantly compared to the control. In the presence of the adipogenic-inducing medium and Bio, the adipogenic differentiation was also significantly increased compared to those in the control.
Conclusion: We therefore suggest that the activation of Wnt pathway by Bio in the ADSCs inhibits the osteogenic but enhances the adipogenic differentiation.
Keywords: ADSCs, Wnt Signaling, Adipogenic and Osteogenic Differentiation

Ps-290: Application of Innovative Orthodontic Devices for Enhancing New Bone Regeneration in Cleft Lip and Palate

Massoud Seifi[1], Negin Sadat Matini[1*], Amir-reza Motabar[2]

1. Department of Orthodontics, Dental School, Shahid Beheshti University Of Medical Sciences, Tehran, Iran
2. Department of Plastic surgery, Bou-Ali Hospital, Azad Islamic Medical University, Tehran, Iran
Email: negin.matini@hotmail.com

Background and Aim: Tissue engineering strategies hold great potential for the repair of alveolar clefts in patients with craniofacial syndromes. However, most of these treatments with the help of stem cells and various designs of scaffolds are complicated and expensive. Orthodontic devices can be used as an alternative strategy in the therapy of alveolar clefts. The aim of this study is to evaluate the feasibility of innovative orthodontic devices in combination with alveolar grafts in order to provide better treatment outcomes in cleft lip and palate.
Methods: The study protocol was based on PRISMA statement for systematic review. Electronic and manual searches for literatures since 2011 were conducted. PubMed and Medline databases were accessed. Data extraction and analysis was performed by two independent individuals. Studies investigated various orthodontic devices which were established on the affected area pre-surgical and post-surgical. The amount of alveolar gap reduction were evaluated.

Results: Bone grafting for alveolar cleft defects and the bone graft survival was satisfactory. Although application of orthodontic devices with the combination of bone grafts were not a simple strategy but it was more cost-effective and had shown highly-predictable outcomes.
Conclusion: Orthodontic devices with innovative configurations can be useful in treatment of cleft defects and provide better outcome.
Keywords: Orthodontic, Cleft Lip, Cleft Palate, Active Appliance, Tissue Engineering

Ps-291: Biomimetic Constructs Comprising a Biodegradable Fibrous Complex and Nanoscopic Particles for Cell-Based Bone Therapy and Tissue Engineering

Mehdi Sadat-Shojai*

Department of Chemistry, College of Sciences, Shiraz University, Shiraz, I.R. Iran
Email: ms.shojai@shirazu.ac.ir

Background and Aim: In recent years, interdisciplinary methods based on tissue engineering have been proposed as an effective approach for bone therapy. Regenerative medicine mainly focuses on finding appropriate scaffolds to serve as a biodegradable template. Among various scaffolds, the ones having a fibrous structure resembling the extracellular matrix (ECM) have exhibited some promising properties. On the other hand, human bone can be structurally considered as a natural nanocomposite consisting of hydroxyapatite (HAp) and collagen-based polymer. Some research groups have therefore tried to combine biocompatible materials in a single construct to fabricate a biomimetic scaffold suitable for bone regenerative medicine. However, the fabricated systems usually show some drawbacks including low functionality, low mechanical properties, difficulty to seed cells deep into the construct, and low osteogenic potential. Hence, it was tried to develop new architectures suitable for bone tissue regeneration and cell-based bone therapy. For this, biodegradable polymer-based fibers were embedded within a model hydrogel system which was incorporated with 2D calcium phosphate (CaP) nanoparticles. As the biological CaP is nanoscopic rod-like crystals, the fabricated ECM-like constructs are expected to form a basis for new approaches in bone treatment.
Methods: The CaP nanoparticles were hydrothermally synthesized in-house through an optimization study. A model hydrogel system was chemically treated to synthesize a crosslinkable hydrogel which can be soluble in water before in-situ molding. Dispersion of some polyhydroxyalkanoates (PHAs) and nanoparticles were then prepared in chloroform by sonication and gentle heating, followed by electrospinning of the resulting viscous mixture at a fixed flow rate, voltage, and working distance. Some fabricated porous sheets were then embedded within a homogenous solution/dispersion of hydrogel/initiator/particles under optimized conditions to be molded under UV light.
Results: It has been well documented that HAp nanoparticles can significantly increase the bioactivity of man-made biomaterials. On the other hand, by controlling the electrospinning parameters, polymeric fibers containing nanoparticles can be fabricated to meet the requirements of bone regeneration applications. The results of this study demonstrated that ultrafine fibers without major defects can be fabricated via electrospinning when processing parameters are set at relatively optimum conditions. Moreover, the results suggested that nanoparticles can be distributed inside and outside the fibrous PHA. In this study to reconstruct the complexity of the ECM, integration of the fibrous sheets with the model hydrogel was also studied. Moreover, the integrated model hydrogel was used to encapsulate the cell populations required for cell-based therapy. According to the results, the fabricated constructs mimics the ECM more closely than the traditional scaffolds. Indeed, the strong fibers provided the required mechanical properties while the hydrogel acted as a cell carrier and provided an ECM-like environment necessary for the cells to proliferate and differentiate. Moreover, the presence of the bioactive nanoparticles resulted in a significant increase in the bioactivity of the system during the studied time period.
Conclusion: The present study showed that the new generation of constructs based on PHAs, biominerals and hydrogels can provide a promising opportunity to create strong and biocompatible systems for both the cell-based therapy and the tissue engineering in the future.

Keywords: Tissue Engineering, Nanoparticles, Bone, Regenerative Medicine, Extracellular Matrix

Ps-292: Lipopolysaccharide Preconditioning Refines the Performance of Mesenchymal Stem Cells Transfusion in Infectious Diseases

Pardis Saeedi, Raheleh Halabian, Abbas Ali Imani Fooladi*

Applied Microbiology Research Center, Medical Science of Baqiyatallah University, Tehran, Iran
Email: saeedi.p@gmail.com

Background and Aim: Mesenchymal Stem Cells (MSCs)-based regenerative therapy is currently considered as an alternative strategy to retrieve the infectious diseases. However, the efficiency of MSCs transplantation is limited by their high vulnerability and lower survival rate during the early-engrafted days. We proposed that lipopolysaccharide (LPS) preconditioning could enhance MSCs therapeutic efficacy and resistance against intensive stresses imposed by the microenvironment. Therefore, in the present study, LPS preconditioned MSCs resistance were compared with MSCs in vitro. Both were transplanted into the live bacterial model of sepsis, and the survival and bacterial clearance capacity of MSCs was observed. Furthermore, we tried to explore the underlying correlation between the MSC preconditioning and the severity of inflammation rate, the splenic volume and spleen regeneration.
Methods: Isolated and expanded MSCs were initially preconditioned with low dose E. coli derived LPS. MSCs and Pre-MSCs were exposed to hypoxia and serum deprivation (H/SD), and then the cell viability was determined by MTT assay. The live bacterial model of sepsis was developed by intra peritoneal (IP) injection of live E. coli. Mice (c57bl/6) were randomly divided into three treatment groups of; PBS (control group), MSCs (MSCs group) and LPS-preconditioned MSCs (LPS-MSCs group) and given an intra venous injection. Observing mice survival, identifying the inflammation. The morphology and size of spleen at necropsy and the number of neutrophils were also evaluated by complete blood count (CBC).
Results: Cell survival assay by MTT showed that LPS-MSCs were considerably more resistance than MSCs. The infected mice engrafted with LPS-MSCs exhibited raised survival rate and so diminished inflammation compared with other two groups. Splenic volume reduced with increasing severity of infection. MSCs could increase splenic volume (splenomegaly) following increasing neutrophils infiltration. LPS-MSCs, for higher survival and regenerative potential, could regenerate spleen more than un-preconditioned MSCs.
Conclusion: These findings suggest that LPS preconditioning has protective effects on in vitro survival of MSCs in H/SD states. LPS preconditioning before MSCs transplantation, effectively increases survival of engrafted MSCs and stimulates regeneration of spleen. Furthermore, our findings highlight the potentials of using LPS preconditioned MSCs, as an effective biological graft, could be a promising approach for infectious diseases treatment. These results would be informative and help refining future MSC-based therapies.
Keywords: Mesenchymal Stem Cells, LPS Preconditioning, Transplantation, Regenerative Therapy, Infection, Inflammation

Ps-293: Putative Cancer Stem Cell Marker CD44, Is Associated With More Aggressive Behavior and Poor Survival in Clear Cell Renal Cell Carcinoma

Leili Saeednejad Zanjani, Zahra Madjd*, Mojgan Asgari, Maryam Abolhasani, Arezoo Rasti

Oncopathology Research Centre, Iran University of Medical Sciences (IUMS), Tehran, Iran
Email: saeednejadleily@yahoo.com

Background and Aim: Renal cell carcinoma (RCC) is a common form of urologic tumor and accounts for approximately 3.8% of total adult human malignancies with an annually increasing incidence. Recent investigations have shown that in several human cancers, including renal carcinomas, a small population of tumor cells have the ability to initiate and maintain grow of tumor. The most important features of these cells, are self-renewal, asymmetric cell division, the ability to differentiate, so they are called cancer stem cells (CSCs). CD44 is a multifunctional class I transmembrane glycoprotein and is also as a potential surface markers in identification and isolation of CSC, in dif-

ferent cancers. Although CD44 suggested as a prognostic marker in RCC, the prognostic significance of this marker in three main subtypes of RCC is still unclear. The aim of this study was to evaluate the expression and prognostic significance of the putative CSC marker CD44 in different histological subtypes of RCC.
Methods: CD44 expression was evaluated by immunohistochemistry in 206 well-defined renal tumor samples on tissue microarrays, including 136 (66%) clear cell renal cell carcinomas (ccRCCs), 40 (19.4%) papillary RCCs, and 30 (14.6%) chromophobe RCCs. The association between CD44 expression, clinicopathological features as well as patients survival outcomes were determined.
Results: There was a statistically significant difference between CD44 expression among the different RCC subtypes examined ($P < 0.001$). In ccRCC, higher expression of CD44 was significantly correlated with increased grade, stage and microvascular invasion (MVI), lymph node invasion (LNI) and tumor necrosis. Increased expression of CD44 was significantly associated with poor overall survival ($P < 0.001$). However, in papillary and chromophobe RCC, CD44 expression did not show any significant correlation with prognosis.
Conclusion: We showed that higher CD44 expression was associated with more aggressive behavior, tumor progression and worse prognosis in ccRCC but not in the other examined subtypes. Therefore, we suggest that the CSC marker CD44 may be a promising target for cancer treatment only in ccRCC.
Keywords: CD44; Renal Cell Carcinoma (RCC), Cancer Stem Cells (Cscs), Tissue Microarray (TMA)

Ps-294: Assessment of the Clinical and Prognostic Significance of CD105 in Different Histological Subtypes of Renal Cell Carcinoma

Leili Saeednejad Zanjani, Zahra Madjd*, Mojgan Asgari, Maryam Abolhasani, Arezoo Rasti

Oncopathology Research Centre, Iran University of Medical Sciences (IUMS), Tehran, Iran.
Email: saeednejadleily@yahoo.com

Background and Aim: Renal cell carcinoma (RCC) accounts for 90% of adult renal malignancies and is the most lethal urological cancer with an annually increasing incidence. CD105 (endoglin) is a cell transmembrane glycoprotein and receptor for transforming growth factor (TGF) that participated in TGF-β signaling by interacting with TGF-β receptors I and/or II. CD105 is also a marker involved in angiogenesis and has been recently described as a cancer stem cell (CSC) marker in clear cell renal cell carcinomas (ccRCCs). This study was designed to evaluate the expression and prognostic significance of the CSC marker CD105 in different histological subtypes of RCC.
Methods: Tumoral CD105 expression was evaluated by immunohistochemistry in 186 well-defined renal tumor samples on tissue microarrays, including 120 (64.5%) ccRCCs, 36 (19.4%) papillary RCCs, and 30 (16.1%) chromophobe RCCs. The association between CD105 expression, clinicopathological features as well as patients survival outcomes were determined.
Results: There was a statistically significant difference between CD105 expression among the different RCC subtypes examined ($P < 0.001$). In ccRCC, higher expression of CD105 in cytoplasm was significantly correlated with increased grade, stage and microvascular invasion (MVI). Tumoral CD105 expression was found to be a predictor of poor overall survival ($P = 0.032$) in univariate analysis. However, in papillary and chromophobe RCC, CD105 expression did not show any significant correlation with prognosis.
Conclusion: We showed that high tumoral CD105 expression was associated with more aggressive behavior, tumor progression and poor OS in ccRCC patients but not in papillary and chromophobe RCC. Therefore, we consider the CSC marker CD105 as a useful prognostic molecular marker only in ccRCC, but not in other subtypes of RCC. We recommend the use of CD105 as a targeted therapy only in ccRCC cases.
Keywords: CD105, Renal Cell Carcinoma (RCC), Cancer Stem Cells (Cscs), Tissue Microarray (TMA).

Ps-295: Expression of CD133 in the Cytoplasm Is Associated With Cancer Progression but Is Not a Poor Prognostic Marker in Clear Cell Renal Cell Carcinoma

Leili Saeednejad Zanjani, Zahra Madjd*, Mojgan Asgari, Maryam Abolhasani, Arezoo Rasti

Oncopathology Research Centre, Iran University of Medical Sciences (IUMS), Tehran, Iran
Email: saeednejadleily@yahoo.com

Background and Aim: CD133 (Prominin-1) is one of the most commonly used markers of cancer stem cells (CSCs), which are characterized by their ability for self-renewal and tumorigenicity. However, their actual clinical and prognostic significance in renal cell carcinoma (RCC) remains disputable. The aim of this study was to investigate the expression and prognostic significance of the stem cell marker CD133 in different histological subtypes of RCC.
Methods: CD133 expression was evaluated using immunohistochemistry in 193 well-defined renal tumor samples on tissue microarrays, including 136 (70.5%) clear cell renal cell carcinomas (ccRCCs), 26 (13.5%) papillary RCCs, and 31 (16.1%) chromophobe RCCs. The association between CD133 expression and clinicopathological features as well as patients survival outcomes were determined.
Results: There was a statistically significant difference between CD133 expression among the different RCC subtypes examined. In addition, more invasive histological subtypes of RCC showed high cytoplasmic CD133 expression. In ccRCC, higher cytoplasmic expression of CD133 was significantly associated with increased grade, stage, microvascular invasion (MVI) and lymph node invasion (LNI) while in membranous expression we could not find these associations. Kaplan-Meier survival curves showed that patients with higher cytoplasmic CD133 expression tended to have a worse prognosis than membranous CD133 expression, although this was not statistically significant. We showed that higher cytoplasmic CD133 expression was associated with more aggressive behavior and tumor progression in ccRCC.
Conclusion: Our results demonstrated that cytoplasmic CD133 expression has a clinical significance in ccRCC and is associated with increased tumor invasiveness and metastasis; therefore evaluation of the pattern of CD133 expression in cytoplasm is useful for predicting progression, rather than membranous expression in these patients but not in other subtypes of RCC.
Keywords: CD133, Renal Cell Carcinoma (RCC), Cancer Stem Cells (CSCs), Tissue Microarray (TMA).

Ps-296: Biological and Regenerative Effect of Emu Oil: A Potential Supplement for Maintaining Stemness of Mesenchymal Stem Cells in Vitro

Khatereh Saei Arezoumand[1,2], Effat Alizadeh[1,2], Mohammad Esmaeillou[3], Maryam Ghasemi[1], Nosratollah Zarghami[1,2*]

1. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
2. The Umbilical Cord Stem Cell Research Center (UCSRC), Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Medical Biotechnologies, Universita degli Studi di siena, Siena, Italy
Email: Zarghami@tbzmed.ac.ir

Background and Aim: Emu oil contains fatty acids and antioxidants, which showed proliferative and anti-inflammatory effects on keratinocytes. The aim of this research is to investigate the effect of emu oil on stemness properties of multipotent mesenchymal stem cells (MSCs).
Methods: Isolation, cultivation, immunophenotyping, and differentiation checking of human adipose tissue derived mesenchymal stem cells (HAT-MSCs) were performed. After treatments of the HAT-MSCs with emulsified emu oil, the proliferation, cell cycle analysis, colony forming potential, healing potential and expression of stemness marker genes (Sox2, Oct4, Nanog, and Nestin) were studied.
Results: According to flow cytometry results, isolated HAT-MSCs in passage 3 were positive for CD90 and CD105 but negative for CD45 and CD34. Also, osteogenic potential of HAT-MSCs was confirmed. Treatment with 1.25 mg/mL emu oil in 5 and 9 days, significantly ($p<0.001$), increased HAT-MSCs proliferation, CFU-F and the expression of stem cell markers (Sox2, Oct4, Nanog, and Nestin). Emu oil caused an increase (2 times) of HAT-MSCs in S phase and also improved potential of in-vitro scratch wound healing.
Conclusion: Emu oil improved stem cells properties and stemness markers in HAT-MSCs at in-vitro system and can be used as a potential supplement in cultivation media.

Keywords: Emu Oil, Stemness, Cell Proliferation, Gene Expression, Mesenchymal Stem Cells

Ps-297: Enhancement of Cell Ingrowth Using Stimulation by Biophysical Factors: Comparison among Low Intensity Pulsed Ultrasound, Vibration and Magnetic Field in Static Culture

Mohammadreza Safari Hajat Aghaei[1], Ghassem Amoabediny[2*], Hadi Tabesh[1], Behrouz Zandieh Doulaby[3], Alireza Saatchi[2]

1. Faculty of New Sciences and Technologies (FNST), University of Tehran, Tehran, Iran
2. Department of Chemical Engineering, University of Tehran, Tehran, Iran
3. Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam-Universiteit van Amsterdam and Vrije Universiteit, Amsterdam, The Netherlands
Email: safari_mr@ut.ac.ir

Background and Aim: It has shown that biophysical factors stimulate bone cells in literature. However the detailed mechanism is has been elusive. Osteoblast cells response to mechanical stress because of mechanosensivity. Mechanical loading by biophysical factors such as low intensity pulsed ultrasound (LIPUS), magnetic field and vibration accelerate proliferation and differentiation by enhancing bone formation and inhibiting disuse osteoporosis. The aim of present study was performed to investigate the osteogenic effect of LIPUS, magnetic field and vibration on proliferation of G 292 preosteoblast cells and compare them to find the best
Methods: Hence, we investigate G 292 cell responses to LIPUS at frequency 1.5 MHz and intensity 30 mW/cm3 by EXOGEN device (Smith & Nephew Inc., Memphis, TN, USA) and also for vibration at frequency of 50-400 Hz and amplitude 0.9 G that applied by vibration motor (Pico Vibe™, 10mm Vibration Motor - 3mm Type, Model 310-003) that used in vibration setup (Research Center of New Technologies in Life Science Engineering (UTLSE), University of Tehran, Iran) and for magnetic field at range of 4-11 mT (Nano magnetic separation setup, Research Center of New Technologies in Life Science Engineering (UTLSE), University of Tehran, Iran). The exposure time was 20 min/day for all of them. G 292 preosteoblast cells (Pasteur cell bank) were seeded on 35 mm petri dishes and cultured for 1, 2 and 3 days (37 ºC, 5% CO2, 95% humidity) and Initial cell seeding was 3*105 cells/dish and 1.5*105 cells/dish. After 1, 2 and 3 days culture and exposure by mechanical factors the cells were counted.
Results: The results shown that after mechanical loading by LIPUS, vibration and magnetic field the proliferation after 2 days culture for 3*105 initial cell seeding was 1.25 fold of control but we can't see significantly different among biophysical factors effect because of the high initial cell seeding so we use 1.5 *105 cells/dish for initial cell seeding. In this step after 2 days culture for LIPUS we observe 56% and for vibration and magnetic field both 37.5% increase in number of cells compare to control and after 3 days culture we observe 42.8% for LIPUS and 28.5% for magnetic field and also 10.4% for vibration increase in number of cells compare to the control ($p<0.001$). The maximum proliferation was in LIPUS
Conclusion: Our data demonstrates that the stimulatory effect of LIPUS for G 292 cells and increasing the number of the cells was more than stimulation by vibration and magnetic field. Stimulation by LIPUS offer a tool for applying controlled mechanical force to G 292 cells for cell ingrowth and has potential to use in 3D bone tissue engineering for enhancement of proliferation and it can be used when the use of grows factors are limited. It can be used for targeted differentiation of stem cells.
Keywords: ultrasound vibration magnetic field bone cells

Ps-298: The Effect of Zingiber officinale Extract on Tumor Necrosis Factor-alpha in Mesenchymal Stem Cells

Zeinab Sahraian[*], Afsaneh nazari[2] Sanaz Mahmazi[2]

1. Department of biology, Biology Research Center, Islamic Azad University, Zanjan Branch, zanjan, Iran
2. Department of Genetic, Zanjan Branch, Islamic Azad University, Zanjan, Iran
Email: biosahraeian58@gmail.com

Background and Aim: Rheumatoid arthritis (RA) is an autoimmune disease particularly affecting elderly people which leads to massive bone destruction with

consequent inflammation, pain, and debility. Cytokines of the tumor necrosis factor –alfa (TNF-α) family play an important role in the regulation of inflammation. Zingiber officinale is a plant belonging to the Zingiberaceae family, which has traditionally been used for treatment of RA in alternative medicines of many countries. In this study the effect of different doses of ginger hydro-alcoholic extract on changing the expression of TNF-α in human bone marrow-derived mesenchymal stem cells (MSCs) was investigated.
Methods: MSCs in both treatment and control groups were studied with 10 and 100µg /ml doses of ginger hydro-alcoholic extract in times of 2, 16 and 24-hours, RNA was extracted from cells and after cDNA synthesis. The TNF-α gene expression was evaluated by Real Time PCR was used for the expression levels measurment and the results was evaluated by t-test.
Results: Samples treated with the extract of ginger in 50 and 100 µg/ml doses for 16 and 24 hours showed a significant decrease for TNF-a gene ($p<0.05$). According to the results obtained in 50 and 100 µg/ml doses for 2 hours showed a non-significant increase for TNF-agene.
Conclusion: Our results demonstrated that TNF-α gene expression is dose and time-dependent. These findings suggest that in vitro control of inflammatory cytokines may be important in stem cell therapy. As the development of RA is a complex process, further research should be continued towards elucidating the molecular details leading to RA and drugs that can stop or reverse these processes by phytoconstituents of ginger.
Keywords: Ginger, Tumor Necrosis Factor-alfa, Mesenchymal Stem Cells, Inflammation, Rheumatoid Arthritis

Ps-299: Human Adipose-Derived Mesenchymal Stem Cells Attenuate Liver Ischemia-Reperfusion Injury and Promote Liver Regeneration

Reza Saidi*

Surgery Department, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran
Email: rfsaidi@hotmail.com

Background and Aim: Ischemia-reperfusion injury (IRI) of the liver is a well-known cause of morbidity and mortality after liver transplantation. Effective treatment strategies aimed at decreasing hepatic IRI injury and accelerating liver regeneration could offer major benefits in liver transplantation, especially in the case of partial allografts. Human adipose-derived mesenchymal stem cells (HADMSCs) are an attractive source for regenerative medicine because of their anti-inflammatory and regenerative properties. We hypothesized that HADMSCs attenuate IRI and promote liver regeneration.
Methods: Mice were subjected to 60 minutes of partial IRI with or without 70% partial hepatectomy. Animals were treated with HADMSCs. Liver IRI was evaluated with serum levels of alanine aminotransferase, serum interleukin-6, and histopathology. Liver samples were stained for specific markers of liver regeneration.
Results: Histology, serum interleukin-6, and alanine aminotransferase release revealed that treatment with HADMSCs attenuated liver injury compared with control patients. Improved animal survival and increased number of regenerating cells were observed in HADMSC-treated animals who underwent IRI and partial hepatectomy compared with the control group.
Conclusion: HADMSC represents a potential therapeutic strategy to decrease IRI and promote regeneration in liver transplantation.
Keywords: Human Adipose-Derived Mesenchymal Stem Cells, Liver Ischemia-Reperfusion Injury, Liver Regeneration

Ps-300: Synthesis and Characterization of Novel Scaffolds Based on Polycaprolactone - Hydroxy Apatite Nanocomposites for Bone Tissue Engineering Using Human Dental Pulp Stem Cells

Vahideh Raeisdasteh Hokmabad[1], Roya Salehi[2*]

1. Department of Chemistry, University of Zanjan, Zanjan, Iran
2. Drug applied Research Center and Department of Medical Nanotechnology Faculty of advanced Medical Science, Tabriz, Iran
Email: salehiro@tbzmed.ac.ir

Background and Aim: Biocomposite scaffolds of poly(ε-caprolactone)/gelatin with hydroxyapatite (HA), SiO2 and Elaeagnus angustifolia Fruit Extract were prepared by electrospinning to show that they supports DPSCs cell attachment, migration and differentiation.
Methods: The polymer was synthesized with ring opening polymerization method, and its nanofiber scaffold with gelatin and biofactors was prepared by electrospinning method. Four different kinds of scaffolds were obtained by adjustment of spinning conditions, which were characterized as SEM, water contact angle measurements. Human DPSCs were then cultured on the scaffolds to compare the effect of three types of biofactors on nanofibrous scaffold. Scanning electron microscopy (SEM) was used to assess cell attachment, migration and differentiation.
Results: The fabricated scaffolds shows uniform pore morphologies. Cell growth and viability studies show that the scaffolds are able to support cell attachment and growth. The results showed that significant higher results demonstrated higher adhesive behavior, viability, alizarin red activity, and dentin specific gene expression on scaffolds.
Conclusion: Nanofibrous scaffolds seeded hDPSCs were cultivated in the presence of HA, SiO2, or Elaeagnus angustifolia Fruit Extract, and all showed supportive or encouraging effects on dental/bone tissues. Our results confirmed the adhesive and odontogenic encouraging properties on all scaffolds. Therefore, it can be concluded that using both improved ECM and signaling molecules could enhance the yield of regenerative tissue engineering.
Keywords: Elaeagnus Angustifolia Fruit Extract Hydroxyapatite Nanofibrous Scaffolds

Ps-301: Synthesis and Characterization of Thermosensitive Hydrogel Scaffolds Based on Polycaprolactone Copolymers for Use in Cartilage Tissue Engineering

Solmaz Sagheb[1], Roya Salehi[2*]

1. Department of Chemistry, University of Zanjan, Zanjan, Iran
2. Drug Applied Research Center, Stem cell research Center, Department of Medical Nanotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: salehiro@tbzmed.ac.ir

Background and Aim: Thermosensitive hydrogel based scaffolds composed of poly (PNIPAAm-PCL-PEG-PCL-PNIPAAm)/gelatin were prepared by thermally induced phase separation and emulsion freeze drying combinatin methods for cartilage tissue engineering application.
Methods: At first, PCL-PEG-PCL block copolymer was synthesized with ring opening polymerization method. Then diacrylate was designed by the reaction of PCL –PEG-PCL block copolymer with acryloyl chloride as crosslinker. Finally, biodegradable hydrogel based on NIPAAm and PCL–PEG-PCL-diacrylate were prepared via free radical polymerization method. Its nanofiber scaffold with gelatin was prepared by thermally induced phase separation and emulsion freeze drying combinatin methods. Prepared scaffolds were characterized by HNMR, FTIR, Zeta potential, SEM and water contact angle measurements. Chondrocytes cells were then cultured on the scaffolds to study the effect of nanofibrous scaffold on cartilage tissue Engineering. Scanning electron microscopy (SEM) was used to assess cell attachment, migration and differentiation.
Results: The fabricated scaffolds shows uniform pore morphologies. Cell growth and viability studies show that the scaffolds are able to support cell attachment and growth. The results showed that significant higher results demonstrated higher adhesive behavior, viability, and cartilage specific gene expression on scaffolds.
Conclusion: Nanofibrous scaffolds cultivated chondrocytes cells were showed supportive or encouraging effects on cartilage tissues. Therefore, it can be concluded that using both improved ECM and signaling molecules could enhance the yield of regenerative tissue engineering.
Keywords: Thermosensitive Hydrogel, Catilage, Tissue Engineering, Thermally Induced Phase Separation

Ps-302: Egg Shell Membrane Nanofibers for Preparation of Biodegradable Scaffolds in Tissue Engineering Application

Leila Mohammadzadeh[1], Roya Salehi[2*]

1. Chemistry Department, Azarbaijan Shahid Madani University, Tabriz, Iran
2. Drug Applied Research Center and Faculty of advanced Medical Science, Tabriz University of Medical Sciences, Tabriz, Iran
Email: salehiro@tbzmed.ac.ir

Background and Aim: Protein-based electrospun nanofibers have a potential to be used in wound dressing, medical implant materials, drug delivery, and tissue engineering applications. In this study, we produced a new type of nanofibrous membrane based on Eggshell membrane and silk fibrin by the combination of SEP preparation and electrospinning technique.
Methods: SEP was prepared by dissolving raw ESM powder in aqueous 3-mercaptopropionic acid and acetic acid followed by neutralizing of medium. We first studied the dissolution process using 3-mercaptopropionic acid as a reductive reagent to cleave the disulfide bonds existing in ESM. The results are: Both temperature and concentrations of 3-mercaptopropionic acid and acetic acid can all affect the time taken for dissolution and sometimes the yield. S-ESM blends fibers containing PVA, silk fibroin and SEP and poly caprolacton was prepared by electrospinning. Adipose stem cells were cultured on this scaffold and its differentiation to skin tissue in the presence of appropriate growth factors was investigated.
Results: The fabricated scaffolds showed uniform pore morphologies with fibers diameter in the range of 50-100. The results showed significant higher adhesive behavior, viability, and skin specific gene expression on scaffolds cultivated with adipose stem cell.
Conclusion: Nanofibrous scaffolds cultivated adipose stem cells were showed supportive or encouraging effects on skin tissues. Therefore, it can be concluded that using both improved ECM and signaling molecules could enhance the yield of regenerative tissue engineering.
Keywords: Egg Shell Membrane, Silk Fibrin, Skin Tissue Engineering, Electrospining

Ps-303: The Effect of HI-LNC25 Overexpression on Differentiation of HU-MSCs into Insulin Producing Cells

Saeede Salehi[1,2], Negar Azarpira[3], M.N. Taheri[1,2], G.R. Rafiei[1], A. Zare3, M.A. Okhovat[1], A. Behzad-Behbahani[1,2*]

1. Diagnosis Laboratory Sciences and Technology Research Center, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
2. Departments of Medical Biotechnology, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
3. Transplant Research Center, Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran
Email: saeede.salehi@yahoo.com

Background and Aim: Type 1 diabetes is an autoimmune disease characterized by deficient insulin production. One potential approach to cope with the disease is cell therapy, hampered by the scarcity donor pancreases. Generation of β cells from human umbilical cord derived mesenchymal stem cells (HU-MSCs) can be a possible solution to overcome this hurdle. Long non-coding RNAs (lncRNAs) are considered as potential candidates for differentiating HU-MSCs into insulin producing cells (IPCs). In this study, the effect of HI-lnc25 overexpression on differentiation of HU-MSCs into IPCs was investigated.
Methods: HU-MSCs were obtained and analyzed regarding their surface markers and differentiation capability. The cells were then transduced using a lentiviral vector harboring HI-LNC25 and cultured in complete medium for 21 days. Differentiated cells were analyzed for typical IPCs features including storage of Zn+2 and beta cell-related gene expression by qRT-PCR.
Results: The first islet-like clusters began to appear by the 5th day and continued to increase in number until day 21, but the cells could not be stained well. The GLIS3 and HI-LNC25 transcripts were detected in days 7, 14 and 21, albeit the insulin and pdx1 transcripts could not be detected.
Conclusion: As combinatorial regulation of gene expression implies, HI-lnc25 overexpression alone in not capable of differentiating MSCs into functional β-cells without synergistic effects of other regulatory factors. However, our data show that HI-lnc25 triggers IPC-like morphological changes in MSCs by up-regulating GLIS3 transcription factor. Furthermore, this

study shows that HI-lnc25 is an important component in β-cell differentiation network.
Keywords: Type 1 Diabetes HU-MSCs lncRNAs

Ps-305: Enrichment of Cancer Stem-Like Cells from Colorectal Cancer Cell Line HT29 with Induction of Epithelial-Mesenchymal Transition (EMT) Using Lentiviral Vector Carrying E-Cadherin ShRNA

Zohreh Saltanatpour[1*], Behrooz Nikbin Moghaddam[2], Behrooz Johari[3], Faezeh Maghsood[3]

1. Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Immunology and Immunohematology, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Biochemistry, Pasteur Institute of Iran, Tehran
Email: zohre_saltanatpour@yahoo.com

Background and Aim: The cancer stem cell (CSC) hypothesis posits CSCs are a subpopulation cancer cell that have the potential for self-renewal, drive tumor growth, tumorgenicity and are resistant to many current anticancer treatments. The better under¬standing of CSCs depend on isolation and enrichment methodology and scientist have conducted extensive research in this field. Epithelial-mesenchymal transition (EMT) is important in the tissue differentiation. Moreover, it has a crucial role in the invasion and metastasis in cancer. E-cadherin is an important molecule in cancer progression and EMT process. A critical molecular feature of EMT is the down regulation of E-cadherin. The aim of study was to create a model of CSC enrichment via the induction of EMT in CRC cell line. We examined whether the knockdown of E-cadherin leads to significant EMT-like alterations and acquirement of most the properties of CSCs.
Methods: We performed knockdown of E-cadherin in the human colorectal cancer cell line HT29 using Lentiviral Vector Carrying shRNA. Following vector transduction, we analyzed EMT markers such as E-cadhern and vimentin using Real time-PCR. Moreover, CSC markers and properties such as CD44, CD133, proliferation rat, and sphere formation ability were analyzed using flow cytometry, MTT assay and sphere assay. During study, cell morphology changes were monitored using light and fluorescent microscope. We used parental HT29 and HT29 cells transduced with Scramble Vector as controls.
Results: We established an EMT model using the HT29 cell line by CDH1 knockdown. Colorectal CSC markers were enriched in the CDH1 knockdown cells. The cells exhibited mesenchymal morphology and expressed high levels of EMT-related proteins, which confirmed that these cells had undergone EMT.
Conclusion: Down regulation of E-cadherin cased induction of EMT in the human colorectal cancer cell line and created enriched cells with cancer stem cell properties in this cell line.
Keywords: Cancer Stem Cells (CSC), epithelial-mesenchymal transition (EMT), E-Cadherin, Lentiviral Vector

Ps-306: Targeting Pluripotency Genes Bmi-1, Sox-2, Nanog and Oct-4 with Fish-Oil Derived Eicosapentaenoic Acid Contributes to Caspase-3 Dependent Apoptosis in Colorectal Cancer Stem-Like Cells

Mohammad Reza Sam[*]

Department of Cellular and Molecular Biotechnology, Institute of Biotechnology, Urmia University, Urmia, Iran
Email: kalbkhani_fahimeh@yahoo.com

Background and Aim: Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide. Colorectal cancer stem cells (CCSCs) are thought to drive tumor growth, metastasis and chemoresistance. Yet, no modulating drug for targeting pluripotency genes in CCSCs with low toxicity and high efficacy has been approved for clinical application in CRC. With this in mind, Targeting pluripotency genes in CCSCs with natural compounds may provide promising therapeutic application in CRC treatment. Fish-oil derived eicosapentaenoic acid (EPA) has been reported to induce apoptosis in different kinds of cancer cells. However, little is known regarding the evaluation of EPA as a safe compound on the expression of pluripotency genes Bmi-1, Sox-2, Nanog and Oct-4 as well as induction of apoptosis in CCSCs.

Methods: LS174T cells as a model for CRC with stem-like cells properties were treated with 50, 100, 150 and 200 µM of EPA (equal to plasma levels achievable in the human body following supplementation of the diet with polyunsaturated fatty acids) after which Bmi-1, Sox-2, Nanog and Oct-4 expression, caspase-3 activation as well as apoptotic-rates were evaluated using real-time RT-PCR, Caspase-3 colorimetric assay and flow cytometry respectively 48-h post treatments.
Results: EPA decreased expression of Bmi-1 and Sox-2. After a 48-h treatment with 50, 100, 150 and 200 µM of EPA, pluripotency genes expression levels were measured to be 98%, 90%, 42% and 25% for Bmi-1, and 54%, 60%, 45% and 61% for Sox-2 respectively compared to untreated. At the same conditions, EPA decreased expression of Nanog and Oct-4 genes only at the concentration of 200 µM and expression levels were measured to be 30% and 29% respectively compared to untreated cells. Increase in the caspase-3 activation level and number of apoptotic cells was also observed with increasing EPA concentrations.
Conclusion: Our observations provide the first evidence that targeting pluripotency genes using fish-oil derived EPA induces caspase-3 activation and apoptosis in CRC stem-like cells and this strategy may open up avenues to new therapeutic strategies for CRC-directed therapy with low toxicity and high efficacy.
Keywords: Colorectal Cancer, Colorectal Cancer Stem Cells, Pluripotency Genes, Bmi-1, Sox-2, Nanog; Oct-4, Caspase-3, Apoptosis

Ps-307: Computational Exploring of the Mirnas Potentially Involved in HSC Differentiation

Tayebeh Sardarzadeh, Sadat Dokanehiifard*

Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
Email: tt.sardar@yahoo.com

Background and Aim: MiRNAs are small non-coding RNA molecules that function in gene expression regulation via mRNA degradation or protein translation inhibition by mostly binding to the 3'UTR of their target genes. These molecules play key roles in a variety of biological processes, including development, proliferation, and regulation of stem cell differentiation and reprogramming. Red Blood Cells are continuously restoration; their half-life in human is about 120 days. Hematopoietic stem cells (HSCs) are multipotent, self-renewing progenitor cells that differentiate into all blood cells from lymphoid and myeloid lineages and have been used to replace unhealthy bone marrow for patients with cancers and blood disorders.
Methods: To better understand whether miRNAs are involved in the control of HSC differentiation to RBCs, we examined miRNAs which could target mRNAs involved in this differentiation. For this, a list of 11 effective genes that promote the differentiation of the RBCs was provided as reported in KEGG pathway database. All possible miRNAs which target 3'UTR of these genes were predicted by using miRWalk database, and then organized based on the miRNAs which could simultaneously target several interested genes.
Results: Here, we have found 8 possible miRNAs which target 5 genes in this pathway.
Conclusion: To better understand whether miRNAs are involved in the control of HSC differentiation to RBCs, we examined miRNAs which could target mRNAs involved in this differentiation. Here, we have found 8 possible miRNAs which target 5 genes in this pathway.
Keywords: Hematopoietic Stem Cell (HSC), RBC, MiRNA, Bioinformatics analysis

Ps-308: Evaluation of the Effect of Zataria Multiflora Boissis on Proliferation and Aging of Rat Adipose or Bone Mesenchymal Stem Cells

Manizhe Sarikhani[1], Effat Alizadeh[2*], Masomeh Firuzamandi[1], Marzie Hejazy[1], Mahla Deylam[1], Roya Herizchi[3]

1. Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
2. The Umbilical Cord Stem Cell Research Center (UCSRC), Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
Email: m_sarikhani@yahoo.com

Background and Aim: Nowadays utilization of industrial antioxidants is increasing in the chemical products, pharmaceutical and cosmetic. Because, these materials have the side effects, study and search for natural material instead of chemical antioxidant is necessary. Zataria multiflora Boissis a spice plant belonging to the Laminaceae family that geographically grows only in Iran, Pakistan, and Afghanistan. This plant known as Avishan-e-Shirazi (in Iran) is used as a flavor agent in a variety of foods in Iran and has different effects such as antiseptic, anesthetic, antispasmodic, antinociceptive, and antibacterial. Methanolic extract of the leaves and flowers of Zataria multiflora also have antioxidant effects. The aim of this study is to evaluate the effect of methanolic extract of the endemic plant, Zataria multifloraon on viability and proliferation of rat adipose/ bone marrow derived stem cells.
Methods: The mesenchymal stem cells were isolated from either adipose (rASCs) or bone (rBSCs) marrow of 3 weeks rat. The DMEM medium enriched with 10%fetal bovine serum was used for cultivation of rASCs or rBSCs. The surface markers of rASCs or rBSCs were studied by flow cytometry. The differentiation potential of rASCs or rBSCs were analyzed by osteogenic or adipogenic differentiation. The rASCs or rBSCs were treated with methanolic extract ofZataria-multiflora in different concentrations (1 to 320µg/ml) in 96-well plates. Finally, MTT assay was performed after 48, 72hours incubation. The aging of rASCs or rBSCs after Zataria multiflora treatment was studied by beta-galactosidase staining.
Results: Our results showed that Zataria multiflora significantly enhanced the viability and proliferation of rASCs as well as rBSCs ($p<0.05$). Interestingly, rASCs were more sensitive to Zataria multiflora, and the rASCs showed 1.25 times more proliferation than rBMSCs. Also, rASCs or rBSCs treated with Zataria multiflora showed less beta-galactosidase positive cells as compared to non-treated controls.
Conclusion: The results of our study revealed some possible proliferative and anti-aging potential for Zataria multiflora. Our findings could be applicable in safe herbal therapy by stem cells.
Keywords: Rat Mscs, Zataria Multiflora, MTT Assay

Ps-309: Fatty Acid Activated Pparγ Promotes Tumorigenicity of Prostate Cancer Stem Cells by Up Regulating VEGF via PPAR Responsive Elements of the Promoter

Farzad Seyed Forootan[1*], Youqiang Ke[2], Shiva Seyed Forootan[2]

1. Department of Cellular Biotechnology, Royan Institute of Biotechnology, ACECR, Isfahan, Iran
2. Molecular Pathology Laboratory, Department of Molecular and Clinical Cancer Medicine, Liverpool University, Liverpool L69 3GA, United Kingdom
Email: fsforootan@gmail.com

Background and Aim: In previous work, it is suggested that the excessive amount of fatty acids transported by FABP5 may facilitate the malignant progression of prostate cancer cells through a FABP5-PPARγ-VEGF signal transduction axis to increase angiogenesis.
Methods: To further functionally characterise the FABP5-PPARγ-VEGF signal transduction pathway, we have, in this work, investigated the molecular mechanisms involved in its tumorigenicity promoting role in prostate cancer.
Results: Suppression of PPARγ in highly malignant prostate cancer cells produced a significant reduction (up to 53%) in their proliferation rate, invasiveness (up to 89%) and anchorage-independent growth (up to 94%) in vitro. Knockdown of PPARγ gene in PC3-M cells by siRNA significantly reduced the average size of tumours formed in nude mice by 99% and tumour incidence by 90%, and significantly prolonged the latent period by 3.5 fold. Results in this study combined with some previous results suggested that FABP5 promoted VEGF expression and angiogenesis through PPARγ which was activated by fatty acids transported by FABP5. Further investigations showed that PPARγ up-regulated VEGF expression through acting with the PPAR-responsive elements in the promoter region of VEGF gene in prostate cancer cells. Although androgen can modulate VEGF expression through Sp1/Sp3 binding site on VEGF promoter in androgen-dependent prostate cancer cells, this route, disappeared as the cells gradually lost their androgen dependency; was replaced by the FABP5- PPARγ -VEGF signalling pathway.
Conclusion: These results suggested that the FABP5-PPARγ-VEGF signal transduction axis, rather than androgen modulated route, may be a more important

novel therapeutic target for angiogenesis-suppression treatment of castration resistant prostate cancer.
Keywords: Prostate Cancer, FABP5, PPARγ, PPRE, VEGF

Ps-310: More Effective Skin Rejuvenation Face Cream Using Ipscs-Shed Microvesicles

Najmeh Seyed-Katouli[1], Behnaz Bakhshandeh[1*], Zohreh Jahanafrooz[2], PegahTaklifi[1], Shiva Allahdadi[2], Shiva Daryani[2]

1. Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
2. School of Biology, College of Science, University of Tehran, Tehran, Iran
Email: n.katooli@ut.ac.ir

Background and Aim: Healthy skin integrity is maintained by epidermal stem cells which renew and produce cells undergo terminal differentiation. Furthermore, in response to injury and aging, these stem cells start to proliferate in order to form new tissue and cytokines/growth factors expression. Some successful pharmaceutical companies utilize stem cells in marketable face creams for better effects.Microvesicles are extracellular vesicles shed by most kind of the cells containing mRNA, noncoding RNA and proteins essential for functional and morphological regulations. We hypothesizedthat involved factors in wound healing and skin rejuvenationcould be found in microvesicles derived from the stem cells near the defect. So, they might be synthesized in order to employ in synthetic medicine with omitting the risk of stem cell application which may lead to carcinogenicity. Prior to microvesicle synthesis, the content of the microvesicles should be determined and the hypothetic function should be confirmed both in vitro and in vivo. Due to the potentials of the induced pluripotent stem cells (iPS cells) in personalized medicine, we collected microvesicles from the condition media of iPS cells cultures and identified the mRNA content. Some genes involved in fibroblast proliferation and collagen synthesis including PPARD, SRAT3, SEPT, INTEGRIN, FGF2, FGF7, GAPDH, AQP3, Col2A, FGFR2 were investigated. In continuation, we can synthesize a cream mixture involved microvesicle-like liposome containing mRNA of our interest for wound healing evaluation in rat models.
Methods: Prior to microvesicle synthesis, the content of the microvesicles should be determined and the hypothetic function should be confirmed both in vitro and in vivo. Due to the potentials of the induced pluripotent stem cells (iPS cells) in personalized medicine, we collected microvesicles from the condition media of iPS cells cultures and identified the mRNA content. Some genes involved in fibroblast proliferation and collagen synthesis including PPARD, SRAT3, SEPT, INTEGRIN, FGF2, FGF7, GAPDH, AQP3, Col2A, FGFR2 were investigated
Results: In continuation, we can synthesize a cream mixture involved microvesicle-like liposome containing mRNA of our interest for wound healing evaluation in rat models.
Conclusion: In continuation, we can synthesize a cream mixture involved microvesicle-like liposome containing mRNA of our interest for wound healing evaluation in rat models.
Keywords: Microvesicle, IPS Cells, Skin Rejuvenation, Wound Healing, Face Cream

Ps-311: Applications of CRISPR/Cas9 for Genome Editing of Cancer Stem Cell

Fateme Shaabanpour Aghamaleki, Shirin Farivar*

Department of Cellular and Molecular Biology, Faculty of Biology and Biotechnology Shahid Beheshti University G.C. Tehran, Iran
Email: s_farivar@sbu.ac.ir

Background and Aim: Medulloblastoma is a common malignant brain tumor in children. It is composed of small round undifferentiated, proliferating embryonic stem cells with the ability to undergo neuronal differentiation and metastasize within the central nervous system. The stem-like appearance of medulloblastoma cells support the cancer stem cell (CSC) hypothesis, i.e. CSCs are the origin of cancer and are required for tumor ongoing growth, although CSCs do not necessarily always come from normal stem cells as they may originate from progenitor cells or differentiated tumor cells which gain stem cell properties due to genetic or epigenetic changes. Dysregulation of signaling path-

ways, such as Hedgehog, Wnt, and Notch that regulate normal stem cell development, may contribute to medulloblastoma CSC formation or tumor initiation and propagation. Correction of the dysregulation in these signaling pathways may target medulloblastoma CSCs and eliminate tumor cells completely. CRISPR/Cas9 is one of technique that can be used for regulating of pathways. A simple version of the CRISPR/Cas9 system, CRISPR/Cas9, has been modified to edit genomes. By delivering the Cas9 nuclease complexed with a synthetic guide RNA (gRNA) into a cell, the cell's genome can be cut at the desired location, allowing existing genes to be removed and/or new ones added. CRISPR/Cas9 genome editing techniques have many potential applications, including medicine and crop seed enhancement.
Methods: Genes expression profiling by array of 19 human primary medulloblastoma and 3 human neural stem cells were extracted from Geo datasets. Genes are compared with logfc for their expression in normal neural stem cells and medulloblastoma and sorted by their p-value. Also, the most related pathway, genes function, and ontology are identified by DAVID database. Finally, genes with most modification and related to the critical pathway in CSC are selected and their best gRNAs (guide RNA) in CRISPR system are identified for editing by CHOPCHOP datasets.
Results: COMT gene has hyperexpression in medulloblastoma patients whereas GLI3 and WNT7B have hyperexpression in neural stem cells. The best gRNA of these genes in CRISPR/Cas9 system are identified that are located within promoter so they can regulate their expression. Genes that have hyperexpression in medulloblastoma play a key role in DNA damage and genes with hyperexpression in neural stem cells play a critical role in WNT signaling and homeobox. Also, important pathways of genes that have hyperexpression in medulloblastoma, are spliceosome and homologous recombination but important pathways of hyper-expressed genes in neural stem cells are cancer pathways, basal cell carcinoma and TGF-β signaling.
Conclusion: CRISPR/Cas9 system is the most recent method in genetic engineering that use gRNA for targeting impaired genes in various cell types like stem cells. Any genome editing in stem cells genome can inherit to daughter cells because of their pluripotency so this system can be used as therapeutic approach instead of common stem cells transplantation.

Keywords: Medulloblastoma, Neural Stem Cell, CRISPR/Cas9, Gene Editing

Ps-312: Comparison of Effects of Activated Omental Cell with Fat Derived Stem Cell on Rat Limbal Corneal Alkaline Injury

Athar Shadmani[1], Masoomeh Eghtedari[2*], Mahboobeh Razmkhah[1]

1. Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Ophthalmology, Shiraz University of Medical Sciences, Shiraz, Iran
Email: drshadmani@gmail.com

Background and Aim: To investigate whether autologous activated omental cells are as effective as fat derived stem cells, in healing process of alkaline burn in limbus of rats.
Methods: Corneal alkaline burn was created in three groups of six rats :Group 1:control group with topical medication, Group 2: subconjunctivally injected by autologous activated omental cell(AAOC) after alkaline burn induction , Group 3:subconjunctivally injected by non-autologous abdominal fat derived stem cells(FDSC).Clinical outcome was evaluated after 2 months by corneal reepithelization, corneal opacity and neovascularization. Pathologic study was done to asses corneal integrity and cell proliferation.
Results: After three weeks all three groups had relatively same degree of corneal neovascularization and opacity. But at the time of 2-month follow-up, Group3 rats showed the best clinical results with a clearer healed cornea compared with other groups. All rats on group 1 developed severe central corneal neovascularization and opacity with no healing. Auto evisceration was done in two of them. IN group 2:most cases achieved a clear cornea with peripheral neovascularization two of them developed central corneal neovascularization, but in group three, non of rats developed central corneal neovascularization and cornea was relatively clear in all member with no epithelial defects.
Conclusion: Also activated omental cells are effective in prevention of corneal neovascularization after alkaline burn, but Fat derived stem cells have the best result in corneal healing process

Keywords: Corneal Alkaline Burn, Fat Derived Stem Cell, Transplantation, Activated Omental Cells

Ps-313: Comparison of Amniotic Membrane and Adipose Tissue Derived Mesenchymal Stem Cells Characteristics

Hajar Shafaei1[1,2*], Jafar Soleimani Rad[1,2], Hojjatollah Nozad[1,2], Saba Shafaei[2], Khadijeh Dizaji Asl[1]

1. Department of Anatomical Science, Tabriz University of Medical Sciences, Tabriz, Iran
2. Stem cell research center, Tabriz University of Medical Sciences, Tabriz, Iran
3. School of Pharmacy ,Zanjan University of Medical Sciences, Zanjan, Iran
Email: shafaei49@gmail.com

Background and Aim: Mesenchymal stem cells (MSCs) are ideal candidates for treatment of diseases. Amniotic membranes are an inexpensive source of MSCs (AM-MSC) without any donor site morbidity in cell therapy. Adipose tissue derived stem cells (ASCs) are also suitable cells for cell therapy. There is discrepancy in CD271 expression among MSCs from different sources. Therefore, we compared the characteristics of AM-MSC and ASCs and CD271 expression.
Methods: Adult adipose tissue samples were obtained from patients undergoing elective surgical procedure, and samples of amniotic membrane were collected immediately after caesarean operation. After isolation and expansion of MSCs, the proliferation rate and viability of cells were evaluated through calculating DT and MTT assay. Expression of routine mesenchymal specific surface antigens of MSCs and CD271 was evaluated by flow cytometry for both types of cells.
Results: DT and MTT assay results revealed that the growth rate and viability of the MSCs from the amniotic membrane was significantly higher compared with the ASCs. The low expression of CD14 and CD45 indicated that AM-MSC and ASCs are non-hematopoietic cells, and both cell types expressed high percentages of CD44, CD105. The results revealed that AM-MSC and ASCs express no CD271 on their surface.
Conclusion: This study showed that amniotic membrane is a suitable cell source for cell therapy, and CD271 is a negative marker for MSCs identification from amniotic membrane and adipose tissue.
Keywords: Adipose Tissue, Amniotic Membrane, CD 271, Mesenchymal Stem Cells

Ps-314: Transplantation of Autologous Adipose Derived Mesenchymal Stem Cells for Improvement of Quality Of Life in Osteoarthritis Patients

Hajar Shafaei[1*], Hassan Bagernajad[2], Kosar Baghernezhad[2]

1. Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Bonyan Cellule Kosar company, East Azarbaijan Science and Technology Park, Tabriz, Iran
Email: shafaei49@gmail.com

Background and Aim: Osteoarthritis (OA) has been recognized, as the most common inflammatory disease in the world. Adipose stem cells (ASCs), as a new feasible source with high numbers of stem cells and proliferative capacity have been used for regenerative medicine. Based immunomodulatory and chondrogenic properties of adipose stem cells (ASCs), this study aims to assess intra articular injection of ASC effect on improvement of osteoarthritis signs.
Methods: Adipose tissue samples were obtained from subcutaneous of abdomen (IRCT2015072523342N1). ASCs were isolated and cultured for at least three passages in culture media containing autologous serum and expanded them to 15-20 ×106 cell. The morphology and proliferative potency of ASCs were determined. Immunophenotype characteristics of ASCs were analyzed by flow-cytometry. Then cell suspensions were injected into knee articular spaces. After 6 months the function of knee was assessed by WOMAC, KOOS, Lysholm and Lequesne indexes.
Results: the results of this study showed that homogenous spindle-shape ASCs expanded rapidly with low doubling time. The low expression of CD14 and CD45 indicated that ASCs are non hematopoietic cells and expressed high percentages of CD44, CD105 and CD90. Our results showed that injected ASCs were effective in improvement of OA by scoring systems for evaluation of pain, joint movements and daily physical activities

were significantly changed due to injection of stem cells. Osteoarthritis severity indexes means of WOMAC andLequesne were decreased from 53 to 12.3 and 15.1 to 2.1 respectively. Also osteoarthritis improvement indexes Lysholm ,KOOS means were significantly increased from 35 to 15.1 and 70 to 126.7 respectively. In six months follow up of intraarticular injection of ASCs, we observed no local or systemic side effect. After ASCs injection walking distance considerably increased. The flexion angle of knee improved by 20-30 degrees relation to before of treatment.
Conclusion: Autologous ASCs injection resulted in increasing of knee function, alleviated of pain and quality of life improvement.
Keywords: ASC, Osteoarthritis, Stem Cell Therapy, Alleviation of Pain, Intra Articular Injection

Ps-315: Intraarticular Injection of Cocultured Adipose Stem Cells by Chondrons for Repair of Articular Cartilage of Induced Osteoarthritis

Hajar Shafaei*, Sorayya Jacer, Jafar Soleimani Rad, Elaham Hosseinzadeh

Department of Anatomical Sciences, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Email: shafaei49@gmail.com

Background and Aim: Osteoarthritis (OA) has been recognized, as the most common inflammatory disease in the world. Adipose stem cells (ASCs), as a new feasible source have been used for regenerative medicine. Chondrogenic induction of ASCs by chemical and physical factors resulted in nonfunctional chondrocyte. Isolated chondrocyte and pericellular matrix (PCM) is called chondron which produce natural signals to induce chondrogenesis. This study aimed to assess intraarticular injection of cocultured ASCs with chondrons on improvement of induced osteoarthritis.
Methods: ASCs of rats were isolated and cultured for at least three passages in culture media. Floating chondrons in cultures from rats is applied for injection to preserve their PCM. OA was created by intraarticular injection of type II collagenase in rats. Rats were divided into 5 groups: control (C), osteoarthritic (OA), chondron (CHN), ASC and cocultured (CO). After 4 weeks, treatments were done by single intraarticular injection of 10^7 cells in CHN and ASC groups and in CO group (106 of chondron with 5×10^6 of ASC. The effects of the treatment were monitored macroscopically and microscopically after three months Sections were stained with toluidine blue and H&E.
Results: We found that collagenase type II destroyed articular cartilage and resulted in thickness of synovial membrane in OA group in comparison with control groups. Intraarticular injection of each group of cells repaired damaged articular cartilage. In ASC group, isogenous chondrocytes were more than controls. In CO group, high collagen type I is observed in repaired tissue that indicated spontaneous fibrocaltilage formation. Cell therapy has no improvement on thickness of synovial membrane.
Conclusion: Intraarticular injection improves histological appearance of articular hyaline cartilage in induced OA.
Keywords: ASC, Cell therapy, Chondron, Coculture, Induced Osteoarthritis

Ps-316: Design and Development of Conductive PLGA Microsphere for Biomedical Application

Akram Shafiee[1*], Fatemeh Atyabi[1], Rassoul Dinarvand[1], Massoud Soleimani[2]

1. Deparment of Pharmaceutics, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Hematology and Blood Banking, Faculty of Medicine, Tarbiat Modaress University, Tehran, Iran
Email: akmshafiee@gmail.com

Background and Aim: Electrically conductive systems enthuse researchers to study on their biomedical application, such as drug delivery systems, biosensors, biomedical implants and tissue engineering. In fact bioelectricity exists during major cellular events such as cell division, migration, and maintaining normal biological functions in many organs such as: nervous system, cardiac and other muscles. Additionally, researchers have used electrical signals to control the release of molecules via conducting drug delivery systems. Conducting systems are classified into two major groups, including the first one conductive and electroactive pol-

ymers and second one conductive polymer composites. These composites are made of carbon filler based such as carbon black, carbon fiber, Carbon nanotube (CNT), Graphene, and Graphene oxide or non-carbon filler based, such as gold nanoparticles, copper nanowires, and silver. The aim of current work was to prepare and characterize conductive microspheres with PLGA and functionalized carbon nanotube.
Methods: Double emulsion technique was used for conductive PLGA microsphere preparation: 100 mg PLGA was dissolved in 1ml DCM containing span 20 (1%) as emulsifying agent. 100 µl of various concentrations of CNT into PBS was emulsified in PLGA solution using probe sonicator for 3 minuts. Produced emulsion was drop wise added to 200 ml PVA 0.5% in water under overhead stirrer (300rpm). Produced microspheres was stirred for 4h to evaporate internal solvent. Morphology and size of microspheres was investigated using scanning electron microscopy (SEM) and master sizer respectively. Conductivity was determined using two probe electrical conductivity meter. To realize the presence of carbon nanotube into microsphere structure Raman spectroscopy was used.
Results: Conductive microspheres were prepared in three levels ((2.5, 5, and 10 %) of functionalized CNT in two groups (140-165 and 180-200 µm). The results demonstrated an increase in the microspheres size and CNT concentration raise conductivity. Raman spectroscopy confirmed presence of CNT into PLGA microsphere. The porosity of microspheres was evident from SEM.
Conclusion: Composition of PLGA and CNT's provide a promising candidate for developing conductive microspheres which are potentially used in electrically triggered drug delivery systems and tissue engineering as conductive scaffold.
Keywords: Microsphere Conductive PLGA Carbon Nano Tube

Ps-317: Electrospun Polycaprolactone/Carbon Nanotube Scaffold for Bone Tissue Engineering

Hadi Tohidloo[1,2], Seyedeh Sara Shafiei[1*], Mehrnoosh Fathi[1]

1. Stem Cell and Regenerative Medicine Department, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Iran
2. Islamic Azad University of Damghan, Damghan, Iran
Email: sarashafiei@aut.ac.ir

Background and Aim: Bone tissue engineering with the aim of repairing bone defects and bone injuries has been trying to design an appropriate scaffold with optimal mechanical and biological properties that can play an important role in this regard.
Methods: In this study, different amounts of amine-functionalized single-walled carbon nanotubes (SWCNTs-amine) were composite with a biocompatible polymer, polycaprolacton, to enhance biological and mechanical properties of scaffolds. PCL-SWCNTs composite nanofibers with the 0, 0.0, 0.0 and 0.0 weight percentages (%wt) were prepared by electrospinning method. The attachment, proliferation, differentiation and growth of rat bone marrow derived mesenchymal stem cells (BMSCs) on the scaffolds were analyzed by scanning electron microscopy (SEM), MTT, live-dead and alkaline phosphatase activity assays. The morphology and mechanical properties of the scaffolds, using the SEM and tensie strenght test, were characterized and the bioactivity of the scaffolds in simulated body fluid (SBF) was assessed.
Results: The results indicated that PCL-SWCNTs 0.0 wt. % had the highest tensile strength (about 00 MPa) and showed a significant increase in compared with pure PCL. Moreover, no toxicity were reported after 0, 3 and 0 days after cell seeding on scaffolds. In addition, surveys carried out by SEM showed that the use of single-walled carbon nanotubes, had promote cell attachment on the scaffold fibers. This increase was more considerable in PCL-SWCNTs 0.0 wt. %. Furthermore, alkaline phosphatase activity demonstrated enhanced proliferation and differentiation of cells on scaffolds containing nanoparticles in comparison with pure PCL.
Conclusion: It is concluded that electrospund SWCNTs/PCL nanofibers with the optimum concenteration can be a good candidate for bone tissue engineering applications.
Keywords: Polycaprolactone, Single-Walled Carbon Nanotubes, Electrospinning, Nanocomposite, Bone Tissue Engineering

Ps-318: Infants Hypoxia Effect on Hematopoietic Stem Cells Line of Cord Blood

Ahmad Shah Farhat[1], Abolfazl Nosrati Tirkani[2*], Dariyuosh Hamidi Alamdari[2], Mohammadhassan Arjmand[2], Sima Tavakolinezhad[2]

1. Pediatrics Ward Neonatal Research Center, Mahhhad, Iran
2. Department of Medical Biochemistry, Mashhad University of Medical Sciences, Mashhad, Iran
Email: farhata@mums.ac.ir

Background and Aim: Background: Prenatal asphyxia or birth asphyxia is the medical situation resulting from deprivation of oxygen to a newborn infant that lasts long enough during the birth process to cause physical harm usually to the brain. Human umbilical cord blood (UCB) is a well-established source of hematopoietic stem/progenitor cells (HSPCs) for allogeneic stem cell transplantation. These can be used clinically to care for children with several disease such as malignant hematologic metabolic and immunologic diseases. In asphyxiated neonates brain and other organs will damaged by hypoxia, but we do not knowe about hypoxia effect on umbilical cord hemopoietic stem celles thus we studied the hypoia effect of asphyxiated neonates on umbilical cord hemopoietic stem celles.
Methods: the cord blood of 11 infants with 3-5 Apgar score or need to cardiac pulmonary Resuscitation as an asphyxia group and ten normal infants with more than 8 Apgar score as normal group was collected and after isolating hematopoietic stem cells, the cells were cultured in enrich media for 14 days to compare the numbers of colonies by microscope.
Results: There was a significant difference of number of RBC precursor's colonies (red colonies) in cultured media with 107 cord blood hematopoietic stem cells of infants who exposed to hypoxemia in two well of palate. There was not a significant difference in number of white cells colonies in two groups in two well of plate.
Conclusion: hypoxia in the perinatal period can cause the increase of hematopoietic stem cells of cord blood special red precursors stem cells in vitro like increase of red blood cell in the body when is exposed to low oxygen condition. But did not have effect on WBC
Keywords: Stem cells, Asphyxia, Hypoxia, Infants

Ps-319: Biocompatible and Biodegradable Poly (Tetramethylene Ether) Glycol /Poly (ε-Caprolactone Diol) Based Polyurethanes for Tissue Engineering Scaffolds

Ehsan Shahrousvand[1], Mohsen Shahrousvand[2*], Mohammad Shahrousvand[2], Marzieh Ghollasi[3], Ali Salimi[4]

1. Department of Chemistry, Malek-ashtar University of Technology, Shahin-shahr, Iran
2. Department of Polymer Engineering & Color Technology, Amirkabir University of Technology, P.O. Box 15875/4413, Tehran, Iran
3. Department of Cell and Molecular Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
4. Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
Email: ehsan.shahruz@gmail.com

Background and Aim: The cells as a tissue component need to viscoelastic, biocompatible, biodegradable and wettable extracellular matrix (ECM) for their biological activity. In this study, in order to prepare biomedical polyurethane elastomers with good mechanical behavior and biodegradability, a series of novel polyester-polyether based polyurethanes (PUs) were synthesized using a two-step bulk reaction by melting pre-polymer method, taking 1, 4-butanediol (BDO) as chain extender, hexamethylene diisocyanate (HDI) as the hard segment, and poly (tetramethylene ether) glycol (PTMEG) and poly (ε-caprolactone diol) (PCL-Diol) as the soft segment without a catalyst. The soft to the hard segment ratio was kept constant in all samples. Polyurethane characteristics such as thermal and mechanical properties, wettability and water adsorption, biodegradability and cellular behavior changed by changing the ratio of polyether diol to polyester diol composition in the soft segment. Our present work provides a new procedure for the preparation of engineered polyurethanes in surface properties and biodegradability, which could be a good candidate for bone, cartilage and skin tissue engineering.
Methods: Fabrication of polyurethane thin films a 20 % solution (w/w) of the PU was dissolved in DMF. 3 g of the solution was poured in a mold with dimensions of 10 x 10 cm2 which was polished by 800-grit sand-

paper. The template was placed in a vacuum oven at 70 °C for 24 h. PU-films were washed three times with distilled water after separation from the mold. The resulted PU films were optically clear and very thin when viewed under light microscopy. Cell culture & seeding the fibroblast cells (SNL) were kindly supplied by Stem Cell Technology Research Center and maintained in a T-75 culture flask. The cells were cultured in Dulbecco's modified eagle medium (DMEM) with 10% fetal bovine serum (FBS) penicillin G sodium (10 units·ml−1) and streptomycin sulfate (10 mg·ml−1) all from Gibco BRL (NY, USA) in a humidified atmosphere of 5% CO2 at 37 °C. Culture media was replaced every 48 hours until the cells reached 80% confluency and cells at passage 2 were used for the study. The adhesion of SNL cells on PU films were qualitatively evaluated using SEM and OM.

Results: Biodegradable and biocompatible polyurethane films were obtained through the prepolymer method based on PCL and PTMEG as polyol. Different compositions of polyols PTMEG and PCL were used and the reaction proceeded without the use of any catalyst. The structural, mechanical and biological features of synthesized polymers were studied. Based on the FTIR, DSC and tensile results, hydrogen interactions between ether groups in the PTMEG and urethane groups cause the more miscibility in the soft and hard segment; therefore mechanical properties, wettability and biodegradability were changed. Furthermore, the mechanical properties are also an important biomaterial selection. Gradient in mechanical properties of the PU-100 to PU-0 make different and useful perspective for various medical applications. For example, cartilage tissue has an elastic modulus in the range of 0.7–15.3 MPa and a tensile strength of 3.7–10.5 MPa.

Keywords: Biocompatible, Biodegradable, Polyurethanes, Thermo-Mechanical Properties, Biomedical Application

Ps-320: Preparation of PES-PEG Electrospun Fibrous Containing of Zn2SiO4 Bioceramic Nanoparticles for Osteogenic Differentiation hMSCS

Mohammad Shahrousvand[1], Mohsen Shahrousvand[1*], Marzieh Ghollasi[2], Ali Salimi[3]

1. Department of Polymer Engineering and Color Techology, Amirkabir University of Technology, Tehran, Iran
2. Department of Cell and Molecular Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
3. Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Email: mohsen.shahrousvand@gmail.com

Background and Aim: Tissue engineering has attracted a great deal of interest by combining fibrous scaffolds and stem cells regarding bone regeneration applications. In the present study, polymeric fibrous polyethersulphone-polyethylene glycol (PES-PEG) was fabricated by electrospinning. It was then treated with NH3 plasma to enhance surface hydrophilicity, cell attachment, growth and differentiation potential. X-ray photoelectron spectroscopy (XPS) measurements were used to evaluate the modification of the scaffold's surface chemistry. Electrospun scaffolds were coated with willemite (Zn2SiO4) bioceramic nanoparticles. Scaffold characterization was done by scanning electron microscope (SEM), differential scanning calorimetry (DSC), contact angle measurements and tensile analysis. MTT assay was used to assess the biocompatibility of fibrous scaffolds loaded with Zn2SiO4 regarding proliferation support. Osteogenic differentiation of cultured human mesenchymal stem cells (hMSCs) on fibers was evaluated using common osteogenic markers such as alkaline phosphatase (ALP) activity, calcium mineral deposition, quantitative real-time PCR (qPCR) and immunocytochemical analysis (ICC). According to the results, proliferation and osteogenic differentiation of hMSCs were significantly enhanced after coating Zn2SiO4 on fibrous scaffolds. These results were detected by higher ALP activity, biomineralization and expression of osteogenic related genes and proteins in differentiated hMSCs. In conclusion, our results indicated that the combination of Zn2SiO4 nanoparticles and electrospun fibers is able to provide a new, suitable and more efficient matrix to support stem cells differentiation for bone tissue engineering applications.

Methods: Electrospun Scaffold Fabrication Electrospinning has been used for preparation of PES-PEG fibers (34% W and 70/30 ratio). In this method, PES-PEG solution was collected on a collector from a blunted needle syringe pump at a rate of 0.5 ml/h. The collector was a rotating cylindrical drum used to collect the elec-

trospun fibers placed at a distance of 15 cm from the needle. Surface Modifications For plasma treatment, a microwave plasma generator of 2.45 GHz frequency with a cylindrical quartz reactor (Diener Electronics, Germany) was used. NH3 plasma treatment was performed for 5 minutes under conditions of 45 W power and 0.5 mbar pressure, then the glow discharge was ignited for 4 minutes. After that, fiberous scaffolds were immersed in the ethanol solution containing 1 wt. (%) Zn2SiO4 according to the protocol reported by JamshidiAdegani et al. [11] Scaffolds were subsequently rinsed with distilled water and used for cell seeding and surface characterization.
Results: In conclusion, PES-PEG electrospun fibers coated with willemite nanoparticles could be used as an appropriate scaffold for efficient regeneration of bone defects. These synthetic ceramic-coated polymeric fibers show promising applications in bone tissue engineering. In the future, these scaffolds can be used in vivo analysis which will have a positive effect on bone healing in critical-size bone defects.
Conclusion: In conclusion, PES-PEG electrospun fibers coated with willemite nanoparticles could be used as an appropriate scaffold for efficient regeneration of bone defects. These synthetic ceramic-coated polymeric fibers show promising applications in bone tissue engineering. In the future, these scaffolds can be used in vivo analysis which will have a positive effect on bone healing in critical-size bone defects.
Keywords: Osteoblast Differentiation, Mesenchymal Stem Cells, PES-PEG Fibers, Zn2SiO4 Nanoparticles

Ps-321: Osteogenic Differentiation of Hmscs on Polyurethane/Cellulose Nanocrystal Bimodal Foam Nanocomposites

Mohammad Shahrousvand[1], Mohsen Shahrousvand[2*], Ehsan Shahrousvand[3], Hafez Jafari[4], Ali Salimi[5]

1. Department of Polymer Engineering & Color Technology, Amirkabir University of Technology, Tehran, Iran
2. Young Researchers and Elites Club, Science and Research Branch, Islamic Azad University, Tehran, Iran
3. Department of Chemistry, Malek-ashtar University of Technology, Shahin-shahr, Iran
4. Department of Cell and Molecular Biology, Faculty of Biological Science, Kharazmi University, Tehran, Iran
5. Nanobiotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
Email: mohammadshahrousvand@yahoo.com

Background and Aim: Biocompatible and biodegradable polyurethanes (PUs) based on polycaprolactone diol (PCL) were prepared and were filled by cellulose nanowhiskers (CNWs) obtained from wastepaper. Incorporated polyurethane nanocomposites were used for preparation of foamed scaffolds with bimodal cell sizes by solvent casting/particulate leaching method. Sodium chloride and sugar porogens were prepared to fabricate the scaffolds. The mechanical and thermal properties' of PU/CNW nanocomposites were investigated. The advantage of incorporating various CNWs is to have tunable mechanical properties and biodegradability due to variety in their structure. All the bimodal foam nanocomposites were observed to be biodegradable as well as non-cytotoxic as revealed by MTT assay in fibroblast cell line SNLs. The PU/CNW foam scaffolds were used for osteogenic differentiation of human mesenchymal stem cells (hMSCs). Our results suggest that these PU/CNW nanocomposites, in conjunction with hMSCs proliferation, could also support osteogeneses them in three-dimensional synthetic extracellular matrix (ECM).
Methods: Cellulose Nanocrystals were prepared from wastepaper similar to previous work [27]. Briefly, The cellulose microfibers (10 g - based on dry weight) were hydrolyzed in 190 mL phosphoric acid solution (85 wt%) under strong agitation at 80 °C for 30 min. Hydrolysis was terminated by adding 300 mL cold water. The diluted suspension was centrifuged at 10,000 rpm for 5 min to obtain a precipitate. The precipitate was re-suspended in water with strong agitation, followed by centrifugation. This process was repeated until the pH of the suspension reached 5 then dialysis was carried out for 3 days until the pH became constant. Subsequently, the suspension dried at 40 °C for 24 h and was sonicated to disperse the nanofibers in DMF using an ultrasonic homogenizer at 19.5 kHz and 300 W output power (26 mm probe tip diameter, US-300T, Nissei, Japan) for 5 min for further enhancing the CNWs extraction yield.
Results: The aim of the present study was to fabricate bimodal nanocomposite foams based on PU reinforced by cellulose nanocrystals and to investigate its poten-

tial to support the adhesion, proliferation and osteogenic differentiation of hMSCs. The tensile strength and modulus of the high porous PU/CNW nanocomposites were increased effectively by addition of CNW because of both the reinforcing effect from stiff nanowhiskers in the rubbery matrix and strong interfacial adhesion between PU and CNW surfaces. Various analyses such as ALP activity, calcium content, alizarin red, mineralization staining and SEM micrograph have demonstrated which these connective porous nanocomposites are suitable for osteogenesis of hMSCs.
Conclusion: The aim of the present study was to fabricate bimodal nanocomposite foams based on PU reinforced by cellulose nanocrystals and to investigate its potential to support the adhesion, proliferation and osteogenic differentiation of hMSCs. The tensile strength and modulus of the high porous PU/CNW nanocomposites were increased effectively by addition of CNW because of both the reinforcing effect from stiff nanowhiskers in the rubbery matrix and strong interfacial adhesion between PU and CNW surfaces. Various analyses such as ALP activity, calcium content, alizarin red, mineralization staining and SEM micrograph have demonstrated which these connective porous nanocomposites are suitable for osteogenesis of hMSCs.
Keywords: Polyurethane (PU), Cellulose Nanowhiskers (CNWs), Human Mesenchymal Stem Cells (hMSCs), Osteogenic Differentiation, Bimodal Foam Scaffold

Ps-322: Fabrication of Gelatin/ Graphene Nanofibrous Scaffolds for Endothelial Regeneration

Atefeh Shamosi, Alireza Shams*

Department of Anatomy, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
Email: a.shamosi@gmail.com

Background and Aim: Angiogenesis is a complicated process that contains the contribution of endothelial cells and numerous morphogens and angiogenic growth factors. In physiological conditions, it is essential for the growth, development and maintenance, tissue regeneration, as well as in wound healing, and ovulation during menstrual cycle. Nanoscale microenvironment mimics invivo condition for cells repair and regeneration. Nanofibrous scaffolds of gelatin/ graphene nanopowders (GEL/ GNPs) were investigated their capacity for increasing Wharton's Jelly Mesenchymal Stem Cells (WJMSCs) to differentiate into the endothelial-like cells in the presence of FGF-2 and VEGF angiogenic factors for this purpose.
Methods: GEL nanofibrous scaffolds with different concentrations of graphene nanopowders (GNPs) (1, 3, 5, 7 and 9 wt%) were fabricated and assessed their physicochemical properties, as well as the influence of fiber diameter and uniformity on the proliferation and distribution of WJMSCs. Expression of endothelial cell genes, and endothelial CD31, VE-cadherin, and KDR markers in differentiated cells on the GEL/ GNPs scaffolds were evaluated by real-time PCR and immunocytochemistry analysis.
Results: SEM results showed the diameter of nanofiber increased from 160 to 273 nm when the GNPs concentration increased in GEL/ GNPs scaffolds. SEM and MTT analysis denoted that the GEL/ 7% GNPs (7.5 wt % /7 wt %) scaffold supported better adhesion and proliferation of the WJMSCs. Moreover, in vitro study indicated that 7% concentration of GNPs in the GEL/ GNPs nanofibrous scaffold provided a suitable three-dimensional (3D) structure for endothelial cells differentiation from WJMSCs and increased expression of the endothelial CD31, VE-cadherin, and KDR markers.
Conclusion: Gel/ 7 % GNPs nanofibrous scaffold could be supportive for WJMSCs adhesion, proliferation and differentiation which in turn allows for reproducible in vitro and in vivo analyses in further studies.
Keywords: Wharton's Jelly Mesenchymal Stem Cells, Endothelial Cells, Gelatin/ Graphene Nanopowders, Nanofibrous Scaffolds

Ps-323: Could A PLGA – Gelatin Scaffold Prepared by Freeze Casting Method be Suitable for Stem Cell Differentiation in Neuronal Cell Therapy?

Alireza Shams[1*], Atefeh Shamusi[1], Mehdi Abbasi[2]

1. Department of Anatomy, Faculty of Medicine, Alborz University of Medical Sciences, Karaj, Iran
2. Department of Anatomy, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: alirezashams2014@gmail.com

Background and Aim: In U.S has reported about 1.4 million new case of external physical trauma for every year which resulted in about 800000 cerebral and 275000 spinal disabilities. Cell engineering is one of the new medical methods for treatment of disabilities due to neuronal defects. In this study, we followed implantation and differentiation of Bone marrow mesenchymal stem cells (BMMSCs) on PLGA -gelatin scaffolds to neural cells to introduce a new method for cell implantation in neuronal defects.
Methods: The scaffolds was produced by PLGA and gelatin with freeze casting method. BMMSCs were obtained by primary cell culturing from male adult bone marrow by flushing. For this purpose was seeded 5×105 cell on scaffolds in experimental and control groups. The cells was incubated with DMEM/F12+10% FBS& 1% pen/strep. Then BMMSCs seeded on scaffolds and cultured for 7 days. 5 µ mol. Of Retinoic acid and 100 µ mol. of ascorbic acid was added to media for differentiation. The cells were tested by MTT assay for cell survival. Specific gene expression was evaluated by Real Time PCR. At the end the morphology of cell in scaffold were checked and investigated for 15 days. Data were analyzed by analysis of variance and statistical significance was determined by the Tukey's test. A p value <0.05 was considered to be significant.
Results: Viability of cells on scaffold compare to other groups was examined with MTT assay in days of 1, 3 and 5 after incubation. The cell survival rate in 3 and 5 days was significant. Therefore, after 20 days the results showed increasing of expression of genes of Nestin and MAP2 in neuronal cells. Gene expression of cells with PLGA - gelatin scaffold was more significant than cell group without scaffold (P<0.001).
Conclusion: In this research we studied producing of PLGA and gelatin scaffold with freeze casting method. Results showed differentiation of stem cell on it. Gelatin-PLGA freeze casting scaffold can be an excellent candidate for improving repair and regeneration in central nervous system. This scaffold could use as a base for induction of neurogenesis in defected neuronal tissue. It may make a new sight and hopes.
Keywords: Stem Cell, Tissue Engineering, Growth Factor, Scaffold, PLGA – Gelatin Scaffold, Mesenchymal Stem Cell

Ps-324: Preparation and Evaluation of Chitosan and Its Derivatives Scaffolds for Hard Tissue Engineering Application

Fereshteh Sharifi Ferdoey[1], Seyed Mohammad Atyabi[2*], Dariush Norouzian[2], Mojgan Zandi[3], Shiva Irani[1]

1.Department of Biology, School of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
2.Department of Pilot Biotechnology, Pasteur Institute of Iran, Tehran, Iran
3.Department of Biomaterials, Iran Polymer and Petrochemical Institute, Tehran, Iran
Email: fer.sharifi.368@gmail.com

Background and Aim: Electrospinning is a versatile and unique technique which produces fibers with diameters in the range from µm down to nm. This technique can produce fibers with high similarity from aspects of structural and textural to the architecture of natural extracellular matrix (ECM). Therefore, fibers fabricated through electrospinning have great potential for determining cell response in tissue regeneration medicine. Another key factor has unavoidable role is a kind of biomaterial used for fabricating scaffold. One of the most practical and favorable natural biomaterial in biomedical application is chitosan and carboxymethyl chitosan (CMC) derivative. Although these biomaterials have advantages like: biocompatible, biodegradable and versatile, blending these biomaterials with synthetic biopolymer such as poly caprolactone (PCL) may be improve and enhance their stability and mechanical properties. In this literature, we studied the effect of various percentages of natural biomaterial and its derivative blending with PCL on human mesenchymal cells (hMSCs) behavior.
Methods: Blending chitosan-PCL and CMC-PCL were fabricated through electrospinning technique. Nano-fibers morphology was evaluated by scanning electron microscope (SEM). Scaffold biocompatibility and proliferation rate were assessed using inverted microscope and MTT test, respectively.
Results: As expected, the result of SEM image exhibited suitable and applicable fiber morphology. According to biocompatible experiments, designed scaffolds had suitable condition for using in biomedical regeneration. Afterward, the graph obtained from MTT assays

showed that the proliferation rate of hMSCs on CMC-PCL scaffolds significantly better than chitosan-PCL and control group.
Conclusion: Our results demonstrated that blending CMC-PCL would creating condition and situation with highest similarity to the natural ECM.
Keywords: Tissue Engineering, Carboxymethyl Chitosan, Human Mesenchymal Cells, Electrospinning

Ps-325: Preparation of Collagen/Polyurethane/Knitted Silk as a Composite Scaffold for Tendon or Ligament Tissue Engineering

Maryam Sharifi-Aghdam[1], Reza faridi-Majidi[1], Mahmoud Azami[2*], Mohammad Ali Derakhshan[1]

1. Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
Email: marya.sharif90@yahoo.com

Background and Aim: The main objective of this study was to prepare a hybrid three-dimensional scaffold that mimics natural tendon tissues.
Methods: It has been found that a knitted silk shows good mechanical strength, however, cell growth on the bare silk is not desirable. Hence, electrospun Collagen (COL)/polyurethane (PU) combination was used to cover knitted silk. A series of COL and PU solutions (4 -7 %w/v) in aqueous acetic acid were prepared and electrospun. According to obtained SEM images from pure COL and PU nanofibers, a concentration was set constant on 5 (%w/v) for blend solutions of COL/PU. Afterward, blend solutions with the weight ratios of 75/25, 50/50 and 25/75 were electrospun.
Results: SEM images demonstrated the smooth and uniform morphology for the optimized nanofibers. The least fibers diameter among three weight ratios was found for COL/PU (25/75) which was 100.86±40 nm and therefore was selected to be electrospun on the knitted silk. ATR-FTIR spectra confirmed the chemical composition of obtained electrospun nanofibers on the knitted silk. Tensile test of the specimens including blend nanofiber, the knitted silk and commercial tendon substitute examined and indicated that COL/PU coated knitted silk has an appropriate mechanical properties as a scaffold for tendon tissue engineering. Then, Alamar Blue assay of the L929 fibroblast cell line on the prepared scaffolds demonstrated appropriate viability of the cells with a significant proliferation on the scaffold containing more COL content. The results illustrate that the designed structure would be promising for being used as a temporary substitute for tendon repair.
Conclusion: Tendon and ligament tissues have very complicated mechanical properties and unique structure. The complexity of this tissue, addressed us to design a scaffold with a set of features. Electrospun COL nanofibers didn't show desirable feature lonely for tendon or ligament scaffolding, so a combination of COL with strong knitted silk was chosen to provide the superior mechanical properties than pure COL. According to the initial experiment, adding PU was needed to increase adhesion between COL and silk substrate. Thus, knitted silk is covered by blend electrospun nanofibers. COL/PU nanofibrous coating notably provided a better condition for cellular attachment and proliferation. Finally, according to the obtained results, the most appropriate ratio of COL/PU for being coated on the knitted silk was (25/75) and according to the mechanical analysis result, the final product can be potentially used in coated form as a proper scaffold for tendon tissue engineering.
Keywords: Collagen, Polyurethane, Knitted Silk, Tendon, Ligament, Tissue engineering, Electrospinning

Ps-326: Unrestricted Somatic Stem Cells as a Feeder Layer to Support Embryonic Stem Cells

Saeed Heidari-keshe[1*]

Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: Saeedhey@gmail.com

Background and Aim: The use of unrestricted somatic stem cells (USSCs) holds great promise for future clinical applications. Conventionally, mouse embryonic fibroblasts (MEFs) or other animal-based feeder layers are used to support embryonic stem cell (ESC) growth; the use of such feeder cells increases the risk of retro-

viral and other pathogenic infection in clinical trials. Implementation of a human-based feeder layer, such as hUSSCs that are isolated from human sources, lowers such risks.
Methods: Isolated cord blood USSCs derived from various donors were used as a novel, supportive feeder layer for growth of C4mES cells (Royan C4 ESCs). Complete cellular characterization using immunocytochemical and flow cytometric methods were performed on murine ESCs (mESCs) and hUSSCs. mESCs cultured on hUSSCs showed similar cellular morphology and presented the same cell markers of undifferentiated mESC as would have been observed in mESCs grown on MEFs.
Results: Our data revealed these cells had negative expression of Stat3, Sox2, and Fgf4 genes while showing positive expression for Pou5f1, Nanog, Rex1, Brachyury, Lif, Lifr, Tert, B2m, and Bmp4 genes. Moreover, mESCs cultured on hUSSCs exhibited proven differentiation potential to germ cell layers showing normal karyotype. The major advantage of hUSSCs is their ability to be continuously cultured for at least 50 passages. We have also found that hUSSCs have the potential to provide ESC support from the early moments of isolation.
Conclusion: Further study of hUSSC as a novel human feeder layer may lead to their incorporation into clinical methods, making them a vital part of the application of human ESCs in clinical cell therapy.
Keywords: Feeder Layer Embryonic Stem Cell

Ps-327: A New Derivative Indole Affects Expression of SALL4 in the Acute Promylocytic Leukemia Cell Line (NB4)

Zahra Sheikhrezaei*, Parisa Heidari, Gholamhossein Hassanshahi

Department of Hematology and Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Kerman University of Medical Sciences, Kerman, Iran
Email: s.sh.rezaei@gmail.com

Background and Aim: Acute myeloid leukemia (AML) is described as a colonal disorder happening due to bone marrow failure and uncontrolled proliferation of myeloid lineage cells. Acute promyelocyte leukemia (APL) is a subtype of AML; more recently, new treatments have been developed for cancer treatment. Heterocyclic compounds, such as indole, are now considered as attractive candidates for cancer therapy, due to their abundance in nature and known biological activity. Therefore, the aim of this study was to evaluate effects of a new indole derivative (TFPHC) on expression of SALL4, as a zinc finger transcription factor in the multipotency of stem cells, in the NB4 cell line.
Methods: In this experimental study, NB4 cells were cultured and treated with different concentrations (75, 150, and 300 µg/mL) of the new indole derivative and DMSO, as a vehicle control, for 24 and 48 hours. Afterwards, cell proliferation was evaluated by using trypan blue exclusion and MTT (Methyl Thiazol-Tetrazolium) assays. The percentage of apoptotic cells was determined by flowcytometry analysis using the Annexin V/PI apoptosis detection kit; mRNA expression of SALL4 was studied using absolute quantitative real time PCR. Data were analyzed by student's t-test. P values less than 0.05 were considered to be statistically significant.
Results: Our findings demonstrated the effects of new indole derivatives on SALL4 mRNA expression. Expression of SALL4 mRNA significantly decreased at concentrations of 75, 150, and 300 µg/mL.
Conclusion: According to our results, the SALL4 transcript plays a role in the survival of APL cells; in addition, SALL4 expression could be suppressed by the novel indole derivative. This study may also indicate that the SALL4 gene suppression can serve as a target in the APL therapy.
Keywords: AML, APL, SALL4, Indole

Ps-328: Phase I Clinical Trial of Bone Marrow Mesenchymal Stromal Cells in CKD Patients: 18 Months Follow Up

Soroosh Shekarchian[1], Atieh Makhlough[2], Reza Moghadasali[3], Seyedeh Esmat Hosseini[1], Nasser Aghdami[1*]

1. Department of Regenerative Biomedicine, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran Iran
2. Department of Nephrology, Molecular and Cell Biology Research Center, Sari University of Medical Sciences, Sari, Iran

3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
Email: soroosh.shekarchian@gmail.com

Background and Aim: Chronic kidney disease (CKD) affects kidney function and structure which finally result in end stage renal disease. It has been shown that cell-based therapies could improve impaired renal parameters in preclinical models of CKD in a metanalysis report. We had planned to evaluate the safety and tolerability of the bone marrow Mesenchymal stromal cells (BM-MSCs) infusion in a phase I clinical trial in CKD patients.
Methods: Seven CKD eligible patients recruited to the trial according to eligibility criteria. One doses of autologous BMMSCs (1-2* 106 cells/kg) has been infused intravenously. Safety was our main concern and and was measured by number of adverse events (AE). We defined eGFR changes as secondary endpoint. Kidney function changes at 18 months follow up compared to baseline and a year prior to the intervention.
Results: No AE and serious AE related to intervention has been reported during the study.A patient experienced creatinine rise due to single dose Gelofen tablet consumption following 3 months of the intervention. However, this serum creatinine change reached to previous level in two weeks. eGFR (P: 0.89) and serum creatinine (P: 0.18) changes compared to baseline and one year before enrollment were not statistically significant.
Conclusion: This phase I trial revealed safety and tolerability of autologous BM-MSCs infusion in CKD patients. Randomized clinical trials with larger sample size should be implemented for efficacy assessment.
Keywords: Chronic Kidney Disease, Bone Marrow Mesenchymal Stromal Cells, Cell Therapy, Stem Cells

Ps-329: The Effect of Autologous Platelet-Rich Plasma Injection on Hair Loss: A Case Study

Mahdieh Sadat Ghiasi[1], Mohsen Sheykhhasan[2*]

1. Department of Pharmacology, Iran University of Medical Sciences, Tehran, Iran
2. Laboratory of Stem Cell, The Academic Center for Education, Culture and Research, Qom, Iran
Email: mohsen_sheikhhasan@yahoo.com

Background and Aim: The use of platelet-derived biomaterials, plasma proteins and platelets are significantly increasing. Platelet-rich blood derivatives have been widely used in different fields of medicine and stem cell-based tissue engineering. They represent natural cocktails of autologous growth factors, which could provide an alternative for recombinant protein-based approaches. Platelet-rich blood derivatives, such as platelet-rich plasma, have consistently shown to potentiate stem cell proliferation, migration, and differentiation. In this study, autologous platelet-rich plasma is discussed as a case study to examine the effect on hair loss.
Methods: In this study, the 15 cc of blood of patients with severe hair loss were sampled. Then, the blood platelet-rich plasma was isolated in the laboratory and it was injected to different parts of the patient's head with hair loss.
Results: Platelet-rich plasma injection to different areas of the patient's head without hair or less hair after 2 weeks revealed that this action could be caused to decrease in loss of hair and even stimulate hair growth in areas of the head.
Conclusion: The results of this study showed that the use of autologous platelet-rich plasma can be a suitable method to reduce hair loss and even to regenerate of hair in people who are baldness.
Keywords: Platelet-Rich Plasma, Hair Loss, Hair Regeneration

Ps-330: In Silico and Experimental Evaluation of Hsv1-Mir-H2 Effect on Smad2

Zahra Shojaei Jeshvaghani[1], Ehsan Arefian[2*], Masoud Soleimani[3]

1. Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran
2. Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran
3. Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
Email: z.shojae@ut.ac.ir

Background and Aim: During its latency, HSV-1 produces a primary miRNA precursor (LAT) which encodes six distinct miRNAs including H2, H3, H4, H5, H7, and H8 in infected cells. Previous studies have revealed that an HSV-1-encoded miRNA not only regulates viral gene expression but also has the potential to regulate the expression of host cell genes. In silico and empirical investigations have shown that LAT has a regulatory effect on TGF-β pathway. Using a miRNA prediction program, we found a cellular target involved in TGF-β pathway for one of these six miRNAs, and examine whether the miRNA could target the candidate protein in practical.
Methods: In silico evaluation: In order to extract miRNA candidates, we searched for TGF-β pathway-related mRNAs that could interact with each of six miRNAs within LAT individually using TargetScanCustom. Considering the miRNA-targets interactions information Smad2 was selected as a target for mir-H2. Experimental evaluation: miR-H2 was cloned into pCDH-GFP-puro vector. The plasmid was extracted from host cell (stBL4) and purified to an appropriate yield, the integrity of the plasmids were confirmed by agarose gel electrophoresis. One day before transfection, LX-2 cells were plated in a 12-well plate. The miRNA-expressing construct, and the backbone plasmid, as negative control, were transfected individually into LX-2 cells (a highly transfectable human hepatic stellate cell line) with Lipofectamine 2000 according to the protocol. After 48 hours, the total RNA from the H2-transfected cells and control groups was extracted and the cDNAs encoding miR-H2, snRNA snord47 (as internal control), and total cDNA (using Random Hexamer primer) were obtained by RT-PCR. The cDNAs were used as templates for qRT-PCR amplification. Using qRT-PCR, mir-H2 overexpression in treated cells was confirmed & relative expression of Smad2 was measured in both treated and control cells.
Results: The qRT-PCR results analyzed by REST Software confirmed the relative overexpression of miR-H2 treated cells in comparison to control cells (backbone-transfected and none-transfected cells) after 48 hour. The experimental results show that contrary to in silico predictions, mir-H2 can't make any significant difference in the expression level of Smad2 as a TGF-β related protein.
Conclusion: Although miRNA-Target prediction software solutions have become useful tools in biological research, they can not be completely reliable and experimental verification need to be performed to complete the in silico results.
Keywords: Herpes Simplex Virus 1 (HSV-1), Latency Associated Transcript (LAT), Transforming Growth Factor Beta (TGF-β), Mothers against Decapentaplegic Homolog 2 (Smad2)

Ps-331: Effects of Aqueous Extract of Lavandula Officinalis on the Liver and Blood Fat in Balb/C Adult Female Mice

Fatemeh Soheili, Parvin Torabzadeh*, Safoora Saffari

Department of Biology, Karaj Branch, Islamic Azad University, Karaj, Iran
Email: Soheilifatemeh@yahoo.com

Background and Aim: In addition to sedative and analgesic properties, aqueous extract of lavender is effective in the treatment of diabetes and rheumatism with antioxidant properties. However, the effects of extract have not yet been studied on the liver. In this study, effects of aqueous extract of lavender were studied on the liver tissue and blood fat in Balb/C adult female mice.
Methods: Following preparation of aqueous extract, several doses of 500 (A), 300 (B), and 100 (C) mg/kg.bw were determined and injected intraperitoneally to 72 mice during 15 days. The mice were randomly divided into six groups, namely control (normal diet: 6 mice), control (fatty feed: 6 mice), sham (fatty feed and saline injection: 6 mice), and three experimental groups (each group with fatty feed and extract injection: 18 mice). Blood serum was obtained to measure triglyceride, cholesterol, HDL, and LDL levels. After dissection, the livers were removed and processed for sectioning. Data were analyzed using SPSS 21 software and also by Dunnett, Duncan, and Tukey tests providing $P < 0.05$.
Results: According to the results, significant ($P < 0.05$) accumulations of fat were observed in the livers of normal, sham, and Group C compared with the control group. A marked reduction ($P < 0.05$) was also detected in the aggregation of fat liver in Group B. Group A displayed no accumulation of fat compared with the other

groups but it showed significant drops ($P < 0.05$) in triglyceride, LDL, and cholesterol levels.
Conclusion: In general, it can be concluded that high doses of lavender plant extract prevent accumulation of fat in the liver cells, hence, it could be used as a medicine for lowering blood and liver fats in the future.
Keywords: Lavender, Liver, Cholesterol, Triglyceride, Mice

Ps-332: Engineered Mesenchymal Stem Cells Can Release Targeted Exosomes against Her2+ Cell Lines

Hosna Gomari[1], Mahdi Forozandeh Moghadam[1*], Masoud Soleimani[2]

1. Biotechnology group, Medical department, Tarbiat Modares University, Tehran, Iran
2. Hematology Group, Medical Department, Tarbiat Modares University, Tehran, Iran
Email: hosnag@gmail.com

Background and Aim: Exosomes are small vesicles that are secreted by various cell types with a size of 40-100 nm. They are released by the most cell types, which indicating that their important roles in both physiological and pathological processes such as, signaling pathways, cell-to-cell communication, tumor progression and transferring molecules like proteins and RNA. Mesenchymal stem cells (MSCs) have some advantages to produce exosomes among the other cells. For instance, they can release more exosomes and can be modified in vitro and in vivo models; besides, they have more reproducibility compared to the others. As well, MSCs-derived exosomes are more sustainable in human plasma and storage in −20°C and can be well tolerated in different animal models.
Methods: Human Embryonic Kidney HEK293, SKBR3 and MDA-MB231 cell lines were grown in Dulbecco's Modified Eagle Medium (DMEM), MSCs primary cells were culture in Alpha-MEM and 10% FBS, 1% penicillin/streptomycin solution were added to cell culture and incubated at 37°C, 5% CO2 in humid atmosphere. MSCs were characterized with their plastic-adherent when cultured in standard conditions. Also the expression of CD105, CD29 and CD90 and absence of CD45 and CD11b was assessed by fluorescent labeled antibody with flow cytometry. Finally, MSCs were differentiated into osteoblast and adipocyte cells. For stable transduction, virus particles were produced by transfecting two Viral plasmids and pLEX containing targeted gene were transfected into HEK cell line supernatant containing viral particles were collected at 24, 48, 72h and enriched by centrifuging at 20000 g/2h. After that, viruses were added to MSCs and exosomes were isolated by exosome purification kit. These exosomes were labeled with PKH67 and added to SKBR3 as a her2+ and MDA-MB231 as a her2- cell lines.
Results: We produced viral particles in HEK cell line which had been transfected by targeted gene. MSCs were incubated with these viral particles and Exosomes were isolated from engineered MSCs. We produced Exosomes containing targeted gene which specially were uptaken by her2+ cells. Up taking of labeled exosomes was checked by fluorescence microscopy and quantified by flow cytometry.
Conclusion: our results indicate that exosomes derived from transduced MSCs can be used as a targeted delivery systems, since they can specially uptake by Her2+ cell lines regards to Her2- cells.
Keywords: Mesenchymal Stromal Cells, Exosomes, Drug Delivery

Ps-333: The Construction of the Forstreat-Gelatin and the Forstreat Nanoconstruction Scaffold and the Researching and Comparing the Cells Culture MG63 Inthem

Melika Taheri, Sajad Shariat*

Shahreza Education Management, Isafahan, Iran
Email: mahdieh.yoosefi@yahoo.com

Background and Aim: The bone sicks and bone suffers including the trauma are prevalent in all the societies and the broken bone isn't compensated in the most times. The different therapy methods including the bone texture graft in from the Alagraft and the Autograft has manyrisks.
Methods: Therefore it is necessary the bontexture designing and it's graft for the patients .one of the therapy methods is the using of the cell culture on the suitable scaffold and the transferring it to the patient. Therefore in this study, besides the cells culture MG63 in from

the single layer culture and forstreat -Gelatin and forstreat scaffold, we studied the beharior of this cells in the different there type culture. The Forstreat parlicle nano was synthesized and we supplied the forstreat scaffold with and we covered the scaffold with Gelatine and then we transferred MG63 cells to the plate of the culture environment and then to the incubator .we divided the cells to three group .In the first group the cells was cultured in from the frosteat scaffold and in the next group in from the forstreat scaffold and in the other group in from the single layer. Then we studied the cells propagation amount in 2, 4 and 6 days with the cell count methad.
Results: With the cell count the in the increasing scaffold comparing to the forstereat scaffold and it's in creasing comparing to the single layer culture was specified in the cells culture.
Conclusion: Also the adhesion of the most cell to the forstreat-Gelatine scaffold is clear in the electrony microscope picture. The forstreat-Gelatine scaffold supports the propagation and surviving of the cells very well.
Keywords: Single Layer Culture, Bone Compensation Forst

Ps-334: Mesenchymal Stem Cells for Cutaneous Wound Healing

Nasrin Takzaree[1*], Alireza Partoazar[2], Hamidreza Barikani[3], Alireza Takzaree[4]

1. Department of Anatomy and Histology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
2. Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran ,Iran
3. Dental Implant Research Center, Dentistry Research Institute School of Dentistry, Tehran University of Medical Sciences, Tehran ,Iran
4. Childrens Medical Center, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
Email: takzaree@gmail.com

Background and Aim: Wound healing is a process that occurs after skin injury .One of the medical science objectives is attempting to heal a wound in a shorter time span, with fewer side effects. Clinicians have been searching for ways to obtain "super normal" wound healing. MSC populations in cells derived from placental membranes.placental tissue has been studied as an alternative source of MSCs, providing multipotent differentiation. We aimed to evaluate the wound contraction and stem cell properties on managing full-thickness wounds in vivo.
Methods: This experimental study was carried out on 54 adult male Wistar rats weighing 200-250 gr, and ages of 3-4 months. A square 1.5*1.5 wound was made on the back of the neck. The rats were divided into control and two experimental groups. Additionally, the control and experimental groups were separated into three subgroups corresponding to 4, 7, and 14 days of study. Mesenchymal stem cells isolated from PLACENTAL TISSUE, Cell collected and cultured.The control group did not receive any treatment. In first experimental group, MSCS was used once on the wound. The second experimental group received 1% phenytoein cream on the wound. For histological studies, samples were taken from the wound and adjacent skin. This tissue was examined using histological staining (H&E). Wound surface and wound healing were evaluated. Data were analyzed by using one-way ANOVA with post hoc Tukey test and ($P<0.05$) was significant.
Results: The results of microscopic study showed histological parameters in wounds bed (the number of fibroblasts, blood vessels, neutrophils and macrophages) in the experimental group were significantly different than the control group. The macroscopic and microscopic evaluations showed that the percentage of wound healing on different days in the control and experimental group were significant ($P< 0.05$).
Conclusion: The beneficial activity of MSCs in wound healing is complemented by the effects of growth factors and ECM produced by the native placenta tissue cells. Using Mesenchymal stem cells on open wounds will accelerate the healing process.
Keywords: Wound Healing, Open Skin Wound, Rat, Mesenchymal Stem Cells

Ps-335: The Evaluation of the Possible Synergic Effects of Platelet Rich Plasma and Hydroxyapatite/Zirconia in Rabbit Mandible Defect Model

Tahereh Talaei-Khozani[1], Ehsan Aliabadi[2], Shila Shahsavaripour[2], Mona latifi[3], Nehleh Zarei fard1[*]

1. Department of Anatomical Sciences, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Maxillofacial Surgery, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Tissue Engineering, National Institute of Genetic engineering and Biotechnology, Tehran, Iran
Email: zareifard@sums.ac.ir

Background and Aim: A big challenge for maxillofacial surgeons is reconstruction of the mandibular defects that are caused by tumor, trauma, infection or congenital anomalies. Both platelet rich plasma (PRP) and bioceramics such as hydroxyapatite (HA) and zirconia (ZrO2) has been suggested to have osteoconductive capacity. The objectives of this project was to find whether HA/ZrO2 bioceramic and PRP have synergic effects and also compare the osteogenic activity of HA/ZrO2 and PRP.
Methods: A ZrO2 scaffold was constructed by slurry method and coated by HA with fluroapatite at between to inhibit the interaction of ZrO2 with HA. Then, it was impregnated by PRP/heparin sulfate (HS). The scaffold was transplanted in a rabbit mandibular defect model and the osteoconductivity property was compared with HA/ZrO2 or PRP-treated defects. Radiological and histological studies were performed after the follow-up period, 6, 8 and 12 weeks.
Results: The results indicated that after two weeks, the percent of the surface occupied by bone were significantly higher in HA/ZrO2/PRP- and HA/ZrO2-treated than PRP-treated defects with a bit higher in HA/ZrO2/PRP group. The number of osteoblasts and osteocytes were higher significantly in PRP-treated group; however, the cells had not started matrix formation in large scale and just small islands of oeteoid that surrounded osteocytes were observed. For longer period of time, the regenerative potential of HA/ZrO2/PRP, HA/ZrO2 and PRP scaffolds were the same. Radiological funding showed that the presence of PRP had no effect on scaffold absorption.
Conclusion: In conclusion, both HA/ZrO2 scaffolds with or without PRP/HS showed a superior osteoconductive capacity for short term.
Keywords: Hydroxyapatite, ZrO2, Platelet Rich Plasma, Heparin Sulfate, Bone, Osteogenesis

Ps-336: Changing in the Biliary System Volume and Oval Cells in Ccl4-Induced Liver Cirrhosis by Platelet Rich Plasma Injection

Tahereh Talaei-Khozani*, Afsaneh Mafi, Farzaneh Dehghani, Abbas Moghadam, Zahra Vojdani, Ali Noorafshan

Anatomy Department, Shiraz Medical School, Shiraz University of Medical Sciences (SUMS), Shiraz, Iran
Email: talaeit@sums.ac.ir

Background and Aim: Liver cirrhosis is a response to the chronic liver damage with an increase in connective tissue content that lead to dysfunction of the organ. The number of oval cells, liver stem cells, increases in CCl4-induced liver cirrhosis. Platelet rich plasma (PRP) is a rich source of growth factors that involves in liver regeneration. Intravascular injection of the PRP led to a decrease in scar formation along with hepatocyte number. This study was designated to find the effect of intravascular injection of PRP on oval cells and biliary system of CCl4-induced liver cirrhosis.
Methods: The experimental design was included the induction of liver cirrhosis by oral administration of 4mg/Kg/week of CCl4 for 10 weeks. Twenty eight animals were then divided into 4 groups; normal control, cirrhosis animals without any treatment; cirrhosis animals received PRP or normal saline. All animals underwent surgery and PRP injected via anterior mesenteric vein. One week later, the animals were killed and the liver was prepared histologically. To estimate the small bile duct volume, serological analysis was performed.
Results: The results indicated that CCL4 led to a significant increase in the volume of small bile ducts compared with non-treated control ($P<0.001$). The bile duct volume was the same statistically in PRP injected with non-treated hepatotoxic animals. However, the volume was significantly higher than normal control. In the CCl4-induced liver cirrhosis group, histopathological analysis showed ductular reaction in portal spaces and oval cells were located around the bile ducts and seem to migrate into the liver parenchyma. The oval cells presented in all CCl4-treated animals and PRP did not exert any adverse effect on the oval cell population.
Conclusion: In conclusion, PRP treatment may have a beneficial effect on CCl4-induced liver cirrhosis by

increase in the volume of small biliary system where the niche of oval cells is and by this way may have a beneficial effect on liver regeneration.
Keywords: Biliary System, Oval Cells, CCl4-Induced Liver Cirrhosis, Platelet Rich Plasma

Ps-337: The Impact of Bone Marrow Microenvironment on Bim and Mcl-1 Gene Expression in U266 Cell Line Following Treatment with Decitabine

Sara Talebi[1*], Pegah Ghoraeian[1], Ali Dehghanifard[2]

1. Tehran Medical Science Branch, Islamic Azad University,Tehran, Iran
2. Tarbiat Modares University, Department of Hematology, Tehran, Iran
Email: ingeniousgirl71@yahoo.com

Background and Aim: Multiple myeloma is a kind of hematologic malignancy in monoclonal plasma cells that characterized by accumulation of myeloma cells over 10% in bone marrow microenvironment. Dependency of myeloma cells to bone marrow microenvironment (MSC) is imprtant factor in proliferation, relapse, survival, migration and drug resistance in malignant plasma cells. Keeping the balance between antiapoptotic factors like Mcl-1 and proapoptotic factors like Bim plays an important role in progression of malignant plasma cells toward apoptosis or survival. On the other side, Decitabine is a kind of methyltransferase inhibitor and also induces apoptosis. Therefore, it can involve in treatment of multiple myeloma by controlling methylation and inducing apoptosis. Our goal in this study was evaluating the effect of Decitabine on alteration of Bim and Mcl-1expression through apoptosis pathway in U266 (a multiple myeloma cell line) and also investigating the impact of bone marrow microenvironment in this alternation.
Methods: In this experimental study after isolation of mesenchymal stem cells from 3 multiple myeloma patients and 3 normal individuals, we assembled a co-culture model of u266 with MSCs from normal donors (ND-MSCs) and MM patients (MM-MSCs) and then treated with Decitabine. After RNA extraction and cDNA synthesis, Mcl-1 and Bim expression was analysed by Real time PCR.
Results: Our results showed that Decitabine, affected the expression of Bim in U266 cell line so that it was significantly increased after treatment with Decitabine ($p<0.05$). In this group upregulation of Mcl-1was also observed. Furthermore, in U266 cells that were co-cultured with ND-MSCs and were treated with Decitabine, expression of Mcl-1 and Bim was significantly increased ($p<0.05$). Finally in U266 cells that were co-cultured with MM-MSCs following treatment with Decitabine, a significant decrease and increase was found in expression of Mcl-1 and Bim, respectively ($p<0.05$).
Conclusion: On the basis of our results from this study, it can be concluded that Decitabine as an epigenetic modulatory drug has altered the expression of our candidate genes through epigenetic methyltransferase inhibitory mechanism and consequently use of this drug as a single drug can be appropriate for suppress or increase of apoptotic marker's expression. Also because there was a significant difference between candidate genes regarding the presence of ND-MSCs and MM-MSCs, it seems that multiple myeloma microenvironment plays an important role in progression of the disease and targeting surrounding BM microenvironment in addition to plasma cells colony should be considered in therapeutic strategies of multiple myeloma in order to inhibit progression of disease.
Keywords: Multiple Myeloma, Decitabine, U266 Cell Line, Mesenchymal Stem Cells, Mcl-1 Gene, Bim Gene

Ps-338: Study the Anticancer Effect of Aqueous Aloe Vera Extract on Expression of Bax/Bcl-2 Genes in AGS Cell Lines

Nazila Tariverdi, Zahra Deilami Khiabani*

Basic Science faculty, Islamic Azad University, Zanjan Branch, Zanjan, Iran
Email: Nazilatariverdi68@gmail.com

Background and Aim: Gastric cancer is a major cause of cancer death. In terms of incidence is the fourth most common cancer and the second leading cause of cancer death in the world. Aloe Vera plant belongs to the Liliaceae family and contain a variety of valuable minerals, vitamins, amino acids and antioxidant. The aim of this study was investigate changes in gene expression of Bax and Bcl-2 in human AGS adenocarcinoma cells,

which treated with aqueous extract of Aloe Vera, using Real-Time PCR quantitative methods.
Methods: The present study is case-control. The aqueous extract of Aloe Vera were prepared in different concentrations. AGS adenocarcinoma cells were treated with aloe Vera aqueous extract in different groups and times. RNA extraction and cDNA synthesis was performed, and gene expression of BCL-2 and BAX was evaluated by Real time PCR. Finally the obtained results were analyzed by statistical software.
Results: BAX and BCL-2 expression at 72 and 48 hours, showed significant changes only in 800µg/ml dose. The Bax gene showed significant increase at the 72h. BCL-2 gene showed significant reduce at 48h and significant increase and at 72h.
Conclusion: Aloe Vera extract increases the expression of Bax to Bcl-2 in 48 hours and this change leading the gastric cancer cells to apoptosis. The results of Bax/Bcl-2 was significant at 800µg/ml dose, and can be effective in improving gastric cancer.
Keywords: Aloe Vera, Bax, Bcl-2, Gastric Cancer

Ps-339: Osteogenic Differentiation of Dental Pulp Stem Cells under the Influence of Phenytoin

Zahra Tayarani-Najaran[1*], Mitra Asgharian-Rezaee[2]

1. Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Pharmaceutics Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.
Email: tayaraninz@mums.ac.ir

Background and Aim: Some of the adverse effect of chronic treatment of epilepsy with Phenytoin (diphenylhydantoin, DPH) treatment is Hyperglycemia, gingival hyperplasia and enlargement of facial features. Although there are some reports on anabolic action of DPH on bone cells, the osteogenic potential of DPH on mesanchymal stem cell has not been studied. The purpose of this study was to evaluate the osteogenic potential of DPH on dental pulp stem cells (DPSCs). Human DPSCs were isolated and characterized by flow cytometry.
Methods: Cells were differentiated either in conventional osteogenic medium or medium containing different concentration of phenytoin instead of dexamethasone. The flowcytometric analysis for presence of CD29 and CD44 and negative presence of CD34 and CD45 were performed to confirm the mesanchymal stem cells. Alizarin red S staining were done to measure the mineralization of cells and the specific proteins of osteogenic differentiation including RUNX2, osteopontin and alkaline phosphatase were compared with control untreated cells.
Results: Cells treated with phenytoin showed morphological changes and mineralized which was comparable with dexamethasone. In addition western blot analysis showed the increase in alkaline phosphatase (ALP), RUNX2 and osteopontin (OP) in comparison with control untreated cells.
Conclusion: The data of present study shows the potential activity of phenytoin as osteogenic factor which could be used for osteogenic differentiation of dental pulp stem cells.
Keywords: Dental pulp Stem Cells, Osteogenic Differentiation, Bone Mineralization, Phenytoin

Ps-340: Comparison of Different Concentrations of Sodium Dodecyl Sulfate Detergent in Lung Decellularization

Hamid Tebyanian[1], Ali Karami[1], Ebrahim Motavalian[2], Jafar Aslani[3], Mohammad Reza Nourani[3*]

1. Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
2. Department of General Surgery, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
Email: tebyan.hamid@yahoo.com

Background and Aim: Pulmonary disease is a worldwide public health problem that reduces the life quality and increases the need for hospital admissions as well as the risk of premature death. In the event that lung diseases turn to severe complications, the patient might end up with transplantation as the only chance for survival. In this regard, a common problem is the significant shortage of lungs for transplantation. Tissue engi-

neering techniques have offered a promising alternative for transplantation from donors: engineered lung tissue. In this study, we want to find the best decellularization approach for maintaining three-dimensional lung architecture and extracellular matrix (ECM) proteins composition which has significant roles in differentiation and migration of cells.
Methods: In this study, PBS, Heparin 5000u/ml, and 1% penicillin and streptomycin perfused via heart into the organ and whole body to remove blood. Animals sacrificed with ketamine and xylazine (100 mg/kg and 10 mg/kg). They were identified and fixed with catheters before harvesting lung to use in decellularization process. Different concentrations were used for decellularizing rat lungs for maintaining three-dimensional lung architecture and ECM protein composition which have significant roles in differentiation and migration of cells. All procedures performed in a sterile condition. Finally, decellularized lungs were evaluated by Hematoxylin and Eosin staining, Trichrome-Masson staining, and Elastin staining.
Results: Results showed that 0.05% to 0.2% concentration of SDS could completely remove cells from tissue in 48 hours decellularization but methods 0.05% to 0.2% could maintain the three-dimensional (3D) lung architecture in 24 hours decellularization better than others. It can be understood that the 3D lungs were destroyed by increasing the concentration of SDS to 0.2%. Collagen and elastin were significantly decreased by increasing times and the concentration and times. SDS 0.05% could remove cells in 48 hours decellularization and also, could maintain 3D, collagen and elastin better than other approaches.
Conclusion: We conclude that these approaches can help to achieve three-dimensional architecture and ECM protein composition of the lung with minimum destruction for next steps such as recellularization and in-vivo study.
Keywords: Lung, Decellularization, SDS, Triton X-100

Ps-341: Evaluating the Impact of MSC-Conditioned Media and MSC Derived Exosomes on Inflammatory Cytokine Secretion from Monocytes

Samaneh Tokhanbigli[1], Kaveh Baghaei[1*], Ali Assadi Rad[2], Aysan Sani Khalilnejad[3], Seyed Mahmoud Hashemi[2], Hamid Assadzadeh[1], Mohammadreza Zali[1]

1. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Islamic Azad University Tehran North Branch, Tehran, Iran
Email: Tokhanbigli.s@gmail.com

Background and Aim: Multipotent Stromal Stem Cells (MSCs) represent a multi lineage population of adult stem cells. In addition to their stem/progenitor properties, they possess immunomodulatory features that have an effect on both innate and adaptive immune responses. Many studies have shown an active interaction between MSCs and innate immune system by direct cell-to-cell contacts, secretion of cytokines and/or by a combination of both mechanisms results in anti-inflammatory or pro-inflammatory effects. Within the innate immune system. Monocytes/macrophages are the main keys in initiating and controlling of inflammation that can be influenced by the present of MSCs. The content of accumulated paracrine secretion from MSC in culture media consists of all immunomodulatory cytokines, exosomes and etc. Exosomes are microvesicles secreted from a broad range of cell types including stem cells. These vesicles contain proteins, lipids, RNAs and many signaling molecules cause communication between cells. In this study, we investigated the effect of conditioned media collected from cultured MSCs and isolated Exosomes from this conditioned media on PBMNCs in order to compare their impact on cytokine secretion of monocytes.
Methods: Mononuclear cells were isolated from PBMNCs using ficoll and cultured in RPMI1641 for 24 hours. After removing lymphocytes from media, monocytes were divided into three groups, control, treated with MSC conditioned media, and treated with exosomes derived from MSCs. After 72 hours treatment RNA extraction and cDNA synthesis were performed. In the next step gene expression of inflammatory cytokines were evaluated by real time PCR.

Results: Collected data from Real time PCR were analyzed by REST software Qiagen. Secreted Inflammatory cytokines from monocytes treated with collected conditioned media from MSC and MSC-derived Exosomes showed a significant difference in compare with control groups. But there were no clear differences between conditioned media treated group and the Exosome ones.
Conclusion: According to proven role and effect of exosomes and their content in cell behavior and gene expression, with considering the collected data from two different treated groups and their impact on cytokine secretion, we came to the conclusion that exosomes were possibly the effective factor on gene expression variation of immunomodulators in conditioned media of treated group.
Keywords: Mesenchymal Stem Cells, MSC Conditioned Media, Exosome, Inflammatory Cytokines, Monocytes

Ps-342: Platelet- Rich Plasma (PRP) In Sinus Augmentation in Dental Implant Treatments

Mehrnaz Karimi Afshar[1*], Molook Torabi[2], Naghmeh Bahrami[3]

1. School of Dentistry, Tehran Medical Science University. Tehran,Iran
2. Oral Pathologydental School Kerman Medical University, kerman, Iran
3. Maxillofacial Surgery Research Center, Dental school, Tehran Medical University Tehran,Iran
Email: m.torabi.p@gmail.com

Background and Aim: In recent decades dental implantology has developed into a prospering component of dentistry and ongoing a popular treatment for tooth loss replacement. Maxillary posterior tooth loss leads to bone loss in the maxillary sinus floor and increases pneumatization of the maxillary sinus. Placement and osteointegration of implants in the maxillary posterior edentulous area requires sinus floor augmentation. Autogenous bone, allograft, xenograft, synthetics, demineralized autogenous teeth and a combination of various materials are the various techniques for sinus floor augmentation
Methods: The problems related to these methods such as resorption and limited amounts donor sites of autolouges bone are still unresolved. PRP (platelet- rich plasma) method demonstrated bone formation in maxillary sinus
Results: The problems related to these methods such as resorption and limited amounts donor sites of autolouges bone are still unresolved. PRP method demonstrated bone formation in maxillary sinus
Conclusion: The aim of the present study is review of articles about role of PRP with and without grafts in sinus augmentation
Keywords: Dental Implant, Sinus, PRP, Augmentation, Osteointegration

Ps-343: Mir-224 Delivery into Murine Ovarian Cells by Intraovarian Injection and Subsequent In Vivo Electroporation

Maryam Vahdat Lasemi[1,2*], Mohammad Salehi[1], Sara Hosseini[3]

1. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Cellular And Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Anatomy Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: m.vahdat@ymail.com

Background and Aim: Gene delivery to ovarian cells (including follicular cells and oocytes) appears to be important for the in vivo analysis of genes related to oogenesis. MicroRNA-mediated post-transcriptional gene regulation is a good way in gene analyses. Mir-224 is involved in follicular granulosa cell (GC) growth.
Methods: In this study we describe the use of direct injection of linear vector containing mir-224 and subsequent in vivo electroporation (EP) for efficient gene delivery to the ovarian cells, including follicular cells and oocytes of mice.
Results: Our findings suggest that a solution introduced inside the ovary is rapidly dispersed to each follicle.
Conclusion: Moreover, indicate that miR-224 may affect ovulation and subsequent embryo development and suggesting potential roles for miRNAs in offering new

treatments for ovulation disorder-associated infertility, or,conversely, designing new contraceptives
Keywords: Mir-224, Gene Transfer, Electroporation, Ovary

Ps-344: The Effect of Different Doses of Ovarian Follicular Fluid on Expression of Survivin and C-MYC Genes on Umbilical Cord Blood Hematopoietic Stem Cells

Bahare Vahidianfar, Saeed Abroun*, Mojtaba Rezazade

Faculty of Medicine, Tarbiat Modares University, Tehran, Iran
Email: baharevahidianfar@gmail.com

Background and Aim: Hematopoietic stem cells play a key role in production of mature blood cells and transplant clinical outcomes. Hematopoietic stem cells can be isolated from various sources, such as bone marrow, peripheral blood and umbilical cord blood. Over the past two decades, an important source of hematopoietic stem cell for transplantation and research is umbilical cord blood. To date, more than 400,000 unrelated cord blood transplants have been done worldwide as a treatment for patients with malignant or non-malignant blood disorders. Absolute count of hematopoietic stem cells in a unit of cord blood is low and it is a limiting factor in transplant adult recipients. Follicular Fluid (FF) is a product of both the transfer of blood plasma constituents that cross the blood follicular barrier and of the secretory activity of granulosa and thecal cells and it is important for oocyte development has provided microenvironment. The aim of this study was to evaluate the effect of follicular fluid on the expression of surviving and c-MYC genes on umbilical cord blood hematopoietic stem cells.
Methods: Ovarian follicular fluid sterile samples were received from the Royan IVF center and bags of cord blood were received from the umbilical cord blood bank. Cord blood mononuclear cells were isolated using Ficoll Solution. Hematopoietic stem cells isolated by MACS column. Flowcytometry was performed to confirm the purity of the hematopoietic cells. The isolated cells at two different dose groups of follicular fluid (25%, 50%) and fetal bovine serum (FBS) were cultured for 7 days. At day 0, 3, 5 and 7 RNA was extracted then cDNA synthesis was performed with Thermo kit. Then, for evaluation the expression of c-MYC and surviving genes, PCR and electrophoresis techniques were used.
Results: On day 3, the expression of survivin gene in a dose of 100% FBS and 50% FF was lower compare to day 0. On day 5, only 25% FF dose and 50% FF survivin gene was expressed, but the expression was lower compared to day 0. On the seventh day following culture, the expression of this gene at doses of 25% FF and 50% FF was the same as day 0. On third day following treatment, the group was treated with 50%FF dose expressed c-MYC and it was lower than day 0. On fifth day, the groups were treated with 25%FF and 50%FF doses expressed c-MYC. Expression of this gene on the seventh day of culture at doses of 25% FF and 50% FF was the same as day 0. C-MYC wasn't expressed in the group treated with 100% FBS dose.
Conclusion: According to the results it can be concluded, doses of 25% FF and 50% FF to 100% FBS dose increases expression of survivin and c-MYC genes.
Keywords: Hematopoietic Stem Cells, Ovarian Follicular Fluid, Survivin, C-MYC

Ps-345: Effect of a Chimeric Vaccine against Colorectal Cancer

Ziba Vaise Malekshahi[1*], Babak Negahdari[1], Jafar Amani[2], Masoumeh Rajabibazl[3]

1. Department of medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2. Applied Microbiology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Department of Clinical Biochemistry, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: zvmalekshahi@yahoo.com

Background and Aim: Colorectal cancer (CRC) is one of the leading causes of cancer related deaths in world. The most common tumor markers of colorectal cancer are carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19.9). Here we explored the protective potency of CE-CA vaccine and investigated the possi-

bility of the protective application of CE-CA as a new vaccine Candidate in mice bearing colorectal cancer.
Methods: The desired structure (CE-CA) was synthesized and received as a clone into the pET28a vector. The recombinant CE-CA fusion protein with molecular weights of 67kDa were expressed and confirmed by anti-CEA western analysis. Proper folding, expression levels and immune reactivity were assessed by ELISA and immunohistochemistry.
Results: The finding demonstrated that injection of CE-CA recombinant protein could elicit strong T-helper 1 (Th1) immunity against antigen, as indicated by the specific high level production of IFN-γ and IL-2 using spleen lymphocytes and lymphoproliferative responses.
Conclusion: Overlay our results indicate that CE-CA might be a promising vaccine candidate for colorectal cancer.
Keywords: Colorectal Cancer, CE-CA, Vaccine, Therapy

Ps-346: In Vitro Biocompatibility of a Compliant, Blood Compatible and Biodegradable Nanofibrous Scaffold for Vascular Tissue Engineering

Elham Vatankhah[1*], Seeram Ramakrishna[2]

1. Department of New Technologies, Shahid Beheshti University, Zirab Campus, Mazandaran, Iran
2. Department of Mechanical Engineering, Faculty of Engineering, National University of Singapore, Singapore 117576, Singapore
Email: e_atankhah@sbu.ac.ir

Background and Aim: The rational design of a scaffold depends on its ability to mimic the native extracellular matrix (ECM) as much as possible in terms of physical, chemical, and mechanical features. Development of an optimal scaffold plays a significant role in vascular tissue engineering. Bioengineered scaffolds serve as the temporary matrix and provide structural, mechanical, and biological support throughout the tissue formation assisting the process of tissue remodeling. A vascular scaffold must not only support appropriate structural integrity until neotissue can form, but also closely mimic the strength and compliance of native blood vessels. Blood compatibility is also clearly a crucial factor to raise success of the engineered construct since the vascular scaffold comes in contact with blood. The degradation profile of the scaffold is another important criterion to consider for successful applications in tissue engineering of load-bearing structures like blood vessel tissues. A tissue-engineered vascular graft requires complete scaffold degradation with well-defined cellular organization and tissue remodeling. Among the numerous techniques used to fabricate scaffolds, electrospinning has been widely used because of the high porosity, large surface area, and nanofibrous structure of electrospun scaffolds mimicking the physical nano features of native ECM.
Methods: To meet all the foregoing requisites, we utilized a blend electrospinning approach to fabricate nanofibers of poly(L-lactide acid-co-poly ε-caprolactone) (PLCL), a biodegradable and compliant polymer, gelatin (Gel), a biodegradable and commercially available natural biopolymer possessing many integrin binding sites (such as RGD) for cell adhesion, and Tecophilic (TP), a hydrophilic, elastic and hemocompatible polyether-based thermoplastic aliphatic polyurethane, with a weight ratio of 60:20:20 (PGT;60/20/20). Polymer blending, one of the most effective methods for providing amalgamated properties of several polymeric materials of the blend, resulted in production of biodegradable and compliant PGT scaffolds to mimic the fibrous structural and mechanical properties of native arteries. Blood compatibility of the scaffolds was prioritized and considered through selecting TP, a blood compatible polyurethane, as a main component of the scaffolds. The nanofibrous structure of the scaffold was visualized using scanning electron microscopy (SEM) and to confirm the presence of all components within the fibers, surface characterization of the scaffold was carried out using ATR-FTIR spectroscopic analysis. For evaluating the potential of electrospun PGT; 60/20/20 scaffold as a substrate for vascular regeneration, human aortic smooth muscle cells (SMCs) were cultured on the scaffold and the biocompatibility of the structure was studied by performing the proliferation assay and cell morphology assessment.
Results: SEM images demonstrated that electrospun PGT; 60/20/20 nanofibers were successfully produced with a fiber diameter of 459±198 nm which revealed a significant reduction in fiber diameter compared to fiber diameter of electrospun pure PLCL and pure TP.

The surface characterization of scaffold was carried out using ATR-FTIR spectroscopic analysis confirming the presence of all components within the fibers. Comparing these results with results of cell behavior on electrospun PLCL and TP scaffolds confirmed the potential use of PGT; 60/20/20 nanofibers in blood vessel tissue engineering.
Conclusion: Therefore, this study approves the feasibility of electrospun PTG scaffold for development of a functional vascular graft.
Keywords: Electrospun Scaffold, Vascular Tissue Engineering, Smooth Muscle Cells

Ps-347: Effect of Long Chain Omega-3 Fatty Acids on Ovine Mesenchymal Stem Cells Differentiation and Adiposity: Possible Role of Mir-103 in Adipogenesis

Arash Veshkini[1], Aliakbar Khadem[1], Abdollah Mohammadi-sangcheshmeh[1], Ali Assadi-Alamouti[1*], Fatemeh Kouhkan[2], Abdolreza Salehi[1], Masoud Soleimani[3]

1. Department of Animal and Poultry Science, College of Aburaihan, University of Tehran, Pakdasht, Tehran, Iran
2. Stem Cell Technology Research Center, Tehran, Iran
3. Department of Hematology, Faculty of Medical Science, Tarbiat Modares University, Tehran, Iran
Email: Arash.veshkini@yahoo.com

Background and Aim: Excessive caloric intake promotes adiposity by conversion of mesenchymal stem cells (MSCs) to preadipocytes that differentiate into adipocytes. Evidences suggested that omega-3 long chain polyunsaturated fatty acids (ω -3 LCPUFA), especially EPA and DHA could possibly inhibit adipogenesis and improve the overall capacity of LCFA oxidation. Just recently, it has been revealed that PPARγ, key nuclear factor regulating lipid metabolism and adipocytes differentiation, targeted by miR-103. So in this study, we aimed to investigate the effect of Fish Oil (as a rich source of ω -3 LCPUFA) supplementation on ovine MSC differentiation and adiposity.
Methods: Fish oil (50μM) was added to cultured media of the MSCs and its effects on proliferation were investigated with cell cycle analysis. MiR-103 as adipogenic marker and its downstream gene PPARγ were also evaluated by QRT-PCR. Differentiation to adipocyte were induced in MSCs with 0.5 mM hydrocortisone, 60 mM indomethacine, and 0.5 mM isobutylmethylxanthine for 21 days in the presence of fish oil (50μM). Next, adipocytic marker have been investigated through in situ Oil Red O (ORO) staining.
Results: Cell cycle analysis of MSCs by flow cytometry indicate that fish oil triggered more accumulation of MSC cells at the s phase. Real-time PCR designated that the expression of miR-103 was downregulated while the expression of its target gene PPARγ was upregulated in fish oil treated cells compared to the controls. In adipogenesis, the number of cells with fat vesicles decreased significantly in fish oil treated group as indicated by ORO staining.
Conclusion: Collectively, the results revealed that fish oil (ω -3 LCPUFA) treatment could inhibit adiposity through downregulation of miR-103 and over expression of key adipogenesis regulatory gene PPARγ.
Keywords: Fish Oil, MSC, Adiposity, Microrna, Differentiation

Ps-348: Fetal Microchimerism in Mouse Caerulein-Induced Pancreatitis Model

Zahra Vojdani[1*], Jafar Bagheri[1], Tahereh talaei-Khozani[1], Negar Azarpira[2], Mahin Salmannzhad[3], Ali Farrokhi[4]

1. Laboratory for Stem Cell Research, Anatomy Department, Medical school, Shiraz University of Medical Sciences, Shiraz, Iran
2. Transplantation Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Laboratory for Stem Cell Research, Anatomy Department, Medical school, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Bioloy and Technology, ACECR, Tehran, Iran
Email: vojdaniz@sums.ac.ir

Background and Aim: Introduction: Fetal microchimerism is the persistence of allogenic cell population that transfer from the fetus to the mother. The aim of this study was to evaluate the presence of fetal micro-

chimerism in the pancreas of the mice with acute pancreatitis (AP).
Methods: Material and methods: The female wild-type mice were mated with male EGFP+. AP model was obtained by injection of caerulein two days after delivery. Sixty mice were divided into 3 groups: the virgin pancreatitis-induced animals, pregnant pancreatitis-induced animals mated with transgenic GFP mice, and pregnant sham animals. To prove pancreatitis induction, the blood amylase and lipase were assessed; and pancreas was removed from a subpopulation of each group for histopathological examinations after 6 h. The remaining mice were kept for 3 weeks and histopathological exanimation, immunohistochemistry and PCR were performed.
Results: Results: EGFP+ cells were found in acini and around the blood vessels in the pancreas of pregnant pancreatitis-induced animals. They differentiated to acinar, adipocyte-like, and mesenchymal-like cells. PCR showed that 20% of the pregnant pancreatitis-induced animals were EGFP+. The histophatological study showed improvement in pancreatitis scores in the mice with pregnancy experience.
Conclusion: Conclusion: There is a correlation between the decrease in pancreatitis score and the presence of fetal microchimerism.
Keywords: Fetal Microchimerism, Acute Pancreatitis, EGFP, Mouse

Ps-349: The Prediction of Mir-221 and Mir-124 Role in Dopaminergic Cell Death Pathways in Parkinson's Disease

Elaheh Yadegari[1], Kamran Ghaedi[2*]

1. Biology Department, School of Sciences, University of Isfahan, Isfahan, Iran
2. Department of Biology, Division of Cellular and Molecular Biology, Faculty of Sciences, University of Isfahan
Email: yadegari.elaheh@gmail.com

Background and Aim: MicroRNAs (miRNAs) are endogenous non-coding RNAs that control gene expression at the posttranscriptional level. The role of miRNAs in the maintenance of normal cellular functions and in disease-inducing pathways has been increasingly demonstrated. miRNAs play important roles in neuronal patterning and cell differentiation. miRNAs also have been reported to be involved in neurodegenerative disorders including Parkinson's disease (PD). PD is the most prevalent movement disorder characterized by selective loss of midbrain dopaminergic (DA) neurons.
Methods: In this study, network-based systems biology tools including Pathway Studio 9.0 and 3Omics were used to identify PD critical molecular players. Utilizing currently available and frequently used computational tools for miRNA target prediction,i.e., PicTar, TargetScan, DIANA-microT, miRanda and miRWalk2.0 databases to predict miRNA-mRNA interaction, we investigated probable interaction of miRNAs and genes that participate in dopaminergic cell death pathways in PD.
Results: we have predicted possible role of two miRNAs, miR-221 and miR- 124 which could control dopaminergic cell death pathways in PD so possibly effect on PD onset and progression.
Conclusion: several therapeutic approaches may be considered for these miRNAs besides of their application as a valuable prognostic or diagnostic biomarkers in PD.Although miRNA-based diagnostics and gene therapy are still in their infancy, their huge potentials will meet our need for future disease diagnostics and gene therapy.
Keywords: MicroRNA, Parkinson's Disease, Dopaminergic Neurons, Gene Therapy

Ps-350: Construction Recombinant Plasmids Expressing Different Suicide Genes and Comparison Effects on Cancer Cell

Hajar Yaghoobi[1*], Mojgan Bandehpour[2], Bahram Kazemi[3], Ali Ghanbari Asad[4]

1. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
2. Department of Medical Biotechnology, School of Advanced Technologies in Medicine Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences,
4. Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran
Email: hajar.yaghoobi64@gmail.com

Background and Aim: Suicide gene therapy is one of the emerging methods of cancer treatment in which lethal genes introduced into cancer cells and then suicide gene expression leads to apoptosis and necrosis of cancer cell. Numerous suicide gene therapy systems have been developed recently, including HSV-TK / GCV, pro-apoptotic genes and bacterial toxins. In this investigation three recombinant plasmids expressing CdtB (bacterial toxin) Bax (pro-apoptotic gene) and TK (HSV-TK / GCV) were constructed and their toxic effects on cancer cells was compared.
Methods: The gene of Bax and TK and cdtB were cloned into pcDNA3.1 vectors. Afterward, genes expression was evaluated through RT-PCR, then the MTT assay was subjected for cell viability. In addition their function were determined through a DNA fragmentation test and flow cytometry.
Results: All the genes exhibit time-dependent cytotoxic effects however they indicated different mechanism of action. On the other hand, CdtB induced significantly higher apoptosis rate in comparison with Bax and TK transfection.
Conclusion: Consequently, CdtB represents a promising therapeutic option for a suicide gene therapy of cancer cells in future studies, leading to rapid and effective tumor cell killing in vitro.
Keywords: Suicide Gene Therapy, Cancer, Pro-Apoptotic, Bacterial Toxins

Ps-351: Lavandula Angustifolia Extract Improves the Result of Human Umbilical Mesenchymal Wharton's Jelly Stem Cell Transplantation after Contusive Spinal Cord Injury in Wistar Rats

Kayvan Yaghoobi*, Gholamreza Kaka

Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran 19568-37173, Iran
Email: k1.neuroscience@gmail.com

Background and Aim: The primary trauma of spinal cord injury (SCI) results in severe damage to nervous functions. At the cellular level, SCI causes astrogliosis. Human umbilical mesenchymal stem cells (HUMSCs), isolated from Wharton's jelly of the umbilical cord, can be easily obtained. Previously, we showed that the neuroprotective effects of Lavandula angustifolia canleadtoimprovement in a contusive SCI model in rats. Objective. The aim of this study was to investigate the effect of L. angustifolia (Lav) on HUMSC transplantation after acute SCI.
Methods: Sixty adult female rats were randomly divided into eight groups. Every week after SCI onset, all animals were evaluated for behavior outcomes. H&E staining was performed to examine the lesions after injury. GFAP expression was assessed for astrogliosis. Somatosensory evoked potential (SEP) testing was performed to detect the recovery of neural conduction.
Results: Behavioral tests showed that the HUMSC group improved in comparison with the SCI group, but HUMSC + Lav 400 was very effective, resulting in a significant increase in locomotion activity. Sensory tests and histomorphological and immunohistochemistry analyses verified the potentiation effects of Lav extract on HUMSC treatment.
Conclusion: Transplantation of HUMSCs is beneficial for SCI in rats, and Lav extract can potentiate the functional and cellular recovery with HUMSC treatment in rats after SCI.
Keywords: spinal cord injury (SCI)و Human umbilical mesenchymal stem cells (HUMSCs)

Ps-352: Assessment of Changes in white Blood Cells in Leukemia Patients after Bone Marrow Transplant Using Random Effects Model

Somayeh Yazdani[1], Farid Zayeri[1*], Maryam Dehghani[1], Soraya Moamer[1], Naser Ahmadi[1], Fatemeh Mahmodi[2]

1. Department of Biostatistics, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Department of Health Safety Environment, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: somayeh.yazdani.1991@gmail.com

Background and Aim: The aim of this study was to Assessment of changes in white blood cells in leukemia patients after bone marrow transplant using random effects model.
Methods: In this study, 50 patients with leukemia (26 women and 24 men, mean age: 42.6±13.39 and

35.5±15.29 years, respectively) were received transplants from Taleghani hospital in Tehran, Iran, during 2007-2014. Data was collected from patients' files and white blood cells was considered as a response variable. Explanatory variables included Disease type, age. Random effects model was applied to using SAS software.
Results: The random effect model showed that Disease type (P<0.001) and Time trend (P<0.001) had significant effect on changes in white blood cells.
Conclusion: According to this study, white blood cells was associated with individual characteristics such as Disease type, and Time trend. Applying random effects model could give more accurate estimates of the factors affecting the white blood cells , thereby, providing a better understanding of the state of disease.
Keywords: Random Effects Model, White Blood Cells

Ps-353: Role of Mir-1246 and Mir-10a in Differentiation of Megakaryocyte from Umbilical Cord Blood Hematopoietic Stem Cells

Narjes Yazdi[1], Mohammad Houshmand[2], Alireza Kazemi[3], Mahin Nikougoftar Zarif[2*]

1. Department of Molecular Genetics, Tehran Medical Branch, Islamic Azad University, Tehran, Iran
2. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
3. Department of Hematology and Blood Banking, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: donya.yazdi@gmail.com

Background and Aim: MicroRNAs (miRNA) are small non-coding RNAs with 18-25 nucleotides which become an indispensable part of studying diverse biological process from differentiation to proliferation of cells and it becomes evident that these RNAs play a key role in different diseases like hematologic malignancies. Studies show that some miRNAs can regulate megakaryocyte differentiation. Owing to this matter, we determined to measure the role of miR-1246 and miR-10a in the differentiation of megakaryocyte from umbilical cord blood hematopoietic stem cells.
Methods: Human CD133+ hematopoietic stem cells were collected from umbilical cord blood, differentiated to megakaryocytic lineage and characterized by flow cytometry, CFU-assay, and ploidy analysis then qRT-PCR was performed to measure miR-1246 and miR10a in differentiated cells.
Results: Our study demonstrated up-regulation of miR-1246 and down-regulation of miR-10a during megakaryocyte differentiation
Conclusion: These microRNAs have a role in megakaryocyte differentiation, and they can be used as a target to change the rate of differentiation and evaluating the biology of megakaryocyte commitment.
Keywords: Hematopoietic Stem Cell, Megakaryocyte, Micrornas, Mir-10a, Mir-1246

Ps-354: Effects of Nano-Cucurcomin on T Regulatory Population in Co-Culture of Pbmc and Adipose-Derived Mesenchymal Stem Cells from Multiple Sclerosis Patients

Forouzan Yousefi[*]

Department of Immunology, Mashhad University of Medical ScienceImmunology center, BuAli institute, Mashhad, Iran
Email: forouzan.mania@gmail.com

Background and Aim: Multiple sclerosis (MS) is an inflammatory mediated demyelinating disease of the human central nervous system. There is enhanced-emerging treatment for multiple sclerosis but conventional disease modifying therapy are not divisive cure for progressive phase of multiple sclerosis. Curcumin has been used in traditional medicine as an effective drug for a variety of diseases. Stem cell therapy as a new therapeutic approach also has attracted interests worldwide. Anti-inflammatory and neuroprotective effects of curcumin was established in older studies. Corcumin can enhance usability of other drugs such as stem cell therapy for MS. Different formulations of curcumin are introduced to increase its stability and effectiveness. Here we have examined the effect of nano miscel form of curcumin on T regulatory population in co-culture with adipose-derived mesenchymal stem cells from multiple sclerosis patients
Methods: We removed abdominal fat mesenchymal stem cells (AT-MSCs) form multiple sclerosis patients in secondary progressive phase, cultured in 6-wells plate and treated them with 100µg/ml, 50 µg/ml, 25 µg/ml and 12 µg/ml of nano-curcumin for 24 and 48h after

treatment, Sixth well was remained untreated. Then we assayed Treg percentage n medium with T regulatory in co-culture of adipose-derived mesenchymal stem cells and pbmc from multiple sclerosis patients by flowcytometry technique.
Results: all concentration of curcumin in 24 and 48h post treatment increase T regulatory population in co-culture of AT-MSC and pbmc
Conclusion: nano curcumin can be used as a potential treatment to increase capability of mesenchymal stem cell therapy in autoimmune diseases specially multiple sclerosis
Keywords: Key words: nano-curcumin, mesenchymal stem cells, multiple sclerosis, T regulatory

Ps-355: Creation of Micro-Topography on the Surface of 3D-Printed Polycaprolactone Scaffold: Enhanced Attachment of MC3T3-E1 Pre-Osteoblasts

Yasaman Zamani[1], Ghassem Amoabediny[2*], Jenneke Klein-Nulend[3], Behrouz ZandiehDoulabi[3], Marco N Helder[4]

1. Department of Life Science Engineering, Faculty of New Sciences and Technologies, and Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran
2. Department of Chemical Engineering, College of Engineering, and Research Center for New Technologies in Life Science Engineering, University of Tehran, Tehran, Iran
3. Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, MOVE Research Institute Amsterdam, Amsterdam, The Netherlands
4. Department of Orthopedic Surgery, MOVE Research Institute Amsterdam, VU University Medical Center, Amsterdam, The Netherlands
Email: y.zamani@ut.ac.ir

Background and Aim: Polycaprolactone (PCL; FDA approved) is among the most widely used polymers in fabrication of scaffolds for bone tissue engineering. When PCL is 3D-printed, smooth strands with no topography are created. This, together with hydrophobic nature of PCL, results in poor cell attachment. Aim: The aim of this study was to enhance MC3T3-E1 pre-osteoblast attachment to 3D-printed PCL scaffold.
Methods: PCL scaffold was fabricated using a 3D-bioprinter device (RegenHU; CH). Cubic scaffolds (0.8×0.8×0.8 cm) were plotted layer-by-layer with a layer deposition angle of 90°. The pore size was ~300 µm. Surface modification was performed by treatment with 3 M NaOH for 24 and 72 h. The morphology of scaffolds was observed by Scanning Electron Microscopy. MC3T3-E1 pre-osteoblasts were seeded onto the modified and unmodified PCL scaffolds and cell attachment was studied by SEM.
Results: SEM images showed that when the PCL scaffold was treated with NaOH for 24 h, an etching effect occurred on the PCL strand and created a honeycomb topography with oval pores of about 0.7 µm wide and 2.5 µm high. Treatment with NaOH for 72 h also created a honeycomb topography with oval pores of about 2.2 µm wide and 7 µm high. MC3T3-E1 pre-osteoblasts had slightly spherical morphology on the surface of untreated PCL scaffold and it seemed that they are not attached firmly to the surface. On the other hand, it was observed that cells could attach better to the surface of PCL scaffold treated with NaOH for 24 h anchoring their filopodia to the topographical features created on the surface. Finally, cells completely covered the micro-pores created on the surface of the PCL scaffold treated with NaOH for 72 h.
Conclusion: In this study, we showed that it is possible to create topographical features as micro-pores on the smooth surface of PCL when it is 3D-printed. Dimension of the micro-pores are increased by increasing NaOH treatment time. Therefore, it is possible to control the size of the micro-pores according to the required function. MC3T3-E1 pre-osteoblast attachment was enhanced on the surface of the NaOH treated PCL scaffolds. Cells had better attachment to the PCL scaffold treated with NaOH for 72 h compared to the scaffold treated with NaOH for 24 h.
Keywords: Polycaprolactone, 3D Printing, Topography, NaOH, MC3T3-E1 Preosteoblast Attachment

Ps-356: Human Skin Keratinocyte Cells Culture for Induced Pluripotent Stem Cells Generation

Sona Zare[1,2], Fardin Fathi[3*], Mohammad Ali Nilforoushzadeh[1], Faeze Jahangiri[1], Atieh Sedighian[4], Sahar Jahtalab[4]

1. Skin and Stem Cell Research Center, Tehran University of Medical Science, Tehran, Iran
2. Department of Biology, Faculty of Basic Science, Islamic Azad University, Hamedan branch, Hamedan, Iran
3. Cellular and Molecular Research Center, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.
4. Faculty of Basic Science, Islamic Azad University East Tehran Branch, Tehran, Iran
Email: sonazarebio@gmail.com

Background and Aim: Today cultured keratinocyte cells used to help repair skin damages such burns, chronic wounds caused by diabetes or surgery, plastic surgery, tissue engineering, gene therapy and induced pluripotent stem cells generation. In this study, we isolated, cultured and transfection of these cells from foreskin with a very accurate method and were introduced standard culture conditions for these cells and iPS cells generation.
Methods: Keratinocyte cells were transfected with using effectene transfection reagent and by pSIN4-EF2-O2S. 16 h after, transfection solution was removed and keratinocyte cells medium were added to cells. 48 h after transfection, cells were trypsinazed and transferred onto fresh feeder layer cells and iPS cells medium was used for culture these cells, Also Valporic acid was used in the iPS cell culture medium for 2 weeks. Immunofluorescence, alkaline phosphatase and RT-PCR methods is used for iPS cells characterization.
Results: The results of this study show that the protocol used for the isolation and cultivation and transfection of these cells can be used as a reliable, efficient and rapid method for generation of these cells and used for iPS cells generation studies from patient's skin somatic cells, and used applicable investigations in the experimental conditions.
Conclusion: Obviously, there cellular with easy access and the Quick cell culture in the laboratory for studies of cell therapy are necessary. Human keratinocyte cells are valuable cells that used them in burn research, restoration, tissue engineering and gene therapy. Also these cells are in addition to therapeutic applications, as one of the best research models to understand the mechanisms reprogramming. In the present study, we have shown our method is the fastest and safest protocol for isolation and culture of these cells in the laboratory. Also we demonstrate effectene and lipofection methods are the proper techniques and safety procedures for gene transfer into these cells. We believe keratinocyte cell culture can be used as a appropriate model and an ideal target for gene therapy studies and generation of patient-specific iPS cells.
Keywords: Keratinocyte, Culture, Transfection, iPS Cells

Ps-357: An Anilinic Electroactive Biocompatible Platform for Neural Tissue Engineering

Payam Zarrintaj[1*], Vahabodin Goodarzi[2], Mohamad Reza Saeb[3], Masoud Mozaffari[4]

1. School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
2. Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Department of Resin and Additives, Institute for Color Science and Technology, Tehran, Iran
4. Nanotechnology and Advanced Materials Department, Materials and Energy Research Center (MERC), Tehran, Iran
Email: payam.zarrintaj@gmail.com

Background and Aim: Design and synthesis of biomimic scaffold has opened new horizons in tissue engineering nowadays. In this study, a conductive gelatin was elaborated as an outstanding platform for neural cell growth. First, aniline dimers were added to amine groups of gelatin to yield an aniline-trimer gelatin hydrogel. Then, the synthesized anilinic oligomer was characterized by FTIR and NMR analyses, followed by investigation of their conductivity and electroavtivity nature. Eventually, the potential of products to cell viability and cell adhesion was explored through MTT and SEM measurements, respectively. The developed aniline-trimer gelatin hydrogel are distinguished among such family of products in view of their proper cell growth, which was fueled by neural cells tendency toward the conductive substrates. The best coupling ability was attained via optimizing the structure of scaffolds toward higher cell growth potential and mimics

the tissue. The optimized scaffold can compensate the gelatin isolation to bring about a better cell growth.
Methods: Aniline-dimer was carboxylated, and then reacted with gelatin to reach a conductive hydrogel. FTIR and NMR tests were proved the conductive hydrogel formation. Conductivity measurement was measured based on four-probe method, while electroactivity was evaluated using cyclic voltammetry. Swelling test was also performed to determine the water uptake capacity. Eventually, MTT test was carried out to determine the cell viability, and made evident with SEM images, which exhibited the cell attachment.
Results: FTIR and NMR illustrated the formation of conductive hydrogel. It is well-documented that a desired conductivity for tissue takes a value in the range of 10-7-10-3 S/cm. The achieved hydrogel conductivity in this work was ca. 10-5, emphasizing usefulness toward tissue engineering. Cyclic voltammetry showed two peaks of reduction/oxidation as a result of the aniline trimer inability to transmit in three states. Swelling ratio was around 500%. PC12 cells were cultured on the samples, where the sample with 2.5% aniline trimer exhibited the proper cell viability. In agreement with all analyses, the SEM image indicated the cell adhesion. Due to these results, it is supposed that the scaffold is biomimetic one which it can be used in tissue engineering
Conclusion: In conclusion, the aniline-trimer gelatin (AT-G) exhibited proper behavior as a scaffold suited to a proper tissue engineering. AT-G because of biocompatible nature and conductivity can mimic the neural tissues. MTT and Cell culture studies reveal that the AT-G has a good biocompatiblity and cell attachment, thus, it can be used in neural tissue engineering.
Keywords: Electro-Conductivity, Neural Tissue Engineering, Biocompatible Scaffold, Gelatin, Aniline trimer

Ps-358: Evaluation of Effect of Indoleamine 2-3 Dioxygenase Enzyme (IDO) Overexpressed in Mesenchymal Stem Cells on Rat B lymphocytes activity

Mahnaz Zeighami, Esmaee[1] Sadroddiny[*]

Tehran University of Medical Science, School of Advanced Technologies in Medicine, Tehran, Iran

Email: zeighami.mahnaz@yahoo.com

Background and Aim: The induction immunosuppression and the use of immunomodulatory reform is one of the strategies in the treatment of autoimmune diseases and transplant rejection prevention. Mesenchymal stem cells through the production of a number of factors solution such as PGE2, NO, IL6 and IDO1 can be a process to prevent an immune response. In this study, using rat mesenchymal stem cells transfected by lentivirus expressing IDO gene and overexpression and induced enzyme IDO, by the antibiotic doxycycline, The effect of the enzyme on B cells, the main cell of humoral immunity, is investigated, Hoping to be effective in reducing the activity of the enzyme to the cell line as an option in the treatment of autoimmune diseases and transplant used to ward off issues.
Methods: In this study, three cell lines, engineered mesenchymal stem cells (TMSC), normal mesenchymal stem cells (MSC) and a mouse B cell lines were used as BCL1. In this regard TMSC + BCL1 co-cultured as the goal, MSC + BCl1 to compare and BCL1 only as controls. Then the rate of growth of B cells in all three groups was evaluated using the test MTT, Differentiation was investigated by measuring the expression of Blimp-1 gene by Real-time PCR and apoptosis were analyzed using flow cytometry.
Results: B cells compared to normal cells, the growth rate dropped in MSC group, and the growth rate in TMSC group was lower compared to two other groups. Blimp1 gene expression in B cells in group TMSC + BCL1 down-regulate and the amount of apoptosis increased in the target group.
Conclusion: It seems to be effective to use enzymes IDO1 as a suitable candidate for inhibiting the immune system by acting on B cells and a favorable option for the prevention of transplant rejection and treatment of autoimmune diseases.
Keywords: Immune System, Mesenchymal Stem Cells, B Cells, Enzymes IDO1

Ps-359: Cloning of Human THAP11 Gene in Lentiviral Vector

Saeid Ziaei[1*], Mostafa Rezae[2], Masoud Soleimani[1]

1. Stem Cell Technology Research Center, Tehran, Iran

2. Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email: sziaee@gmail.com

Background and Aim: Thap11, the most recent described member of THAP domain family, which is involved in cell proliferation and described as one of the key pluripotency gene. Despite of very good research projects established to elucidate real role of Thap11 in cell, there is still inconsistency among researches about the exact role of thap11 in cells. In this research we are over expressed the thap11 gene in human primary fibroblast cells to find that how this gene could alter the cells behavior during expression.
Methods: Human Genome was extracted from human blood sample with Qiagen DNA extraction kit. The THAP11 primers designed according to THAP11 sequence in NCBI nucleotide database and PCR amplification was done with phusion DNA polymerase. The PCR product cloned into PCDH vector by Xba1 and EcoR1 restriction enzyme Digestion followed by purification and ligation steps according to standard protocols and the validity of cloning is proved by Colony PCR and DNA sequencing.
Results: PCDH letiviral vector containing THAP11 was constructed and the sequencing analysis proved the sequence of the THAP11 gene.
Conclusion: Thap11 gene is one of the recently founded pluripotency genes which is very important in stem cells survival and embryo development. So it will be subject to many researches in stem cell and related areas.
Keywords: Thap11, Ronin, Cloning

Ps-360: Human Wharton's jelly Mesenchymal Stem Cells Can Show Some Characteristics of Germ Cells in Vitro Even without Differentiation

Mona Zolfaghar[1], Rouhollah Fathi[2*]

1. Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University (Iaups), Tehran, Iran

2. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
Email: mona_zolfaghar@yahoo.com

Background and Aim: WJ-MSCs derived from proximal epiblast in day 13 of the embryogenesis, hence have same origin with PGCs so it supposed WJ-MSCs may have some characteristics of germ cells. The purpose of present study was to evaluate germ cell markers expression in WJ-MSCs.
Methods: WJ-MSCs were isolated through explant method, then characterized by flowcytometry in the third passage also differentiated to adipocytes and osteocytes. Then WJ-MSCs were Cultured in α-MEM containing 10% FBS for 21 days. Genes expression of ZP1, ZP2, ZP3, GDF-9, VASA, C-KIT, and SYCP3 (oocyte and germ cells specific markers) were evaluated under Real-Time PCR analysis on days 0, 7, 14 and 21 of culturing. Also on day 21 of culturing ZP3, GDF-9, VASA, and SYCP3 proteins were investigated by Immunofleurescent assay.
Results: Flowcytometry analysis indicated that isolated cells could express CD73, CD90 and CD105 (MSCs markers) but don't express CD34 and CD45 (hematopoietic markers) also could differentiate into adipocytes and osteocytes. Immunofleurescent technique and Real time PCR analysis revealed WJ-MSCs, could express some oocyte and germ cells specific markers.
Conclusion: The present study demonstrated that WJ-MSCs could express oocyte and germ cells markers at low levels without no differentiation medium so it shows WJ-MSCs could maintain their germ cell memory. In accordance to this capability of WJ-MSCs, it seems they could provide prominent source for cell therapy protocols in reproductive biomedicine especially for who has deficiency or lack of germ cells.
Keywords: WJ-MSCs, Proximal Epiblast, Germ Cells Memory, Reproductive Biomedicine

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