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Letter

From a Chemical Matrix to Biologically/Biomechanically-Defined Matrices-Optimizing/Correlating Growth Rate and Differentiation Potential of Human Adipose-Derived Mesenchymal Stem Cells

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Letter to the editor in response to: Rezaei S, Shakibaie M, Kabirsalmani M, Moghaddam MS, Rezvani M, Shahali M, *et al*. Improving the Growth Rate of Human Adipose-Derived Mesenchymal Stem Cells in Alginate/Gelatin Versus Alginate Hydrogels. *Iranian J. Biotech.* 2016;14: 1-8.

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Use of Adipose Stem Cells (ADSCs), obtained easily in a relatively less invasive manner (abdominoplasty) and characterized by flow cytometry, is a classical approach in stem cell research and clinical aspects. Other techniques such as isolation of the cells from bone marrow aspirates (1) are rather more invasive. Further, it is pertinent to point out that growth rate, differentiation potential and functions are better in abdominal Adipose Stem Cells (ADSCs), in comparison with those isolated from the Visceral Omental Pads. Such changes are attributable to these cells retaining the memory of their sites of origin (2). Furthermore, their other features of having the potential to differentiate into a number of lineages coupled with their immunomodulatory, angiogenic, anti-inflammatory and anti-apoptotic effects puts ADSCs as the prominent cell type in stem cell research and clinical aspects. The authors have chosen alginate (specific M/G ratio)-gelatin composite hydrogel, with favourable charge properties, to demonstrate an increased growth rate and differentiation potential (50:50 ratio better than the 70:30 ratio) (1) Gelatin is widely accepted as a biocompatible, biodegradable, cost-effective substratum for cell growth. It is also widely believed that it is non-immunogenic. From the commercial point of view, GELFILM™ and GELFOAM™ provide us with evidence of its tremendous utility as scaffold composites. Additionally, these products are amenable to be remodeled *in vivo* by collagenase digestion[2]. However, there are certain reports, which state that its possible allergenicity may depend on its molecular weight (3) and the methodology adopted for crosslinking (4). Hence, these results provide an impetus to thoroughly/more closely evaluate the possible allergenicity of their composite cell-based construct in suitable model systems. In addition to the likelihood of being allergenic, gelatin is thermo-susceptible and can lose its tertiary structure due to heatinduced alterations. In this regard, collagen type I hydrogel might be a better choice than gelatin in producing bone from mesenchymal stem cells (5). Some attempts have been made to improve the mechanical characteristics of gelatin via the removal of divalent cations (6).

This improvement in the mechanical properties of gelatin can possibly contribute to an improved growth rate of ADSCs, despite the known advantages of using calcium instead of chemical cross-linkers[6]. Further, porosity percentage data as well as internal pore size information can add more information to the experimental data generated (7). Such additional information would enable us to better visualize the entrapment and encapsulation efficiency. This approach will allow us to better exploit the angiogenic properties of the ADMSCs to possibly create an "in built" vasculature that can be improved in terms of mass and gas transfer. The excellent biodegradation rate of gelatin provides an

impetus for comparative resorption rate measurements with other biocompatible natural/synthetic biopolymers such as Matrigel in suitable model systems. Further, the long term stability of the cell-matrix construct should also be studied. It is widely accepted that the matrix stiffness gradients, in combination with ligand density, determines the adipose stem cell fate by contributing to the mechano-transduction-mediated cell signalling (outside-in as well as inside-out) (8). More specifically, after the initial commitment stage of cell differentiation/ reprogramming (elasticity-insensitive stage of cell differentiation), lineage specification (after several weeks of culturing) is controlled, in major part, by the rigidity of the matrix (9). In this regard, matrices with a stiffness of 34 kPa are ideal for osteogenic lineage cells in comparison with the soft matrices (0.1-1 kPa) producing neurogenic cells (9). Cells with a stiffness of 11 kPa produce myogenic cells (9). The authors approach to use alginate-gelatin combination is an effective empirical strategy to produce cells of the desired lineage as demonstrated by positive staining with Nile Red and Alizarin Red. However, the need to define such matrices warrants stress-strain moduli measurements (indices of the stiffness of the matrix) for optimizing the growth rate and differentiation potential (extent and efficiency of differentiation) of their ADSCs. Further, their strategy should also recapitulate and regulate biochemical gradients. Recapitulation of such gradients may require transient or long-term dosing of signalling molecules at defined time periods. Such an approach can further enhance the quality of the existing ECM models, wherein space constraints (can improve reprogramming efficiency) have been imposed by microfluidics. It has been demonstrated that such models are able to provide meaningful information about the migratory behaviour of certain cell types (10) In this regard, the incorporation of specific peptide motifs (mimicking certain ECM ligands) (*e.g.*, RGDS binding site for the β(1)-integrin and β(3)-integrin) and TMKIIPFNRLTIGG (a ligand for Mac-1, a β(2)-integrin) in this hydrogel has improved cell adhesion and spreading (11). Similarly, incorporation of hyaluronic acid in the matrix will ensure preferential binding of stem cells that express the hyaluronic acid receptor, apart from its ability to swell upon exposure to water. Control over the nanoscale architecture of the ECM microenvironment is necessary for proper alignment of the actin fibers intracellularly, since this aspect positively regulates cellular behavior (12). Therefore, defined matrices that are tunable based on their unique physicochemical and/or physical properties, without compromising on stability and safety, are ideal for optimal viability and differentiation. In this regard, while the move towards cost-effective matrices offers exciting possibilities (as has been done in this paper), it is imperative that the matrix properties be compared with reference matrices with defined bioactive properties (like Matrigel) in terms of possible temporal and spatial variations in molecular profiling in real time correlated with the differentiation status and cytotoxicity profile of these cells. Subsequently, *in vivo* homing/engraftment potential (efficiency and rate) of these cells should be determined.

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