Effect of Pressure Stresses on Cell Viability and Protein Expression of Fascial Fibroblast

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Background: Many physical and mechanical phenomena occur during the acupuncture and tuina regime, and pressure is one of the most basic mechanical phenomena. Objectives: To understand the cellular bio-physical mechanism of basic mechanical stimulation via acupuncture and tuina by investigating the effect of different in vitro pressures on the cell viability and protein expression differences that originate from the facial fibroblasts around the meridians.

Materials and Methods: In vitro culture of the facial fibroblasts around the meridians was conducted using different pressures to perform single and multiple stimulation(s) on the cells. Thus, the changes in the fibroblast cell viability (cell viability rate and diameter) were tested, and changes in the fibroblast protein expression were observed.

Results: We found that the pressure stimulation may excite the fascial fibroblast viability at the acupoint and increase cell viability. Two interactive factors are involved: the pressure intensity and the number of pressure stimulations. In addition, we found that all three pressures lead to significant regulation effects on the protein expression of the meridian-related fascial tissue fibroblasts, and clustering analysis revealed that 100 kPa pressure stimulation exhibits the most evident effect on the protein expression which is the pressure inducing the most differentiated protein expression.

Conclusions: During the in vitro pressure process, the difference in the cell viability rate and protein expression of the facial fibroblasts around the meridians may (from a cell mechanics’ point-of-view) reveal the cytobiological and therapeutic mechanism of the basic mechanical stimulation via acupuncture and tuina on the facial fibroblasts around the meridians.

Keywords: Cell Survival; Fascia; Fibroblasts; Meridians

1. Background
Many physical and mechanical phenomena occur during the acupuncture and tuina regime, and pressure is one of the most basic mechanical phenomena (1, 2). Our team’s pilot study showed that cells that are under pressure, which was the basic mechanical factor that was applied during the in vitro simulation during acupuncture and tuina, may not only promote the release of multiple biochemically active substances, such as NO, PGE2, MMP-1, TIMP-1, IL-1, IL-6, etc., but also down-regulate and/or maintain the synthesis of certain biochemically active substances, such as IGF-1 (3-5). These changes in the biochemically active substances occur in the meridians’ acupoint region where the fascial connective tissue fibroblast originated, and the region receives and transfers the pressure stimulation via the filaments within the β1 integrin and cytoskeleton. However, neurological functions and other complex body fluid factors are not affected. The potential therapeutic and regulatory functions of the functional proteins occur, and this is the effect of “meridian patency, vital energy and blood smoothing, and vital qi strengthening” (6, 7). Unfortunately, currently, it is unclear how pressure stimulation can influence the cell viability rate of the meridian-related fascial fibroblasts, what may occur to the synthesis of more functional proteins inside the cells after the cells receive the stress.
and how the stability is maintained and how down-regulation is inhibited.

2. Objectives
Therefore, the present study focuses again on the meridians’ acupoint-related fascial connective tissue fibroblast. Different pressures will be applied to further explore the in vitro effect of this type of stress on the biological behaviors of the meridian-related fascial connective tissue fibroblasts by observing the difference in the meridian-related fascial connective tissue fibroblast viability rates and protein expression. The results may provide an experimental understanding of the cellulae bio-physical mechanism that occurs due to basic mechanical stimulation during acupuncture and tuina.

3. Materials and Methods
3.1. Cultures of Primary and Passage Facial Fibroblasts around the Meridians
Use of animals was approved by the Ethic Committees of Guiyang University of Chinese Medicine and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (Publication No. 85-23, revised 1996).

Pregnant (14 days pregnant) Kuming mice (Chongqing Tengxin Biotechnology Co., Ltd., China animal license No.: SCXK (Army) 2012-0011) were adopted. The pregnant mice were sacrificed via cervical dislocation, the abdomen was dissected, the uterus was cut open, and the fetus was exposed. The skin and subcutaneous tissue on and within 2 mm of the governor meridian (posterior midline, from neck to lumbosacral area) of the fetal mouse were taken, the fascial connective tissues were scraped with a scalpel, and the fat and blood vessels were removed. All of these operations were performed under a dissecting microscope. The subcutaneous tissues were placed in a culture dish and cut into slurry with an ophthalmic scissor. Three milliliters of 0.1% type I collagenase were added. We followed the general in vitro experimental protocols for extraction, cultivation, and passage to amplify the fibroblast.

3.2. Pressure Experiment on the Meridian-related Fascial Connective Tissue Fibroblast
The 5th to 8th generations of the meridian-related fascial connective tissue fibroblasts were used, and 0.25% pancreatin and 0.5 mL of EDTA were added to prepare the cellular suspension. The cell density was adjusted to 1×10^6/mL for inoculation in the 6-orifice plate, and then they were placed in the incubator. After 2-4 hours, 2 mL of serum-free DMEM was added to the 6-orifice plate, and the incubator cultivation continued for another 24 hours. We replaced the two ml of DMEM with 10% of FCS after cell synchronization.

3.3. Sample Preparation and Tests for the Fibroblast Viability Rate
The stressed cells were placed into the incubator for another four hours of cultivation. The culture medium was absorbed and removed, and three cycles of PBS washing was performed for 5 min/cycle. Then, 0.25% pancreatin and 200 μL of EDTA was added, and the cell culture plate was gently shaken to sufficiently cover the entire bottom of the culture plate. The culture was then placed in the incubator for five minutes for digestion. Then, we added 800 μL of 10% FBS and DMEM to stop the digestion. We evenly distributed the cells on the culture plate and moved it into the viability rate meter. The stressed cells were placed into the incubator for the pressure experiments ended.

3.4. Sample Preparation and Protein Expression of the Fibroblast
As shown above, the culture was then placed into the incubator for five minutes of digestion, and we added 800 μL of 10% FBS and DMEM to stop the digestion in addition to three cycles of PBS washing and centrifugation (1000 rmp/min for 8 min). The resulting cells were collected and frozen for later inspection. After the six samples from each group were mixed evenly, the AAM-BLM-1 antibody chip assays (Fig. S1; Table S1) by RayBiotech (Georgia, USA) were used. Detailed protocols referred to the Instruction for Use (IFU) of the assay kits.
3.5. Real-time Quantitative PCR
Total RNA was isolated from cells by RNA isolation Kit (Tiangen, Beijing, China) and reverse-transcribed into cDNA by M-MLV reverse transcriptase (Promega, Madison, WI). Real-time PCR was quantified by SYBR green mix (Takara, Dalian, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control to check the efficiency of cDNA synthesis and PCR amplification. The sequence of primers used are: IFN-gamma, F, 5'-GCCACGGCACAAGTCTTTGA-3', R, 5'-TGCTGTGGCCCTATGCTT-3'; CD30, 5'-CCTTCCCCACGATCGACC-3', 5'-CCACTGTGGTGAAAGCAAT-3'; EDAR, 5'-CCACGTCTTTGACGTAGTAGT-3'; NOV/CCN3, 5'-AGTGGCCCAATATATCACCG-3', 5'-TGGTCTACAGTAGGACACCA-3'; P-Selectin, 5'-CCCTGGCAACAGCCTTCAG-3', 5'-TGGTCTACAGTAGGACACCA-3'; GAPDH, 5'-AGGTTCGGTGTGAAACGGATTG-3', 5'-GGGGTCGTGATGGCAAC-3'.

3.6. Statistical Analysis
All data are expressed as means ± SD. We used factorial ANOVA in the SPSS19.0 statistics software to determine if there was any statistical significance between the experimental group and the control. In the fibroblast protein expression investigation, we determined and counted the proteins that changed more than two magnitudes (up-regulation) and less than 0.5 magnitude (down-regulation) using a chemiluminescence imaging system. We used radiographic images that were obtained from a laser confocal scanner to quantify the grey scale for the original signal value, which, was standardized after correction with a positive protein to obtain the standard value. For the calculated data, we used the SPSS19.0 statistical software to perform row × column \( \chi^2 \) inspections to compare the cell protein expression of each group under different stimulation intensities and determine if there was any statistical difference for the inter-group changes. Finally, the clustering analysis software, Treeview, was used to export the clustering analysis chart to analyze the effect of different pressure stimulation intensities on the biological behaviors of the facial fibroblasts around the meridians.

4. Results

4.1. Effect of In vitro Pressure Stimulation on the Meridian-related Fascial Connective Tissue Fibroblast Viability Rate
As shown in the factorial ANOVA analysis, for the two 0 kPa groups that involved single and multiple stimulation(s), the cell viability rate was kept stable during the experiment. As the pressure was increased, the cell viability rate trends for each single stimulation group first increased and then decreased, and the cell viability rate for the 100 kPa group was the highest. For the multiple stimulation groups, the cell viability rate of the 50 kPa group was the highest, the cell viability rate of the 100 kPa decreased, and the cell viability rate of the 200 kPa group was the lowest. The different pressures were evidently higher than that of the control group (P < 0.01). The different number of stimulations had a significant effect on the viability rate of the meridian-related fascial connective tissue fibroblasts (P < 0.05), and the intensity of pressure stimulation combined with the number of stimulations had a significant effect on the viability rate of the meridian-related fascial connective tissue fibroblasts (P < 0.05) (Tables 1 and 2).

Table 1. Effect of in vitro pressure stimulation on the meridian-related fascial connective tissue fibroblast viability rate

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 stimulation Viability rate (%)</th>
<th>3 stimulations Viability rate (%)</th>
<th>Total Viability rate (%)</th>
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</thead>
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<tr>
<td>n</td>
<td></td>
<td>n</td>
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</tr>
<tr>
<td>0 kPa</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>50 kPa</td>
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<tr>
<td>100 kPa</td>
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<td>3</td>
</tr>
<tr>
<td>200 kPa</td>
<td>3</td>
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<td>3</td>
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Table 2. Tests of between-subjects effects Dependent Variable: viability rate

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<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
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<td>7.995</td>
<td>0.000</td>
<td>0.778</td>
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<tr>
<td>Intercept</td>
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<td>155719.260</td>
<td>29888.534</td>
<td>0.000</td>
<td>0.999</td>
</tr>
<tr>
<td>The times of stimulation</td>
<td>41.082</td>
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<td>41.082</td>
<td>7.885</td>
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<td>0.330</td>
</tr>
<tr>
<td>The intensity of stimulation</td>
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<td>61.621</td>
<td>11.827</td>
<td>0.000</td>
<td>0.689</td>
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<tr>
<td>The times * the intensity</td>
<td>65.635</td>
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<td>21.878</td>
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\( R^2 = 0.778 \) (Adjusted R Squared = 0.680).
4.2. Effect of in vitro Pressure Stimulation on the Diameter of the Meridian-related Fascial Connective Tissue Fibroblasts

As shown in the factorial ANOVA analysis, for the two 0 kPa groups that involved single and multiple stimulation(s), the cells kept growing and the diameter increased during the experiment. As the pressure increased, the cell diameter trend for each single stimulation group first increased and then decreased. The cell diameters in the 100 kPa group were the highest. As the pressure increased, the cell diameters in the multiple stimulations groups gradually increased, and the cell diameters in 200 kPa group were the highest. However, when compared with the control group, the different pressures pressure stimulation did not have a significant effect on the diameters of the meridian-related fascial connective tissue fibroblasts (P>0.05). Additionally, the different number of stimulations did not have a significant effect on the diameter of the meridian-related fascial connective tissue fibroblasts (P>0.05). The pressure and the number of stimulations combined did not result in any significant differences in the diameters of the meridian-related fascial connective tissue fibroblasts (P>0.05) (Tables 3 and 4).

Table 3. Effect of in vitro pressure stimulation on the diameter of the meridian-related fascial connective tissue fibroblasts

<table>
<thead>
<tr>
<th>Groups</th>
<th>1 stimulation</th>
<th>3 stimulations</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>Cell diameter (μm)</td>
<td>n</td>
</tr>
<tr>
<td>0 kPa</td>
<td>3 14.4933 ± 0.45982</td>
<td>3 14.8067 ± 0.34530</td>
<td>6 14.6500 ± 0.40214</td>
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<tr>
<td>50 kPa</td>
<td>3 14.5967 ± 0.65577</td>
<td>3 14.9267 ± 0.26633</td>
<td>6 14.7617 ± 0.48276</td>
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<tr>
<td>100 kPa</td>
<td>3 14.7033 ± 0.33171</td>
<td>3 14.9467 ± 0.38837</td>
<td>6 14.8250 ± 0.32390</td>
</tr>
<tr>
<td>200 kPa</td>
<td>3 14.5433 ± 0.20744</td>
<td>3 15.0200 ± 0.33151</td>
<td>6 14.7817 ± 0.35963</td>
</tr>
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Table 4. Tests of between-subjects effects dependent variable: cell diameter

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<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
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<td>33674.808</td>
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<td>1.000</td>
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<td>0.508</td>
<td>3.260</td>
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<tr>
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4.3. Effect of the in vitro Pressure Stimulation on the Protein Expression of the Meridian-related Fascial Connective Tissue Fibroblasts

For this experiment, we chose a biotin-marked mouse antibody chip that can detect 308 proteins simultaneously. The antibody chip detection results were shown in Figure 1A. In detail, the positive control points are visible, whereas the negative control points were not detected, and the remaining protein points are irregularly presented, indicating that the quality control is accurate and the result is confident.

Figure 1. Effect of the in vitro pressure stimulation on the protein expression of the meridian-related fascial connective tissue fibroblasts. (A). A biotin-marked mouse antibody chip was chosen for this study, positive control strains were visible, and negative control strains were not detected. The remaining proteins were irregularly presented. (B). Real-time quantification PCR was used to verify the chip screening to confirm the observed variation in cell protein expressions. In all 3 strain groups, we observed upregulated proteins IFN-gamma, CD30, and EDAR; additionally, we also observed downregulated proteins NOV/CCN3 and P-Selectin.

The three pressures resulted in a clear regulatory effect on the protein expression of the meridian-related fascial tissue fibroblasts; the comparison of the three interregulation groups showed a $\chi^2 = 59.005$ with P<0.0005.
The overall regulation (including up-regulation and down-regulation) in the 50 kPa group was the smallest, and up-regulation prevailed. The magnitude of regulation in the 100 kPa group was the highest with the amount of protein that was up-regulated and down-regulated was the most of the three groups, and the amount of up-regulation was the highest. For the 200 kPa group, the magnitude of regulation was in between the other two groups, and up-regulation also prevailed. However, for the 200 kPa group, the amount of down-regulation was the lowest of the three groups (Table S2, Table S3 and Table S4).

We used real-time quantitative PCR to verify the mRNA levels in these three groups. The results showed that compared to the control group the expression of the IFN-gamma, CD30, and EDAR in the pressure groups increased, whereas the expression of the NOV/CCN3 and P-Selectin in the pressure groups were significantly lower, which further confirmed the accuracy of the chip results (Fig. 1B).

4.4. Clustering Analysis of the Effect of Different Pressures on the Protein Expression of the Fibroblasts

The clustering analysis revealed pressure stimulation that the 200 kPa pressure had the lowest influence on the regulation of the fibroblast protein expression, followed by the 50 kPa pressure. The influence of the 100 kPa pressure stimulation on the protein expression was the highest, which is the pressure inducing the most differentiated protein expression (Fig. 2).

5. Discussion

The primitive stimulation approach is to press, squeeze, massage, and rub with the hands or fingers the body's meridians and acupuncture points. Therefore, pressure stimulation is one of the most primitive mechanical stimulations in acupuncture and tuina. From the perspective of modern medical physics and its mechanical analysis, no matter how the acupuncture and tuina approaches have evolved, pressure stimulation has remained the most basic mechanical stimulation. Cell viability refers to the biological status and functions of the cells. There are various parameters that define the cell viability, such as vital cell ratio, cell size, oxidation-reduction potential of the cell mass, integrity of the cytomembrane, the enzymatic activity of the cells (e.g., esterase), etc. These parameters that provide a measurable and comparative metric for the health of the cells may be adopted separately or combined into cell viability research. Modern investigations show that the mechanical factor is also one of the major factors that impacts cell viability. In this study, we chose the cell viability rate and cell diameter as the metrics for cell viability. The results showed that pressure stimulation can produce an overall increase in the acupoint fascial fibroblast viability by increasing the cell viability rate. Moreover, single (i.e., immediate effect) pressure stimulation and multiple (i.e., accumulative effect) pressure stresses resulted in different cell viability rate trends. For single load stimulation, the cells are sensitive to a medium load, whereas for multiple load stimulations, the cells are sensitive to a light load. The pressure stimulation encouraged the overall growth (i.e., increase in diameter) of the acupoint fascial fibroblast, but the load intensity, number of independent load stimulations, and the two factors combined all resulted in a statistical difference in the cell growth. Therefore, the pressure can increase the cell viability, and its effects on the cell viability are primarily due to the increase in the cell viability rate.

The clustering analysis revealed pressure stimulation that the 200 kPa pressure had the lowest influence on the regulation of the fibroblast protein expression, and the influence of the 100 kPa pressure stimulation on the protein expression was the highest.

Meanwhile, we used AAM-BLM-1 antibody chips from RayBiotech to detect the expression changes of 308 proteins. The results showed that a low pressure stimulation (50 kPa) up-regulated the expression of 106 proteins, maintained the expression of 130 proteins, and down-regulated the expression of 72 proteins. The medium pressure stimulation (100 kPa) up-regulated the expression of 170 proteins, maintained the expression of 83 proteins, and down-regulated the expression of 55 proteins. The 200 kPa pressure up-regulated the expression of 161 proteins, maintained the expression of 128 proteins, and down-regulated the expression of 19 proteins. The pressure stimulation of all three groups imposed evident regulatory effects on the expression of the facial fibroblasts around the meridians protein. The lowest pressure caused the lowest overall regulation (including up-regulation and down-regulation), with up-regulation prevailing. The medium pressure caused the maximum regulation, and there were more proteins that were up-regulated than in the other two groups. With this group, up-regulation also prevailed. The regulation of the heavy pressure group was similar to that of the medium pressure group. In addition, the clustering analysis revealed pressure stimulation that the medium pressure stimulation had the most influence on the protein expression, being the greatest differentiated protein expression among the clustering analysis.
Figure 2. Clustering analysis of the effect of different pressures on the protein expression of the fibroblasts

According to our experiments, during *in vitro* pressure stress, the cell viability and protein expression of the meridian-related fascial connective tissue fibroblasts may change accordingly. All of these changes may (from a cellular mechanics’ point-of-view) reveal the cyto biological and therapeutic feedback mechanism that occurs the meridian-related fascial connective tissue fibroblasts via simple mechanical stimulation (pure pressure stress) that occurs during acupuncture and  tuina. Based on the mechanical intensity tests of this study, we observed that a medium pressure stimulation may activate the cell viability and protein synthesis/regulation functions the most. However, our study also shows that low and medium pressures contribute to the accumulated activation effects of pure pressure stimulation on the cell viability, whereas medium and heavy pressures contribute to the accumulated regulation effects on the cell protein expression. This difference requires further investigation to determine the cause of this phenomenon.
Acknowledgements
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Disclosure of Conflict of Interest
None.

References

Figure S1. AAM-BLM-1 antibody chip assays were used to detect the fibroblast protein expression.

Table S1. Location and name of 308 proteins in antibody chip

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<td>1-3</td>
<td>28-30</td>
<td>P (average)</td>
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stimulation on release of PGE2 and IL-6 in fibroblasts in the rat "Zusanli" and its adjacent areas]. Zhongguo Zhen Jiu. 2007;27(2):135-140. pmid: 17370500
1.2 10 Activin A 9,10 27 G1TR Ligand / TNFSF18 21,22 8 MCP-1
1.2 11 Activin C 9,10 28 Glut2 21,22 9 MCP-5
1.2 12 Activin RIB / ALK-4 9,10 29 GM-CSF 21,22 10 M-CSF
1.2 13 Adiponectin / Acrp30 9,10 30 Granocyte B 21,22 11 MDC
1.2 24 AgRP 11,12 1 Granocyte D 21,22 12 MFG-E8
1.2 25 ALCAM 11,12 2 Granocyte G 21,22 13 MFRP
1.2 26 Angiopoietin-like 2 11,12 3 Gremlin 21,22 16 MIG
1.2 27 Angiopoietin-like 5 11,12 4 Growth Hormone R 21,22 17 MIP-1 alpha
1.2 28 AR (Amphiregulin) 11,12 5 HGF-R 21,22 18 MIP-1 gamma
1.2 29 Artemin 11,12 6 HGF 21,22 19 MIP-2
1.2 30 Ax 11,12 7 HVEM / TNFRSF14 21,22 20 MIP-3 alpha
3.4 9 b FGFR 11,12 8 ICAM-1 21,22 21 MIP-3 beta
3.4 10 B7-1 / CD80 11,12 9 ICAM-2 / CD102 21,22 22 MMP-2
3.4 11 BAFF-R / TNFRSF13C 11,12 10 ICAM-5 21,22 23 MMP-3
3.4 12 BCMA / TNFRSF17 11,12 11 ICK 21,22 24 MMP-9
3.4 13 beta-Catenin 11,12 12 IFN-alpha / beta R1 21,22 25 MMP-12
3.4 24 BLC 11,12 13 IFN-alpha / beta R2 21,22 26 MMP-14 / LEM-2
3.4 25 BTC (Betacellulin) 11,12 16 IFN-beta 21,22 27 MMP-24 / MTS-MMP
3.4 26 Cardiotrophin-1 11,12 17 IFN-gamma 21,22 28 Neuregulin-3 / NRG3
3.4 27 CCL1 / 1-80 / TCA-3 11,12 18 IFN-gamma R1 21,22 29 Neurturin
3.4 28 CCL4 / MIP-1 beta 11,12 19 HGF-BP-1 21,22 30 NGF-R / TNFRSF16
3.4 29 CCL4 / MIP-1 beta 11,12 20 HGF-BP-2 23,24 1 NOV / CCN3
3.4 30 CCL7 / MCP-3 / MARC 11,12 21 HGF-BP-3 23,24 2 Osteoactivin / GPNMB
5.6 1 CCL8 / MCP-2 11,12 22 HGF-BP-5 23,24 3 Osteopontin
5.6 2 CCR10 11,12 23 HGF-BP-6 23,24 4 Osteoprotegerin
5.6 3 CCR3 11,12 24 HGF-BP-p1 / HGF-BP-7 23,24 5 OX40 Ligand / TNFSF4
5.6 4 CCR4 11,12 25 IGF-1 23,24 6 PDGF C
5.6 5 CCR6 11,12 26 IGF-II 23,24 7 PDGF R alpha
5.6 6 CCR7 11,12 27 IL-1 alpha 23,24 8 PDGF R beta
5.6 7 CCR9 11,12 28 IL-1 beta 23,24 9 Pentraxin3 / TSG-14
5.6 8 CD11a 11,12 29 IL-1 R / ST2 23,24 10 PP-4
5.6 9 CD14 11,12 30 IL-1 R6 / IL-1 R R2p2 23,24 11 PIGF-2
5.6 10 CD195 11,12 31 IL-1 R9 23,24 12 Progranulin
5.6 11 CD27 / TNFRSF7 13,14 10 IL-1 R1 23,24 13 Prolinectin
5.6 12 CD27 Ligand / TNFSF7 13,14 11 IL-1 RII 23,24 14 P-Selectin
5.6 13 CD30L 13,14 12 IL-2 23,24 17 RAGE
5.6 16 CD30 13,14 13 IL-2 R alpha 23,24 18 RANTES
5.6 17 CD40 13,14 24 IL-2 R beta 23,24 19 RELM beta
5.6 18 CD40 Ligand / TNFSF5 13,14 25 IL-3 23,24 20 Resistin
5.6 19 Cerberus 1 13,14 26 IL-3 R alpha 23,24 21 S100A10
5.6 20 Chordin-Like 2 13,14 27 IL-3 R beta 23,24 22 SCF
5.6 21 Coagulation Factor III / Tissue Factor 13,14 28 IL-4 23,24 23 SCF R / c-kit
5.6 22 Common gamma Chain / II-2 R gamma 13,14 29 IL-4 R 23,24 24 SDF-1
5.6 23 CRG-2 13,14 30 IL-5 23,24 25 Serum Amyloid A1
5.6 24 Cripto 15,16 9 IL-5 R alpha 23,24 26 Shh-N
5.6 25 Crossveinless-2 15,16 10 IL-6 23,24 27 SgIRR
5.6 26 Cryptic 15,16 11 IL-6 R 23,24 28 SLPI
5.6 27 Csk 15,16 12 IL-7 23,24 29 Soggy-1
5.6 28 CTACK 15,16 13 IL-7 R alpha 23,24 30 SPARC
5.6 29 CTLA-4 / CD152 15,16 24 IL-9 25,26 1 Spinexin Ectodomain
5.6 30 CXCL14 / BRAK 15,16 25 IL-9 R 25,26 2 TACI / TNFRSF13B
7.8 1 CXCL16 15,16 26 IL-10 25,26 3 TARC
7.8 2 CXCR2 / IL-8 RB 15,16 27 IL-10 R alpha 25,26 4 TCA-3
7.8 3 CXCR3 15,16 28 IL-11 25,26 5 TCCR / WSS1
7.8 4 CXCR4 15,16 29 IL-12 p40 / p70 25,26 6 TECK
7.8 5 CXCR6 15,16 30 IL-12 p70 25,26 7 TFF1
7.8 6 DAN 17,18 9 IL-12 R beta 1 25,26 8 TGF-beta 1
7.8 7 Decorin 17,18 10 IL-13 25,26 9 TGF-beta 2
7.8 8 DKK-1 17,18 11 IL-13 R alpha 2 25,26 10 TGF-beta 3
7.8 9 Dkk-3 17,18 12 IL-15 25,26 11 TGF-beta RI / ALK-5
7.8 10 Dkk-4 17,18 13 IL-15 R alpha 25,26 12 TGF-beta RII
7.8 11 DPPIV / CD26 17,18 24 IL-16 25,26 13 Thrombospondin
7.8 12 DR3 / TNFRSF25 17,18 25 IL-17 25,26 16 Thymus Chemokine-1
7.8 14 Dmpk 17,18 26 IL-17R 25,26 17 Tic-2
7.8 16 EDAR 17,18 27 IL-17C 25,26 18 TIMP-1
7.8 17 EGF R 17,18 28 IL-17D 25,26 19 TIMP-2
Table S2. Effect of the in vitro pressure stimulation on the protein expression of the meridian-related fascial connective tissue fibroblasts

<table>
<thead>
<tr>
<th>Groups</th>
<th>Numbers of upregulated proteins</th>
<th>Number of nonregulated proteins</th>
<th>Numbers of downregulated proteins</th>
<th>Total numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 kPa</td>
<td>106</td>
<td>130</td>
<td>72</td>
<td>308</td>
</tr>
<tr>
<td>100 kPa</td>
<td>170</td>
<td>83</td>
<td>55</td>
<td>308</td>
</tr>
<tr>
<td>200 kPa</td>
<td>161</td>
<td>128</td>
<td>19</td>
<td>308</td>
</tr>
</tbody>
</table>

Table S3. The effect of the in vitro pressure stimulation on the protein upregulation of the meridian-related fascial connective tissue fibroblasts

<table>
<thead>
<tr>
<th>Groups</th>
<th>Protein name</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 kPa</td>
<td>IfNgamma, CD30, CD40, EDAR, IL-5 R alpha, Endostatin, PIGF-2, TGF-beta 2, NGF R / TNFRSF16, VEGF R2, MCP-1, TECK, CCRX3, CCRX6, E-Selectin, FADD, SLPI, Soggy-1</td>
<td>1</td>
</tr>
<tr>
<td>100 kPa</td>
<td>IL-15, IL-2, IL-23, IL-24, IL-7, IL-1 R9, IL-1 R1, IL-1 RII, IL-13 R alpha 2, IL-2 R alpha, IL-6 R, IL-7 R alpha, IFN-alpha, IFN-alpha / beta R1, IFN-alpha / beta R2, IFN-gamma R1, CD27 Ligand / TNFSF7, OX40 Ligand / TNFSF4, TNF-beta / TNFSF18, TWEAK / TNFSF12, BAFF R / TNFSF13, BCMA / TNFSF17, CD27 / TNFRSF7, CD30 L, DR5 / TNFRSF25, HVEM / TNFRSF14, TWEAK R / TNFRSF12, G-CSF, Activin A, EGF R, EG-FGF / PIK1, Endostatin / CD105, FGF-21, Fshr-3 Ligand, FLRG (Follistatin), Follistatin-like 1, GDF-1, GDF-3, Growth Hormone R, HGF, IGFBP-1, IGFBP-2, IGFBP-3, PDGF C, TGF-beta 1, TGF-beta 3, VEGF, HGF R, PDGF R alpha, PDGF R beta, TGF-beta RI / ALK-5, TGF-beta RI, VEGF R1, Fractalkine, MIP-3 alpha, PF-4, TARC, CCR10, CCR6, CCRX4, CD11b, CD195, MacaCAM-1, B7-1 / CD80, ICAM-1, ICAM-2 / CD102, ICAM-5, VEG-CaFiberin, TIMP-2, beta-Catenin, Frizzled-1, Frizzled-6, Frizzled-7, Dkk-1, Dkk-3, Dkk-4, Resistin, CD14, DPPIV / CD26, DAN, Decorin, Dkk, Endocan, Osteopontin, Pentraxin 3 / TSG-14, SPARC, TFPI, Thrombomodulin</td>
<td>2</td>
</tr>
<tr>
<td>200 kPa</td>
<td>IL-13, IL-21, IL-22, IL-22BP, IL-6, Common gamma Chain / IL-2 R gamma, IL-12 R beta, IL-15 R alpha, IL-21 R, TCCR / WSX-1, TSLP R, IFN-gamma, CD30, CD40, Ligand / TNFSF15, TRAIL / TNFSF10, EDAR, TPO, IL-5 R alpha, Endostatin, Epirufinin, Erythropoietin (EPO), GDF-5, GDF-8, GDF-9, GFR alpha 2 / GDNF R alpha 2, PIGF-2, TGF-beta 2, NGF R / TNFRSF16, Eotaxin, MCP-1, MIP-1 alpha, RANTES, TACA-3, TECK, CCR9,CXCR3, CXCR6, E-Selectin, FADD, S100A10, SLPI, Soggy-1, Coagulation Factor III / Tissue Factor, Crossveinless 2, Glut2, Insulin, Neuritin, Ubiquitin</td>
<td>1</td>
</tr>
</tbody>
</table>

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1, the expression of proteins was upregulated by more than 2 folds. 2, the expression of protein was negative in control group, and was positive in pressure groups.

**Table S4. The effect of the in vitro pressure stimulation on the protein downregulation of the meridian-related fascial connective tissue fibroblasts**

<table>
<thead>
<tr>
<th>Groups No.</th>
<th>Protein name</th>
</tr>
</thead>
<tbody>
<tr>
<td>50kPa VS 0kPa</td>
<td>IL-1 alpha, IL-10, IL-13, IL-22, IL-1 R6 / IL-1 R1 R2 / IL-10 R alpha, IL-21 R, IL-9 R, GHR, IL-3, IL-5, M-CSF, SCF, IL-3 R alpha, IL-3 R beta, SCF R / c-kit, Activin C, GFR alpha-3 / CDGNF R alpha-3, GFR alpha-4 / GDNF R alpha-4, IGF-1, IGF-2, MPG-8, Progranulin, TMEFF1 / Tomoregulin-1, Activin RIB / ALK-4, CCL-1 / TAC-1, CCL28, CXCL14 / BRAK, 1-TAC, KC, MCP-5, MDC, MIG, MIP-1 alpha, MIP-2, Thymus Chemokine-1, Integrin beta 2 / CD18, TREM-1, MMP-12, MMP-2, MMP-3, MMP-9, TIMP-1, SI010A10, SIGIRR, WIF-1, Serum Amyloid A1, TLR4, Cerebus 1, Chordin Like-2, Granzyme B, Kremen-1, Kremen-2, Lefty-1, MFRP, Prolactin, Shh-N</td>
</tr>
<tr>
<td>50kPa VS 0kPa</td>
<td>IL-21, IL-31, IL-12 R beta 1, IL-31 RA, NOV / CCN3, VEGF-B, VEGFC, VEGF-D, MIP-1 gamma, RAGE, RANTES, P-Selectin, Adiponectin / Acrp30, Insulin, Spineos Ectodomain</td>
</tr>
<tr>
<td>100 kPa VS 0kPa</td>
<td>Cardiotrophin-1, IL-12 p70, IL-16, IL-17C, IL-17D, IL-17E, IL-17F, IL-17F / IL-17 R2, IL-10 R alpha, IL-17R, Fas / TNFSF6, TROY, IL-5, SCF R / c-kit, AR (Amphiregulin), TMEFF1 / Tomoregulin-1, VEGF-B, VEGF-D, VEGF R3, BLC, CCL1 / TAC-3, CCL28, CCL4 / MIP-1 beta, CCL7 / MCP-3, MARC, CXCL14 / BRAK-I-TAC, KC, SAF-1, CCR4, P-Selectin, MMP-12, MPP-24 / MT5-MMP, MMP-3, MMP-9, WIF-1, TLR3, 6Ry, AGFR, Granzyne B, Kremen-1, Kremen-2, Lefty-1, Leptin R, LEPTIN (OB), Shh-N</td>
</tr>
<tr>
<td>100 kPa VS 0kPa</td>
<td>Angiopoeitin-like 2, Angiopoeitin-like 3, BTC (Betacellulin), NOV / CCN3, VEGFC, VEGF R2, ALCAM, Adiponectin / Acrp30, Spineos Ectodomain</td>
</tr>
<tr>
<td>200 kPa VS 0kPa</td>
<td>NOV / CCN3, MIG, MPG-8, CCR7, Progranulin, TRAIL R2 / TNFRSF10B, MRFP, P-Selectin, MMP-24 / MT5-MMP, CCR4, Cerebus 1, RAGE, CCR9</td>
</tr>
<tr>
<td>200 kPa VS 0kPa</td>
<td>IL-13, CXCR3, IL-6, IL-5 R alpha, Spineos Ectodomain, IL-12 R beta 1</td>
</tr>
</tbody>
</table>

3, the expression of proteins was downregulated by more than 2 folds. 4, the expression of protein was positive in control group, and was negative in pressure groups.