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Abrasion Stimulations on Biological Behaviors of Fascial Fibroblasts

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Sibu Ma[1, 2, #], Minghong Luo[3, #], Bo Chen[3, *, #], Lei Chen[3], Yalin Wang[3], Weiyin Cheng[3], Feng Liu[4], Jiangyan Xiong[5], Ying Jia[6]
[1], College of Humanities, Guiyang University of Chinese Medicine
[2], Institute of Traditional Chinese Medicine of Minorities, Central University for Nationalities
[3], College of Acupuncture and Massage, Guiyang University of Chinese Medicine
[4], Emergency Department, Affiliated Hospital of Jiujiang University
[5], College of Nursing, Guiyang University of Chinese Medicine
[6], Department of Stomatology, Guizhou Medical University
**Address for correspondence to: Prof. Bo Chen, College of Acupuncture and Massage, Guiyang University of Chinese Medicine, Dongqing South Road Guiyang, Guiyang City, Guizhou, 550025, People 's Republic of China. Tel: +86 130 0784 3202. Fax: +86 0851 8565 2189. E-mail: chenbo_med@163.com*
#, Sibu Ma, Minghong Luo and Bo Chen contribute equally to this work

Abstract

Objective: To investigate the influence of different in vitro abrasions on cell viability and protein expression and to provide experimental foundation for cellular and biophysical mechanism of basic mechanical stimulation via acupuncture and tuina.

Methods: Fibroblasts from the meridian-related fascial connective tissue were cultured in vitro, and abrasion was applied onto the study subjects with different intensities. The deviations of cell viability and protein expression in the fibroblasts were observed.

Results: We found that abrasions may decrease the viability of fibroblasts from the meridian-related fascia, resulting in reduced cell viability rate and roundness. Abrasion intensity is the factor that inhibits the cell viability rate and roundness, while the number of stimulations is not. These two factors (abrasion intensity and the number of stimulations) are not integrative. Furthermore, the abrasion stimulations from all 3 groups result in a clear adjustment of protein expression of the fibroblasts. We found that abrasion stimulations with medium intensity have maximum effect on the protein expression, as supported by the clustering analysis.

Conclusions: We found that abrasion stimulations with medium intensity may have maximum role in inhibition of cell viability and protein synthesis/adjustment.

Keywords: Abrasion stimulation; meridian; fascia fibroblast; cell viability; protein expression.

1. Introduction

During the acupuncture and tuina therapy, a significant number of biophysical and mechanical phenomena occur. Similar as pressure stimulation, abrasion is a common mechanical phenomenon that occurs during acupuncture and tuina (XU, J. & al [1], PASYK, K. A. & al [2], BO CHEN, & al [3]). Our previous studies showed that, after in vitro simulation of basic mechanical factors (e.g., pressure and abrasion) from acupuncture and tuina was applied, fascial connective tissue fibroblasts that originate from the meridian acupoint region received and transferred pressure and abrasion stimulations via the β1 integrin and cytoskeleton filaments (BO CHEN, & al [4]). Pressure stimulation may promote cell proliferation through releasing a number of bioactive matters such as NO, PGE2, MMP-1, TIMP-1, IL-1, and IL-6, as well as down-regulate and/or maintain the stable synthesis of certain biochemically active compounds, such as IGF-1, thus delivering proteins with therapeutic potential and regulatory functions (BO CHEN, & al [5], BO CHEN, & al [6], BO

CHEN, & al [7]). However, several questions remain unknown, such as how abrasion stimulations affect the viability of fibroblasts from the meridian-related fascia, how functional proteins within the cells are functioned after the abrasion stimulation, or how protein synthesis stability is maintained.

Considering the abovementioned information, we chose fibroblasts derived from the meridian-related fascial connective tissue as the study subjects. The stimulations were applied via shearing force from shaking fluids with different intensities (BO CHEN, & al [8]). The deviations in cell viability and protein expression in the fibroblasts were observed to further explore the in vitro influence of abrasion stimulations on the biological behaviors of the fibroblasts (FENG LIU, & al [9], WISE, G. E. & al [10]). Our study provides new experimental foundations for the cell therapy via mechanical stimulation during acupuncture and tuina.

2. Materials and Methods

Reagents and Equipments

For the experiments, we used a CO2 incubator (Type MCO-15AC, SANYO, Japan), an inverted phase contrast microscope and an imaging group system (Olympus, 1×71, Japan), a vertical shaking table (SYC2101, Crystal, USA), a 6-orifice cell culture plate (UAS Corning Co., Newyork, USA), and a cell viability analysis meter (Type VICELL AUTO100/240, USA BECKMAN Co.,).

Primary culture of the fibroblasts from the meridian-related fascial connective tissue

Pregnant mice (Kunming Species, 14th day of pregnancy) (Chongqing Tengxin Biotechnology Co., Ltd., animal license No.: SCXK(Army)2012-0011) were sacrificed via cervical dislocation. The abdominal cavity was cut open to expose the fetus by cutting open the uterus with scissors. Then, the sacrificed animal was placed under the dissecting microscope. The skin and subcutaneous tissue of the fetus were obtained from the area around the governor meridian (posterior midline, from neck to lumbosacral area) with a width of around 2 mm. We scrapped away the subcutaneous fascial connective tissues with a scalpel and removed fat and blood vessels. The resulting subcutaneous tissues were placed into the culture dish and were cut into a slurry using ophthalmic scissors. In total, 3 ml of 0.1% Ⅰ type collagenase was added to the extract to cultivate and amplify the number of cells using the general in vitro experiment protocol.

Abrasion loading tests on the fibroblasts

We collected the 5th-8th generation of the fibroblasts from the meridian-related fascial connective tissue and added an appropriate volume of 0.25% pancreatin + 0.5 ml EDTA to prepare the cell suspension. The cell density was adjusted to 1×104/ml for inoculation in the 6-orifice plate. Then, the plate was placed into an incubator at 37℃, 5% CO2, and 100% humidity. After 2-4 hours, serum-free 2 ml DMEM was added to the 6-orifice plate for another 24 hours of cultivation. After the cells were synchronized, we replaced the solution with 2 ml of 10% FCS of DMEM substrate.

The expression of cell proteins was randomized according to the force magnitude into the blank control group, minor, medium, and heavy abrasion stimulation groups. There were 6 samples in each group. In addition, for the cell viability investigation, each stimulation group was further randomized into the single force subgroup (i.e., single force stimulation) and multiple force subgroup (i.e., 3 cycles of force stimulation), according to the number of force applications. There were 3 samples in each subgroup.

The cells from the abrasion stimulation groups were placed on the horizontal shaking table, and then moved into the incubator. The speeds of the horizontal shaking table were adjusted

to 30, 60, and 120 turns/min for the minor, medium, and heavy abrasion stimulation groups, respectively. The force application was maintained for 2 hours, with 24 hours intervals in between force application for the multiple force subgroup. Normal incubation was maintained in the incubator during these force intervals. Four hours after the end of force application, the cells were collected for later inspection. The blank control group was placed into the incubator for regular incubation without any stimulation. The samples from this group were collected and inspected along with the samples from the other stimulation groups.

Fibroblast viability test

After force application, the cells were placed into the incubator for another 4 hours. Then, the substrate was removed and the plate was washed with PBS 3 times (5 min per cycle). Following this, we added an appropriate volume of 0.25% pancreatin + 200 μL EDTA and gently shook the culture plate. Thus, the cells covered the entire bottom of the plate. Then, the plate was placed into the incubator for 5 minutes of digestion. Following this, an appropriate volume of added 10% FBS of 800 μL DMEM was added to stop the digestion, and the cells were evenly distributed on the culture plate. The entire setup was moved into the measuring cup that was specially designed for the viability testing device, and then placed into the cell viability meter for detection.

Protein expression test of the fibroblasts

After the force application, the cells were placed into the incubator for another 4 hours. Then, the substrate was removed, and the plate was washed 3 times with PBS (5 min per cycle). Following this, we added an appropriate volume of 0.25% pancreatin + 200 μL EDTA and gently shook the culture plate, and the cells covered the entire bottom of the plate. Then, the plate was placed into the incubator for 5 minutes of digestion. Following this, we added an appropriate volume of 10% FBS of DMEM to stop the digestion. Then, the plate was repeatedly washed with a PBS solution, followed by 3 cycles of centrifugation (1000 rmp, 8 min). After this, we collected the cells, froze and stored them for later inspection. For the AAM-BLM-1 antibody chip (supplied by RayBiotech) assays (Figure S1), 6 samples from each group were evenly mixed, and we followed the manufacturer instructions of the kit for specific procedures.

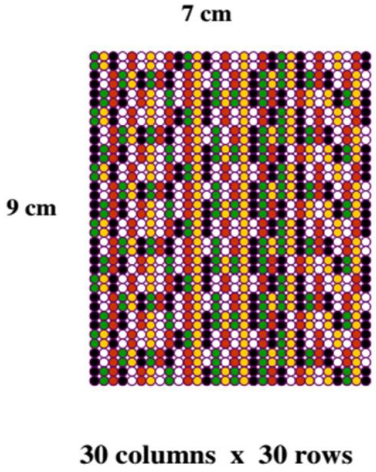

Figure S1. AAM-BLM-1 antibody chip assays were used to detect the fibroblast protein expression.

Real-time quantification 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). Glyceral-

dehyde-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: IL-15, F, 5'-CATCCATCTCGTGCTACTTGTG-3', R, 5'-GCCTCTGTTTTAGGGAGACCT-3'; G-CSF, 5'-ATGGCTCAACTTTCTGCCCAG -3', 5'-CTGACAGTGACCAGGGGAAC-3'; beta-catenin, 5'-ATGGAGCCGGACAGAAAAGC-3', 5'-TGGGAGGTGTCAACATCTTCTT-3'; angiopoietin-like 2, 5'-CCACCTCGGGTCTACCAAC-3', 5'-CTTGCAGGCAGTCTCTCCAT-3'; CCL4/MIP-1 beta, 5'-TTCCTGCTGTTTCTCTTACACCT-3', 5'-CTGTCTGCCTCTTTTGGTCAG-3'; AR (Amphiregulin), 5'-GGTCTTAGGCTCAGGCCATTA-3', 5'-CGCTTATGGTGGAAACCTCTC-3'; GAPDH, 5'-AGGTCGGTGTGAACGGATTTG-3', 5'-GGGGTCGTTGATGGCAACA-3'.

Statistics

All data were expressed as the mean ± standard deviation. We used the factorial ANOVA analysis in the SPSS19.0 software to compare variations between the test groups and the control group to determine whether there was any statistical significance. For the fibroblast protein expression investigation, we adopted the chemiluminescence imaging system and obtained exposure images using a laser confocal scanner. From these images, the raw signal value was obtained from the grey scale quantification, and the signal value was obtained from the grey scale quantification. From these standard values, we identified and counted proteins with the magnitude of change greater than 2 times (defined as up-regulation) and less than 0.5 times (defined as down-regulation). The counts were analyzed using the SPSS19.0 software as the row × column □2 testing to compare the cell protein expression of each group under different stimulations and to determine whether there was any statistical difference in the inter-group changes. Finally, the clustering analysis software Treeview was used to export the clustering analysis chart. Thus, the effects of different pressure stimulations on the biological behavior of fibroblasts from the meridian-related fascial connective tissue could be analyzed.

3. Results and discussion

Influence of abrasion stimulations on the survival rate of meridian-related fascial fibroblast

As seen in Tables 1 and 2, the factorial ANOVA analysis showed that during the experiments, the cell survival rates of both single and multiple abrasion blank control groups were approximately stable. In each single stimulation subgroup, the survival rate reflected a downward trend with increased stimulation intensity (abrasion), whereby the cell survival rates in medium and heavy abrasion groups were the lowest. In each multiple stimulation subgroup, the survival rate also reflected a downward trend with increased abrasion intensity, similar to the lowest cell survival rates in medium and heavy abrasion groups. Different abrasion stimulation intensities caused the survival rate of the fibroblasts to be significantly different from those of the blank control group ($P=0.036<0.05$). However, a different number of stimulations may not significantly affect the survival rate of the fibroblasts ($P=0.953>0.05$). Combination of these two factors (abrasion stimulation intensity and the number of stimulations) did not show a synergistic effect on the survival rate of the meridian-related fascial connective tissue fibroblasts ($P=0.051>0.05$).

Table 1 Influence of in vitro abrasion stimulations on fibroblast viability

Groups	1 stimulation		3 stimulations		Total	
	n	viability rate (%)	n	viability rate (%)	n	viability rate (%)
Control	3	86.7333±1.87705	3	86.4667±2.70617	6	86.6000±3.13050
Minor	3	83.1667±6.24046	3	81.0667±2.51462	6	82.1167±6.80688
Medium	3	81.8000±6.53835	3	78.7000±4.44410	6	80.2500±5.28044

Heavy	3	80.7000±2.65141	3	78.8000±5.55608	6	79.7500±4.03026

Table 2 Tests of Between-Subjects Effects

Dependent Variable: viability rate

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	400.863(a)	7	57.266	2.927	0.036	0.562
Intercept	154385.000	1	154385.000	7891.213	0.000	0.998
The times of stimulation	0.070	1	0.070	0.004	0.953	0.000
The intensity of stimulation	212.591	3	70.864	3.622	0.036	0.404
The times * the intensity	188.201	3	62.734	3.207	0.051	0.375
Error	313.027	16	19.564			
Total	155098.890	24				
Corrected Total	713.890	23				

a R Squared =0.562 (Adjusted R Squared =0.370)

Influence of abrasion stimulations on the morphology of the acupoint fascia fibroblast

As seen in Table 3 and Table 4, the factorial ANOVA analysis showed that during the experiments, the roundness of cells in both single and multiple abrasion blank control groups was approximately stable. In each subgroup with single stimulation, the changes in the roundness of cells from minor and medium abrasion groups were the lowest. Likewise, in each subgroup with multiple stimulations, the changes in the roundness of cells from the minor and medium abrasion groups were also the lowest. Different abrasion stimulation intensities caused the cell roundness of the meridian-related fascial connective tissue fibroblasts to be significantly different from that of the blank control group ($P=0.008<0.01$). Different number of stimulations did not significantly affect the cell roundness of the meridian-related fascial connective tissue fibroblasts ($P=0.260>0.05$). The combination of the above 2 factors (abrasion stimulation intensity and the number of stimulations) did not provide a significantly different effect on the cell roundness of the meridian- related fascial connective tissue fibroblast ($P=0.911>0.05$).

Table 3 Influence of in vitro abrasion stimulations on cell roundness

Groups	1 stimulation		3 stimulations		Total	
	n	Roundness	n	Roundness	n	Roundness
Control	3	0.7200±0.01000	3	0.7233±0.01100	6	0.7217±0.00894
Minor	3	0.7067±0.01732	3	0.6900±0.01732	6	0.6984±0.01643
Medium	3	0.7000±0.01000	3	0.6933±0.00577	6	0.6967±0.00816
Heavy	3	0.7133±0.00577	3	0.7067±0.01528	6	0.7100±0.01095

Table 4 Tests of Between-Subjects Effects

Dependent Variable: *cell roundness*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	0.003(a)	7	0.000	2.663	0.050	0.538
Intercept	11.943	1	11.943	79618.028	0.000	1.000
The times of stimulation	0.000	1	0.000	1.361	0.260	0.078
The intensity of stimulation	0.003	3	0.001	5.583	0.008	0.511
The times * the intensity	7.92E-005	3	2.64E-005	0.176	0.911	0.032
Error	0.002	16	0.000			
Total	11.948	24				

Corrected Total	0.005	23	

a R Squared =0.538 (Adjusted R Squared =0.336)

Influence of abrasion stimulations on the protein expression of the acupoint fascial fibroblasts

We used the biotin-labled mouse antibody chip, and the results are shown in Figure 1. The positive control stains were visible and negative control stains were not detected. The remaining proteins were irregularly presented, which suggests that the quality control was accurate and the results were valid.

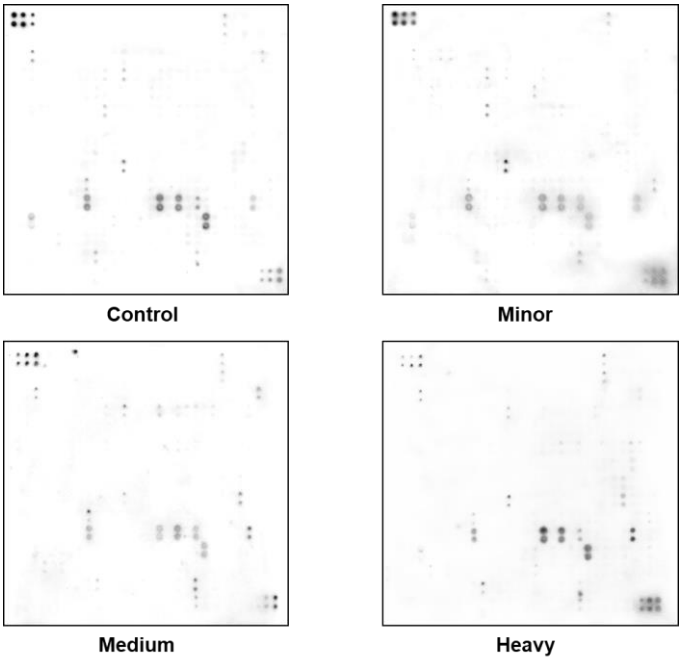

Figure 1. Biotin-marked mouse antibody chip was used, the positive control stains were visible and negative control stains were not detected. The remaining proteins were irregularly presented, which suggests that the quality control was accurate and the results were valid.

The abrasion stimulation for all 3 groups indicated clear regulatory effects on the protein expression from the acupoint-localized fascial tissues, with 3 groups comparing at □2=109.047, P＜0.0005. Among them, the abrasion group with minor intensity had the lowest overall effect (including up-regulation and down-regulation), with down-regulation being prevalent. The abrasion group with medium intensity had the highest effect and the most quantity of down-regulated proteins among the 3 groups, as well as the least quantity of up-regulated proteins among the 3 groups, with down-regulation being prevalent. The effect of the abrasion group with high intensity was medium between the other 2 groups, and the regulation trends resembled those of the abrasion group with medium intensity, and down-regulation was also prevalent. (Table 5)

Table 5. Difference in the in vitro abrasion stimulations on fibroblast protein expression

Groups	Numbers of downregulated proteins	Number of non-regulated proteins	Numbers of upregulated proteins	Total numbers
Minor VS Control	76	164	68	308
Medium VS Control	201	82	25	308
Heavy VS Control	119	143	46	308

Meanwhile, we used real-time quantification PCR to confirm the chip results. The chip results showed that the significantly down-regulated molecules IL-15, G-CSF, and beta-

catenin from the abrasion groups were also presented as down-regulated in the real-time quantification PCR results. The significantly up-regulated molecules angiopoietin-like 2, CCL4/MIP-1 beta, and AR (Amphiregulin) from the test groups in the chip results were also presented as up-regulated in the real-time quantification PCR results (Figure 2). This further supported our results from in the chip experiment.

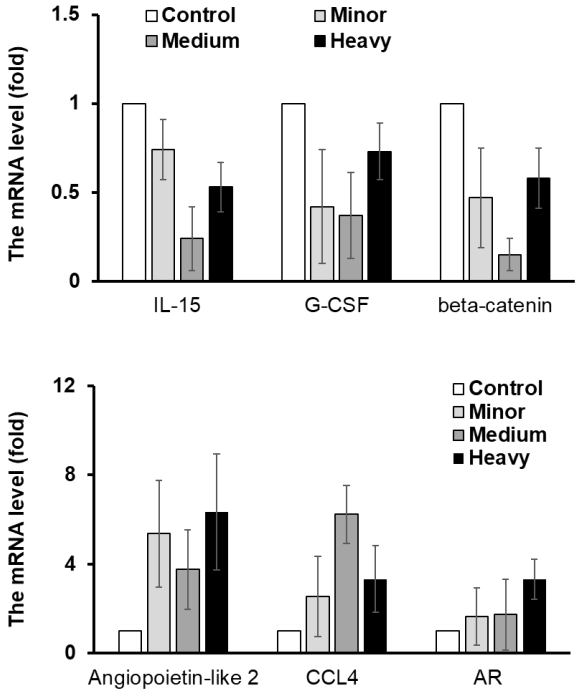

Figure 2. Real-time quantification PCR assay was used to confirm the chip results. The chip results showed that the significantly down-regulated molecules IL-15, G-CSF, and beta-catenin from the abrasion groups were also presented as down-regulated. The significantly up-regulated molecules angiopoietin-like 2, CCL4/MIP-1 beta, and AR (Amphiregulin) from the test groups in the chip results were also presented as up-regulated.

The clustering analysis showed that the effects of abrasion stimulations with different intensities on the protein expression of fibroblasts from the acupoint fascial tissue are similar as the influence with a minor abrasion. Abrasion with high intensity may share the same category and have a small effect. However, the effect on the protein expression of the acupoint by the medium abrasion was the highest, because it had the highest variation in the clustering analysis (Figure 3).

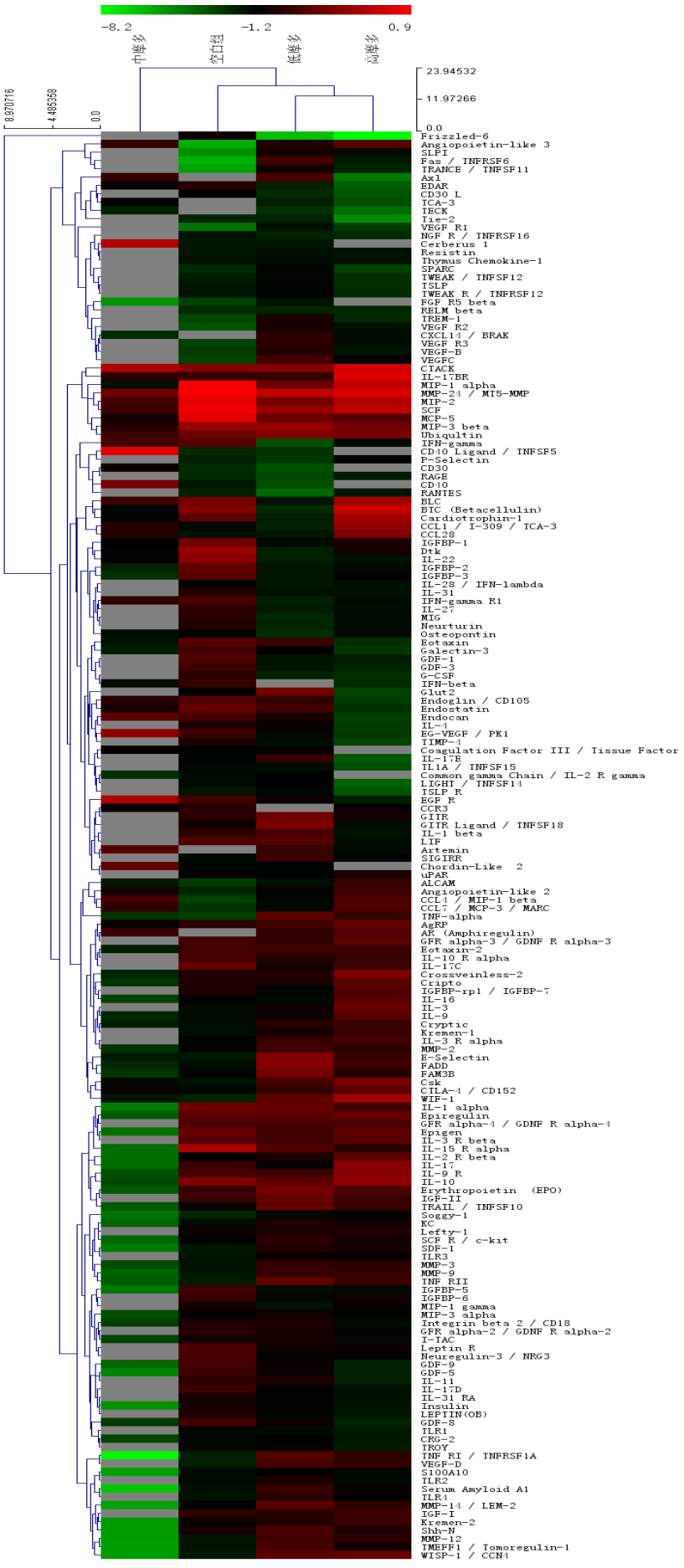
-8.2
-1.2
0.9
中等多
空白组
低等多
高等多
23.94532
11.97266
0.0
8.970716
4.485368
0.0
Frizzled-6
Angiopoietin-like 3
SLPI
Fas / TNFRSF6
TRANCE / TNFSF11
Axl
EDAR
CD30 L
TCA-3
TECK
Tie-2
VEGF R1
NGF R / TNFRSF16
Cerberus 1
Resistin
Thymus Chemokine-1
SPARC
TWEAK / TNFSF12
TSLP
TWEAK R / TNFRSF12
FGF R5 beta
RELM beta
TREM-1
VEGF R2
CXCL14 / BRAK
VEGF R3
VEGF-B
VEGFC
CTACK
IL-17BR
MIP-1 alpha
MMP-24 / MT5-MMP
MIP-2
SCF
MCP-5
MIP-3 beta
Ubiquitin
IFN-gamma
CD40 Ligand / TNFSF5
P-Selectin
CD30
RAGE
CD40
RANTES
BLC
BTC (Betacellulin)
Cardiotrophin-1
CCL1 / I-309 / TCA-3
CCL28
IGFBP-1
Dtk
IL-22
IGFBP-2
IGFBP-3
IL-28 / IFN-lambda
IL-31
IFN-gamma R1
IL-27
MIG
Neurturin
Osteopontin
Eotaxin
Galectin-3
GDF-1
GDF-3
G-CSF
IFN-beta
Glut2
Endoglin / CD105
Endostatin
Endocan
IL-4
EG-VEGF / PK1
TIMP-4
Coagulation Factor III / Tissue Factor
IL-17E
IL1A / TNFSF15
Common gamma Chain / IL-2 R gamma
LIGHT / TNFSF14
ISLP R
EGF R
CCR3
GITR
GITR Ligand / TNFSF18
IL-1 beta
LIF
Artemin
SIGIRR
Chordin-Like 2
uPAR
ALCAM
Angiopoietin-like 2
CCL4 / MIP-1 beta
CCL7 / MCP-3 / MARC
TNF-alpha
AgRP
AR (Amphiregulin)
GFR alpha-3 / GDNF R alpha-3
Eotaxin-2
IL-10 R alpha
IL-17C
Crossveinless-2
Cripto
IGFBP-rp1 / IGFBP-7
IL-16
IL-3
IL-9
Cryptic
Kremen-1
IL-3 R alpha
MMP-2
E-Selectin
FADD
FAM3B
Csk
CTLA-4 / CD152
WIF-1
IL-1 alpha
Epiregulin
GFR alpha-4 / GDNF R alpha-4
Epigen
IL-3 R beta
IL-15 R alpha
IL-2 R beta
IL-17
IL-9 R
IL-10
Erythropoietin (EPO)
IGF-II
TRAIL / TNFSF10
Soggy-1
KC
Lefty-1
SCF R / c-kit
SDF-1
TLR3
MMP-3
MMP-9
TNF RII
IGFBP-5
IGFBP-6
MIP-1 gamma
MIP-3 alpha
Integrin beta 2 / CD18
GFR alpha-2 / GDNF R alpha-2
I-TAC
Leptin R
Neuregulin-3 / NRG3
GDF-9
GDF-5
IL-11
IL-17D
IL-31 RA
Insulin
LEPTIN(OB)
GDF-8
TLR1
CRG-2
TROY
TNF RI / TNFRSF1A
VEGF-D
S100A10
TLR2
Serum Amyloid A1
TLR4
MMP-14 / LEM-2
IGF-I
Kremen-2
Shh-N
MMP-12
TMEFF1 / Tomoregulin-1
WISP-1 / CCN4

Figure 3. The clustering analysis showed the effects of different abrasion stimulations on the protein expression of fibroblasts from the acupoint fascial tissue as the influence on the protein expression of fibroblasts from the acupoint fascial tissue by a minor abrasion. Columns from left to right were: Column 1, Medium group; Column 2, Control group; Column 3, Minor group; Column 4, Heavy group.

The primitive stimulation approach of meridians and acupuncture points of the human body is performed with hands or fingers by pressing, squeezing, massaging, and rubbing. Therefore, the abrasion stimulation is one of the most primitive mechanical stimulations in acupuncture and tuina. Regardless of how the acupuncture and tuina approaches have evolved, the mechanical analysis of abrasion stimulation remains one of the most basic mechanical stimulations in acupuncture and tuina.

Abrasion exists during the filiform needle perforation, when the needle body is rubbing the tissue cells surrounding the acupuncture points, and also during the body surface abrasion therapy of tuina such as massaging, rubbing, pushing, scrubbing, and applying. Moreover, when human body receives other stimulation approaches, such as moxibustion and tuina (pressing and pushing technique, swinging technique, shaking technique, and knocking technique), the tissue fluid flow caused by the heat balance effect and mechanical vibration effect may also incur a fluid shearing force on the tissue cells. This forms stimulation that we call the inner abrasion effect. Therefore, abrasion stimulation is actually a widespread mechanical stimulation in acupuncture and tuina.

Currently, studies of the influence of abrasion on tissue cells are primarily performed via the fluid shearing force loading (i.e., inner abrasion). Specifically, the flowing substrate is used to produce shearing stimulations on the cells in the culture device. This type of force has 3 loading methods: parallel plate flow chamber loading, tapper plate flow chamber loading, and horizontal shaking table loading. The advantage of the parallel plate flow chamber loading and the tapper plate flow chamber loading is the accurate and measurable force application. However, the parallel plate flow chamber and the tapper plate flow chamber are large, expensive, complex in operation, have a higher demand on the substrates, and are prone to contamination. These are the reasons why we rejected them for this study. For this study, we adopted the 3rd method, which may, via different shaking frequencies of the horizontal shaking table, cause the substrate and the cells growing on the wall of the 6-orifice plate to produce abrasion stimulations with different intensities of shearing force. Although the obtained abrasion was not sufficiently accurate, the devices were simple and easy to operate, and satisfied the requirements for performing a semi-quantitative study on heavy, medium, and minor stimulations for this project.

In this study, we adopted the cell survival rate and cell roundness (cytomembrane integrity in the cell roundness reaction) as indices for measuring cell viability. The results showed that within the scope of force stimulation intensities and the number of stimulations during this study, the abrasion could inhibit the overall survival rate of acupoint fascial fibroblast, with decreased cell survival percentage. Here, the force intensity became the only factor that effectively inhibited the cell survival rate, and both factors (force intensity and the numbers of force applications) were not synergistic. The abrasion stimulation may decrease the overall roundness of acupoint fascial fibroblast. Here, the force intensity was an effective factor, and both factors (force intensity and numbers of force applications) were not synergistic. Therefore, the abrasion may inhibit the overall viability of cells. Moreover, minor abrasion stimulations may result in the up-regulation of 68 proteins, maintenance of 164 proteins, and down-regulation of 76 proteins. Abrasion stimulation with medium intensity may result in the up-regulation of 25 proteins, maintenance of 82 proteins, and down-regulation of 201 proteins. Abrasion stimulation with high intensity may result in the up-regulation of 46

proteins, maintenance of 143 proteins, and down-regulation of 119 proteins. Abrasion stimulations from all 3 groups result in a clear adjustment of protein expression of fibroblasts from the meridian-related fascial tissue, with 3 groups comparing at □2=109.047, P＜0.0005. The minor abrasion groups had the lowest overall regulation (including up-regulation and down-regulation), with the down-regulation being prevalent. Abrasion stimulation with medium intensity group had the highest regulation and the highest quantity of down-regulated proteins as well as the smallest quantity of up-regulated proteins among the 3 groups, with the down-regulation being prevalent. The effect of abrasion stimulation with high intensity was close to that of the minor abrasion group. The clustering analysis showed the influence pattern of different abrasion stimulations on the protein expression of fibroblasts from acupoint fascial tissues. Specifically, the influence on the protein expression of the acupoint by the medium abrasion was the highest, because it had the highest variation in the clustering analysis.

4. Conclusion

Therefore, this study proves that during the in vitro abrasion stimulation, the cell viability and protein expression of fibroblasts from the meridian-related fascial connective tissue may change accordingly. In terms of the cell mechanics, these changes may represent the cytobiological and therapeutic feedback of the fibroblasts from the meridian-related fascial connective tissue due to the basic mechanical stimulation (pure abrasion stimulations) during acupuncture and tuina. Additionally, the study indicates that, within a given scope of force intensity, abrasion stimulation with medium intensity may maximize cell viability and protein synthesis.

5.Acknowledgements

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