Supplementary Information S1-12

Static Pressure Induced Neural Differentiation of Mesenchymal Stem Cells

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S11. The process of neural differentiation of MSCs under static pressure mediated LiNbO3 wafers. Movie S2

S12. The process of neural differentiation of MSCs under patterned static pressure for 21h. Movie S3

S1. Surface topography and cytocompatibility of FSG

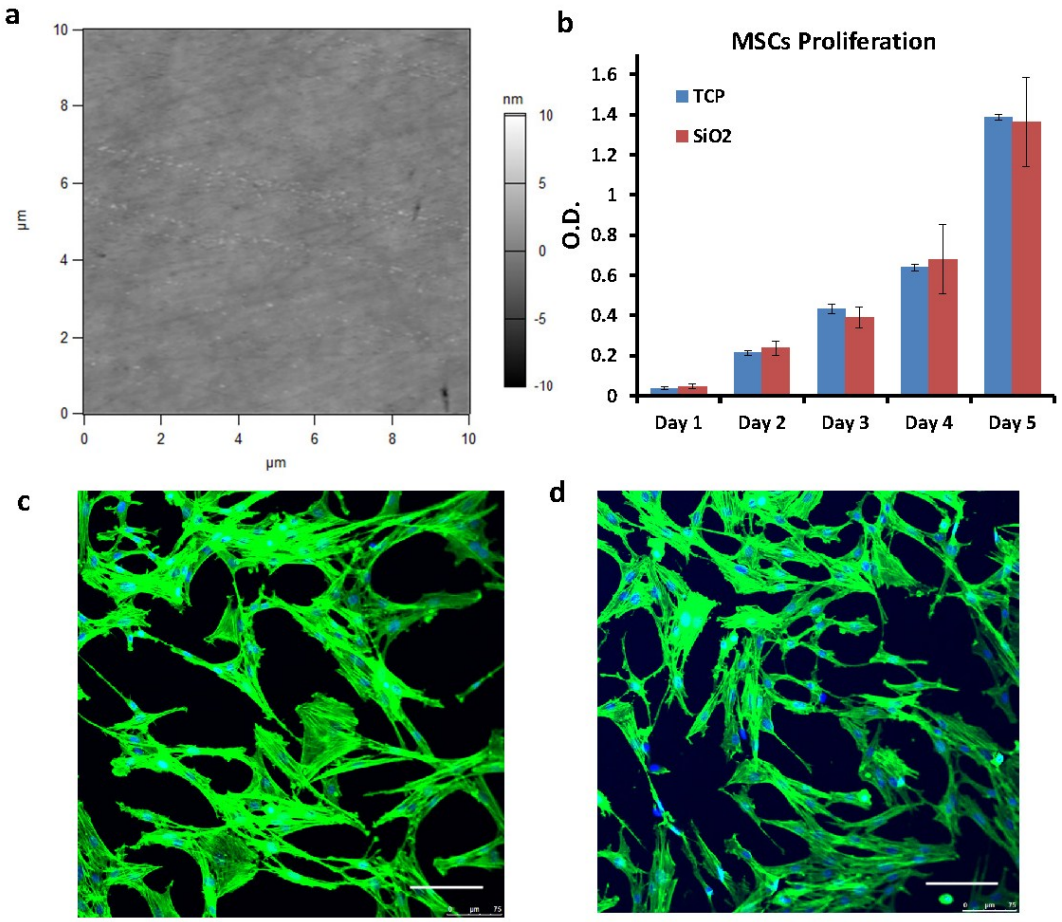


Fig. S1. Characterization of Surface topography and cytocompatibility of FSG. (a) The roughness of FSG surface was measured by atomic force microscope (AFM) (Asylum Research, MFP-3D-SA), and the average of roughness of FSG is 413.825 pm. (b) MSCs proliferation was detected on two substrates. All data represented the mean ± standard deviation (n=3, statistics by ANOVA). (c, d) F-actin of MSCs on FSG (c) and TCP (d) was stained by Alexa Fluor 488 conjugated phalloidin (green) and nuclei were stained by DAPI (blue). Scale bars: 100 μm.

To exclude the effect of FSG surface topography on MSCs, we used polished FSG, and the result of AFM showed smooth surface of FSG. To test the cytocompatibility of FSG, we have detected the proliferation of MSCs on FSG, using a Cell Count Kit-8 (Dojindo Molecular Technology), according to manufacturer' instructions. The results indicated that the culturing of MSCs on FSG did not perturb mitochondrial function, and there was no significantly different proliferation compare to culturing on TCP (Fig. S2b). MSCs culturing on two substrates were stained with F-actin by Alexa Fluor 488 conjugated phalloidin (Life Technology). The data showed that FSG has no negative effects on cell shape and cell spreading compare to on TCP. These results showed that FSG had good cytocompatibility.

S2. Calculation the value of static pressure with different thickness FSG

Because it is very difficult to measure the pressure, we calculate the pressure applied on the cells by dividing difference of pressure force and buoyant force of FSG slide by the total area of stem cells, which support the covered FSG slide.

The formula: $Pressure = \frac{G-F}{S1} = \frac{\rho 1ghS2 - \rho 2ghS2}{S1} = \frac{S2}{S1} \times h \times 1.176 \times 10^4 Pa$

G, gravity of fused silica glass; F, buoyant force of fused silica glass; S_1, size of MSCs adhesion area; S_2, size of fused silica glass; S_1/S_2, the percent of MSCs spread on FSG, measured by Image J software; , the density of fused silica glass=2.2^3 kg/m^3; , the density of water=1^3 kg/m^3; g =9.8 N/kg; h, the thickness of fused silica glass (m).

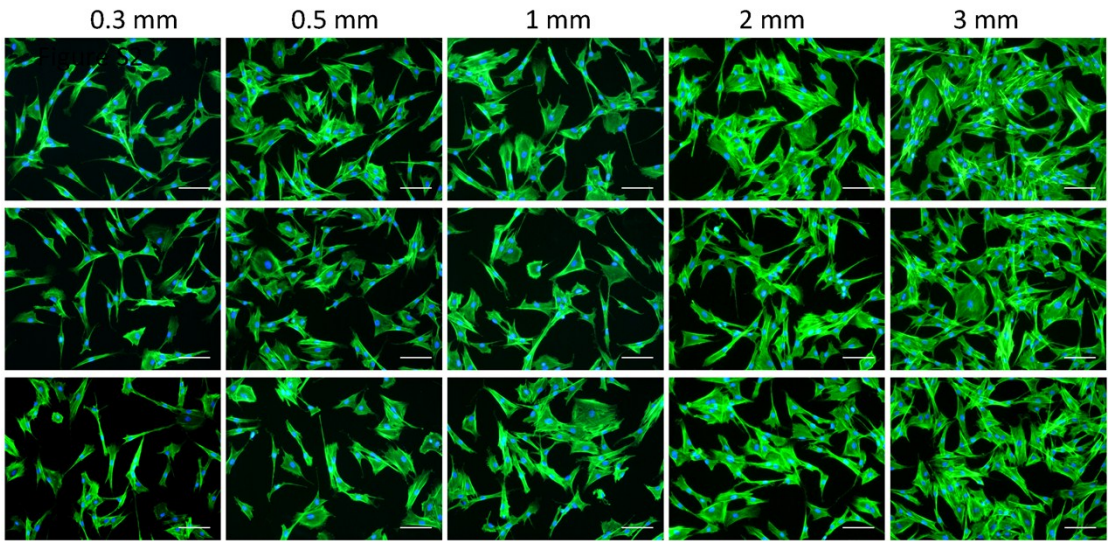


Fig. S2. F-actin of MSCs on different thickness FSG was stained by Alexa Fluor 488 conjugated phalloidin (green) and nuclei were stained by DAPI (blue). Scale bars: 100 μm.

After carefully measurement and calculation, the pressure applied on the stem cells by covering FSG slides with different thickness were estimated, which are shown in Table S1.

Table S1. The value of different thickness pressure

Thickness	**0.3 mm**	**0.5 mm**	**1 mm**	**2 mm**	**3 mm**
S_1/S_2-1	0.2841	0.4305	0.3419	0.4710	0.6546
S_1/S_2-2	0.2344	0.3840	0.2621	0.3454	0.5908
S_1/S_2-3	0.2952	0.2566	0.3320	0.3735	0.5904
Ave S_1/S_2	0.2712	0.3570	0.3120	0.3966	0.6119
S_2/S_1	1/0.2712	1/0.3570	1/0.3120	1/0.3966	1/0.6119
H /m	3-4	5-4	1-3	2-3	3-3
Pressure/Pa	~13	~16.5	~37.7	~42.9	~57.7

S3. Static pressure induced MSCs morphology changes

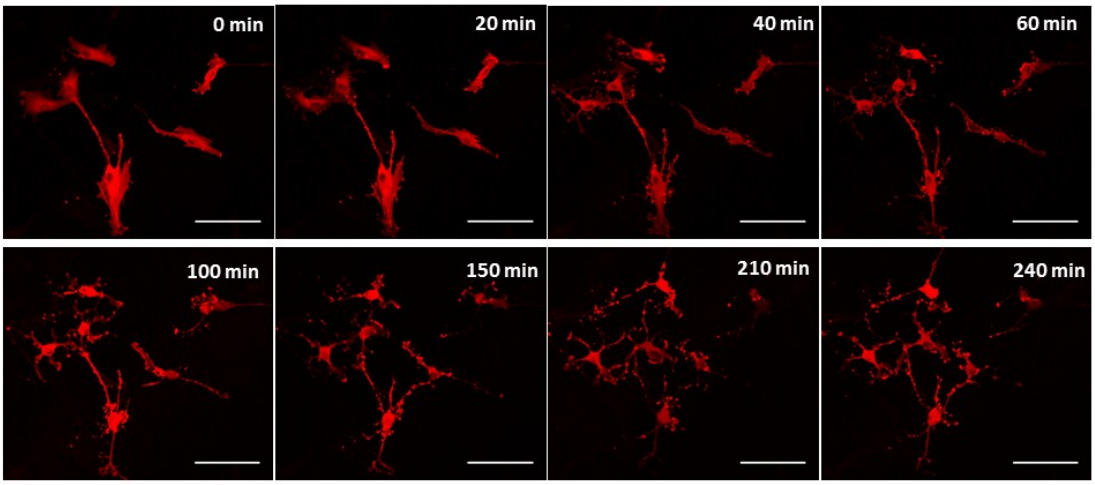


Fig. S3. Time-lapse microscopy of static pressure induced morphology changes. The MSCs was stained by commercial product Actin-RFP overnight, and took images by Leica Laser Confocal Scanning Microscope SP8 for recoding the changes of morphology in situ. Scale bar: 100 μm.

The figure shows that under static pressure, the shape of spindle-like MSCs changed gradually, neuritis grow, extend, connect with each other, and finally form neural network in the first 240 min.

S4. Different stem cells response to the static pressure

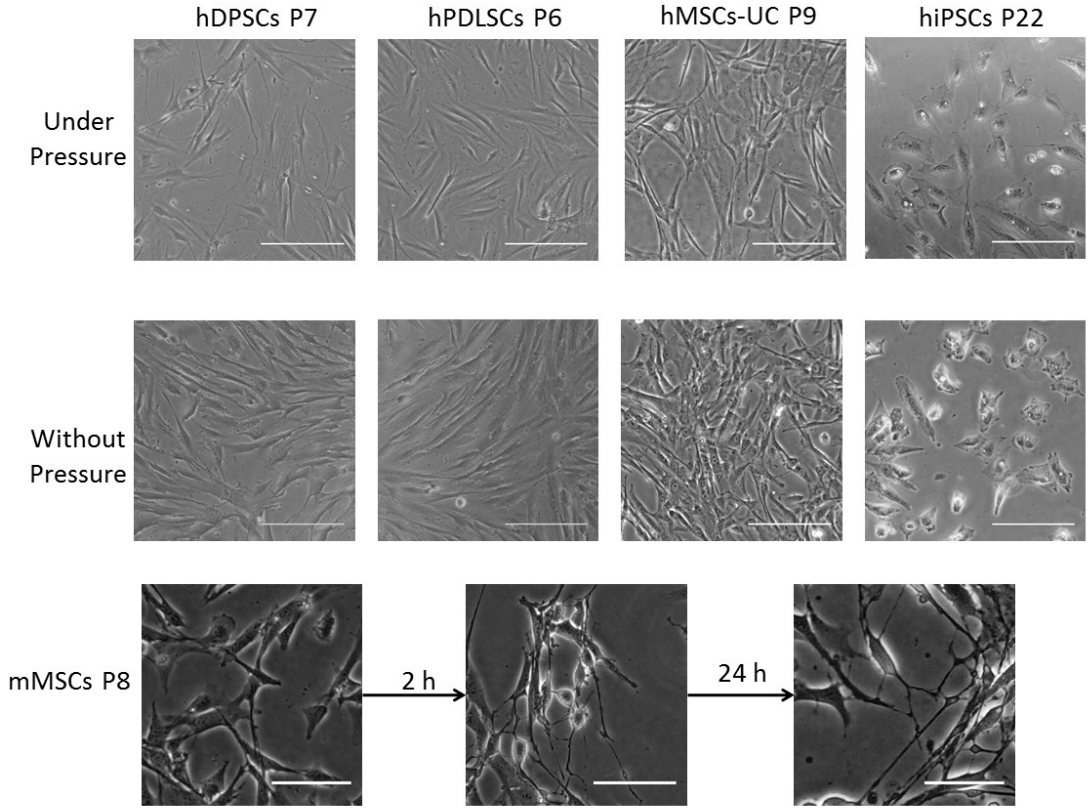


Fig. S4. The morphology of different stem cells under static pressure. hDPSCs (human dental pulp stem cells), hPDLSCs (human periodontal ligament stem cells), hMSCs-UB (human mesenchymal stem cells derived umbilical cord)), hiPSCs (human induced pluripotent stem cells) and mMSCs (mouse mesenchymal stem cells derived bone marrow). Scale bars, 100 μm.

To investigate whether static pressure can induce some other stem cells differentiation into neural cells, we used other stem cells (hDPSCs, hPDLSCs, hMSCs-UB and hiPSCs) to test the effect of static pressure. hDPSCs, hPDLSCs and hMSCs-UB were gifts from Shandong University School of Stomatology, and hiPSCs was a kindness gift from Institute of Biophysics, Chinese Academy of Sciences. mMSCs is a commercial product from Cyagen Company (cat. No. MUBMX-01001). All the cells were cultured as previously described[1-4]. After 24 hours applying static pressure to hDPSCs, hPDLSC and hMSCs-UB, the results showed that cell number was less than that without pressure and cell proliferation was hindered. Compared

with the normal culture cells, the morphology of cells under pressure looked like a little shrinkage, but not like neural cells. For hiPSCs differentiation, we changed MSCs culture medium for hiPSC induction. When applying static pressure to hiPSCs for 24 hours, the morphology of hiPSC looked like enhancement of cell area, more like fibroblastic cells. For mMSCs, it is significantly different from other stem cell lines, a neural-like morphology under pressure. These results demonstrated that different stem cells could respond the static pressure with different changes, but neural cells differentiation was specific for MSCs from bone marrow.

S5. Global gene-expression analysis of microarrays

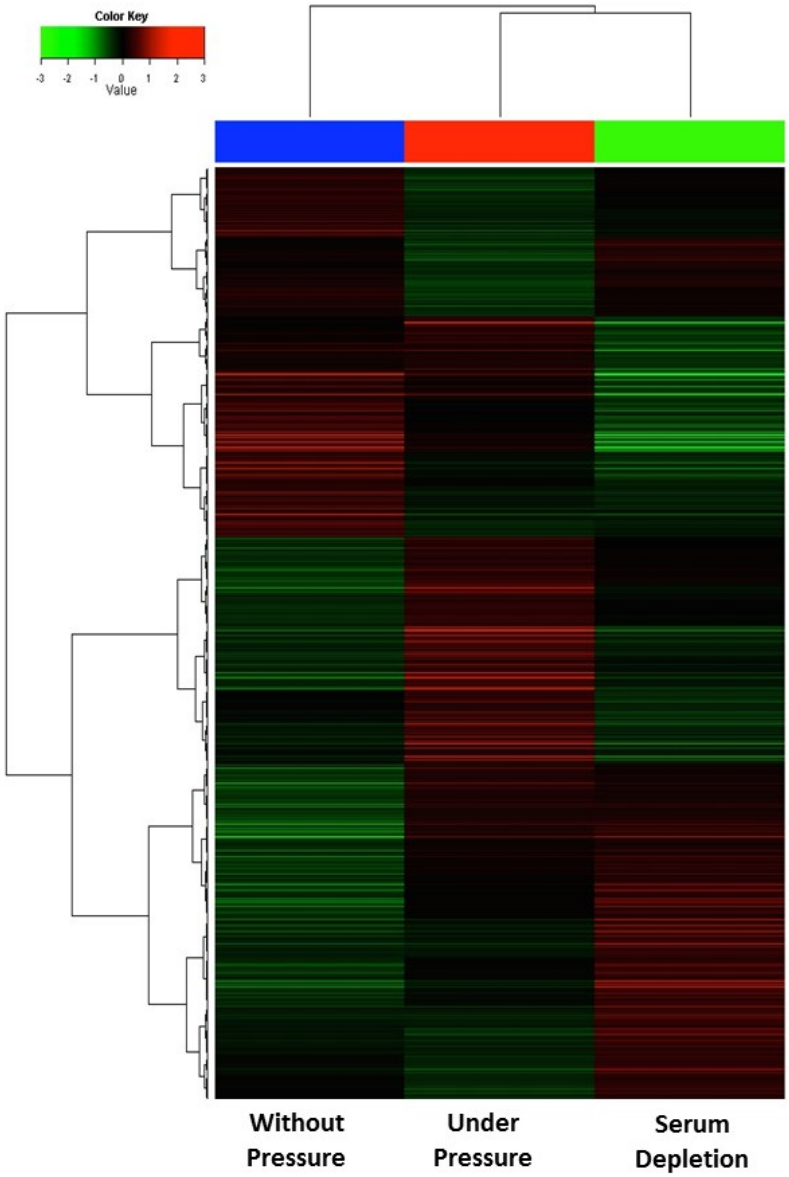


Fig. S5. Microarray analysis. Cluster analysis for MSCs cultured without pressure, under pressure, and serum depletion. Expression levels are colored green for down-regulation (>2 folds), red for up-regulation (>2 folds) and black for no change.

S6. Neuronal specific genes regulation by microarrays analysis

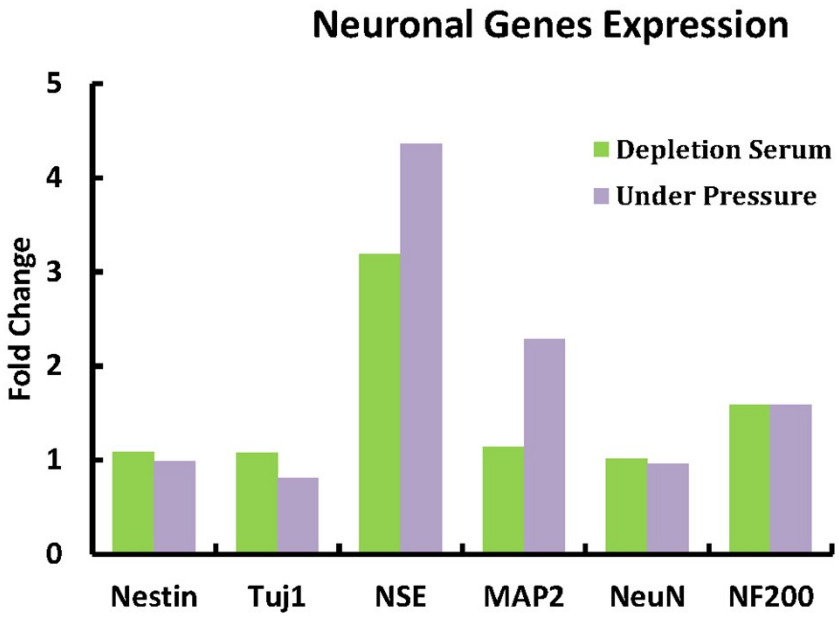


Fig. S6. Quantification of neuronal specific genes regulation from microarrays of MSCs on static pressure and on serum depletion condition. NeuN (neuronal nuclei), NF200 (Neurofilament heavy polypeptide).

In some chemicals induction MSCs into neuronal differentiation, serum depletion-preinduction is necessary. And we also analyzed the global gene profile of MSCs under serum depletion. The results showed some neuronal marker were up-regulation under serum depletion (NSE, MAP2 and NF200, Fig. S6), and serum depletion could promote MSCs differentiation into neuronal cells. For MSCs under static pressure, the result of MAP2 and NSE expression level was consistent with qPCR, but Nestin and Tuj2 was a little down-regulation, maybe the different accuracy between the two methods.

S7. Signaling pathway analysis

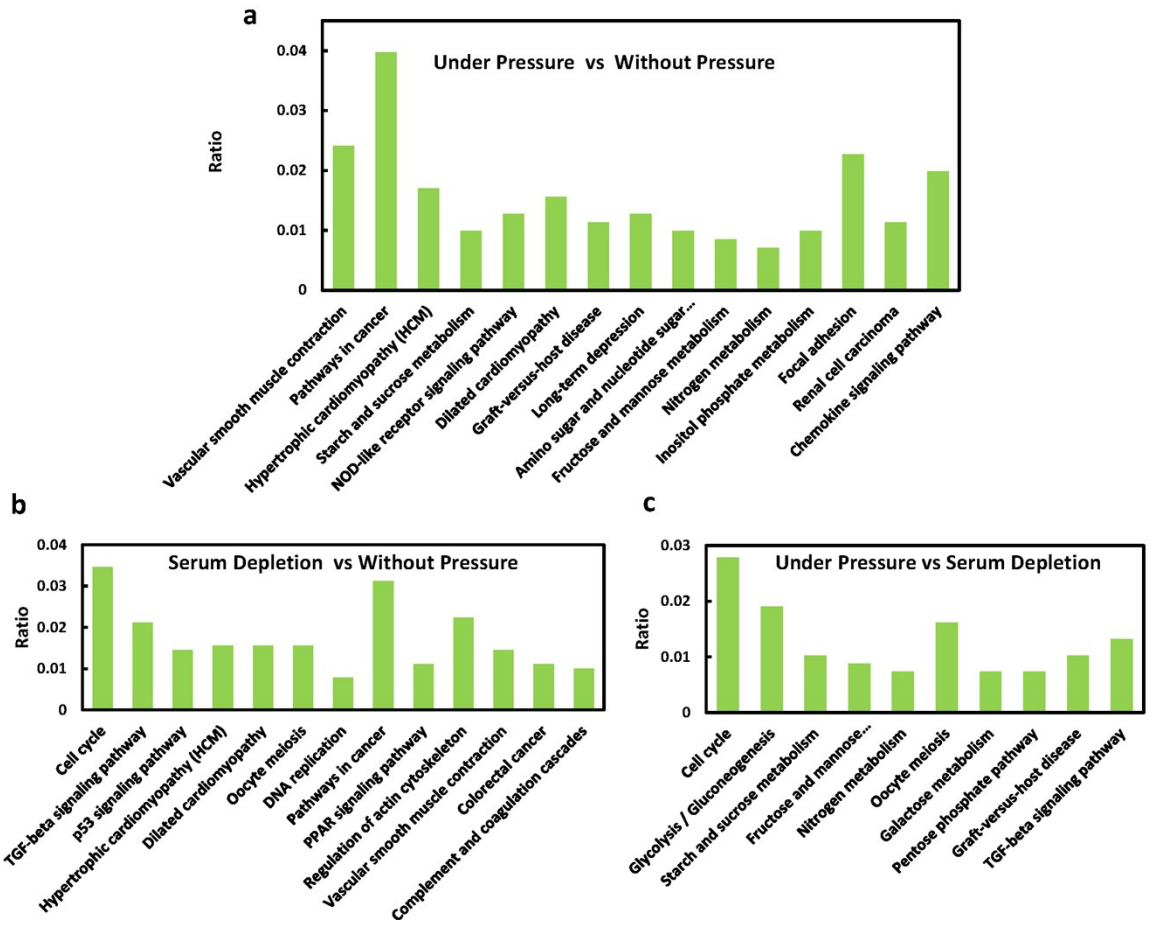
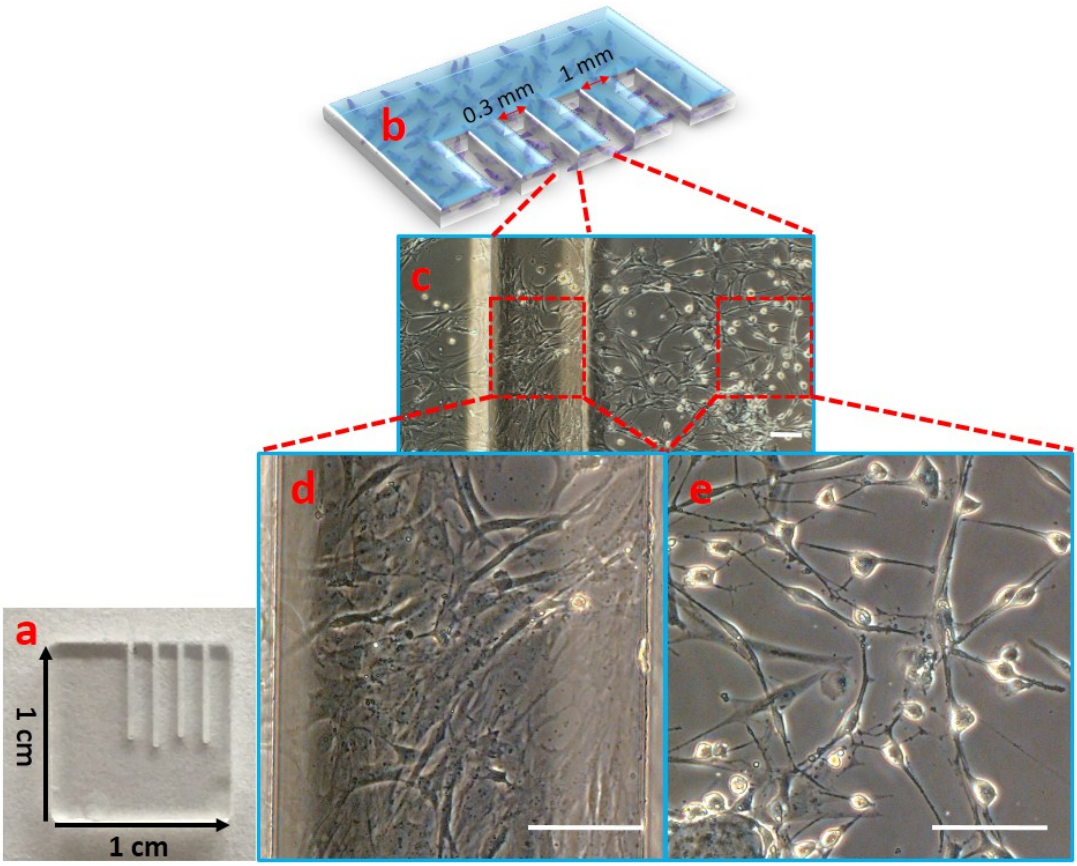


Fig. S7. MAS for gene microarray analysis. Canonical pathways for MSCs under pressure compared with normal cultured MSCs (a), MSCs starvation for depletion serum compared with normal cultured MSCs (b), MSCs under pressure compared with MSCs starvation for depletion serum (c). Threshold refers to cutoff $p<0.05$.

In this study, we used static pressure to influence the MSCs differentiation into neuronal cells. It is a very simple method, but can induce very complex changes for cell microenvironment. When adding substrate to cells surface, physical force was applied to cells, but also formed a nutrient-poor environment for cells. In the chemical induction of MSC differentiation into neuronal –like cells, starvation is necessary[5,6]. For example, Woodbury et al. have used several chemicals to induce MSCs differentiation into neurons in vitro, but before adding the chemicals, it also needed

serum depletion in differentiation[5]. Huang et al. could not induce MSCs into neural cells using resveratrol without starvation[6]. Under this static condition, microarray results showed MSCs were also undergoing metabolism depression and pathways in cancer, compared with normal cultured MSCs (Fig. S7a). Serum depletion condition could cause MSCs cytoskeleton contraction and regulation (Fig. S7b), which is necessary to induce MSCs differentiation into neural like cells. The microenvironment formed by using a solid substrate to apply static pressure to MSCs is enough to differentiate MSCs into neural-like cells, and takes too less time than the chemical induction methods.



S8. The neuronal-like differentiation of MSCs under patterned static pressure.

Fig. S8. Patterned FSG and MSCs differentiated into neuronal-like morphology in different regions of FSG. (a) Digital picture of grooved FSG slide. The width of square FSG is 10mm, and each groove is 300 μm in width. (b) Schematic diagram of FSG with grooves. (c) The image shows the morphology of MSCs with or without static pressure under patterned groove FSG for 24h. The magnified views show two static pressure status-without pressure (d) and with pressure (e). Scale bars: 100 μm

To further observe MSCs differentiation with/without static pressure simultaneously, 1 mm thick FSG was etched by laser to form a comb-like structure. After MSCs were proliferated on TCP for three days, the grooved FSG was put upon MSCs directly. MSCs under groove were not subjected to static pressure and MSCs under the rest region of FSG underwent static pressure. After 24h, the MSCs in groove remained their typical spindle shape (Fig. S8d) but MSCs under pressure transformed into neuron-like cells (Fig. S8e).

S9. Primers sequences of qPCR

Table S2

Sequence of Real-Time PCR Primers			
Gene	5'-3'	Sequence	Size (bp)
ACTB	F	GGCCGTCTTCCCCTCCATCG	154
	R	CCAGTTGGTGACAATGCCGTGT	
Nestin	F	AGAGTCAGATCGCTCAGATC	93
	R	GCAGAGTCCTGTATGTAGCCAC	
Tuj1	F	TAGACCCCAGCGGCAACTAT	127
	R	GTTCCAGGCTCCAGGTCCACC	
NSE/Eno2	F	GATGGGGACAAACAGCGTTAC	102
	R	CACAGAGAGGCCTGAGCTGAT	
MAP2	F	GCCAGCATCAGAACAAACAG	146
	R	AAGGTCTTGGGAGGGAAGAAC	
GFAP	F	CGGAGACGTATCACCTCTG	123
	R	TGGAGGCGTCATTCGAGACAA	
GAPDH	F	GCCTCGTCTCATAGACAAGATGGT	142
	R	GAAGGCAGCCCTGGTAACC	

S10. The process of neuronal-like differentiation of MSCs under static pressure mediated FSG.

Movie S1.

S11. The process of neuronal-like differentiation of MSCs under static pressure mediated LiNbO3 wafers.

Movie S2.

S12. The process of neuronal-like differentiation of MSCs under patterned static pressure for 21h. Movie S3

References:

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