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Modulation of Mesenchymal Stem Cell Migration using Programmable Polymer Sheet Actuators

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ABSTRACT

Recruitment of mesenchymal stem cells (MSCs) to damaged tissue is a crucial step to modulate tissue regeneration. Here, the migration of human adipose-derived stem cells (hADSCs) responding to thermal and mechanical stimuli was investigated using programmable shape-memory polymer actuator (SMPA) sheets. Changing the temperature repetitively between 10 and 37 °C, the SMPA sheets are capable of reversibly changing between two different pre-defined shapes like an artificial muscle. Compared to non-actuating sheets, the cells cultured on the programmed actuating sheets presented a higher migration velocity (0.32 ± 0.1 vs. 0.57 ± 0.2 μm/min). These results could motivate the next scientific steps, for example, to investigate the MSCs pre-loaded in organoids towards their migration potential.

INTRODUCTION

Recruitment of progenitor cells at lesion points is the initial and crucial step for endogenous tissue regeneration. Mesenchymal stem cells (MSCs) represent a promising source for regenerative medicine applications not only owing to the differentiation potential, but also due to their homing capacity[1-3]. However, the major challenge for an effective MSC-based therapy remains the low infiltration of transplanted cells at the injury site [4, 5]. Improving the migration ability by preconditioning the MSCs prior to transplantation could be a strategy to circumvent this limitation.

Growth factors such as bFGF, VEGF, HGF, IGF and TGF- β1 are typical enhancers of MSC migration[6-10]. Increasing of CXC chemokine receptor 4 expression

on MSC surface via small molecule stimulation or gene transfection can promote cell recruitment [11-14]. Besides biochemical factors, it has been reported that the cell migration ability can also be modulated by mechanical cues. For instance, the rigidity of extracellular matrix (ECM) can regulate cell migration by actin and microtubule cytoskeleton assembly and remodeling [15-17]. Compared to stiff cell culture substrates (30 and 600 kPa), a higher cell migration speed can be achieved in MSCs from a soft substrate (3 kPa) due to their weaker formation of focal adhesion complexes [18]. Transient calcium influx, which is highly dependent on the activity of a stretch-activated cation channel was observed in migrating keratinocytes and fibroblasts over a decade ago [19, 20]. The application of mechanical strain (ranging from 5 to 10%) on MSCs efficiently promoted stem cell migration in recent studies [21, 22]. The mechanical stimuli can provide a simple, safe, cost effective and well-defined physical approach for the reinforcement of stem cell migration compared to established strategies relying on small molecule compounds and transgene expression.

Temperature-controlled programmable shape-memory polymer sheet actuators (SMPA) have been applied as a platform to autonomously apply cyclic mechanical strain to MSCs to direct the cell fate [23]. Cells cultured on programed SMPA under cyclic temperature change can sense both the thermal stimulus (ΔT) and the mechanical stimulus ($\Delta\varepsilon$). The 50 × 50 μm grids on the bottom side of the sheet enabled the visualization of SMPA deformation, without influencing the cells on the topside. During SMPA actuation, more than 10% of material elongation could stimulate hADSC response and influence their behavior, such as proliferation and differentiation [23]. Here, we study the influence of this material as well as the thermal and synchronized mechanical stimuli on stem cell migration. SMPA sheets were utilized under three distinct conditions: i) programmed SMPA (p-SMPA) which exerted a 2D actuation when exposed to cyclic temperature changes between 37 °C and 10 °C (SMPA, ΔT $\Delta\varepsilon$); ii) non-programmed (np-SMPA) SMPA with cyclic temperature changes between 37 °C and 10 °C (SMPA, ΔT); iii) non-programmed SMPA at a constant temperature of 37 °C (SMPA, 37 °C). Standard tissue culture plates (TCP) were used as reference material (Fig. 1A). Cell morphology, migration speed, cytoskeleton organization and integrin mediated mechanical transduction signaling of human adipose-derived stem cells (hADSCs) were investigated to explore the potential molecular mechanism responsible for the influence of SMPA on MSC migration.

EXPERIMENTAL DETAILS

Preparation of SMPA sheets

SMPA sheets containing poly (ε-caprolactone) domains as actuating unit were prepared according to our previous report [23]. The sheets were programmed by stretching to a strain (ε_{prog}) of 60% at 50 °C before cooling to –20 °C under constant strain. The circular SMPA specimens with a diameter of 10 mm were punched out and put into the 24-well standard tissue culture plate (TCP).

Cyclic temperature change

Computer-controlled thermochambers (Instec, Colordao, USA), supplied with 5 % (v/v) CO_2, were used for realizing the cyclic temperature changes between 37 °C and 10

°C [23]. The time for each cycle was set to 60 minutes (8 minutes from 37 °C to 10 °C, 22 minutes at 37 °C, 8 minutes from 10 °C to 37 °C, and 22 minutes at 10 °C).

Cell culture

hADSCs were isolated from human adipose tissue after informed consent (No.: EA2/127/07; Ethics Committee of the Charité – Universitätsmedizin Berlin, approval from 17.10.2008) [24]. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, Germany) containing 10% (v/v) fetal bovine serum (FBS, Sigma-Aldrich, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Merck Millipore, Germany), and incubated at 37 °C containing 5% (v/v) CO_2. The medium was changed every two days.

Cell migration

5×10^3 /cm^2 hADSCs were seeded on SMPA sheets and TCP, followed by 3 days of cultivation under different conditions. The CellMask™ Deep Red plasma membrane staining kit was applied for visualization of living cells. Cell nuclei were stained using Hoechst 33342 (Thermo Fisher Scientific, Schwerte, Germany). Cells were then transferred into the cage incubator (37 °C, 5% (v/v) CO_2) equipped on a time-lapse microscope (Olympus, Hamburg, Germany). The cell movement was recorded for 10 hours with 10 minutes intervals, and the cell migration was analyzed by tracing the cell nuclei. Data analysis was performed using ImageJ software (NIH, USA) suppled with the plugins of Manual Tracking (Fabrice Cordelieres, Institut Curie, Orsay, France) and Chemotaxis tool (ibidi GmbH, Gräfelfing, Germany).

Cell staining

For immunocytochemical staining, samples were washed with PBS and fixed with 4% (w/v) paraformaldehyde (Sigma-Aldrich, Hamburg, Germany), permeabilized with 0.25% (v/v) Triton X-100 (Sigma-Aldrich, Hamburg, Germany), and blocked with 5% (v/v) normal goat serum (Thermo Fisher Scientific, Schwerte, Germany). The samples were then incubated with primary antibodies overnight at 4 °C and treated with secondary antibodies (Thermo Fisher Scientific, Schwerte, Germany) for 1 hour at room temperature. The following primary antibodies were used: β-Tubulin (mouse, Thermo Fisher Scientific, Schwerte, Germany), phospho-Myosin Light Chain 2 (Ser19) (mouse, Cell Signaling Technology, Frankfurt am Main, Germany), AlexaFluor® 647 conjugated anti-Integrin β1 (activated, clone HUTS-4, Merck, Darmstadt, Germany). Cell nuclei and F-actin were stained with Hoechst 33342 and ActinRed™ 555 ReadyProbes (Thermo Fisher Scientific, Schwerte, Germany), respectively. The images were taken using a laser scanning confocal microscope (LSM780, Carl Zeiss, Jena, Germany) and analyzed with ImageJ software (NIH, USA).

Statistics

All data were from at least three independent experiments and presented as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA with post-hoc Tukey test, and a p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

SMPA deformation and hADSC morphology

In a previous report, we have demonstrated the great compatibility of SMPA for hADSCs at the changed temperature from 37 °C to 10 °C, as evidenced by a high cell survive rate after a long term cultivation [23]. Here, we studied the cell morphology at different stimuli and included standard TCP as a reference material. No obvious morphology change was observed in hADSCs cultured in different conditions for 3 days (Fig. 1B). The cells on SMPA sheets exhibited typical spindle-shaped morphology, which was similar to those on standard TCP. Cyclic temperature changes did not affect the cell morphology on both TCP and SMPA sheets. Comparison between the cells on p-SMPA and np-SMPA under the changed temperature suggested that the SMPA actuation had no visible effect on cell morphology.

However, compared to the cells on TCP, the cells on SMPA sheets were less spreaded. This result suggested that the cells might form a looser attachment on SMPA than on TCP, which could be attributed to the difference of chemical and physical properties between these two surfaces. In addition, the stretching force during SMPA actuation might also contribute to low cell anchoring from the material surface. Since the migration of anchorage-dependent cells are highly dependent on the cell attachment and spreading, one could expect that hADSCs may exhibit different migration capacities on SMPA and TCP [18, 25].

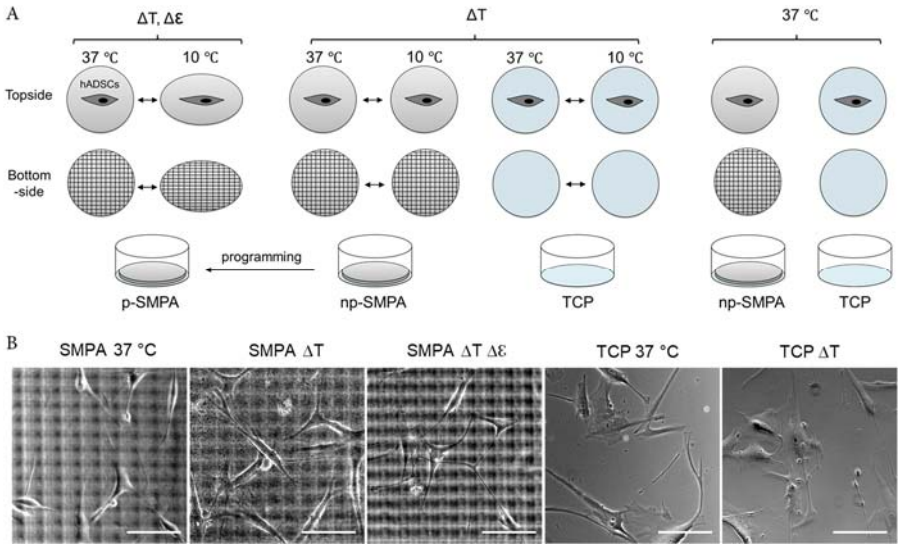

Fig. 1 (A) Schematic illustration of applying different stimuli on hADSCs. When changing the temperature, thermal and mechanical stimuli were generated by using p-SMPA, while only a thermal stimulus was apparent when using np-SMPA sheet or TCP. Neither thermal nor mechanical stimuli were present at 37 °C. (B) Morphology of hADSCs cultured on SMPA sheets and TCP under different stimuli for 3 days. Scale bar = 100 μm.

SMPA actuation promotes hADSC migration

In order to evaluate the migration of hADSCs, the cells were cultured under different conditions and then were observed with a time-lapse microscope to record their movement for 10 hours. The cells exhibited a faster migration velocity on SMPA sheets than on TCP (Fig. 2), which could be attributed to the relatively looser attachment and less spreading on SMPA as discussed above. The cyclic temperature change resulted in the significant decrease of cell migration velocity on SMPA sheets. However, such an effect was not observed on TCP, suggesting the influence of temperature change on hADSC migration was depent on the materials. The temperature change in this study (60 min for each cycle) might be too fast for cells, cultured on TCP with relatively tighter attachment, to response and alter their migration behavior. Interestingly, hADSC migration was significantly promoted by SMPA actuation. Compared to the cells on SMPA with single thermal stimulus (0.32 ± 0.14 μm/min), the cells exposed to thermal and mechanical dual stimuli showed almost doubled migration velocity (0.57 ± 0.2 μm/min) (Fig. 2B).

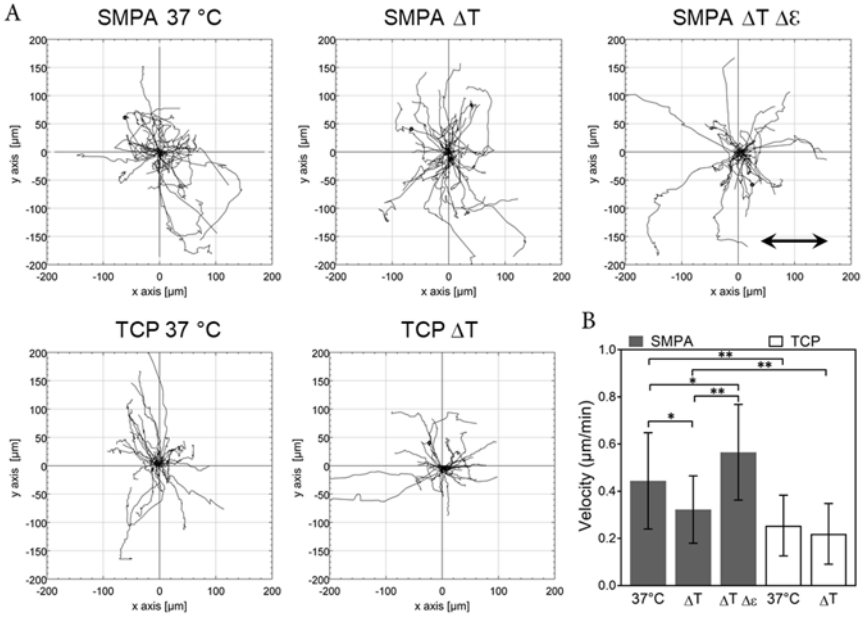

Fig. 2 Migration of hADSCs on SMPA sheets and TCP under different stimuli. The cells were cultured on the material surfaces for 3 days and the migration was recorded for 10 hours using a time-lapse microscope to generate the trajectories of hADSCs (A) and calculate the migration velocity (B, n ≥ 26 cells for each group, $*p < 0.05$ and $**p < 0.01$). The black arrow in (A) indicates the direction of p-SMPA elongation during temperature change.

Thermal and mechanical stimuli regulate cytoskeleton and cell adhesion

The migration of anchorage-dependent cells is a complex process, which is regulated by the dynamics of cytoskeleton organization and the transduction of spatial and temporal signals [26]. During cell migration, the protrusive and contractile force is generated from actin cytoskeleton, while the formation of the polarized network allowing organelle and protein movement is relying on the microtubules [27]. Crosstalk between the actin cytoskeleton and microtubules are essential for cell migration [28]. In order to investigate the mechanism, through which cells respond to the external stimuli, we cultured the cells under different conditions for 3 days and then performed immunostaining of the key components for regulating cell migration.

Cells exposed only to thermal stimulus presented decreased tubulin and enhanced F-actin compared to the cells without stimuli (SMPA, 37 °C). Exposure of cells to dual stimuli increased the F-actin level but had no obvious effect on tubulin level. SMPA actuation could enhance the tubulin level, as shown by the higher fluorescence intensity in the group with dual stimuli than that with single thermal stimulus (Fig. 3). These results suggested that both thermal and mechanical stimuli could regulate the cytoskeleton organization.

Integrin is a transmembrane receptor and primary mechanosensor of cells, which plays a critical role to mediate signal transduction in response to various mechanical stimuli. Upon external stimulation, integrins can be activated by changing their conformation and affinity, which allows the recruitment of several cytoplasmic proteins including focal adhesions and their variants mediating cell-ECM adhesion and cell migration [29, 30]. As one of the downstream molecules of integrin signalling and a motor protein, myosin can bind to actin filaments to regulate actin movement to generate contractile force. The activity of myosin was found to be highly dependent on its light chain phosphorylation [31, 32].

After 3 days of cultivation on different conditions, increase of pMLC and decrease of integrin activation were observed in cells with only thermal stimulus (SMPA, ΔT), in comparison to cells without stimulus (SMPA, 37 °C). In contrast, the cells exposed to thermal and mechanical dual stimuli (SMPA, ΔT $\Delta\varepsilon$) showed the similar level of activated integrin and enhanced MLC phosphorylation. Higher levels of activated integrin and pMLC were found in cells with dual stimuli compared to the cells on SMPA with single thermal stimulus (Fig. 4). These data indicated that the mechanical cue could be sensed by cells and transduced intracellularly, inducing integrin activation and MLC phosphorylation to mediate cell migration. In summary, thermal and mechanical stimuli could affect cell migration through the regulation of cytoskeleton organization and cell adhesion.

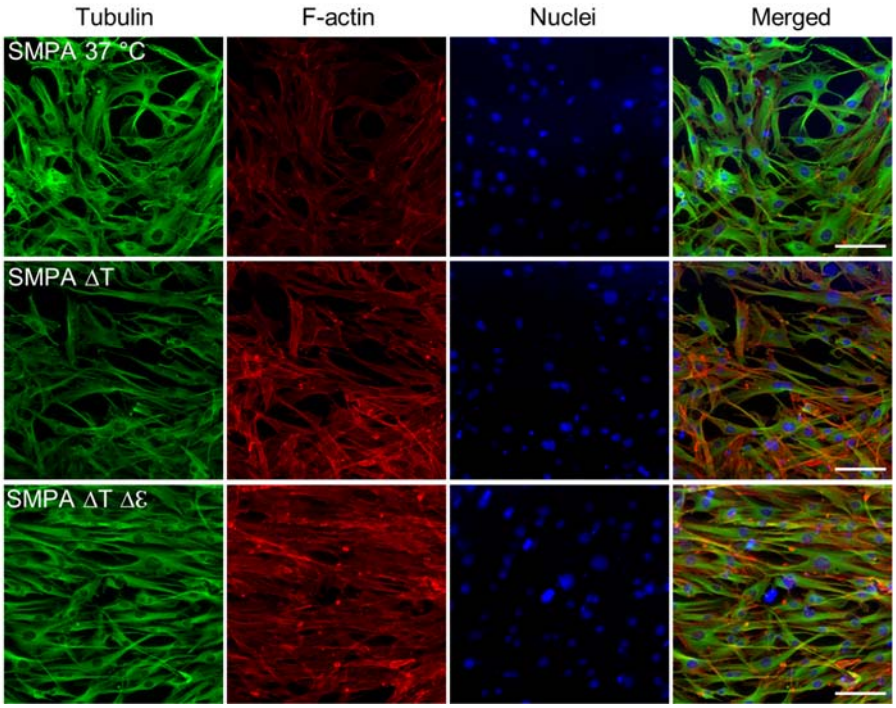

Fig. 3 Representative images of Tubulin (green) and F-actin (red) of hADSCs cultured on SMPA sheets for 3 days under indicated stimuli. Cell nuclei were stained with Hoechst 33342. Scale bar = 100 μm.

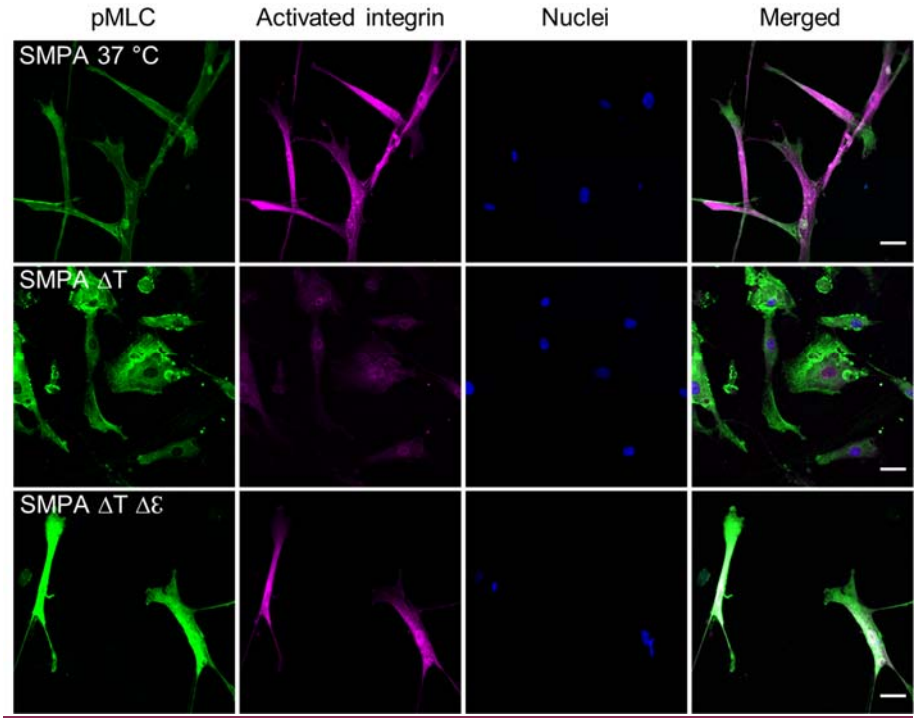

Fig. 4 Representative images of pMLC (green) and activated integrin (purple) of hADSCs. The cells were cultured on SMPA sheets for 3 days under different stimuli. Cell nuclei were stained with Hoechst 33342. Scale bar = 50 μm.

CONCLUSIONS

The influence of thermal and mechanical stimuli on hADSCs migration was investigated using the SMPA sheets in comparison to TCP. The cells on SMPA sheets exhibited faster migration velocity than on TCP. The mechanical stimulus from the actuation of SMPA sheets could significantly enhance cell migration capacity. Both thermal and mechanical stimuli could regulate the cytoskeleton organization and cell adhesion. This study demonstrated the interplay of different external stimuli for regulating stem cell migration, and provided a noval approach for improving MSCs homing capacity by a pretreatment with physical cues.

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