Strain Responsive Surface Patterns to Dynamically Control Cell and Nuclear Alignment

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Statement of Purpose: The interaction of cells (e.g., attachment, proliferation, differentiation) strongly depends on the chemistry, mechanics, and topography of a biomaterial. Topographical cues are shown to strongly influence nuclear organization and alter the cell alignment and function.[1,2] However, the majority of reports on surface topography have focused on static micro/nano-patterns and the use of dynamic patterns is limited. In this work, we used dynamic PDMS patterns and investigated the effect of pattern switching on nuclear orientation and stem cell behavior towards a platform material to explore how cells behave with changing topographical cues.

Methods: PDMS precursor (Slygard 184) mixed with curing agent (10:1) was cured between two flat glass slides (separated by a 0.5 mm spacer) at 65°C for 4 h. PDMS films (l_o = 30 mm, w = 30 mm, t = 0.5 mm) were clamped (Fig. 1a) and stretched to an initial strain ε_o = 22.4% strain (l_o = 25 → l = 30 mm) in one direction (Fig.1b). The stretched sample was treated with UV-ozone for 1 h (Fig. 1c) and released gently, which generated uniform lamellar wrinkle patterns (Fig. 1d). AFM images revealed that the lamellar patterns are uniform, having a 25 μm peak to peak distance and 5 μm depth (Fig. 1d). Patterns completely disappeared when the PDMS film was stretched back to ε_o (Fig. 1e). PDMS films were immersed in ethanol-water (70:30 v/v) for 30 min, and rinsed with PBS followed by sterilization under germicidal lamp for 2 h. Films were then incubated in fibronectin-PBS (20 μg/mL) for 18 h, and then in serum containing media for 30 min. Human mesenchymal stem cells (hMSCs) were seeded onto each sample (6.5 x10^3 cells/cm^2) and cultured for 1 or 7 days. For dynamic pattern switching experiments, hMSCs were cultured on pre-stretched and pre-released films for 1 day, and films were either released (for pre-stretched) or stretched (for pre-released) *in situ*, followed by 1 day incubation. Cells were stained with rhodamine-phalloidin (actin) and DAPI (nuclei) for visualization. A MatLab program was used to measure the major and minor axis of each cell nuclei, and nuclei orientation (the angle between the major axis of the nuclei and pattern).

Results: As expected, hMSCs attached and spread randomly on the pre-stretched films (Fig. 1f). On the pre-released films, the cells attached and aligned themselves to track the pattern shape (Fig. 1g). After 1 day for this group almost 80% of the cells had nuclei orientation ≤ 10°, which remained constant after 7 days. For dynamic experiments, hMSCs were cultured onto pre-released (patterned) or pre-stretched (flat) PDMS films for 1 day, and films were either stretched (for pre-released, Fig 1a-b) or released (for pre-stretched, Fig. 2d-e) *in situ*, followed by 1 day incubation. One set of samples was kept in the pre-determined condition, which were used as controls. The nuclear orientation of the cells on the pre-released films changed significantly from aligned to completely random when the film was stretched (Fig 2b-c). Similarly, cells on the pre-stretched film (completely random cellular alignment) became highly aligned when the film was released after 1 day of incubation (Fig 2e-f).

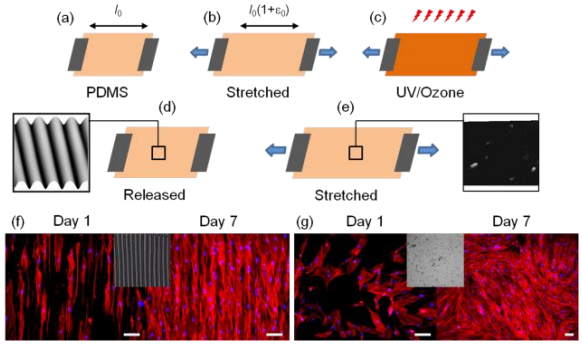

Figure 1. Fabrication of patterned PDMS films (a-c). Switching from patterned (d) to flat surfaces (e) occurs by stretching back to the initial strain. Images of hMSCs on released (patterned) (f) and stretched (flat) surfaces after 1 and 7 days (Insets: Bright-field images of surfaces). Scale bars are 50 microns.

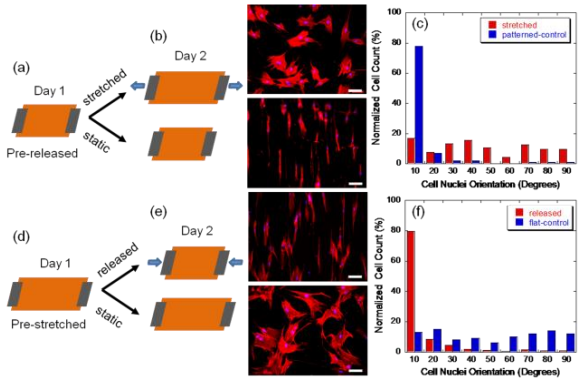

Figure 2. *In situ* switching from patterned to flat (a-b) or from flat to patterned (d-e) surfaces. Images of hMSCs on dynamic or static surfaces after 48 hours. Comparison of nuclear orientation for cells on dynamic and static surfaces (c: dynamic stretch, f: dynamic release). Scale bars are 50 μm.

Conclusions: This study demonstrates that the strain responsive PDMS surface patterns can be used as a powerful tool to dynamically control the cell and nuclear alignment. This dynamic control over cell nuclei provides a novel platform to investigate the relationships between cellular differentiation and nuclear morphology with changing microenvironments (e.g., after disease or injury). Moreover, we can gain valuable information by switching the morphology at different time points during the evolution of differentiation. Ongoing long term studies are enabling us to study the arrangement of matrix generated by aligned and random cells, and the effect of switching on matrix reorganization.

References: [1]Wang N. et al. Nat. Rev. Mol. Cell Biol., 2009, 10, 75-82. [2]Lim E. et al. Exp. Cell Res., 2007, 313, 1820-1829