Dynamic Cell Culture on Shape Changing Micropatterns

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Statement of Purpose: During development and disease progression, the geometry and density of cell adhesion sites change over time [1]. Substrates micropatterned with cell adhesion proteins have been used to investigate how protein density and geometry affect cell behaviors such as cell migration, growth, and differentiation. Such substrates have typically featured micropatterns that are static and unable to change over time [2]. Shape memory polymers (SMPs) are programmable smart materials capable of transitioning from a temporary shape to a permanent shape upon application of a trigger, such as heat. We have recently developed cell culture substrate SMPs that are able to undergo a programmed change in shape under cell compatible conditions [3]. Here we introduce the use of SMP substrates to dynamically control the shape of micropatterns over time under cell compatible conditions.

Methods: An acrylate-based SMP copolymer was cured to produce flat films. The films were heated, uniaxially strained by 20 %, and fixed in the elongated state by cooling to 25 °C. A Sylgard 184 stamp featuring a square-wave pattern of parallel plateaus 15 μm wide, spaced 15 μm apart, and 15 μm high was inked with human TRITC-fibronectin and used to microcontact print parallel, cell-adhesive lines onto the SMP substrates. Human adipose derived stem cells were plated on the micropatterned substrates at 30 °C. Cell nuclear morphology was analyzed before substrate shape change and after recovery of the substrate to its initial, unstretched length, which was triggered by increasing the temperature to 37 °C.

Results: Substrates remained stable at 30 °C for 8 h while cells attached and spread. Upon increasing the temperature to 37 °C, the substrates recovered 96.5 % of the programmed 20 % strain over 16 h, thereby actuating programmed changes in micropattern geometry.

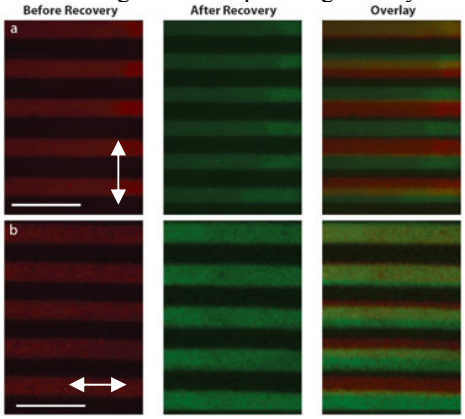

Figure 1. Contracting (a) and expanding (b) micropatterns before (red) and after (green) transition. Arrows indicate direction of substrate contraction. Scale bars are 50 μm.

For micropatterned substrates with patterns oriented perpendicular to the strain axis the pattern wavelength decreased from 29.3 ± 0.5 μm to 24.8 ± 1.5 μm (Figure 1a) following shape recovery, due to uniaxial contraction. For patterns oriented parallel to the strain axis, the pattern wavelength increased from 29.0 ± 0.7 μm to 32.1 ± 0.3 μm (Figure 1b) following recovery, due to Poisson effect expansion. The latter substrates were used for dynamic cell culture. Substrates that were not programmed to change showed no change in micropattern wavelength (data not shown). Before triggering recovery, cells on micropatterned substrates and their nuclei aligned with the micropattern direction and had a nuclear area of 184 ± 8 μm^2 on substrates not programmed to change shape (Figure 2a) and 210 ± 32 μm^2 on substrates programmed to contract (Figure 2c). Cells on substrates uniformly coated with fibronectin showed random orientation and a nuclear area of 340 ± 65 μm^2 (Figure 2e). Following heating to 37 °C and incubation for 16 h, cells on micropatterned substrates that were not programmed to change remained aligned with an aligned nucleus and a nuclear area of 195 ± 5 μm^2 (Figure 2b). Those cells on micropatterns aligned parallel to the strain axis contracted and showed a significantly smaller, round nucleus with a nuclear area of 100 ± 39 μm^2 ($p < 0.02$, Figure 2d) following recovery of the programmed strain. Cells on uniformly coated substrates remained randomly oriented (Figure 2f) with a nuclear area of 300 ± 78 μm^2.

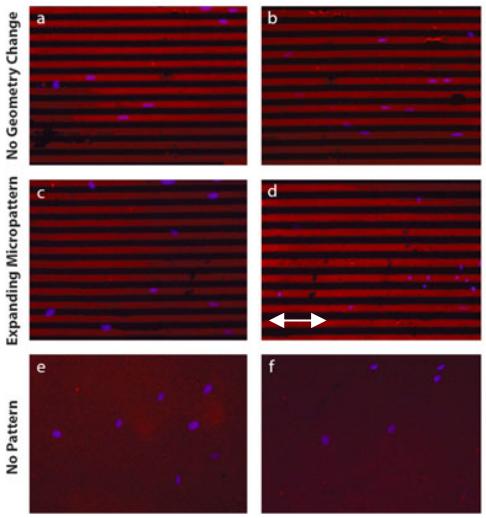

Figure 2. Cell culture on dynamic micropatterns. Cell nuclei (blue) on fibronectin (red) coated substrates. Arrow indicates direction of substrate contraction.

Conclusions: Micropattern geometry was successfully controlled via the shape memory effect. This change in geometry was shown to affect cell behavior. Such programmable control over micropattern geometry has the potential to revolutionize the study of temporal aspects of cell mechanobiology, for example, by allowing triggering of cell differentiation, growth, or death on command.

References: 1. Daley WP. J Cell Sci. 2008;121:255-264. 2. Théry M. J Cell Sci. 2009;123:4201-4213. 3. Davis KA. Biomaterials. 2011;32:2285-2293.