Spontaneous Formation of Stable Wrinkling Patterns in Hydrogels to Control Cellular Morphology

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Purpose: The interaction of cells (e.g., attachment, proliferation, differentiation) strongly depends on the chemistry, mechanics, and topography of a biomaterial.[1] The majority of reports on surface topography have focused on micro- and nano-patterns for a wide range of cell types; however, only a limited variety of patterns and materials are available due to fabrication and stability issues. Typical topographies are grooves and ridges, and materials include elastomers (e.g., polydimethylsilane (PDMS)), hydrophobic polymers (e.g., polystyrene and poly(methyl methacrylate)), and rigid surfaces (e.g., quartz, titanium, glass).[2] One limitation to this work is that the material mechanics and water content are typically very different than the native cellular microenvironment. In this work, we fabricated hydroxyl ethyl methacrylate (HEMA) hydrogel films with stable wrinkling patterns (depending on HEMA gel formulation) to study cellular interactions. These wrinkling patterns can be templated into other hydrogels to decouple material properties from wrinkle dimensions and were used to control cellular morphology.

Methods: HEMA monomer mixed with photoinitiator (3 wt% Darocur 1173) was exposed to UV to form a viscous, partially polymerized solution. A crosslinker (1 wt% ethylene glycol dimethacrylate) was added into the pre-polymer to form a precursor solution, which was cast onto a silicon wafer and exposed to UV to form a crosslinked film. Stable wrinkling patterns were spontaneously formed as the hydrogel film swelled in water (Figure 1a). Replicas of the patterns were fabricated by a micromolding method using the HEMA wrinkles as a master template. Optically transparent PDMS daughter templates were fabricated by covering the master template with PDMS precursor (Slygard 184) mixed with curing agent (10:1) and cured at 65^{o}C for 4 h (Figure 1b). In order to prepare the replicas, a drop of HEMA precursor was put on a silane (3-trimethoxysilyl propyl methacrylate) functionalized glass slide, covered with the PDMS mask, and exposed to UV for 1 min (Figure 1c). Patterned gels were equilibrated in PBS for at least 24 h and immersed in ethanol-water (70:30 v/v) for 1 h. Gels were dried and sterilized under germicidal lamp for 1 h, incubated in fibronectin-PBS (20 μg/mL) for 18 h, and then in serum containing media for 1 h. 3T3 fibroblast cells were seeded onto each sample (5.5 x10^4 cells/cm^2), cultured for 48 h, and stained with FITC-phalloidin and DAPI for visualization.

Results: Wrinkling patterns were easily formed by immersing HEMA gels into water. As the gels swell, lateral confinement coupled with osmotic stress leads to the formation of wrinkling patterns. Pattern morphology and wavelength (λ) were easily tuned by crosslinker concentration and film thickness (t_f). For instance, for 1 wt% EGDMA and t_f = 50 μm the λ was ~60 μm, whereas λ increased to ~180 μm for 400 μm films. When the EGDMA concentration was decreased to 0.4 wt% and t_f was 50 μm, λ was ~100 μm. For cellular interactions studies, we focused on a random grooved geometry with λ ~150 μm and depth ~10 μm. To eliminate variations in HEMA properties with wrinkling morphology, the pattern was templated to PDMS and then to a new HEMA gel where the properties could be decoupled from the pattern. We have formed a range of photocrosslinkable gels with this technique where properties such as mechanics, ligand inclusion, and degradation are readily controlled (results not shown). 3T3 fibroblasts were seeded onto both patterned and flat HEMA gels, where only the surface morphology was different. As expected, cells attached and spread randomly on the flat gel (Figure 2a). On the patterned surfaces, the cells attached inside the grooves, rather than the peaks, and aligned themselves to track the groove shape (Figure 2b). Only a few cells formed bridges between the grooves. This approach provides a facile technique to control cellular alignment (through different wrinkle patterns) apart from material properties and will be useful for controlling stem cell differentiation.

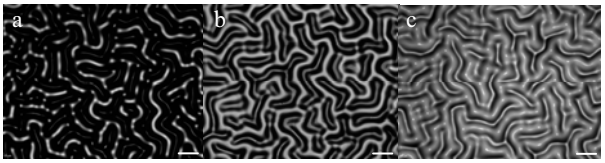

Figure 1. Wrinkling patterns on (a) HEMA hydrogels, (b) PDMS template, and (c) HEMA gel replica. Images are not from same location. Scale bars = 100 μm.

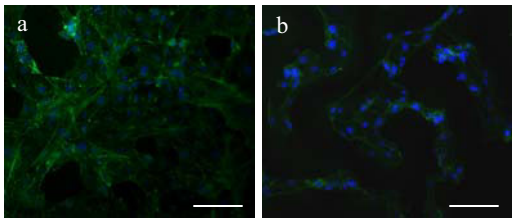

Figure 2. Images of fibroblasts on (a) flat and (b) patterned HEMA gels after 48 hours stained for nuclei (blue) and actin (green). Scale bars = 100 μm.

Conclusions: HEMA films photocrosslinked with EGDMA on silicon wafers formed a range of wrinkling patterns when swollen in water, where pattern morphology and dimensions were controlled by film thickness and crosslinker concentration. 3T3 cells attached to templated wrinkled surfaces and aligned themselves to take the pattern shape, whereas they randomly aligned on flat surfaces. This precise control over cell morphology, which is decoupled from material properties, provides a means to investigate relationships between cellular differentiation and morphology.

References: [1]Curtis A.Trends in Biotechnology : 2001 : 19 : 97-101. [2]Flemming RG.Biomaterials : 1999 : 573-588.