PEGDA Hydrogels with Patterned Elasticity for the Study of Cell Response to Substrate Stiffness

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Introduction: Cells are able to sense and respond to substrate mechanical properties and may exhibit differences in attachment, spreading, proliferation, migration, and differentiation based on substrate rigidity.[1] Knowing how cells sense and respond to substrate rigidity may aid in biomaterial design as well as the study of both normal development and mechanisms of disease. Using poly(ethylene glycol)-diacrylate (PEGDA) hydrogels with varying polymer chain length and photolithographic patterning techniques, we are able to provide substrates with patterned and gradient elasticity for the study of cell response to substrate rigidity. Additionally, the excellent biocompatibility of this polymer system makes it ideal for use in tissue engineering applications.

Materials and Methods:

Hydrogel preparation: Base hydrogels were prepared by photocrosslinking 20 kDa using an acetophenone photoinitiator. Hydrogels were then incubated in a 3.4 kDa PEGDA solution containing photoinitiator. This was allowed to diffuse into the hydrogel. To generate patterns, photocrosslinking was performed through a photomask, then unreacted polymer was allowed to diffuse out of the hydrogel.

Hydrogels with gradient elasticity were formed using a gradient maker. The two reservoirs of the gradient maker contained PEGDA (3.4 and 20 kDa, respectively). The resulting polymer gradient was dripped into a rectangular glass mold and crosslinked with long-wavelength UV light. Hydrogels were removed from molds and placed in HEPES-buffered saline to swell prior to mechanical testing.

Prior to cell studies, acryloyl-PEG-RGDS was covalently grafted to the hydrogel surfaces.

Mechanical Testing: Hydrogel samples were subjected to tensile testing using an Instron Model 3340. Striped hydrogels were tested parallel and perpendicular to stripe axis. Prior to mechanical testing, hydrogels with gradient elasticity were sliced into sequential sections along the length of the gradient.

Cell Studies: Smooth muscle cells (SMC) were seeded on hydrogels at 5,000 cells/cm^2. Nonadherent cells were rinsed off after 3 hr, and cells were cultured for 12 to 24 hr prior to fixation.

Imaging: Hydrogels with elasticity patterned in stripes were soaked in solutions of fluorescent dextran, rinsed, and imaged. Cells on all hydrogels were fixed after 12 or 24 hr, then stained with DAPI and phalloidin.

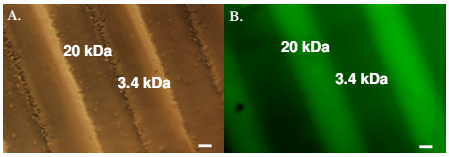

Figure 1: (A) Striped patterned PEGDA hydrogel. Surface patterning controlled by photomask geometry. (B) Patterning confirmed by exclusion of fluorescently-labeled 70 kDa dextran from stiffer stripes.

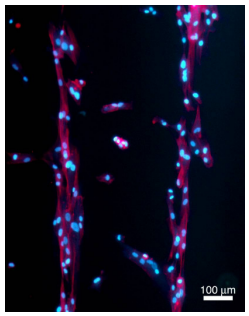

Figure 2: DAPI (blue) and phalloidin (red) staining of SMCs 24 hrs after seeding on hydrogel with rigidity patterned in stripes.

Results and Discussion: Hydrogel patterning was confirmed by exclusion of 70 kDa dextran-fluorescein from patterned areas (Fig 1). Tensile testing of striped hydrogels revealed pattern-dependent changes in elasticity and anisotropy.

24 hr after seeding on hydrogels, SMCs had aligned along stiff stripes with little attachment or spreading on softer stripes (Fig 2), even though the adhesive peptide RGDS was present in both regions.

Tensile testing of sequential cross-sections of gradient hydrogels verified a gradient of elastic moduli in one axis only, with good repeatability between hydrogels (Fig 3). 12 hr after seeding on hydrogels, SMC density was found to increase with hydrogel stiffness.

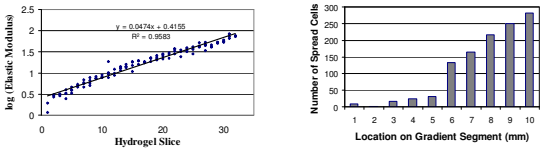

Figure 3: (left) Tensile testing of sequential sections of gradient hydrogel confirms a gradient of elastic moduli. (right) Analysis of cell location after 12 hr on gradient hydrogel shows SMC density increasing with stiffness.

Conclusions / Summary: PEGDA hydrogels with patterned and gradient elasticity are attractive substrates for the study of cell response to substrate rigidity. Using PEGDA hydrogels with tailored mechanical profiles, we are able to show differences in SMC behavior with substrate elasticity within a single sample. These substrates allow for the investigation of edge effects between substrates of different elasticity, effects of anisotropy on cellular behavior, screening of the effects of a wide range of substrate elasticity on cellular behavior, and investigation of cellular migration in response to a gradient of elasticity. PEGDA hydrogels also present an advantage over other systems used to study cell response to substrate rigidity due to their high degree of biocompatibility, which renders them readily translatable into clinical applications.

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References:

1. Discher, D.E., et al. Science, **310**(5751) 2005.