Cellular Behavior on Hydrogels with Patterned Elasticity Is Influenced by Substrate Mechanics and Ligand Density

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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. 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 show differential macrophage response to substrate rigidity and ligand density.

Materials and Methods:

Polymer preparation: PEGDA was prepared by combining 0.1 mmol/mL dry PEG (3.4 or 20 kDa) with 0.4 mmol/mL acryloyl chloride and 0.2 mmol/mL triethylamine in anhydrous dichloromethane under argon, stirring overnight. The resulting PEGDA was washed with K_2CO_3, dried with anhydrous $MgSO_4$, and precipitated in diethyl ether, then filtered and dried *in vacuo*. PEG-RGDS was prepared by combining acryloyl-PEG-SCM with RGDS peptide and DIPEA in anhydrous DMSO. The product was dialyzed against ultrapure water using a 2000 MWCO membrane, then lyophilized and stored at -20 ºC until use.

Hydrogel preparation: Base hydrogels were prepared with 20 kDa PEGDA with 0, 1, or 3 mM PEG-RGDS and were photocrosslinked using an acetophenone photoinitiator. Hydrogels were then incubated in a 3.4 kDa PEGDA solution containing photoinitiator and fluorescein-o-acrylate, which was allowed to diffuse into the base hydrogel. To generate patterns, photocrosslinking was performed through a photomask then unreacted polymer and fluorescein was allowed to diffuse out of the hydrogel.

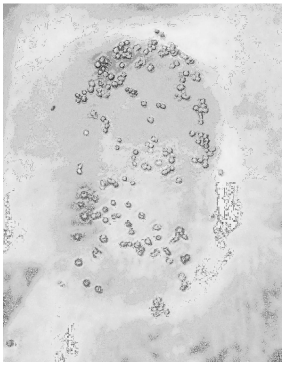

Figure 1: Macrophages are preferentially located on stiff areas of patterned hydrogel (oval) at 48 hours.

Cell Studies: RAW 264.7 macrophages (50,000 cells/cm^2) were seeded on patterned hydrogels. Nonadherent cells were rinsed off after 4 hr, and hydrogels were imaged after 4, 24 and 48 hr.

Results and Discussion: After 24 hours on hydrogels with patterned rigidity, macrophages were preferentially located on stiff areas, with few cells adherent to softer regions of the substrate (Fig 1). This was true for hydrogels with 1 and 3mM PEG-RGDS and continued out to 48 hr (Fig 2). Greater initial attachment of cells to stiff areas and greater proliferation of cells on those areas appear to be the greatest contributors to the differential behavior seen, as no significant migration of cells or apoptosis of cells on softer areas was observed.

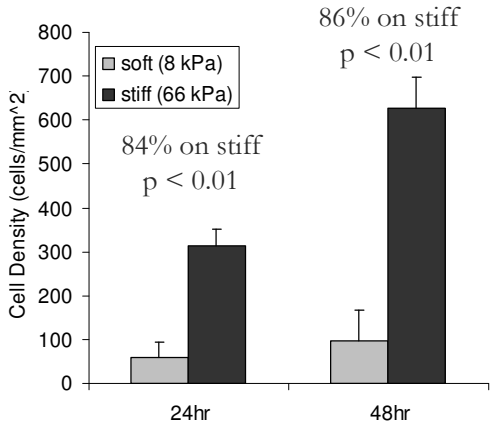

Figure 2: After both 24 and 48 hours, macrophages are preferentially located on stiffer areas of the hydrogel. This difference is more pronounced on hydrogels with 3 mM PEG-RGDS (above) than on those with 1 mM PEG-RGDS.

Conclusions / Summary: PEGDA hydrogels with patterned 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 cellular behavior with substrate elasticity within a single sample. These substrates also allow us to investigate cell sensitivity to substrate mechanics and ligand density independently. Furthermore, PEGDA hydrogels 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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