Multiple Length-Scale Spherical Indentation of PEG and Alginate-Based Hydrogels

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Statement of Purpose: Hydrogels are popular materials for cell and tissue culture substrates as they can absorb substantial quantities of water and have mechanical properties that can be tailored via alteration of fabrication parameters. Mechanical cues from substrates are becoming increasingly recognized as important signaling sources for cells in biological applications. Thus far substrate stiffness has been the primary mechanical property of interest. Other cell-relevant mechanical properties, including time-dependent relaxation, have largely been overlooked. In addition, measurements of the bulk systems do not reflect the mechanics on cell-relevant scales. Spherical indentation, a modification of compression testing, is non-destructive and allows for localized measurements at various length-scales. Additionally, time-dependent behavior can be extracted from load-time data via viscoelastic analysis frameworks. Such information provides a more complete view of possible mechanical stimuli for cellular responses.

Methods: Poly(ethylene glycol) dimethacrylate (PEGDMA), alginate, and PEGDMA-alginate composite systems were fabricated with varying molecular weights and total polymer concentrations (*%T*). Hydrogels were characterized for their mechanical and chemical properties using ATR-FTIR spectroscopy, swelling measurements, gel fraction, and cryo-scanning electron microscopy (SEM). Spherical indentation load-relaxation data were obtained at two length-scales using a fluid cell to maintain equilibrium swelling. An Asylum Research MFP3D AFM (Santa Barbara, CA) with gold colloidal probe tips (nominal radius = 3 μm) was used for microindentation. Bulk mechanical properties were obtained using an Instron 5544 universal testing frame (Canton, MA) with 4.7 mm radius stainless steel indenter. Load-relaxation data were fit to extract time-dependent shear moduli. MC3T3 cells (ATCC, Manassas, VA) from a mouse pre-osteoblast cell line were seeded onto the hydrogels and an MTT assay (Promega, Madison, WI) was used to evaluate changes in proliferation and viability of the cells based upon gel mechanical properties.

Results: Hydrogels were fabricated from pure PEGDMA, pure alginate, and a composite system of the two. Equilibrium swelling measurements of the gels showed that alginate gels swelled up to 30 times their dry weight in water, whereas PEGDMA gels and the composites swelled up to ten times their dry weight. Swelling was inversely related to *%T* and temperature.

In all spherical indentation measurements, increasing *%T* increased the shear modulus for each hydrogel system tested. In addition, the degree of viscoelasticity of the hydrogels was purely material-dependent and independent of *%T*. Overall, single-component PEGDMA, alginate, and composite gels were fabricated that exhibit long-term shear moduli of ~25 kPa, with viscoelastic ratios in the range of ~0.3-0.8, indicating that multiple gel systems with the same stiffness but variable time-dependent properties can be fabricated. Preliminary cell viability and proliferation results indicate that small *%T* alginate and composite gels best support cell growth.

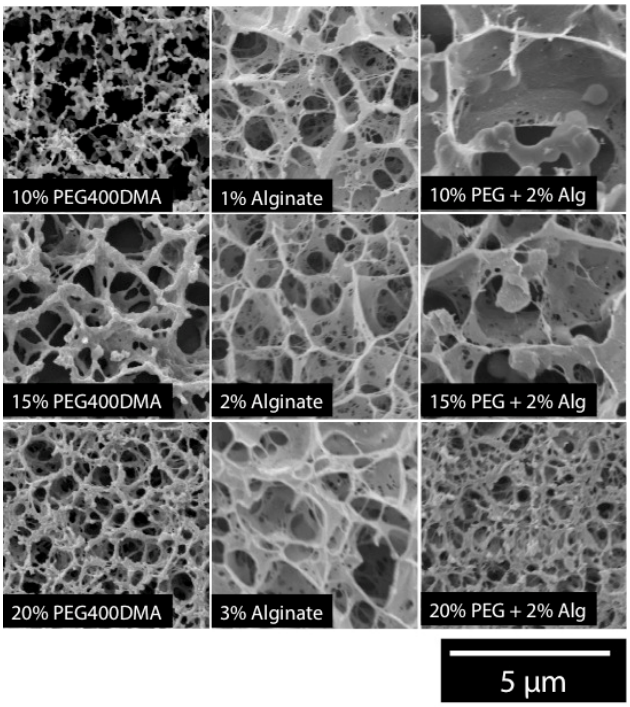

Figure 1. Cryo-SEM images of PEGDMA, alginate, and composite hydrogels.

Conclusions: PEGDMA, alginate, and composite hydrogels were fabricated with a range of shear moduli, time-dependent behaviors, and other mechanical properties. Spherical indentation at multiple length-scales allowed for improved measurement of these properties and aided in the understanding of the behavior of hydrogel substrates at cell-relevant length-scales. This can ultimately better inform material design for tissue engineering substrates.