Superswelling and Patterned Dual-Crosslinked Hyaluronic Acid Hydrogels : Zonal Distribution of Hydrogel Properties for Tissue Engineering Applications

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Statement of Purpose: Tissue engineering focuses on synthesizing scaffolds that can replace damaged tissues. Native tissues exhibit heterogeneity at both macro- and micro-scales. For example, cartilage is a highly organized yet heterogeneous tissue. The organization of cartilage tissue can be divided into several zones and each zone has different water content, mechanical properties, and mesh size. These heterogeneities are vital to the tensile and load bearing properties of cartilage[1]. Unlike native cartilage, typical hydrogel scaffolds uniform bulk properties with chemical homogeneity. Therefore, it is necessary to fabricate hydrogels with zonal distributions of material properties for producing the next generation of tissue engineering constructs. High concentration hyaluronic acid hydrogels were prepared with superswelling (Fig. 1) and patterned anisotropic swelling (Fig. 2). Chemical crosslinking and photocrosslinking were combined at macro- and micro-scales in single bulk hydrogel to yield zonal distributions of hydrogel properties like water content, mesh size, and viscoelasticity.

Figure 1. Images of chemically crosslinked super-swelling hydrogels of hyaluronic acid demonstrating the ability to maintain shape during swelling. Hand-shaped button that was used to cast the PDMS mold (left), hydrogel after swelling in saline (middle), and hydrogel after swelling in water (right). Scale bar = 1 cm.

Methods: Photopolymerizable HA (GMHA) was synthesized by modifying it with glycidyl methacrylate as described previously.[2] HA (10 wt% and 20 wt%) was chemically crosslinked using butanediol diglycidyl ether (BDDE) in presence of 0.2 M NaOH for 8hrs at 40°C. Photocrosslinked hydrogels were prepared by dissolving GMHA in I2959 (photocrosslinker) and exposing the resulting solution to UV light for appropriate time. Dual-crosslinked hydrogels combine two kinds of crosslinks: chemical crosslinks and photocrosslinks. First, photocrosslinks were formed by exposure to UV light and then chemical crosslinks were formed by adjusting pH and temperature. Patterned dual-crosslinked hydrogels were prepared by using a photomask during photocrosslinking. Chemically crosslinked, photocrosslinked, and dual-crosslinked hydrogels were characterized and compared using rheology, swelling studies, and HAase degradation studies. Viscoelastic properties of gels were determined by rheology experiments which provide a measure of stiffness and degree of crosslinking.

Results/Discussion: Chemically crosslinked and photocrosslinked hydrogels were successfully synthesized and the two types of crosslinking were combined to make dual crosslinked hydrogels. Rheology results demonstrated that the storage modulus for the dual-crosslinked hydrogels was much higher than either chemically crosslinked or photocrosslinked hydrogels. Chemically crosslinked hydrogels exhibited superswelling with shape maintenance (Fig. 1). Patterned dual-crosslinked hydrogels of HA were prepared with high swelling chemically crosslinked regions and low swelling photocrosslinked regions (Fig. 2). All hydrogels were biodegradable by the enzyme HAase.

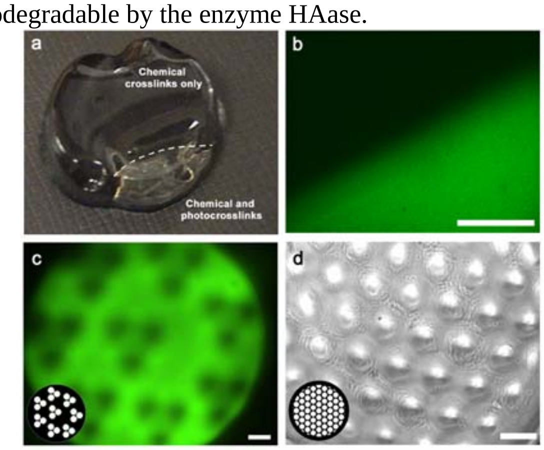

Figure 2. Patterned anisotropic dual-crosslinked hydrogels. (**a**) An anisotropically swelling hydrogel that had half its area exposed to UV and half unexposed. The UV exposed (i.e., dual-crosslinked) region exhibited low swelling and the non-exposed (i.e., chemically-crosslinked) region exhibited high swelling. (**b**) Fluorescent image of the hydrogel in (a) depicting the interface between the two crosslinking regions. The photocrosslinked region incorporated fluorescein-acrylate during photopolymerization. (**c**) Hydrogel demonstrating micro-patterning of dual-crosslinked and chemically-crosslinked regions. The photomask used to pattern the photocrosslinks is displayed in the bottom left corner. The fluorescent regions of the hydrogel correspond to dual-crosslinked regions that incorporated fluorescein-acrylate. (**d**) A dual-crosslinked hydrogel depicting micro-patterning with a hexagonal lattice photomask. The regions coinciding with the mask have a high crosslink density and low swelling. The regions coinciding with the interstices have a low crosslink density and high swelling. The result is a hydrogel surface with regular repeating bumps arising from anisotropic swelling.

Conclusions: We have engineered novel HA hydrogels that exhibit superswelling and patterned anisotropic swelling. The unique features of the method are that we have combined two different methods of crosslinking within a single bulk hydrogel and have synthesized hydrogels with macro- and micro-patterned distributions of hydrogel properties. These hydrogels are composed of a biodegradable glycosaminoglycan that is well suited for drug delivery and tissue engineering applications.

References:

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