Biodegradable Hyaluronic Acid Hydrogels with Tunable Properties and Encapsulated Microspheres for Wound Repair

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Statement of Purpose: Hyaluronic acid (HA) is a high molecular weight polysaccharide that plays a large role in wound healing by modulating cell behavior, as well as maintaining tissue homeostasis and biomechanical integrity [1]. For exogenous HA delivery, we have designed an injectable and tunable biodegradable hydrogel system through introduction of either methacrylates (Me) or hydroxyethylmethacrylate (HeMA) onto the HA backbone to capitalize on both enzymatic and hydrolytic gel degradation. Furthermore, integration of poly (DL- lactide-co-glycolide) (PLGA) microspheres to our system adds an additional structural cue for fibrous tissue formation upon degradation of the hydrogel [2]. Here, we characterize Me-HA, HeMA-HA and copolymer hydrogels with and without microspheres, with respect to mechanics, degradation, and tissue formation.

Methods: MeHA (Figure 1) was synthesized as previously described using methacrylic anhydride at pH 8.0 for 24 hours followed by dialysis [3]. HeMA-HA (Figure 1) was synthesized by reacting HeMA-COOH and HA (Lifecore, 59 kDa) with 4-dimethylaminopyridine and ditertbutyldicarbonate (BOC_2O) at 45ºC for 21 hours, dialyzing against DI-H_2O for 15 hours, and precipitating in acetone. Methacrylation was adjusted by varying HeMA-COOH and BOC_2O and assessed with ^1H NMR.

PLGA (Lactel Absorbable Polymers) microspheres were synthesized using a glass-capillary microfluidic device with a flow focusing geometry [4]. 4 wt% PLGA in DCM was emulsified in a 2 wt% poly(vinyl alcohol) solution. Syringe pumps controlled the delivery to the device (Q_{PLGA}= 2.0 mL/hr and Q_{PVA}=10mL/ hr).

Macromers were dissolved in PBS at 4 wt% and crosslinked by redox reaction (i.e., mixing solutions of 5 mM tetramethylethylenediamine (TEMED) and 5 mM ammonium persulfate (APS)). Microspheres were added to some mixtures at either 10 mg/mL and 75 mg/mL. The macromer solutions were injected in between two glass slides with a 1 mm spacer and incubated at 37ºC for 30 minutes. Compression testing was performed on samples (n=3-4) using a Dynamic Mechanical Analyzer (Q800 TA Instruments) at a strain rate of 10%/ min. Moduli were calculated at strain from 10-20%. Degradation was monitored in PBS at 37 ºC.

Results: Varying the concentration of HeMA-COOH and BOC_2O allowed for changes in HeMA-HA modification. In general, as the degree of methacrylation increased, moduli and degradation rates both increased (Figure 1). These properties were also adjusted by forming copolymers with the non-hydrolytically degradable MeHA. HeMA-HA (20% modification) and MeHA (100% modification) were used to form homopolymers or a 75:25 copolymer. Compared to MeHA alone, the compressive modulus of the two HeMA-HA gels significantly decreases with time. Thus, these gels are tunable with respect to degradation and moduli.

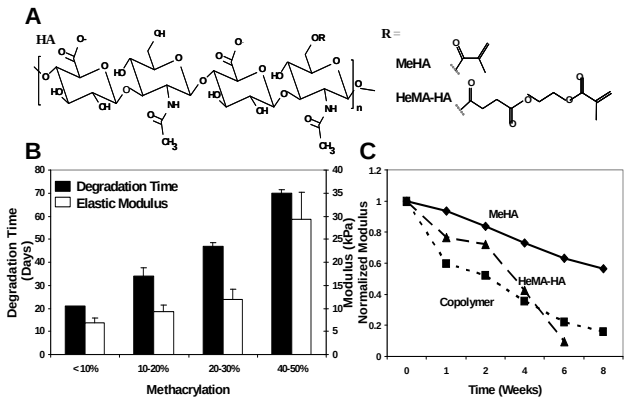

Figure 1: The chemical structures of HeMA-HA and MeHA (A). Degradation time and compressive modulus for 4 different modifications of 4 wt% gels of HeMA-HA (B). Normalized compressive modulus data of copolymers with degradation (C). All results used 4 wt % gels and 5 mM APS and TEMED.

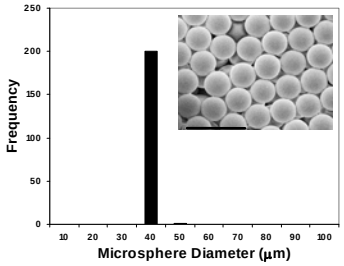

Figure 2: Frequency distribution of PLGA spheres. Inset: SEM of ~40 μm microspheres. Scale bar=100μm.

It has been shown previously that smooth, spherical microspheres 30-60 μm in diameter can direct tissue formation [2]. We fabricated PLGA microspheres (Figure 2) that meet these requirements. When encapsulated, the microspheres only significantly increased the modulus at the 75 mg/mL, but not the 10 mg/ml concentration. HA hydrogels have been recently implanted subcutaneously in rats without microspheres and with microspheres at 10 mg/mL and 75mg/ mL. Ongoing work will investigate the tissue growth around these implants at 2 and 4 weeks with changes in both the polymer degradation rates and microsphere incorporation.

Conclusions: We have successfully developed a HA hydrogel system with tunable mechanics and degradation through variations in the methacrylation and macromer concentrations. The addition of microspheres to our system allows us to analyze microsphere contribution to gel function, in addition to microsphere involvement in the wound healing response. This work will provide a platform for a wide range of applications where alterations in the cellular response are desired in the wound healing cascade using an injectable gel composite.

References:

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