Influence of Injectable Hyaluronic Acid Hydrogel Degradation Behavior on Myocardial Infarct Repair

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Statement of Purpose: Recently, acellular injectable biomaterials have been shown to attenuate left ventricular remodeling after myocardial infarction by reducing stresses in the heart wall [1]. Although studies have shown that the mechanics of the material plays a role in altering geometry changes that contribute to maladaptive remodeling, few studies have been able to systematically evaluate both the network mechanics and degradation of injectable hydrogels[2]. This is the focus of the present study, in order to gain insight into material design.

Hyaluronic acid (HA), is a native extracellular matrix molecule that can be functionalized to be either primarily enzymatically degradable (addition of a methacrylate, MeHA) or both enzymatically and hydrolytically degradable (addition of a hydroxy ethyl methacrylate, HeMA) and can be injectable via a radical polymerization (e.g., APS/TEMED redox initiators)[3]. In this work, we investigated gels in both *in vitro* studies and in an ovine infarct model with initial moduli of either approximate that of native myocardium or 4-fold higher that exhibit a range of degradation (~3 weeks and up to little mass loss after 8 weeks).

Methods: Briefly, MeHA (Figure 1) was synthesized by reacting HA (Lifecore, 66 kDa) with methacrylic anhydride and dialyzing[3]. HeMA-HA (Figure 1) was synthesized by reacting HeMA-COOH and HA 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 ^{1}H NMR. Macromers were dissolved in PBS at 4 wt% and crosslinked by redox reaction (APS/TEMED). Gelation was assessed on samples (n=3-4) by monitoring the storage (G') and loss (G") moduli over time with a AR2000ex Rheometer (TA Instruments) at 37°C under 1% strain and 1 Hz using a cone and plate geometry (1°, 20 mm diameter). 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 strains from 10-20%. Degradation was monitored in PBS at 37 ºC using a uronic acid assay or mechanics.

In vivo function (n=6 per group) was accessed in an ovine infarct model. Infarction was induced via ligation of the left anterior descending and 2nd diagonal coronary artery. Thirty minutes after infarction, 0.3cc of the pre-polymer solution was injected at 20 sites in the infarct area. Hemodynamic data and real time 3D echocardiographs were collected before infarction, 30 minutes post-infarction, 30 minutes post-injection, and 2 and 8 weeks after therapy. Animals were sacrificed at 8 weeks and a histological evaluation was performed.

Results: Varying the concentration of HeMA-COOH and BOC_2O or methacrylic anhydride allowed for changes in HeMA-HA or MeHA modification, respectively. This study focused on four macromers (low MeHA: 7.7 kPa, high MeHA: 43.0 kPa, low HeMA-HA: 7.2 kPa, high HeMA-HA: 32.5 kPa) each exhibiting unique mass loss profiles and mechanical changes with time (Figure 1)[2]. In general, lower modification resulted in a faster degradation rate and decrease in mechanics. Furthermore, functionalization with HeMA leads to more significant changes in degradation and mechanics with time than MeHA. It is important to tailor these properties with the biological and mechanical trends found after infarction.

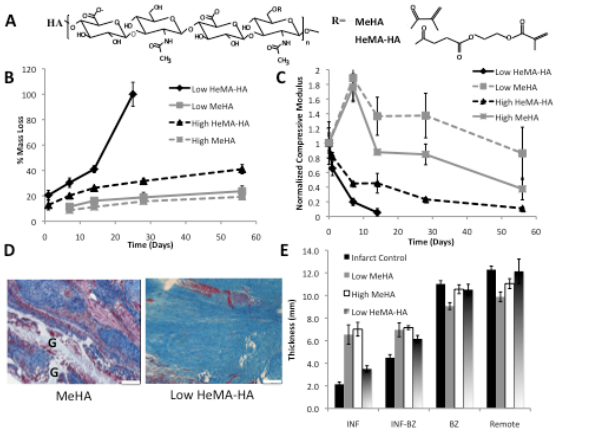

Figure 1: The chemical structure of MeHA and HeMA-HA (A). Degradation rate (B) and normalized compressive moduli (C) with time for low and high HeMA-HA and MeHA gels. Representative Masson's Trichrome staining of MeHA and low HeMA-HA treatments in the apical region of the infarct, scale bar =200μm (D). Myocardial thickness at 8 wks (E).

At the 8 week time point, no hydrogel was present after histological evaluation in low HeMA-HA therapy animals (Figure 1); however, there was a slight increase in apical myocardium thickness (3.5 mm vs 2.1 mm, Figure 1) and decrease in infarct area compared to infarct controls (22.4% vs 28.6 %, data not shown). Gel was still present in both MeHA formulations (Figure 1). Functional data (NESV and NEDV) was similar to our low MeHA therapy study (data not shown). Ongoing *in vivo* work is being performed on the high HeMA-HA therapy group.

Conclusions: We have successfully designed an injectable hydrogel system with tunable mechanics and degradation. This system can be used to systematically evaluate the efficacy of biodegradable HA in attenuating left ventricular remodeling, through timing of various cues (e.g., magnitude of stress reduction) following injury. These results show that a quickly-degrading material can be as effective as a slowly-degrading material with the same initial mechanics. Evaluation of our high therapy animals will provide additional insight regarding the role of material degradation as well as the mechanical effects over time on ventricular remodeling.

References:[1] Christman, KL et al. J Am CollCardiol2006: 907–13. [2] Ifkovits, JL et al. PNAS 2010 : 11507-12. [3] Burdick, JA et al. Biomacromolecules 2005: 386-391.