Tunable and Injectable Hyaluronic Acid Hydrogels to Attenuate Post-Infarction Left Ventricular Remodeling

Jamie L. Ifkovits[1], Elena Tous[1], Masahito Minakawa[2], Masato Morita[2], Joseph H. Gorman[2], Robert C. Gorman[2], Jason A. Burdick[1]

[1]Department of Bioengineering, [2]Harrison Department of Surgical Research, University of Pennsylvania, Philadelphia, PA

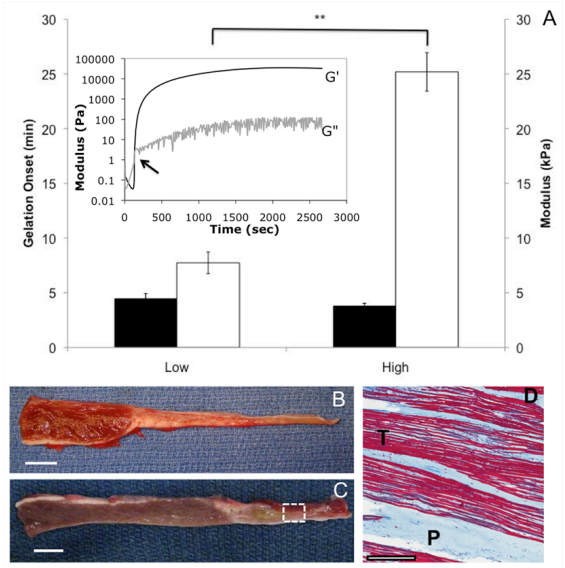

Figure 1. Gelation onset (black) and storage modulus at steady state (white) for the two macromers; representative time sweep curves, the arrow indicates gelation onset (inset) (A). Representative images of infarct control (B) and High MeHA treatment (C). Representative trichrome stain image from the apex of a MeHA (P) treated infarct demonstrating integration with tissue (T) (D). Scale bar = 10 mm (B, C) or 200 μm (D), **p<0.01.

Statement of Purpose: Changes in peak left ventricle (LV) wall stress have been implicated in the pathogenesis of post-infarct LV remodeling. A recent finite element model simulation has confirmed the impact of an acellular, non-contractile material injected into the myocardium after infarction to reduce these stresses and the mechanical burden on the heart [1]. Hyaluronic acid (HA) is a linear polysaccharide, which in addition to playing roles in many biological processes, can be modified to introduce methacrylate groups (MeHA) capable of crosslinking via free radical polymerization [2]. Specifically, stable injectable hydrogels can be formed through the use of the redox initiators ammonium persulfate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED). Using MeHA, we have developed an injectable hydrogel system that is tunable with respect to mechanics in order to investigate the associated salutary effect in the reduction of post-LV remodeling in an established ovine model of infarction.

Methods: MeHA was synthesized as previously described [2]. Briefly, 1 wt% HA (Lifecore, 74 kDa) in DI water was reacted with methacrylic anhydride at pH 8.0 for 24 hours on ice followed by purification via dialysis (MW 6-8 kDa cutoff) for 72 hours and lyophilization. ^{1}H NMR (Bruker) was used to determine % methacrylation. Hydrogels were formed by dissolving MeHA (4 wt%) in saline with APS (5.0 mM, Sigma) and TEMED (5.0 mM, Sigma). The gelation onset (n=3) was determined by monitoring the storage (G') and loss (G") moduli over time using a AR2000ex Rheometer (TA Instruments) at 37°C under 1% strain and 1 Hz in a cone and plate geometry (1°, 20 mm diameter).

Finally, *in vivo* function (n=5 per group) was assessed by injection of 0.3 cc of the combined solution into 20 injection sites into an ovine heart 30 minutes post-infarction. Infarction was induced via ligation of the left anterior descending and 2nd diagonal coronary artery. Hemodynamic data and real time 3D echocardiographs were collected prior to infarction, 30 minutes post-infarction, 30 minutes after injection, and 2 and 8 weeks after therapy. Animals were sacrificed at 8 weeks and processed for histological evaluation.

Results: Two MeHA macromers (~30 % (Low) and ~60% (High) methacrylation) were synthesized. The gelation onset time was approximately 4 minutes, regardless of macromer (Figure 1A). However, G' was significantly different between hydrogels formed from the different macromers (~8 kPa versus ~25 kPa). Our previous data indicates that gelation can occur after injection into the myocardium and that the high modification increases the mechanics of the tissue, whereas the low does not (results not shown).

Echocardiographic data demonstrated a large, dyskinetic anteroapical segment immediately after coronary occlusion. Injection of the hydrogel lead to thickening of the tissue, as evident upon sacrifice at 8 weeks (Figure 1C) when compared to control infarct samples (Figure 1B). The regional thickness from the apex to the base was quantified and demonstrated significant tissue thickness in the apex and borderzone regions (6.3, 7.3 mm, respectively) compared to control infarct samples (2.1, 4.5 mm, respectively). Furthermore, this treatment group demonstrated a significant reduction in size of infarct (23.3%) and an improvement in normalized end systolic volume (2.1) and normalized end diastolic volume (1.7) compared to infarct control samples (28.6%, 2.2, 1.8, respectively). The effect of the Low macromer injection *in vivo* is currently ongoing.

Conclusions: Two different MeHA macromers exhibiting different mechanical properties were developed for injection post-infarction to evaluate the associated salutary effect on the remodeling response based on hydrogel properties. Treatment using the High MeHA group thickened the tissue and reduced the associated infarct expansion compared to the infarct control. Ongoing work is evaluating the effect of treatment using the Low MeHA macromer to assess outcomes based on hydrogel parameters. This work will provide fundamental information towards the development of hydrogels for treatment of LV remodeling.

References: [1] Wall, ST et al. Circulation 2006: 114, 2627-35. [2] Burdick, JA et al. Biomacromolecules 2005: 386-391.