Visualization of *In Situ* Forming Hydrogels in the Myocardium using CEST MRI

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Statement of Purpose: *In situ* forming biomaterials have become an attractive approach for many biomedical applications, including to attenuate left ventricular (LV) remodeling after myocardial infarction (MI) by mechanically stabilizing the myocardial wall to reduce stress.[1] Recent studies have used *in situ* forming hydrogels with a range of degradation profiles for post MI repair;[2] however, the degree and duration of stabilization necessary to optimize the mechanical benefit observed remains unclear. One challenge in identifying optimal material degradation is the ability to noninvasively visualize injectable materials over time *in vivo*.

Magnetic resonance imaging (MRI) is particularly well suited for material assessment due to its ability to provide accurate, reproducible images with high spatial resolution in three dimensions.[3] Several studies have used MRI to gain insight into LV remodeling,[4] but none have used it to track cardiac biomaterials *in vivo*. Chemical exchange saturation transfer (CEST) is a new MRI contrast approach that relies on endogenous contrast from protons in specific molecules or metabolites based on their exchange with bulk water protons.[5] This technique allows material visualization without the need to couple an exogenous MRI contrast agent. In this work, *in vitro* CEST MRI experiments were preformed to characterize the CEST effect of *in situ* forming and degradable hyaluronic acid (HA) hydrogels and visualize differences with macromer concentration to mimic degradation.

Methods: Hydroxyethyl methacrylated HA (HeMA-HA) macromers were synthesized and crosslinked into hydrogels using a redox initiator system of APS and TEMED.[2] Degradation (*n=3-4*) was monitored in PBS at 37°C using an uronic acid assay and mechanical testing.

Macromer was dissolved at different concentrations in PBS at pH 7.0 and mixed with initiators. Samples of prepolymer solution were added to test tubes (10mm diameter) and immersed in a PBS phantom, allowing temperature to be maintained at 37±1°C throughout imaging experiments. CEST imaging was performed on 3T and 7T whole body scanners (Siemens) with an 8-channel and 32-channel ^{1}H head coil, respectively. CEST images were acquired from chemical shifts of −4ppm to +4ppm (step=0.2ppm) around the water resonance (0ppm) at varying saturation pulse amplitude (B_{1rms}) and duration to empirically optimize the CEST saturation parameters.

Ex vivo samples were prepared by mixing the macromer and initiators and injecting 0.3 mL of the prepolymer solution after 2 minutes into the LV of porcine explants. Samples were sectioned, placed into test tubes (30 mm diameter), and immersed in PBS at 37±1°C. CEST images were acquired on a 3T clinical whole body scanner (Siemens) with an 8-channel knee coil.

For all CEST scans, B_0 and B_1 maps were acquired to correct for magnetic field inhomogeneity. Data was processed using a custom MATLAB code. Z-spectra asymmetry curves were generated by plotting the relative water signal difference at frequency offsets from 0 to 3.8 ppm. After defining the peak position, the CEST contrast was calculated at ±1ppm using the equation $CEST=100*[S_{-ve}-S_{+ve})/(S_{-ve})]$, where S_{-ve} and S_{+ve} are the B_0 corrected MR signals at -1ppm and +1ppm. The CEST contrast was further corrected for B1 inhomogeneity.

Results: In addition to HA's native enzymatic degradability, crosslinks formed through HeMA enable hydrolytic degradability. Degradation is thus controlled through both macromer concentration (Figure 1B) and extent of HeMA modification on HA (data not shown).

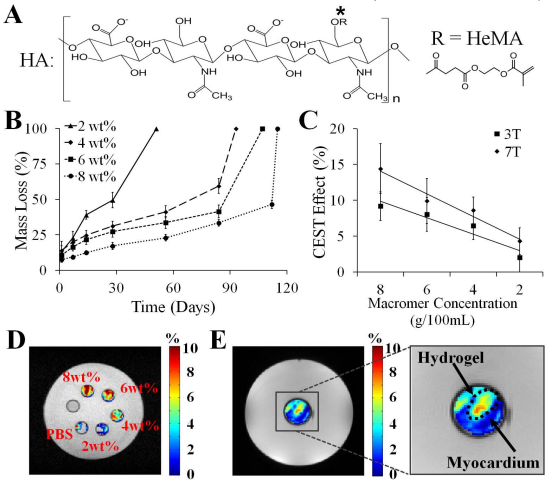

Figure 1: HeMA-HA chemical structure (A). *In vitro* degradation (B). *In vitro* CEST effect (C) and corresponding CEST map at 3T (1.0ppm, B_1=300Hz/300ms) (D). *Ex vivo* CEST effect of 8wt% hydrogel at 3T (1.0ppm, B_1=300Hz/300ms) (E). Data presented as mean ± SD.

The exchangeable hydroxyl protons on the HA backbone (Figure 1A) were shown to generate a CEST effect at a chemical shift of 1.0ppm. Chemical exchange, and consequently CEST contrast, is highly dependent on environmental conditions, such as pH and temperature.[5] To assess differences in CEST contrast with macromer concentration *in vitro*, pH and temperature were maintained at physiological levels. At both 3T and 7T, the CEST effect decreases linearly with decreasing macromer concentration (Figure 1C, 1D). The heterogeneous CEST effect within the samples could be due to inconsistent mixing leading to non-uniform macromer crosslinking (Figure 1D). *Ex vivo* scans demonstrate the ability to distinguish HA hydrogels from surrounding myocardium using CEST (Figure 1E). Work is ongoing to apply CEST methods to assess material degradation over time *in vivo*.

Conclusions: We have successfully applied endogenous CEST contrast MRI to visualize and quantify in situ forming HA hydrogels *in vitro* and *ex vivo*. By visualizing hydrogels in tissue, CEST MRI could become a valuable tool for studying dynamic hydrogel changes (i.e., degradation) *in vivo* and allow further optimization of biomaterial therapies aimed at clinical translation.

References: [1] Christman, KL. J Am Coll Cardiol 2006;48:907-13. [2] Tous, E. Biomacromolecules 2011;12: 4127-35. [3] Dixon, JA. Circ Heart Fail 2009;2:262-71. [4] Reeder, SB. Radiographics 2001;21:1047-74. [5] Kogan, F. Curr Radiol Rep 2013;1:102-14.