Injectable Dual-Crosslinking Hydrogels to Decouple Material Retention and Mechanical Properties *In Vivo*

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Statement of Purpose: Hydrogels suitable for percutaneous delivery (i.e. direct or catheter injection) continue to gain interest due to their ease of clinical application.[1] Translation of synthetically engineered hydrogels remains challenging in part due to crosslinking kinetics. Rapid crosslinking results in delivery failure, while slow crosslinking limits material retention. To overcome this, we utilize supramolecular assembly to localize hydrogels at the injection site with subsequent covalent crosslinking to control final material properties. Supramolecular hydrogels were prepared by modifications of hyaluronic acid (HA) by the guest-host pair cyclodextrin and adamantane. Secondary covalent crosslinking of HA was enabled through Michael-addition. Application of the hydrogel system to a myocardial infarct model showed improved outcomes relative to untreated and supramolecular hydrogel controls, demonstrating its potential in a range of applications where the precise delivery of hydrogels with tunable properties is desired.

Methods: To obtain hydrogel precursors for Michael-addition crosslinking (MA), HA was modified by methacrylation (MeHA) or esterification with 3,3'-dithiodipropionic acid followed by reduction to yield thiolated HA (HA-SH). For guest-host (GH) assembly, HA was modified by coupling of 1-adamantane acetic acid (Ad-HA) or aminated β-cyclodextrin (CD-HA) as previously described.[2] For dual-crosslinking (DC), thiolated Ad-HA and methacrylated CD-HA were prepared by sequential combination of these methods. Hydrogels (3.5wt%) were injected into porcine myocardium and imaged by MRI (9.4T) to confirm retention. Myocardial infarct (MI) was induced in adult male Wistar rats by suture ligation of the LAD. Animals received 6-8 intramyocardial injections (75 μL per animal) of saline, GH, or DC hydrogel into the infarct border zone. Sham procedures received saline injection without ligation. Endpoint analysis was performed at 28 days post-infarct (n≥8 per group). Hydrogels were labeled for imaging with the near-IR dye Cy7.5.

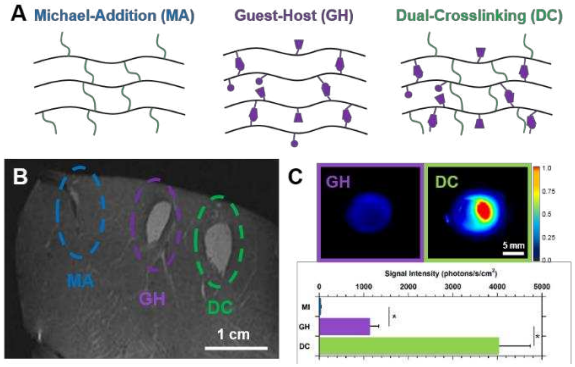

Figure 1. (A) Schematic of Michael-addition (MA), guest-host (GH), and dual-crosslinking (DC) hydrogels. (B) MRI cross-section of porcine myocardium injected with MA, GH, or DC hydrogels as indicated. (C) Fluorescence imaging of hearts explanted at the terminal time point (top) and quantification of fluorescence intensity (bottom; mean±SEM, n>5).

Results: Mixing of HA-SH and MeHA resulted in formation of a covalently crosslinked MA hydrogel (Fig 1A, left), with gelation occurring on timescales suitable for percutaneous delivery (>30min). Following injection into myocardial tissue, observation by MRI (Fig 1B) showed minimal retention in the tissue. In contrast, mixing of Ad-HA and CD-HA resulted in rapid formation of a GH hydrogel composed of non-covalent bonds (Fig 1A, middle). The guest-host hydrogels exhibited shear-yielding for injection and rapid re-assembly at the injection site (>98% retention). DC hydrogels (Fig 1A, right) exhibited assembly characteristics similar to guest-host gels, with an increase in moduli (E=25.0±4.5kPa) observed after injection in situ.

DC hydrogels were investigated for their ability to afford mechanical stabilization following MI. MA gels were excluded, due to their lack of retention in tissue. Both GH and DC hydrogels showed high initial retention with increased retention of the DC hydrogel at 28 days (Fig 1B,C), resulting from a decreased degradation rate. In endpoint analysis, histological evaluation showed a trend toward reduced infarct size for DC relative to GH treatment (Fig 2A). Left ventricle (LV) diameter (Fig 2B) showed decreased dilation of the LV for both treatments, relative to MI control. Functional measures, including cardiac output (Fig 2C), showed improvement over MI controls for both treatments, with a trend for DC to be improved over GH. Measures of end-systolic elastance (Fig 2D) indicate that functional outcomes may be related to preservation of native properties of the myocardium.

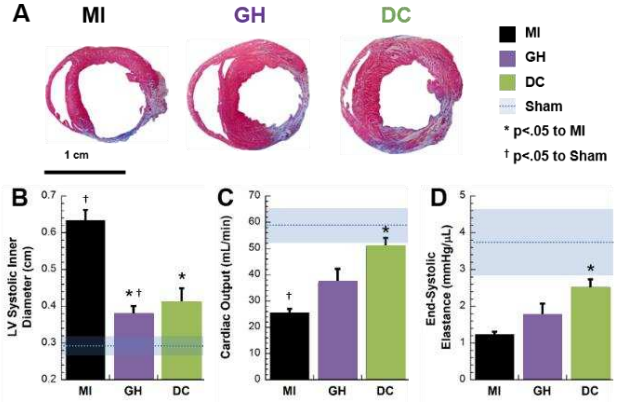

Figure 2. Outcomes of myocardial infarct controls (MI), guest-host (GH) or dual-crosslinking (DC) hydrogel treatments, and sham controls (shaded, blue). Histological cross-sections (A), LV systolic inner diameter (B), cardiac output (C), and end-systolic elastance (D). (mean±SEM; n≥4).

Conclusions: Results demonstrate the ability of dual crosslinking to be achieved on timescales for percutaneous delivery, to be retained at the target site by supramolecular assembly, and to be employed in treatment of MI. Ongoing work includes pursuit of a large animal model of heart failure, enabling longitudinal investigation of treatment efficacy and mechanism.

References: [1]Kretlow JD, et al. *Adv Drug Delivery Rev*. 2007. [2]Rodell CB, et al. *Biomac*. 2013.