Injectable Shear-Thinning Hydrogels for Minimally Invasive Delivery to Infarcted Myocardium to Limit Left Ventricular Remodeling

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Background—Injectable, acellular biomaterials hold promise to limit left ventricular remodeling and heart failure precipitated by infarction through bulking or stiffening the infarct region. A material with tunable properties (eg, mechanics, degradation) that can be delivered percutaneously has not yet been demonstrated. Catheter-deliverable soft hydrogels with in vivo stiffening to enhance therapeutic efficacy achieve these requirements.

Methods and Results—We developed a hyaluronic acid hydrogel that uses a tandem crosslinking approach, where the first crosslinking (guest–host) enabled injection and localized retention of a soft (<1 kPa) hydrogel. A second crosslinking reaction (dual-crosslinking) stiffened the hydrogel (41.4±4.3 kPa) after injection. Posterolateral infarcts were investigated in an ovine model (n=26 per group), with injection of saline (myocardial infarction control), guest–host hydrogels, or dual-crosslinking hydrogels. Computational (day 1), histological (1 day, 8 weeks), morphological, and functional (0, 2, and 8 weeks) outcomes were evaluated. Finite-element modeling projected myofiber stress reduction (>50%; \(P<0.001\)) with dual-crosslinking but not guest–host injection. Remodeling, assessed by infarct thickness and left ventricular volume, was mitigated by hydrogel treatment. Ejection fraction was improved, relative to myocardial infarction at 8 weeks, with dual-crosslinking (37% improvement; \(P=0.014\)) and guest–host (15% improvement; \(P=0.058\)) treatments. Percutaneous delivery via endocardial injection was investigated with fluoroscopic and echocardiographic guidance, with delivery visualized by magnetic resonance imaging.

Conclusions—A percutaneously delivered hydrogel system was developed, and hydrogels with increased stiffness were found to be most effective in ameliorating left ventricular remodeling and preserving function. Ultimately, engineered systems such as these have the potential to provide effective clinical options to limit remodeling in patients after infarction. (Circ Cardiovasc Interv. 2016;9:e004058. DOI: 10.1161/CIRCINTERVENTIONS.116.004058.)

Key Words: heart failure ■ hydrogel ■ myocardial infarction ■ percutaneous treatment ■ remodeling

In the United States, an estimated 785,000 acute myocardial infarctions (MIs) occur annually, and the speed of treatment and use of percutaneous coronary interventions have improved the inhospital survival rate by nearly 40% in recent decades.1,2 However, there remain downstream consequences for these patients because MI is known to be a major contributor to the development of chronic heart failure, which affects an estimated 5.7 million Americans.1 Transplantation remains the only definitive treatment for heart failure, motivating the development of preventative therapies.

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In the case of ischemic heart failure, loss of left ventricular (LV) function is the result of LV remodeling through a deleterious cascade of biological and mechanical events, which ultimately result in geometric reshaping of the LV and loss of contractile function.3–5 It has been recognized that infarct compliance plays a major role in this process because loss of infarct contractility results in increased systolic compliance, creating an energy sink that increases workload on
WHAT IS KNOWN

• Left ventricular remodeling and heart failure after myocardial infarction are characterized by progressive ventricular dilation and decreased ejection fraction.
• Mechanical restraints, including myocardial wraps and injectable biomaterials, may attenuate left ventricular remodeling.

WHAT THE STUDY ADDS

• This report describes the development of 2 distinct injectable materials with differing stiffness and degradation times, where ventricular dilation and loss of ejection fraction are most attenuated by the stiff and slowly degrading material in a large animal model.
• Finite-element modeling demonstrates bulking of the myocardial wall and reduction of potentially damaging myofiber stress both within and surrounding the infarct, dependent on material stiffness.
• Percutaneous intramyocardial injection of the materials is feasible, as demonstrated with catheter guidance by fluoroscopy and intracardiac echocardiography.

extracellular matrix)\(^7\) make them unsuitable for mechanical stabilization of the infarct. It has been demonstrated that supraphysiological hydrogel moduli of \(\approx 40\) kPa effectively attenuate LV remodeling.\(^2\) To address this need, we have developed an injectable hydrogel system based on guest–host (GH) interactions, that is, shear-thinning (ie, flows easily through a syringe or catheter) and self-healing (ie, localizes at the injection site). The hydrogel includes an optional secondary crosslinking (dual-crosslinking [DC]) that occurs in situ to enhance mechanical properties (\(\approx 40\) kPa moduli, motivated by prior results) and prolong degradation. Herein, we demonstrate the utility of these material systems toward attenuating the LV remodeling response post MI and demonstrate the feasibility of percutaneous hydrogel delivery in an ovine model.

Methods

Hydrogel Synthesis and Preparation

Modified hyaluronic acid (HA) polymers were prepared by methods previously described, as detailed in Methods in the Data Supplement.\(^3\)\(^4\) These included HA modified with adamantane (Ad-HA) or \(\beta\)-cyclodextrin (CD-HA) to form GH hydrogels, as well as HA modified with both adamantane and thiols (Ad-HA-SH) or both \(\beta\)-cyclodextrin and methacrylates (CD-MeHA) to form DC hydrogels. GH hydrogels were formed under sterile conditions by separately dissolving the polymers in PBS at 4.5 wt%, mixing of the solutions, and loading into syringes for injection. adamantane (guest, Ad) and \(\beta\)-cyclodextrin (host, CD) were present in equimolar ratios, and the concentration denotes the combined weight percent of both polymers in solution. DC hydrogels were similarly prepared, with the pH of buffers adjusted to obtain pH 5 after mixing.

In Vitro Hydrogel Evaluation

To assess hydrogel mechanical properties, oscillatory rheology was performed (AR2000, TA Instruments; 20-mm-diameter cone plate, 59 min 42 s angle, 27 \(\mu\)m gap, 37°C). GH hydrogel mechanics were determined by frequency (0.01–100 Hz; 1.0% strain) and strain sweeps (1.0 Hz; 0.1%–500% strain). Compressive mechanical analysis (Q800, TA Instruments) of DC hydrogels was performed serially on samples (\(n=6\)) following overnight crosslinking at 37°C, with a rate of 10% strain/min (moduli were calculated from 10% to 20% strain).

To examine hydrogel degradation, 30 \(\mu\)L hydrogels (\(n=5\)) were contained within a 5-mm diameter depression in acrylamide molds. Hydrogels were submerged in 1 mL PBS and stored at 37°C. At set time points, the buffer was collected and replaced. At study completion, hydrogels were degraded in hyaluronidase (1.0 mg/mL) to determine remaining hydrogel content. Degradation was quantified via a uronic acid assay with normalization to cumulative release.

Finite-Element Modeling

Finite-element (FE) modeling of end-diastolic myocardial thickness and corresponding myofiber stress distributions was conducted by adaptation of a method similar to those previously described, as detailed in the Methods in the Data Supplement.\(^5\) Briefly, the LV geometry was approximated by an ellipsoidal model and hydrogel inclusions introduced to displace the myocardial volume (Figure I in the Data Supplement). Myocardial and hydrogel dimensions were acquired via magnetic resonance imaging (MRI; Figure II and Table I in the Data Supplement), and hydrogel moduli represent those determined by oscillatory rheology and compressive mechanical analysis for GH and DC hydrogels, respectively. Pressure (10 mmHg) was applied to the endocardial surface, mimicking that of end-diastolic relaxation, and corresponding regional examination of myofiber stress was performed.
Ovine Infarct Model
Animals in this study were provided care in compliance with the National Institute of Health’s guidelines for the care and use of laboratory animals (NIH Publication 85-23, revised 1996), with protocol approval by the University of Pennsylvania’s Institutional Animal Care and Use Committee. Twenty-two adult male Dorset sheep, ≈45 kg, were subject to infarction and study for 8 weeks. Infarct size was determined by direct epicardial examination and quantification of LV and infarct area (ImageJ) at baseline and terminal time points, respectively, after staining with Masson’s Trichrome and hematoxylin and eosin. To ensure and report as the average of 3 measurements from the base, anterior papillary). From these samples, myocardial thickness was measured and stained with Masson’s Trichrome and hematoxylin and eosin. To evaluate the distribution of the hydrogel in vivo, tissue was analyzed in one GH and one DC hydrogel–injected animal within the first 24 hours post MI.

MRI and Analysis
Image acquisition was performed at 3T (Tim Magnetom Trio Scanner; Siemens, Inc.). For visualization of the hydrogels after in vivo injection, the explant was submerged in saline and imaged via a T2-weighted turbo spin echo pulse sequence. For longitudinal analysis of myocardial geometry and function in vivo, imaging was performed at baseline (immediately before infarct), as well as at 2 and 8 weeks post infarct. Anesthesia was maintained throughout the procedure, and cardiac gating was performed by placement of a pressure catheter (Millar Instruments, Inc.) into the LV. Myocardial geometry was assessed from 2-dimensional CINE images, with additional late gadolinium enhancement imaging to confirm the infarct location (Figure III in the Data Supplement). Imaging parameters and analysis methods are available in Methods in the Data Supplement.

Postmortem Analysis
Animals were euthanized at 8 weeks, the hearts harvested, and long-axis sections were taken through the infarct region (adjacent to the posterior papillary) and from remote sections (adjacent to the anterior papillary). From these samples, myocardial thickness was measured and reported as the average of 3 measurements from the base, infarct (approximately equatorial), and apex for each animal. Sections from these regions were fixed in formalin, paraffin-embedded, and stained with Masson’s Trichrome and hematoxylin and eosin. To evaluate the distribution of the hydrogel in vivo, tissue was analyzed in one GH and one DC hydrogel–injected animal within the first 24 hours post MI.

Percutaneous Intramyocardial Injection
Two healthy adult male Dorset sheep were used, allowing investigation of 2 separate procedural approaches, both using a delivery system (Figure IV in the Data Supplement) comprising an Agilis NxT steerable introducer and a BRK transseptal needle (4 Fr, 90 cm; St Jude Medical) preloaded intraoperatively with sterile hydrogel and a 1-mL syringe containing the desired injection volume (0.3 mL each). Injection position was monitored by fluoroscopy and directly visualized by simultaneous intracardiac echocardiography (AcuNav 8 Fr; Siemens). In the first approach, the introducer was inserted through the internal jugular and passed into the right ventricle over wire. The sheath was deflected to reach various locations, and the needle was advanced 4 to 5 mm into the tissue for injection and subsequently retracted. Four injections were performed into the septal wall. In the second approach, the introducer was similarly passed into the LV with access via the right carotid, and 5 injections were performed into the inferior and anterior walls. After the procedure, injection was confirmed by MRI of the explanted tissue.

Statistical Analysis
Data are presented as mean±standard deviation (for in vitro data) or as mean±standard error of the mean (for in vivo data). Statistical significance was determined by analysis of variance, using repeated measures where appropriate, in conjunction with post hoc Student’s 2-tailed t tests with Bonferroni correction to account for multiple comparisons. Normality of data was confirmed by Shapiro–Wilk test. Significance was determined at α=0.05. For volumetric analysis, outliers were identified within groups by Grubb’s test and excluded from further analysis.

Results
Development of Injectable Hydrogels With Controlled Biophysical Properties
GH hydrogel precursors were prepared with ≈25% of HA repeat units modified with either Ad or CD. On mixing solutions of Ad-HA with CD-HA, GH hydrogels (Figure 1B) rapidly formed through physical interactions. The elastic modulus (E) of GH hydrogels was estimated at 1.6 Hz (corresponding to a heart rate of 100 bpm) to be 799.2 Pa (Figure VA in the Data Supplement). GH hydrogels are known to exhibit shear-thinning (during injection) and self-healing (after injection) properties18,22 necessary for delivery into myocardial tissue (Figure 1D); yet, shear yielding was not observed at physiological myocardial strains (Figure VB in the Data Supplement), indicating stability after reaching the tissue.

DC hydrogels were developed to introduce additional covalent crosslinks into the GH hydrogels to increase their mechanical properties (Figure 1C). Thus, they are likewise injectable via shear-thinning but with increased mechanical strength. DC hydrogels stiffened to 41.4±4.3 kPa moduli within 48 hours. Subsequent softening, significant beyond 2 weeks (Figure 1E), was observed because of hydrogel degradation. GH and DC hydrogel degradation was monitored for 8 weeks (Figure 1F); rapid degradation of the GH hydrogel was observed (>50% degradation), in contrast to the DC hydrogel, which remained stable at 8 weeks (5.1±0.2% degradation).

In vivo examination of hydrogel retention was performed at 24 hours after infarct induction and intramyocardial injection. Both the GH and DC hydrogels were retained as solid, discrete hydrogels within the myocardium (Figure 2A and 2B). MRI of DC hydrogel injection (Figure 2C) demonstrated dispersion of hydrogel throughout the tissue, with a measured volume of 5.1 mL, in agreement with the 4.8 mL of hydrogel injected. Excised DC hydrogels exhibited moduli of 30.3±2.6 kPa, coinciding with measured in vitro moduli at these times (P=0.83) and demonstrating the ability for DC to occur in vivo.

FE Assessment of Myofiber Stress and LV Wall Deformation
FE simulations were conducted to evaluate anticipated myocardial bulking and altered distribution of end-diastolic myofiber stress throughout the LV wall at early times post MI. The end-diastolic wall thickness within the injected regions differed between the control, GH, and DC cases (1.01±0.025, 1.08±0.029, and 1.23±0.023 cm; P<0.09×10−2).
Qualitatively, the myofiber stress distributions throughout the LV were differentially altered by GH (Figure 3A) and DC (Figure 3B) hydrogel injections. The average stress in the myocardium surrounding the DC injection was 2.5±0.13 kPa (27.0±8.0% reduction relative to control; *P*<0.0001), whereas the stress around the GH injection was 3.4±0.28 kPa (P=NS; Figure 3C). Through the transmural dimension, DC injection reduced the myofiber stress by 45.0±1.6% at the epicardium, 26.1±4.5% at mid-myocardium, and 51.4±3.0% at the endocardium compared with the control case (*P*<0.42×10^-6; Figure 3D). Circumferentially, near the edge of the injection region, hydrogels reduced the myofiber stress (Figure 3E) by a maximum of 31.5±8.8% for GH (*P*<0.01 relative to day 0) and 62.0±4.4% for DC (*P*<0.05 relative to DC for all time points beyond day 1).

**Assessment of Myocardial Thickness**

Wall thickness was measured after excision at 8 weeks. Qualitatively, thinning of the infarct region was observed after MI (Figure 4A), which was attenuated by hydrogel injection, and remaining DC hydrogel was observed post mortem in all cases. Histological examination (Figures VII and VIII in the Data Supplement) indicated integration of the DC hydrogel into the tissue, which was not apparent at early time-points (Figure 2B), with minimal chronic inflammation (ie, foreign-body giant cell localization, fibrous encapsulation). Histologically, GH materials were not observed at 8 weeks, consistent with the observed rapid erosion (Figure 1F) and prior in vivo examination.19 Quantitatively, hydrogel injection increased infarct thickness relative to controls (MI, 3.90±0.48 mm; GH, 5.79±0.96 mm, *P*=0.013; DC, 8.92±0.24 mm,
Rodell et al  Catheter Injectable Hydrogels Limit LV Remodeling

$P = 0.46 \times 10^{-6}$ and tended to increase adjacent basilar and apical thicknesses (Figure 4B; Table III in the Data Supplement). Temporal assessment of end-diastolic thickness by MRI (Figure 4C) revealed significant differences between infarct tissue thickness at 2 and 8 weeks, with minimal differences observed in remote thicknesses (Table IV in the Data Supplement). Notably, DC hydrogel injection was observed to maintain wall thickness at 8 weeks (10.02±0.79 mm; $P > 0.35$ relative to 

Figure 2. Material retention in vivo. A and B, Histological image of guest–host (GH; A) and dual-crosslinking (DC; B) hydrogels (indicated, *) within infarct tissue by hematoxylin and eosin (H&E) staining at 1 day post myocardial infarction (MI). For complete image and corresponding trichrome staining, see Figure VI in the Data Supplement. C, Magnetic resonance imaging (MRI) reconstruction of retained DC hydrogel (purple) within the myocardium (red) after initial injection in vivo.

Figure 3. Finite-element analysis of hydrogel injection. A and B, End-diastolic myofiber stress distribution for a left ventricle (LV) with either (A) guest–host (GH) hydrogel injection or (B) dual-crosslinking (DC) hydrogel injection. Note that only a portion of the model is shown to visualize the distribution within the myocardium. C–E, Myofiber stress in elements adjacent to the material (C) or distributed along a transmural (D) or circumferential (E) path in the edge of the injection region. Corresponding regions are indicated for the material region (i) or transmural (ii) and circumferential (iii) paths. Data are presented as means±SD with error bars for all points (some not visible because of low error); $n \geq 6$; *$P < 0.05$ relative to myocardial infarction (MI); **$P < 0.05$ relative to GH.
baseline), in contrast to the observed drastic thinning in both MI (3.56±0.19 mm; \( P = 0.67 \times 10^{-12} \)) and GH (5.51±0.23 mm; \( P = 0.24 \times 10^{-8} \)) groups.

**LV Dilation and Functional Assessment**

MRI was used to assess temporal changes in LV volume and function. The LV progressively dilated, as indicated by a >2-fold increase (\( P < 0.014 \) relative to baseline) in LV end-diastolic volume and LV end-systolic volume in MI controls. At end-diastole (Figure 5A), hydrogel treatment tended toward reduced dilation at 8 weeks. Significant differences were observed between DC and MI at 2 weeks and between DC and both GH and MI groups at 8 weeks at end-systole (Figure 5B). Although not significant, stroke volume (Figure 5C) was increased with DC hydrogel injection. Ejection fraction (Figure 5D) showed a consistent, progressive loss of function after MI, which was moderately attenuated by GH (15% improvement; \( P = 0.058 \)) and significantly attenuated by DC (37% improvement; \( P = 0.014 \)) hydrogel injections at 8 weeks.

**Percutaneous Hydrogel Delivery**

The potential for percutaneous injection of the shear-thinning GH hydrogel was examined using equipment and methods amenable to adaptation in the majority of interventional cardiology units. Hydrogels were prepared as described, and the injection volume (0.3 mL) was loaded into 1-mL Luer-Lock syringes. The desired injection location (ie, septal for right ventricle approach and anterior and posterior wall for LV approach) were identified by fluoroscopy, the introduction sheath positioned, and intracardiac echocardiography used to allow visualization of the needle location (Figure 6A and 6D). Injections were performed by insertion of needle into the LV wall, as visualized by intracardiac echocardiography (Figure 6A, inset). After euthanasia and excision of the heart, hydrogel injections were visualized by MRI. As with syringe injection, discrete hydrogel injections were located along the long axis (Figure 6B and 6E) and short axis (Figure 6C and 6F) of the myocardium.

**Discussion**

Towards abating LV remodeling post MI, epicardial placement of devices, including both mechanical restraints and therapeutic-containing patches, have demonstrated great efficacy in preclinical studies. However, the clinical application of such therapies will likely be limited because of their inherent requirement for open surgical approaches. To address this important consideration, therapeutics that may be delivered via catheter have been investigated (ie, cell therapy). In addition to such approaches, our group and others have used a combination of experimental and computational tactics to explore the capacity for injectable hydrogels to directly alter the mechanical environment both in and around the infarcted region.
The ability of material injection to reduce myofiber stress within the infarct region and its border zone is critical because rapid geometric changes within the infarct (i.e., infarct expansion) and progressive dysfunction of the border zone have been repeatedly implicated in the progression of LV remodeling. GH and DC hydrogels are both shear-thinning and self-healing to permit hydrogel localization in the myocardium (Figure 2; Figure V in the Data Supplement); however, the DC hydrogel exhibits stiffening (>40-fold change) to increase the hydrogel mechanical properties. DC injection showed greater reduction in myofiber stress, relative to MI controls with FE modeling. For DC injections, stress reduction was driven by the preservation of LV shape by maintaining the LV wall thickness. In contrast, GH injections deformed under loading, elongating circumferentially (Figure 3A). Although only an initial snapshot into this mechanism, these results indicate that the hydrogel stiffness is important because it enables reduction in fiber stress through stiffness-induced bulking of the myocardium. Although progressive DC degradation resulted in moduli decline and hydrogel integration with the host tissue, the therapy is intended to intercept the remodeling process early after infarction and before endogenous infarct stiffening, which occurs later because of collagen deposition.27

Myocardial bulking predicted by FE modeling was consistent with in vivo observations, as the thickness of the myocardium was better maintained with hydrogel injection. Notably, DC injection maintained baseline measurements at 2 and 8 weeks (P>0.35 relative to baseline). Importantly, recent analyses have highlighted myocardial thinning as a dominant feature of LV remodeling, consistent across species, making it an attractive therapeutic target.28 In addition to bulking, the FE model predicted alterations in myocardial loading, which translated to attenuation of LV remodeling events in vivo, with DC treatment resulting in significant reduction in LVESV and improvement in ejection fraction at both 2 and 8 weeks. Both of these metrics have been shown to be valuable clinical predictors for survival post MI.29,30 Although decreased LV volume, resulting from tissue displacement by hydrogel, may contribute minimally to changes in ejection fraction, the injection volumes alone (4.8 mL) cannot account for the disparity in LVESV between control and DC cases (44.0±12.8 mL at 8 weeks), and consistent improvement in stroke volume was demonstrated, indicating genuine preservation of both ventricular geometry and function.

To enable formation of injectable hydrogels in vivo for MI applications, both physical and covalent crosslinking have been individually leveraged.11 Yet, only physically crosslinked hydrogels have been delivered percutaneously via catheters. Specifically, calcium crosslinked alginate has been delivered via intracoronary infusion14 and decellularized extracellular matrix,15,31 and pH-responsive poly(ethylene glycol)
assemblies have been delivered via intramyocardial injection. For such physically assembling systems, the range of attainable elastic moduli limit their applicability toward mechanical restraint. Yet, positive results have been demonstrated in porcine models, possibly attributable to biological effects, and these material systems have advanced to clinical trials (Bellerophon, ClinicalTrials.gov Identifier: NCT01226563 and Ventrix, ClinicalTrials.gov Identifier: NCT02305602).

Although physically assembling systems have demonstrated percutaneous delivery with some positive effects on LV remodeling, they have failed to exploit mechanical stabilization in their mechanism of action. Alternatively, covalent crosslinking of hydrogels has been used to achieve mechanical restraint of the infarcted region, with increasing stiffness and prolonged degradation correlated with improved functional outcomes. However, gelation of these systems relies on the mixing of several components, which can be challenging with a catheter, where rapid gelation can clog the catheter and slow gelation can lead to material dispersion in the tissue, compromising hydrogel formation. This challenge has prevented covalently crosslinking hydrogels from being delivered percutaneously.

To address these limitations, we leveraged physical interactions (ie, GH complexes) to enable formation of a soft hydrogel that exhibited fluid-like behavior within the needle or catheter to allow injection. Importantly, the GH hydrogels allowed rapid reassembly within the tissue and, thus, high local retention (Figure 2). Similar to the previously mentioned soft materials (eg, alginate, decellularized extracellular matrix), there was some positive outcomes with the soft GH hydrogel—likely because of the biological effect of injecting a foreign material into the myocardium, which may alter collagen production. Secondary covalent crosslinking, via Michael addition reaction, has been tuned to provide crosslinking on the order of hours under controlled conditions (ie, pH 5) to enable ease of use in a clinical setting. The resulting stiffer DC hydrogels provided tissue bulking to thicken the myocardium, reduce myofiber stress, and attenuate LV remodeling.

Taken together, we have developed a catheter-injectable material system with the ability to mitigate LV remodeling through mechanical restraint afforded by the stiff hydrogel.

The present study has demonstrated that hydrogels can effectively assuage LV remodeling after MI without the need for added therapeutics (ie, cells, drugs) through modulation of the myocardial stresses. The study also demonstrated the feasibility of delivering these materials via catheter-based techniques, because of the independently designed mechanisms for material retention and stiffening. Such materials will facilitate the development of clinically relevant approaches, owing to the relative ease of preparation and potential for minimally invasive delivery. Moreover, the primary constituents (ie, HA and CD) are generally recognized as safe by the US Food and Drug Administration and are industrially well represented in the pharmaceutical and medical device industries. The defined material formulations, therefore, constitute a medical device that holds potential to rapidly progress toward clinical use.

Figure 6. Percutaneous hydrogel injection. A, Internal jugular approach toward right ventricular (RV) injection, with alignment of the steerable introducer (i), intracardiac echocardiograph probe (ICE, ii), and deployment of needle assembly (iii). Inset: corresponding ICE view of deployed needle (indicated, white arrow) entering the myocardial wall (indicated, dashed red line). Long axis (B) and short axis (C) images of hydrogel injection (indicated, white arrows). D, Right carotid approach toward left ventricular (LV) injection, including steerable introducer (i), ICE (ii), and deployment of needle assembly (iii) into the midwall of the LV. Long axis (E) and short axis (F) images of hydrogel (indicated, white arrows).
Despite the positive findings, it is important to address potential limitations in the study. Intervention with hydrogel injection was performed at the time of infarct induction; though, intervention in the 3- to 7-day range may be more clinically feasible (Bellerophon, ClinicalTrials.gov Identifier: NCT01226563). The results of this study, therefore, demonstrate the capacity for the material systems developed to act as a preventative therapy to alter LV remodeling, as well as a delivery approach that could expand the feasibility of injection at later times. Although efficacy of material injection at later time points has been demonstrated in both animal31,37,38 and clinical studies,9 the optimization of injection time for both clinical applicability and efficacy remains an important issue in need of direct examination. These efforts will be aided by percutaneous injection procedures that are accompanied by myocardial mapping. Additionally, animals were only studied out to 8 weeks post MI; longer studies will be required to assess the durability of the therapeutic response at time points beyond the in vivo lifetime of the DC materials. Ultimately, translation of the materials described here will be dependent on future studies that will be focused on timing, dosing, and injection location.

Conclusions

For the first time, we have developed shear-thinning hydrogels with therapeutically relevant properties for delivery via percutaneous intramyocardial injection. Such shear-thinning delivery enabled local hydrogel retention, while secondary covalent crosslinking enhanced mechanically advantageous bulking of the infarct tissue. Importantly, the stiffening reaction occurred autonomously in situ on clinically relevant time scales and further enhanced treatment efficacy through bulking and mechanical stabilization of the infarct. The DC hydrogel system represents the first engineered material designed to specifically and simultaneously address the needs of localized retention, mechanical stabilization, and percutaneous delivery for treatment of MI. The present study establishes the efficacy of the material system as a therapeutic approach toward modifying LV remodeling.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Injectable Shear-Thinning Hydrogels for Minimally Invasive Delivery to Infarcted Myocardium to Limit Left-Ventricular Remodeling

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SUPPLEMENTAL METHODS

Polymer Synthesis.

Sodium hyaluronic acid (HA, 90kDa) was purchased from Lifecore (Chaska, MN). β-cyclodextrin (CD) and 1-adamantane acetic acid (Ad) were purchased from TCI America. All other reagents were purchased from Sigma, unless otherwise indicated. Following modification, modified hyaluronate was purified by dialysis (and vacuum filtration, where necessary to remove insoluble impurities) and recovered by lyophilization. Polymer modifications were determined by 1H NMR acquired at 360MHz (Bruker). Disaccharide modifications were approximately 25% of repeat groups; Ad-HA utilized in percutaneous delivery had a 50% modification.

HA-TBA and MeHA-TBA: To enable anhydrous reactions in DMSO, the tetrabutylammonium (TBA) salt of HA was prepared. The sodium salt of HA or methacrylated HA (MeHA; synthesized by the typical esterification with methacrylic anhydride) was dissolved in DI water at 2.0 wt%, exchanged against Dowex-100 resin, neutralized by tetrabutylammonium hydroxide, and lyophilized.

Ad-HA Synthesis: Ad-HA was prepared by esterification of HA-TBA with Ad via BOC₂O/DMAP catalysis. A round bottom flask was charged with HA-TBA (1 eq), Ad (3 eq), and 4-dimethylaminopyridine (DMAP; 1.5 eq). Anhydrous DMSO was added via cannulation under nitrogen, affording a 2 wt% solution. Di-tert-butyl dicarbonate (BOC₂O) was added (0.5 eq) and the reaction allowed to proceed at 45°C overnight.

CD-HA and CD-MeHA Synthesis. CD-HA was prepared by coupling 6-(6-aminohexyl)amino-6-deoxy-β-cyclodextrin (β-CD-HDA) to HA-TBA via amidation. A round bottom flask was charged with HA-TBA (1 eq) and β-CD-HDA (0.5 eq). Anhydrous DMSO was added via cannulation under nitrogen, affording a 2 wt% solution. Once dissolved, (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP; 0.5 eq) was dissolved in minimal DMSO and transferred to the reaction vessel via cannulation. The reaction was allowed to proceed at room temperature for >2 hours. CD-MeHA was identically prepared, with MeHA-TBA as the starting material.

Ad-HA-SH Synthesis. Ad-HA-SH was prepared by modifying HA with Ad and subsequent thiolation. A portion of Ad-HA was converted to the TBA salt as described above. Esterification of Ad-HA-TBA with 3,3’-dithiopropionic acid (5.0 eq) was performed (2.50 eq DMAP, 1.0 eq BOC₂O) similarly to Ad-HA modification. Following purification, the disulfide was reduced by DTT.

MRI Acquisition Parameters.

$T_2$ weighted turbo spin echo: matrix size = 320 x 256 x 65, voxel size = 0.3125 x 0.3125 x 1.0 mm³, repetition time = 1128 ms, echo time = 71 ms, 4 signal averages.

TrueFISP CINE: field of view = 280x166.25 mm, acquisition matrix = 256 x 152 pixels, repetition time = 27.52 ms, echo time = 1.46 ms, BW = 930 Hz/pixel, slice thickness = 4 mm.

LGE spoiled gradient echo sequence: field of view = 218 x 350, acquisition matrix = 256 x 160, repetition time = 5.50 ms, echo time = 2.42 ms, BW 244 Hz/pixel, slice thickness = 4 mm, 2 signal averages. Acquisition was performed approximately 15 minutes following bolus intravenous injection of 0.1 mmol/kg gadobenate dimeglumine (MultiHance; Bracco Diagnostics, Inc.).
MRI Analysis.

Examination of hydrogel distribution in explanted tissue was performed by 3D reconstruction of the hydrogel and myocardial segments (ITK-Snap\textsuperscript{5}). Longitudinal analysis of infarct thickness was performed from CINE MRI. Three consecutive short axis images were isolated at end-diastole from the infarcted region, immediately sub-papillary where possible. The infarct was confirmed from corresponding LGE images, and the location was maintained across all time points. For each image, 5 radial lines were drawn from epicardium to endocardium and measured (ImageJ; Fig. S3) and reported values represent the average of all 15 measurements for each animal. Analyses were repeated in the remote region along the contralateral LV wall.

Toward assessment of LV dilation and function, volumetric analysis was performed. CINE images were sorted and cropped using customized programming (MATLAB). Through all acquired planes and phases, semi-automated segmentation of the intraventricular space was performed with manual correction as necessary (ITK-Snap). Repartitioning of the images into the time-space and corresponding volumetric reconstruction enabled determination of the LV volume as a function of time, from which the minimum volume (LVESV), maximum volume (LVEDV), SV, and EF were determined.

Finite Element (FE) Modeling.

Myocardial Dimensions. To evaluate the effects of hydrogel injections on myocardial wall stress, finite element (FE) models of the LV were generated. The geometry of the LV wall was based on measurements at end-systole in ovine hearts; images were acquired via TrueFISP CINE sequences. The wall thickness was 1.3 cm, the inner diameter of the endocardial wall near the equator was 4.0 cm, and the distance from base to apex was 6.4 cm. To simulate hydrogel injection, the base model was modified to include 16 injections in a 4-by-4 array within the free wall (Fig. S1). The size and shape of the hydrogel injections were based on MR images of injected explant tissue (Fig. S2, Table S1), where the injections were approximated as ellipsoids where the volume is given by the equation:

\[ V = \frac{4}{3} \pi (a \times b \times c) \] (1)

The spacing between injections was determined to be 1.5 cm from center-to-center, based on expected anatomical measures. Each injection was 0.3 mL, resulting in a total volume of 4.8 mL added to the model. To account for volumetric addition, the myocardial wall thickness throughout the injection regions was increased to 1.5 mm, thus preserving the total volume of myocardium (Fig. S1B). The longitudinal dimensions were unaltered, as was dimensions in remote regions, away from the injection site.

Material Properties and Loading Conditions. The material response of the myocardium was represented using a nearly incompressible, transversely isotopic, hyperelastic constitutive law, which was defined using the strain energy function:

\[ W_{\text{myocardium}} = \frac{C}{2} \left( b_f E_f^2 + b_t (E_{ss}^2 + E_{sn}^2 + E_{ns}^2) + b_{fs} (E_{fs}^2 + E_{ff}^2 + E_{fn}^2 + E_{fn}^2) - 1 \right) + \frac{\kappa}{2} (J - 1)^2 \] (2)

where \( E_{ij} \) are the deviatoric components of the Green-Lagrange strain tensor relative to the myofiber coordinate system (f = fiber direction, s = cross-fiber in-plane direction, n = transverse-fiber direction) and \( J \) is the determinant of the deformation gradient. The diastolic material parameters were assigned to be \( C = 0.51 \text{ kPa}, b_f = 22.84, b_t = 3.45, \) and \( b_{fs} = 12, \) while the bulk modulus was \( \kappa = 1 \times 10^3 \text{ kPa}. \)\textsuperscript{3} Since the model was intended to mimic the initial time frame after infarction, it was assumed that the properties would be unchanged during this timeframe.\textsuperscript{4} Hence, the model was created such that the myocardial material properties around the hydrogel injections were the same as in remote regions. The myofiber orientation was assigned to vary linearly from epicardium to endocardium using the angles of -37 degrees to 83 degrees,
respectively. The material response of the hydrogel injections was represented using a nearly incompressible, isotropic, hyperelastic constitutive law, which was defined using the strain energy function:

\[
W_{\text{Injection}} = \frac{E}{2(1+\nu)} \text{tr}(E^2) + \frac{E}{6(1-2\nu)} \ln(J)^2
\]

where \(E\) is the deviatoric Green-Lagrange strain tensor, \(\text{tr}(\ )\) is the trace operator, and \(\ln(\ )\) is the natural log operator. The material parameters for Young’s modulus \((E)\) were assigned based on the experimental measurements of the GH and DC hydrogels, while the Poison ratio \((\nu)\) was assigned a value of 0.499. A pressure of 10 mmHg was assigned as a boundary condition on the endocardial wall in each of the FE models, simulating end-diastolic loading conditions. For all data presented, the results depict the mean ± standard deviation (SD) of the path length or myofiber stress of adjacent elements \((n\geq 6)\) along the path indicated.

**SUPPLEMENTAL TABLES**

**Table S1.** Ellipsoid dimensions of a 0.3 mL hydrogel injection, based on MRI data.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>6.60mm</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>3.94mm</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>2.73mm</td>
<td></td>
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</table>

**Table S2.** Animal usage and assessment of gross cardiac geometry.

<table>
<thead>
<tr>
<th>Category/Metric</th>
<th>MI</th>
<th>GH</th>
<th>DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal Usage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Included</td>
<td>7</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Weight (kg), Baseline</td>
<td>39.7 ± 1.1</td>
<td>44.4 ± 1.8</td>
<td>43.7 ± 2.0</td>
</tr>
<tr>
<td>Weight (kg), 2 WK</td>
<td>38.9 ± 0.8</td>
<td>42.0 ± 2.3</td>
<td>43.0 ± 1.9</td>
</tr>
<tr>
<td>Weight (kg), 8 WK</td>
<td>46.6 ± 1.0</td>
<td>51.4 ± 1.7</td>
<td>51.3 ± 1.3</td>
</tr>
<tr>
<td>Heart Size, 8 WK</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Mass (g)</td>
<td>274.99 ± 16.20</td>
<td>306.42 ± 13.53</td>
<td>300.63 ± 18.43</td>
</tr>
<tr>
<td>RV Mass (g)</td>
<td>71.03 ± 14.01</td>
<td>58.20 ± 3.59</td>
<td>62.18 ± 4.64</td>
</tr>
<tr>
<td>LV Mass (g)</td>
<td>141.00 ± 18.78</td>
<td>166.84 ± 4.02</td>
<td>169.88 ± 9.02</td>
</tr>
<tr>
<td>Infarct Geometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial Infarct Area (% LV)</td>
<td>19.4 ± 0.6</td>
<td>18.6 ± 0.7</td>
<td>19.3 ± 0.7</td>
</tr>
<tr>
<td>Final LV Area (cm³)</td>
<td>19.35 ± 0.78</td>
<td>18.36 ± 1.22</td>
<td>17.25 ± 1.34</td>
</tr>
<tr>
<td>Final Infarct Area (cm²)</td>
<td>98.56 ± 5.96</td>
<td>98.85 ± 3.85</td>
<td>103.58 ± 4.41</td>
</tr>
<tr>
<td>Final Infarct Size (% LV)</td>
<td>19.31 ± 1.97</td>
<td>18.15 ± 1.43</td>
<td>17.93 ± 1.81</td>
</tr>
</tbody>
</table>

Data provided as mean±SEM.
No differences observed between groups \((P > 0.05\) by ANOVA).
Table S3. Direct measurement of myocardial thickness at 8 weeks post-MI.

<table>
<thead>
<tr>
<th>Treatment Group/Region</th>
<th>Base Thickness (mm)</th>
<th>Infarct Thickness (mm)</th>
<th>Apical Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarcted Region</td>
<td>11.11 ± 0.96</td>
<td>3.90 ± 0.48</td>
<td>8.49 ± 1.08</td>
</tr>
<tr>
<td>Remote Region</td>
<td>13.31 ± 1.04</td>
<td>12.39 ± 1.13</td>
<td>10.51 ± 0.49</td>
</tr>
<tr>
<td>GH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarcted Region</td>
<td>11.25 ± 0.27</td>
<td>5.79 ± 0.96</td>
<td>8.66 ± 0.42</td>
</tr>
<tr>
<td>Remote Region</td>
<td>12.38 ± 0.85</td>
<td>11.13 ± 0.50</td>
<td>9.69 ± 0.74</td>
</tr>
<tr>
<td>DC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infarcted Region</td>
<td>11.92 ± 0.20</td>
<td>9.92 ± 0.24*,#</td>
<td>9.08 ± 0.84</td>
</tr>
<tr>
<td>Remote Region</td>
<td>11.75 ± 0.21</td>
<td>11.10 ± 0.25</td>
<td>9.82 ± 0.32</td>
</tr>
</tbody>
</table>

Data provided as mean±SEM.
* P < 0.05 relative to MI
# P < 0.05 relative to GH

Table S4. MRI assessment of myocardial thickness over time.

<table>
<thead>
<tr>
<th>Treatment Group/Timepoint</th>
<th>Infarct Thickness (mm)</th>
<th>Remote Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>9.44 ± 0.14</td>
<td>9.81 ± 0.28</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>4.39 ± 0.43</td>
<td>9.94 ± 0.34</td>
</tr>
<tr>
<td>8 Weeks</td>
<td>3.56 ± 0.19</td>
<td>10.31 ± 0.22</td>
</tr>
<tr>
<td>GH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>8.92 ± 0.24</td>
<td>9.32 ± 0.18</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>6.98 ± 0.46*</td>
<td>9.76 ± 0.40</td>
</tr>
<tr>
<td>8 Weeks</td>
<td>5.51 ± 0.23*</td>
<td>9.70 ± 0.22</td>
</tr>
<tr>
<td>DC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>10.90 ± 0.44*,#</td>
<td>10.79 ± 0.48</td>
</tr>
<tr>
<td>2 Weeks</td>
<td>11.39 ± 1.16*,#</td>
<td>10.78 ± 0.31</td>
</tr>
<tr>
<td>8 Weeks</td>
<td>10.02 ± 0.79*,#</td>
<td>11.21 ± 0.41*</td>
</tr>
</tbody>
</table>

Data provided as mean±SEM.
* P < 0.05 relative to MI
# P < 0.05 relative to GH
SUPPLEMENTAL FIGURES

Figure S1. (A) Finite element model of ovine LV with 16 hydrogel injections in the free wall. The injections were modeled as ellipsoids, which caused the LV wall to thicken. (B) Short axis view of LV wall. (C) Long axis view of LV wall.

Figure S2. Reconstruction of hydrogel geometry based on MRI data of injections embedded in myocardial wall. Note that the shape is approximately ellipsoidal and a volume of 0.298mL was directly determined from the reconstruction in ITK-Snap.
Figure S3. MRI based determination of myocardial thickness over time. Red and green arrows indicate infarct and remote thickness, respectively, as assessed for MI (left), GH (middle), and DC (right) treatment groups. Measurements were performed (3 slices, n = 5 measurements per slice) at baseline, 2 weeks, and 8 weeks, with delayed contrast enhancement (DCE) used to confirm measurements were made within the infarcted region (myocardial wall in white contrast).
Figure S4. Hydrogel delivery system. The Agilis™ NxT steerable introducer (A) allowed for user-controlled deflection of needle tip (inset) to guide injection location. A BRK™ transseptal needle (B, 4 Fr, 90 cm; St. Jude Medical) was used for injection by insertion through the introducer (C), where injection depth was controlled by the length of needle deployment (inset).
Figure S5. (A) Frequency sweep (1.0 % strain, 37°C) showing frequency dependence of GH hydrogel shear moduli (G’). Relevant moduli were estimated at 1.6 Hz, corresponding to a heart rate of approximately 100 BPM. Elastic moduli (E) estimated assuming an incompressible solid whereby E = 3*G’. (B) Strain sweep (1.0 Hz, 37°C) showing strain dependence of GH hydrogel shear moduli (G’). Shear-induced loss of GH mechanics and corresponding flow is not observed below strains of ~40%. Approximate physiological range (maximum strain of 10-15%)5,6 is indicated (shaded regions).

Figure S6. Representative histological examination of guest-host (GH) and dual-crosslinking (DC) injections within the infarct region at 24 hours post-injection by H&E (top) and trichrome (bottom) with the expanded region indicated.
**Figure S7.** Low magnification histological examination 8 weeks post-MI of myocardial tissue from the infarct region in control (MI), guest-host (GH), and dual-crosslinking (DC) groups with remote section provided for reference. H&E (left) and trichrome (right), with the expanded region (provided in Figure S8) indicated for DC injection.
Figure S8. High magnification histological examination 8 weeks post-MI of myocardial tissue from the infarct region in control (MI), guest-host (GH), and dual-crosslinking (DC) groups with remote section provided for reference. H&E (left) and trichrome (right), where DC hydrogel visible by H&E staining (dark purple) and trichrome (light blue) integrated with viable tissue. Other groups demonstrate fibrosis (trichrome, dark blue) without indication of remaining hydrogel.
SUPPLEMENTAL REFERENCES


