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Oxime Cross-Linked Injectable Hydrogels for Catheter Delivery

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Keywords

Injectable hydrogel; catheter; oxime cross-linking

Heart failure post-myocardial infarction (MI) is the leading cause of cardiovascular related death in the developed world.^[1] The progressive nature of heart failure after MI is due to the limited capability of the heart to regenerate after sustaining an injury. There are no therapies that prevent the negative left ventricular (LV) remodeling process that occurs post-MI, and the only successful therapy for end-stage heart failure is total heart transplantation, which is limited by the number of available donor hearts. One potential new avenue to repair the heart post-MI is through the use of injectable biomaterials, either as acellular scaffolds to encourage endogenous repair or as cell delivery vehicles.^[2] A variety of materials, such as fibrin,^[3–5] collagen,^[5, 6] alginate,^[7, 8] chitosan,^[9] hyaluronic acid,^[10] Matrigel,^[5, 11] myocardial matrix,^[12] self-assembling peptides,^[13] and synthetic polymers derivatives^[14] have demonstrated promise in preventing post-MI heart failure in animal models.

Three general gelation mechanisms are prevalent for these injectable materials: 1) self-assembly or aggregation of macromolecules such as collagen, fibrin, alginate, and poly(*N*-isopropylacrylamide), 2) *in situ* radical polymerization of vinyl-functionalized monomers and cross-linkers, and 3) condensation of prepolymers for example by amidation, Michael type additions, thiolene, Diels-Alder, Huisgen [3+2] cycloaddition, copper-free click, Staudinger ligation, and hydrazone reactions.^[15] All three approaches allow materials to be injectable; however, this does not necessarily translate to minimally invasive delivery via catheter in the heart. Two methods of cardiac catheter delivery have been used for biomaterials, transendocardial injections and intracoronary infusion. The former involves the catheter being placed in the LV chamber and delivery via multiple needle based injections to distribute the material throughout the infarct and borderzone. The latter involves infusion into a vessel in or near the infarcted region.^[16] For injections into the heart using these clinically available cardiac injection catheters, which were initially developed for cell injections, the following requirements must be met. First, the components must be premixed and go through a single barrel. The material must also stay in liquid form while being held at 37 °C for potentially over an hour-long procedure and only form a gel once it enters the tissue. In the case of transendocardial delivery, the material must be capable of being injected multiple times at the site of injury. Finally, for both transendocardial and intracoronary delivery, the material must be hemocompatible since leakage into the systemic circulation occurs with both techniques. The majority of materials tested in small animal MI

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models would not be compatible with cardiac catheter delivery, and in fact, only two materials, alginate^[8] and a decellularized myocardial matrix hydrogel,^[12] have been reported to be delivered in MI models via catheter. Shear thinning materials that self-assemble such as β -sheets^[17] and various peptides^[18] also have the potential for catheter delivery in the heart; however, this has yet to be demonstrated. As such there is a need to develop new approaches to injectable materials capable of delivery by catheter in challenging locations such as the heart.

Schiff base chemistry, the addition of amine nucleophiles to aldehydes/ketones, has been previously used for tissue engineering applications,^[19, 20] and injectable hydrogel systems using hydrazone-cross-linking have shown the ability to have tunable rates of gelation, degradation, and self-healing capabilities.^[19] Herein, we demonstrate a general approach to injectable materials capable of catheter delivery by oxime cross-linking. The oxime bond, condensation of a hydroxylamine with a ketone or aldehyde, is ideally suited to biological systems. Oximes exhibit improved hydrolytic stability over hydrazones and imines with the equilibrium lying far toward the oxime.^[21] It is a chemospecific “click” reaction that is bioorthogonal because the two reaction partners react efficiently and specifically with each other in the presence of other functional groups with the byproduct being water.^[22, 23] The biocompatibility of this reaction has been demonstrated for stem cell encapsulation *in vitro*.^[24] Additionally, oxime bond formation is catalyzed under acidic conditions.^[22] We initially envisioned that this would be a beneficial trait for *in situ* hydrogel injection in the heart due to the acidic environment of the ischemic tissue post-myocardial infarction.^[25]

A polyethylene glycol (PEG) system was investigated as a model system to demonstrate the feasibility of utilizing oxime chemistry for injectable hydrogels for delivery to the heart. Four-armed ketone-PEG (ket-PEG) was synthesized in one step via carbodiimide coupling with levulinic acid in 95.0 % yield with 94.9 % of the PEG functionalized with a ketone (Figure 1A). Percent functionalization was determined by comparison of the singlet of the methylene protons from the pentaerythritol core to the protons of the end-group (Figure S1–2). Analysis by size exclusion chromatography (SEC) with dynamic light scattering indicated a number average molecular weight (M_n) of 20,800 g/mole (polydispersity index (PDI) = 1.08) (Figure S3). Four-armed aminooxy-PEG (AO-PEG) was synthesized in two steps by Mitsunobu reaction of the terminal PEG-alcohols with *N*-hydroxyphthalimide (Figure S4) followed by deprotection with hydrazine (Figure 1B).^[26] ¹H NMR analysis indicated that 95.1 % of the AO-PEG was functionalized with hydroxylamines (Figure S5), while SEC analysis with dynamic light scattering indicated a M_n of 20,420 g/mole (PDI = 1.53) (Figure S6). Mixing of these two four-armed PEG polymers at pH 5.0 resulted in PEG-hydrogels cross-linked by oxime bonds ($n=3$) (Figure 1C). The PEG-oxime hydrogels (50 mg/mL, 400 μ L, pH 5.0) were formed overnight at 37 °C in a mold and then swollen in PBS pH 7.4 for 30 hrs. Analysis by parallel plate rheometry gave similar mechanical properties to PEG-hydrogels formed through amidation cross-linking^[27] with a storage modulus (G') at 1 Hz of 523.6 Pa ($\pm$ 34.2 Pa) and a loss modulus (G'') at 1 Hz of 1.85 Pa ($\pm$ 0.4 Pa) (Figure 1D). The PEG-hydrogels also had a mass ratio of 39.35 ($\pm$ 1.19), a swelling ratio of 22.81 ($\pm$ 3.76), and were 97.45 % ($\pm$ 0.08 %) water by mass.

Oxime bond formation is known to be acid and base catalyzed^[21] and therefore gelation was monitored at different pH values with total PEG content of 50 mg/mL and a ket-PEG:AO-PG mass ratio of 1:1 (Figure 1E). Ket-PEG (100 mg/mL in DI water) and AO-PEG (100 mg/mL in DI water) were mixed in 1:1 volume ratios followed by addition of a 2× buffer at the desired pH, injected into a vial through a 27G needle, and incubated at 37 °C. *In vitro* gelation rates were strongly dependent on pH ranging from 30 minutes at pH 4–4.5 to 50.3 hrs at pH 7.4 (Figure 1E). Rapid gelation at acidic pH 4–4.5 and slow gelation at neutral and physiological pH was consistent with cross-linking occurring via the acid catalyzed oxime bond. Post-gelation the material was frozen, lyophilized, and infrared spectrum recorded (Figure 1F). A new peak was present at $\sim 1670\text{ cm}^{-1}$, which was not seen in the precursor materials, that was consistent with an aliphatic oxime bond. Analysis by ^1H NMR confirmed cross-linking via oxime-bonds by comparison with levulinic acid/hydroxyl amine (Figure S7), ket-PEG/hydroxyl amine (Figure S8), and the hydrogel system in deuterated PBS pH 5.5 (Figure S9). Addition of excess hydrazine to ket-PEG before mixing with AO-PEG prevented gelation (Figure S10). These experiments indicated the ability to form PEG-hydrogels by oxime cross-linking. To assess stability, gels that were pre-swollen in PBS pH 7.4 with volumes of $\sim 1\text{ mL}$ (1.1–1.3 g) were then incubated in 6 mL of PBS pH 6.0, 6.5, and 7.4 ($n=3$ for each pH). Gels were stable through 7 days (Figure S11). Thus oxime bond cross-linking allows for facile tuning of gelation rates *in vitro*, as well as stability of the gel *in vitro*.

After confirming that cross-linking was occurring due to oxime bonds and tunable by adjusting the pH, *in vitro* injection of the PEG-oxime system through the 27G inner nitinol tubing of a Myostar catheter was pursued. A PEG-oxime solution at 50 mg/mL at pH 6.0 was prepared and loaded into a syringe. The material was then pushed through the catheter that was immersed in a 37 °C water bath (Figure S12). PEG-oxime (50–75 μL) was pushed through at 15 min, 30 min, 1 hr, and 2 hrs. The material pushed through the catheter gelled in 4 hrs at 37 °C, consistent with the time previously determined during the gelation kinetics (Figure 2A).

We next investigated injection of the material *in vivo*. Subcutaneous dorsal injections of the PEG-oxime system (100 μL , 50 mg/mL) in Sprague Dawley rats were investigated at pH values of 6.0, 6.5, 6.75, 7.0, 7.4, and 10.5 ($n=2$ for each pH). All formulations quickly formed a bolus upon subcutaneous injection (Figure 2B). Gels were excised after 20 minutes. The excised gels had a mass similar to the pre-injection material and water volume displacement similar to gels formed *in vitro* (Figure S13). Solutions of PEG-oxime were then injected again subcutaneously at pH 4, pH 7.4, and pH 10.5 in volumes of 100 μL and 200 μL for each pH. After 20 minutes the gels were excised (Figure 2C–E). Interestingly, rapid gelation (20 minutes) was observed *in vivo* for all pHs tested while a broad range of gelation times was observed *in vitro* when the pH is varied. The mass and volumes of the *in vivo* gels are similar to *in vitro* gels (Figure S13), indicating that *in vivo* the gels are not swelling within the tissue or becoming more concentrated.

Since gelation occurred rapidly, within 20 minutes, we sought to investigate the cause of this time difference between gelation *in vitro* and *in vivo*. It is possible that the cross-linking was catalyzed by a protein or enzyme found within the tissue. We tested this hypothesis by

monitoring the gelation rate at three different pH values (5.0, 6.5, and 7.4) in the presence of three different proteins; bovine serum albumin (50 mg/mL), lipase (5 mg/mL), and aldolase (5 mg/mL). Bovine serum albumin is found in high concentrations in the blood, and lipases and aldolases are known to have amine residues in their active sites. No change in gelation rate was observed at any of the pH values investigated (pH 5.0, 6.5, and 7.4) with any of the proteins (data not shown). We next investigated if differences in oxygen concentration *in vitro* versus *in vivo* had an effect on gelation rate. Solutions (PEG-oxime pH 6.5) were either bubbled extensively with air or argon and injected into a sealed vial containing air or argon, respectively, and incubated at 37 °C. No change in gelation rate was observed of the PEG-oxime solutions (pH 6.5) in a syringe at 37 °C over the course of two hours did not result in gel formation. Finally, no gels were formed after two hours by dissolving PEG-oxime components in Dulbecco's PBS containing magnesium chloride and calcium chloride at pH 6.5 and 7.4 at 37 °C (data not shown). This difference of gelation rates *in vitro* versus *in vivo* has been seen with other injectable hydrogel systems. For example certain extracellular matrix based hydrogels were shown to be incapable of forming hydrogels *in vitro*; however, these materials formed gels rapidly *in vivo*.^{[29],[30]} These examples taken with the data presented above indicate that slow or no gelation *in vitro* does not necessarily relate to gelation *in vivo*, where the environment is more complex.

Slow *in vitro* gelation of materials could result in new injectable materials for delivery by minimally invasive approaches.^[31] Subcutaneous injection of the PEG oxime system resulted in hydrogels within 20 minutes over a broad range of pH 4–10.5, while *in vitro* gelation at 37 °C occurred from 30 minutes to >2 days. This allows PEG-hydrogels cross-linked by oximes to be held at physiological temperature for extending periods of time, which could facilitate delivery of these materials with a catheter. The ability to obtain rapid gelation *in vivo* over broad range of pH values is a powerful tool for designing injectable material therapies for delivering therapeutics.

A cross-linking mechanism that allows a material to gel within tissue yet withstand long periods at body temperature and physiological pH without gelation (Figure 1E) could open up the possibility of numerous delivery routes, including catheter based, multi-injection delivery in the heart. We therefore tested the ability of the PEG-oxime hydrogel system to form a gel within myocardial tissue. We injected PEG-oxime at pH 7.4 (75 µL, 50 mg/mL) into the left ventricular free wall of Sprague Dawley rats. Twenty minutes post-injection, the heart was excised, frozen, and sectioned. Hematoxylin and eosin (H&E) stained sections demonstrated that the presence of a gel within the myocardium (Figure 2F). As with the subcutaneous injections, the composition at pH 7.4, which gels slowly *in vitro* at 37 °C, is capable of gelling within 20 minutes after injection into the myocardium.

To demonstrate the versatility and applicability of oxime chemistry for *in situ* hydrogel formation, we applied our cross-linking approach to polysaccharides commonly used for tissue engineering and wound healing applications. Numerous examples exist in the literature in which cis-diols of naturally occurring polysaccharides were oxidized with sodium periodate to aldehydes and then cross-linked with multimeric hydrazides.^[32] We amended this approach to oximes because of the greatly improved hydrolytic stability over hydrazones. Commonly utilized polysaccharides, hyaluronic acid (HA) and alginate (Alg)

were oxidized with sodium periodate (Figure 3A). Aldehyde content was quantified by the Purpald assay with oxidized-HA and oxidized-Alg containing 10.17 nmoles aldehyde/mg polysaccharide and 23.96 nmoles aldehyde/mg polysaccharide; respectively. Gelation of both oxidized materials with AO-PEG was pursued at various concentrations at pH 5.5 to determine the concentration of saccharide required for hydrogel formation with an aldehyde:aminoxy ratio of 1:1. It was determined that HA-PEG (3.33 mg/mL HA, 0.713 mg/mL AO-PEG) and Alg-PEG (13.3 mg/mL Alg, 6.71 mg/mL AO-PEG) rapidly formed hydrogels at pH 5.5. The rate of gelation was then monitored for both HA-PEG and Alg-PEG at pH 4.0, 6.0, and 7.0 at 37 °C (Figure 3B). The rate of gelation of HA-PEG and Alg-PEG from pH 4–7 was 1.6–115.0 minutes and 1.95–58.3 minutes, respectively, thus demonstrating the ability to tune the rate of *in vitro* gelation by altering the pH of the system. *In vitro* gelation times for both oxidized-polysaccharide-PEG were faster than the PEG-only system at all pH values investigated. The increased gelation rate is likely the result of using the more electrophilic aldehyde (versus ketone for the PEG-only system) and increased number of reactive groups per macromolecular chain.

Injection through the inner nitinol tubing of the Myostar catheter (37 °C) *in vitro* was pursued using the concentrations determined above for the HA-PEG and Alg-PEG systems. Four injections (50–75 µL) of HA-PEG (pH 7.0) were performed at 30 min, 1 hr, 1.5 hrs, and 1.75 hrs. These time points were chosen because this system has an *in vitro* gelation time at 37 °C of ~2 hrs. After injection through the catheter and incubation at 37 °C, gels were formed in 2 hrs (Figure 3C). Three injections (50–75 µL) of Alg-PEG (pH 7.0) were performed at 15 min, 30 min, and 45 min. These time points were chosen because this system has an *in vitro* gelation time at 37 °C of ~1 hr. After injection through the catheter and incubation at 37 °C gels, were formed in 1 hr (Figure 3D). Subcutaneous injection of both of the oxime-cross-linked polysaccharide-PEG materials resulted in gels within 20 minutes (Figure 3E–F), similar to the PEG only system. This data demonstrates that oxime-based cross-linking could be extended to other injectable biomaterials, such as therapeutically relevant polysaccharides. Furthermore, *in vitro* gelation times directly impact how long the materials can be held at 37 °C and injected through a catheter. Upon injection *in vivo* rapid gelation is observed demonstrating that oxime chemistry can be applied to complex macromolecular systems allowing catheter delivery and hydrogel formation across a range of pHs.

Herein, we have demonstrated a new approach for gelation of injectable materials utilizing oxime cross-linking. Tunable *in vitro* gelation was achieved by the altering the pH with a bioinert-PEG system, as well as with oxidized hyaluronic acid and alginate. Oxime chemistry allowed for these materials to be injected multiple times through a catheter *in vitro* over the course of hours while at 37 °C, mimicking the *in vivo* situation. The PEG and polysaccharide systems form gels within 20 minutes upon injection into the subcutaneous space. The PEG-oxime system was capable of rapid gelation upon injection into myocardial tissue. While the above experiments seek to mimic catheter delivery and *in vivo* gelation, *in vivo* transendocardial catheter injections of these materials into the LV wall in a large animal model still needs to be performed. Overall, the data presented in this paper demonstrated that oxime cross-linking of injectable biomaterials has the potential to open up more difficult

minimally invasive delivery routes that require a material to be held at body temperature for extended periods of time, yet gel quickly once it enters the tissue (i.e catheter based delivery in the heart).

Experimental

Materials

Four-arm polyethylene glycol (20,000 g/mole) was purchased from JemKem and used as received. Hyaluronic acid sodium salt from *Streptococcus equi* sp. (1.63 MDa) and alginic acid sodium salt ($M_n = 120,000$ – $190,000$) were purchased from Sigma and used as received. All other materials and reagents were purchased from Sigma, Fisher, and Acros and used as received.

Methods

NMR spectra were recorded on a 400 MHz Varian Mercury Plus spectrometer. Chemical shifts are reported in δ (ppm). Polymer polydispersity and molecular weights were determined by size-exclusion chromatography (Phenomenex Phenogel 5u 10, 1K–75K, 300×7.80 mm in series with a Phenomenex Phenogel 5u 10, 10K–100K, 300×7.80 mm (0.05 M LiBr in DMF)) using a Jitachi-Elite LaChrom L-2130 pump equipped with a multi-angle light scattering detector (DAWN-HELIOS: Wyatt Technology) and refractive index detector (Hitachi L-2490) normalized to a 30,000 g/mol polystyrene standard. Infrared Spectra were recorded on a Thermo Scientific Nicolet 6700 FTIR with the diamond ATR accessory. Rheological measurements were performed with a TA Instruments ARG2 Rheometer using a parallel-plate geometry (20 mm diameter) at 37 °C with 0.3 N normal force.

Synthesis of ketone-PEG

Alcohol terminated four arm-PEG (2.0 g, 97.3 μ moles) and levulinic acid (398.3 μ L, 3.89 mmoles) were dissolved in dichloromethane (DCM) (10 mL) and then placed in an ice bath. *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (745.7 mg, 3.89 mmoles) was slowly added followed by addition of 4-(dimethylamino)pyridine (1.2 mg, 9.73 μ moles) and the solution was stirred for two days. The polymer was then precipitated into diethyl ether (190 mL). Redissolved in DCM (10 mL) and flushed over a silica plug. The solvent was removed *in vacuo* to afford ketone terminated four arm PEG (1.95 g, 95.0 % yield). ^1H NMR (400 MHz, CDCl_3 , δ): 4.20–4.17 (m, methylene alpha to ester), 3.79–3.76 (m, methylene alpha to pentaerythritol oxygen), 3.67–3.46 (m, methylene protons from ethers), 3.37 (bs, methylene protons from pentaerythritol core), 2.73–2.70 (m, methylene protons alpha to oxygen of ester), 2.58–2.55 (m, methylene protons alpha to ketone), 2.15 (bs, methyl protons alpha to ketone). IR (neat, cm^{-1}): 840, 944, 959, 1060, 1090, 1140, 1240, 1280, 1340, 1360, 1460, 1720, 2870. Size exclusion chromatography (dynamic light scattering): $M_n = 20,800$ g/mole, PDI = 1.08 (molecular weight by ^1H NMR = 20,774 g/mole).

Synthesis of aminoxy-PEG

Alcohol terminated four arm-PEG (2.0 g, 97.3 μ moles) and *N*-hydroxyphthalimide (634.6 mg, 3.89 mmoles) were dissolved in anhydrous DCM (10 mL) in a flame dried flask. Triphenylphosphine (1.02 g, 3.89 mmoles) was added and the flask was placed in an ice

bath. An anhydrous, cold solution (4 °C) of diisopropyl azodicarboxylate (765.9 μ L, 3.89 mmol) in DCM (5 mL) was slowly added and the solution was stirred for 24 hours. The polymer was then precipitated into diethyl ether (190 mL), redissolved in DCM (10 mL) and flushed over a silica plug. The solvent was removed *in vacuo*. *N*-hydroxyphthalimide PEG (0.25 g, 11.8 μ mol) was dissolved in acetonitrile (1 mL). Hydrazine monohydrate (22.9 μ L, 473 μ mol) was added and the solution was stirred for two hours. DCM (9 mL) was added and the mixture was filtered over a plug of celite. The solvent was removed *in vacuo* to afford aminooxy terminated four arm PEG in 91.4 % yield over two steps. ^1H NMR (400 MHz, CDCl_3 , δ): 3.88–3.86 (m, methylene α to hydroxylamine), 3.79–3.76 (m, methylene α to pentaerythritol oxygen), 3.67–3.46 (m, methylene protons from ethers), 3.37 (bs, methylene protons from pentaerythritol core), 1.23–1.21 (bs, $-\text{ONH}_2$). IR (neat, cm^{-1}): 690, 719, 840, 944, 958, 1060, 1090, 1140, 1240, 1340, 1360, 1460, 2870. Size exclusion chromatography (dynamic light scattering): M_n = 20,420 g/mole, PDI = 1.53 (molecular weight by ^1H NMR = 24,920 g/mole).

Oxidation of polysaccharides

Oxidation of polysaccharides was performed following a published procedure with slight modification.^[33] Polysaccharides (0.15 g) were dissolved in deionized water (15 mL) and then sodium periodate (0.8 g, 3.74 mmol) was added. The reaction was quenched after 2 hours with the addition of ethylene glycol (0.04 mL) and stirred for an additional hour. The reaction was dialyzed (MWCO 3,500 g/mol) against water (12 h) and then 0.1 M saline (12 h) cycling for 3.5 days, and then freeze dried. The degree of oxidation was determined using the Purpald® reagent as described by the manufacturer with a standard curve from formaldehyde.

Gelation kinetics of PEG-oxime system

Phosphate/citric acid (2 $\times$) (100 μ L) at various pH values (pH 4, 4.5, 5, 5.5, 6, 6.5, 7, and 7.4) was added to aminooxy-PEG/ketone-PEG (100 mg/mL, 100 μ L). The solution was loaded into a syringe with a 27 G needle and injected to a glass vial. The glass vial was then placed in a 37 °C incubator. Gelation was monitored by inability of the solution to flow when the vial was inverted.

Gelation kinetics of PEG-oxime system in the presence of proteins

Gelation kinetics of the PEG-oxime system at three different pH values (pH 5.0, 6.5, and 7.4) were monitored in the presence of four different proteins in triplicate; bovine serum albumin (50 mg/mL), aldolase (5 mg/mL), and lipase (5 mg/mL).

Rheometry on PEG-oxime system

PEG hydrogels (400 μ L, 50 mg/mL, pH 5.0) were prepared as described above and then pipetted between two glass slides with a spacing of 0.2 cm. The gels were incubated at 37 °C for 12 hrs and then placed into PBS pH 7.4 (5 mL) (n=3) for 48 hrs. The gels were analyzed as previously described.^[27]

Swelling ratio, mass ratio, and water content of PEG-oxime hydrogel

PEG hydrogels (400 μ L, 50 mg/mL, pH 5.0) were prepared as described above and then pipetted between two glass slides with a spacing of 0.2 cm. The gels were incubated at 37 °C for 12 hrs. The gels were then placed into PBS pH 7.4 (5 mL) (n=3) for 48 hrs after which the gels were massed and the dimensions of the gels were measured with calipers. The gels were then lyophilized. The dehydrated gels were massed and the dimensions were measured with calipers. The swelling ratio was calculated as follows: $\text{Volume}(\text{swollen})/\text{Volume}(\text{dehydrated})$. The mass ratio was calculated as follows: $\text{Mass}(\text{swollen})/\text{Mass}(\text{dehydrated})$. The water content of the swollen hydrogels was calculated as follows: $(\text{Mass}(\text{swollen})-\text{Mass}(\text{dehydrated}))/\text{Mass}(\text{swollen})*100\%$

Catheter injection of materials

Polymer solutions were prepared as described above and loaded into a syringe through a 27 G needle. The material was then loaded into the 27 G inner nitinol tubing of the Myostar catheter that was immersed in a 37 °C water bath (Fig. S12). The material was pushed through (50–75 μ L) at different time points.

Animal surgeries

All experiments in this study were performed in accordance with the guidelines established by the Committee on Animal Research at the University of California, San Diego, and the American Association for the Accreditation of Laboratory Animal Care.

Subcutaneous injections

Female Sprague Dawley rats (225–250g) were anesthetized with 5% isoflurane, and maintained at 2.5% isoflurane. Six injections of material (100 μ L or 200 μ L) were performed on the dorsal region (n=2 for each formulation of material injected). After 20 minutes the gels were excised and the animal was euthanized. After excision, the mass and volume (by water displacement) of the gels was recorded.

Intramyocardial Injections

Intramyocardial injections were performed similar to a previously described procedure.^[4, 12, 27] The animal was anesthetized with 5% isoflurane, and maintained at 2.5% isoflurane. An incision was made in the abdomen and the diaphragm was cut to expose the heart. A single 75 μ L injection of material (PEG total concentration 50 mg/mL, PBS pH 7.4) was injected with a 27 G needle into the LV free wall. After 20 minutes the heart was excised and frozen in Tissue Tek OCT freezing medium.

Tissue sectioning and histological analysis

Frozen hearts were sectioned into 10 μ m slices and stained with H&E.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A) Synthesis of four-armed ketone-PEG. B) Synthesis of four-armed aminooxy-PEG. C) Hydrogel formation of PEG by oxime cross-linking. D) Parallel plate rheometry of PEG-oxime hydrogel with 0.3 N normal force at 37 °C (n=3). E) Graph of gelation times *in vitro* at 37 °C of PEG-oxime at different pHs (n=3 for each pH). F) IR spectra of ketone-PEG, aminooxy-PEG, and PEG-oxime hydrogel.

Figure 2.

A) PEG-oxime (pH 6.0) after injection through the inner nitinol tubing of the MyoStar catheter and subsequent incubation for four hours at 37 °C. B) Subcutaneous injection of PEG-oxime (pH 7.4) after 20 minutes. C–E) 200 μ L (left) and 100 μ L (right) PEG-oxime gels excised after 20 minutes (scale bar = 1 cm). C) pH 4.0. D) pH 7.4. E) pH 10.5. F) H&E stained section of heart after injection of PEG-oxime (pH 7.4) (scale bar = 200 μ m). * denotes the region of PEG injection.

Figure 3.

A) Sodium periodate oxidation of the cis-diols to aldehydes of hyaluronic acid. B) Gelation times of oxidized-hyaluronic acid/AO-PEG and oxidized-alginate/AO-PEG at pH 4.0, 6.0, and 7.0; respectively. C) Oxidized-hyaluronic acid/AO-PEG (pH 7.0) after injection through a catheter and incubation at 37 °C. D) Oxidized-alginate/AO-PEG (pH 7.0) after injection through a catheter and incubation at 37 °C. E–F) Subcutaneous injections (100 μ L) excised

after 20 minutes of E) oxidized-hyaluronic acid/AO-PEG (pH 7.4) and F) oxidized-alginate/AO-PEG (pH 7.4) (scale bar = 1 cm).