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The effect of matrix stiffness of injectable hydrogels on the preservation of cardiac function after a heart attack

Marian Plotkin [a,1], Srirangam Ramanujam Vaibavi [a,1], Abdul Jalil Rufaihah [b], Venkateswaran Nithya [b], Jing Wang [b], Yonatan Shachaf [c], Theo Kofidis [b,d,**], Dror Seliktar [a,c,*]

[a] Nanoscience and Nanotechnology Initiative, National University of Singapore, Singapore
[b] Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[c] Faculty of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa, Israel
[d] Department of Cardiac, Thoracic & Vascular Surgery, National University Heart Centre Singapore, National University Health System, Singapore

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ABSTRACT

This study compares the effect of four injectable hydrogels with different mechanical properties on the post-myocardial infarction left ventricle (LV) remodeling process. The bioactive hydrogels were synthesized from Tetronic-fibrinogen (TF) and PEG–fibrinogen (PF) conjugates; each hydrogel was supplemented with two levels of additional cross-linker to increase the matrix stiffness as measured by the shear storage modulus (G′). Infarcts created by ligating the left anterior descending coronary artery in a rodent model were treated with the hydrogels, and all four treatment groups showed an increase in wall thickness, arterial density, and viable cardiac tissue in the peri-infarct areas of the LV. Echocardiography and hemodynamics data of the PF/TF treated groups showed significant improvement of heart function associated with the attenuated effects of the remodeling process. Multi-factorial regression analysis indicated that the group with the highest modulus exhibited the best rescue of heart function and highest neovascularization. The results of this study demonstrate that multiple properties of an injectable bioactive biomaterial, and notably the matrix stiffness, provide the multifaceted stimulation necessary to preserve cardiac function and prevent adverse remodeling following a heart attack.

1. Introduction

Cardiovascular diseases (CVDs) remain the leading cause of death in the western world [1]. Progression of coronary heart disease (CHD) is associated with myocardial infarction (MI), where obstructed coronary perfusion results in myocardial ischemia, necrosis, scarring and irreversible damage to the heart. Current treatment approaches are mostly pharmacological or interventional, such as percutaneous coronary intervention (PCI) and/or stenting. While these strategies may help salvage the injured myocardium, they have limited success in severe cases that lead to end-stage heart failure. These cases require transplantations or the use of ventricular assist devices – highly invasive interventions that are limited in their availability and/or efficacy. Novel approaches to alleviate the burden of end-stage heart failure, such as the use of minimally invasive injectable therapeutic biomaterials, show promise in providing an alternative treatment at the early stages of myocardial intervention [2–4]. Hydrogels are one such class of biomaterial [5], made from crosslinked hydrophilic polymers, that can provide the exogenous stimulation required to treat damaged myocardium immediately following MI [6]. The polymers used for creating hydrogel implants may be natural or synthetic, or a combination of both (i.e. semi-synthetic) [5,7]. Injectable hydrogels can be used immediately after an MI event, by injecting a liquid precursor into the myocardium in a minimally invasive manner [6,8], followed by a non-toxic *in situ* gelation process [9].

Injecting a hydrogel into the wall of the left ventricle (LV) can affect the heart function as elucidated by the law of Laplace [10], which stipulates that wall stress is proportional to pressure and radius, and inversely proportional to the wall thickness. In cardiac

* Corresponding author. Faculty of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa 32000, Israel. Tel.: +972 48294805; fax: +972 48294884.
** Corresponding author. Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore. Tel.: +65 67722059; fax: +65 67766475.
E-mail addresses: surtk@nus.edu.sg (T. Kofidis), dror@bm.technion.ac.il (D. Seliktar).
[1] These authors contributed equally to this work as first author.

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mechanics, the remodeling following MI promotes thinning of the LV wall and thereby increases the wall stresses. This results in apoptotic cell death that progressively enhances adverse remodeling towards scarring and dilated cardiomyopathies [11]. Hence, by increasing wall thickness using an injectable biomaterial, the wall stress can be reduced either by attenuating the adverse cardiac remodeling or increasing the bulk properties of the myocardial tissue (i.e. bulking agent).

In theory, altering cardiac mechanics vis-à-vis the law of Laplace using a cardiac bulking agent can decrease apoptosis and provide a more effective preservation of cardiac function [10]. However, Rane et al. recently demonstrated that passive structural reinforcement alone is not sufficient to prevent adverse remodeling following MI [12], implying that cardiac bulking agents must also bioactively alter cardiac remodeling to attenuate post-MI scar formation. Accordingly, bioactive hydrogels, such as those that can promote cell adhesion, proliferation or inflammatory biodegradation, are likely necessary for post-MI cellular remodeling leading to improvements in cardiac function [13–20]. The role of bioactivity notwithstanding, no studies have yet to examine the biomechanical augmentation on cardiac remodeling using injectable bioactive bulking agents of wide-ranging mechanical properties. Here, we examined how the matrix stiffness of an injectable bioactive hydrogel affects functional cardiac performance following MI, mainly by quantifying key cardiac parameters and correlating these to the hydrogel modulus. Injectable bioactive hydrogels were delivered following Acute MI (AMI), and cardiac performance was quantified thirty days later by measuring ejection fraction, LV fractional area change, LV end diastolic/systolic volumes, LV wall motion, contractility index, stroke volume, mean LV pressure and LV end-diastolic pressure.

The bioactive biomaterials used in this study are semi-synthetic, and derive their bioactivity from a fibrinogen backbone molecule. Fibrinogen and its main byproduct fibrin are extensively used in cardiac bioengineering both as a vehicle for cell delivery and as an implantable biodegradable scaffold for cellular homing [16,17,21–24]. In its hydrogel form, fibrin contains multiple cell signaling domains, cell-adhesion motifs [25], and protease degradation sites [26]; but with limited control over its physical properties, fibrin is rarely used as an effective bioactive cardiac bulking agent. Therefore, fibrin(ogen) was conjugated to a synthetic component providing the material the ability to transform from a liquid to a gel by light-activated crosslinking that also provided a mean of controlling the material stiffness [27–29]. These physical control features were used to evaluate stiffness-dependent post-MI LV remodeling in a rodent model. Specially, two groups of bioactive hydrogels were examined: a Tetronic-fibrinogen (TF) conjugate and a PEG–fibrinogen (PF) conjugate; each was made with two different levels of material stiffness.

2. Materials and methods

2.1. Synthesis of PEG–fibrinogen

Poly(ethylene glycol)–diol (PEG–OH, 10 kDa) was modified with acryloyl chloride to make PEG–diacrylate (PEG–DA) according to published protocols [30]. Bovine fibrinogen (Bovogen Biologicals Pty Ltd, Melbourne, Australia) was covalently conjugated to PEG–DA according to published protocols [28]. Briefly, a 7 mg/ml solution of fibrinogen in 10 mM phosphate-buffered saline (PBS) with 8M urea was prepared with tris (2-carboxyethyl) phosphine hydrochloride (TCEP–HCl) (Sigma); the TCEP–HCL was added at a molar ratio of 1.5:1 TCEP to fibrinogen cysteines. The solution pH was corrected to 8.0 with NaOH. Next, PEG–DA was dissolved in 10 mM PBS and 8M urea (280 mg/mL); after centrifugation the solution containing PEG–DA was added to the solution of dissolved fibrinogen. The molar ratio of PEG to fibrinogen cysteines was 3.7:1 (linear PEG–DA, 10 kDa). Next, the mixture was reacted in a reaction vessel with a thermostatic jacket at a temperature of 22.5 °C for 3 h in the dark. The solution was then diluted with an equal volume of PBS-8M urea and precipitated by adding it into acetone (Aik Moh Paints & Chemicals Pte Ltd., Singapore), at a 4:1 ratio of acetone to solution (v/v). The precipitant was centrifuged, the acetone liquid phase was removed and the precipitant 'cake' was redissolved by adding 1.8 volumes of PBS-8M urea. Tangential flow filtration technique was implemented using Centramate cassette (50 kDa MW cutoff, pall corporation) to purify and concentrate the modified protein against 10 mM of PBS (ratio of 80:1 v/w PBS to precipitant) down to a concentration of 8–12 mg/ml. The final conjugate hydrogel product was comprised of PEG–DA moieties, wherein an acrylate group of each of the PEG–DA moieties was attached to a cysteine residue on the fibrinogen backbone.

2.2. Synthesis of T1307 tetraacrylate-fibrinogen

The acrylation of Tetronic 1307 (T1307, also known as Pluracare1307, O-BASF), MW = 18 kDa was carried out based on a modified protocol used for making the PEG–DA [30]. Briefly, the process was carried out under Argon by reacting T1307-tetraol in a solution of Dichloromethane (Aldrich, Sleeze, Germany) and Toluene (Bio-Lab, Jerusalem, Israel) with acryloyl–chloride (Merck, Darmstadt, Germany) and triethylamine (TEA) (Fluka) at a molar ratio of 150% relative to the hydroxyl groups. The final product was precipitated in ice-cold petroleum ether (40–60). The solid polymer was dried under vacuum for 48 h and characterized by proton NMR in order to determine the average number of acryl groups on the T1307 molecule. Fibrinogen was conjugated to Tetronic-tetraacrylate (T1307-TA) by a Michael-type addition reaction. In order to conjugate fibrinogen to the synthetic polymers, 8.3 mg/ml solution of fibrinogen in PBS (150 mM) with 8M urea was supplemented with tris (2-carboxyethyl) phosphine hydrochloride (TCEP) (Sigma) at a molar ratio of 1.5:1 TCEP to fibrinogen cysteines. After dissolution of Fibrinogen, the functionalized polymer (T1307-TA) in a solution of PBS and 8M urea (260 mg/ml) was added at molar ratio of 4:1 synthetic polymer to fibrinogen cysteines (T1307-TA). The reaction was incubated for 3 h at room temperature and then the volume of the reaction was doubled by adding PBS and 8M urea. The conjugated protein was precipitated by adding 6 volumes of acetone (Bio-Lab) to the final obtained solution. The precipitate was dissolved in PBS containing 8M urea at a protein concentration of 7–9 mg/ml and then dialyzed against PBS at 4 °C for 2 days with two changes of PBS per day (Spectrum, 12–14 kDa MW cutoff). The net fibrinogen concentration in the Tetronic-fibrinogen (TF) precursor was determined using a Nano-drop ND-2000 (Extinction Coefficient (1%) = 15.1, λ_{max} = 280 nm). The final product was characterized according to previously published protocols [28].

2.3. Hydrogel preparation and shear modulus analysis

Photoinitiator stock solution made of 10% w/v Irgacure™ 2959 (Ciba) in 70% ethanol and deionized water. Sterile 15% PEG–DA stock solution (w/v) was prepared by dissolving PEG–DA in PBS and then filtering the dissolved PEG–DA through a 0.2-micron syringe filter. Sterile 15% T1307-TA stock solution (w/v) was prepared by dissolving T1307-TA in PBS and then filtering the dissolved T1307-TA through a 0.2-micron syringe filter. Hydrogel precursor solution was prepared by adding 1% (v/v) of photoinitiator stock solution to the liquid PF. In order to increase the shear modulus of the PF hydrogel, an additional 1% or 2% PEG–DA was added to the PF solution by adding 6.67% or 13.33% from the stock solution to the liquid PF (v/v), respectively. PBS was added to the PF solution resulting in a final dilution of 8 mg/ml of fibrinogen in solution. Shear modulus was measured with an AR-G2 rheometer (TA Instruments). After 1 min of equilibration, the PF solution was exposed to 365 nm UV light from an Omnicure Series 2000 UV light source at an intensity of 5 mW/cm^2. Time-sweep oscillatory tests were performed at room temperature 23 ± 1 °C at a sinusoidal 2% strain rate and a 6 rad s^{-1} angular frequency, within the linear viscoelastic region as determined previously [31]. At the end of the crosslinking reaction, the loss modulus G″ was negligible with respect to storage modulus G′.

TF hydrogel precursor solution was prepared by adding 3% (v/v) of photoinitiator stock solution to the liquid PF. In order to increase the shear modulus of the TF hydrogels, an additional 1% or 2% T1307-TA was added to the liquid TF by adding 6.67% or 13.33% from the stock solution to the TF (v/v), respectively. PBS was added to the TF solution resulting in a final dilution of 7 mg/ml of fibrinogen. Shear modulus was measured as before, with a 50 mm Quartz Plate Geometry on top of a peltier plate set at 37 °C. After 1 min of equilibration, the TF solution was exposed to 365 nm UV LED light source (MDRL-CUV31, Moritex). Time-sweep oscillatory tests were performed at a sinusoidal 2% strain rate and a 6 rad s^{-1} angular frequency. The shear modulus was quantified as the real part (i.e. the storage modulus) G′ as described above.

2.4. Animal preparation and surgical procedure

The animal study and all the related study protocols comply with the "Guide for the Care and Use of Laboratory Animals", published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). The experimental protocol was pre-approved by the National University of Singapore–Institutional Animal Care and Use Committee (IACUC/062/10). SPF Wistar Rats (weighing 350–450 g) were used for our experiments. The body weight of the rat was measured prior to anesthesia. The animal was anesthetized with mixture of 95% oxygen and 5% isoflurane. A ventilator was connected to the 16G JELCO enabling a flow of 3% isoflurane/97% oxygen in tidal volume of 1 ml/100 g body weight, at a rate of 60 breaths/min. Rectal temperature probe was connected to the rectum and the body temperature was monitored. ECG analysis was done to monitor the heart functions before the surgery.