Bioengineered Stromal Cell-Derived Factor-1 α Analogue Delivered as an Angiogenic Therapy Significantly Restores Viscoelastic Material Properties of Infarcted Cardiac Muscle

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Ischemic heart disease is a major health problem worldwide, and current therapies fail to address microvascularization. Previously, our group demonstrated that the sustained release of novel engineered stromal cell-derived factor 1- α analogue (ESA) limits infarct spreading, collagen deposition, improves cardiac function by promoting angiogenesis in the region surrounding the infarct, and restores the tensile properties of infarcted myocardium. In this study, using a well-established rat model of ischemic cardiomyopathy, we describe a novel and innovative method for analyzing the viscoelastic properties of infarcted myocardium. Our results demonstrate that, compared with a saline control group, animals treated with ESA have significantly improved myocardial relaxation rates, while reducing the transition strain, leading to restoration of left ventricular mechanics. [DOI: 10.1115/1.4027731]

Introduction

Heart disease is the leading cause of death worldwide, with coronary heart disease causing one of every six deaths in the United States [1]. Following a myocardial infarction (MI), scar tissue gradually replaces myocardium that is lost through a process of collagen deposition and an increase in tensile strength of the tissue [2]. Borderzone myocardium becomes hypocontractile, leading to left ventricular dilation, increased left ventricle (LV) wall stress, and a decrease in ejection fraction [3]. LV dilation perpetuates infarct expansion, adverse ventricular remodeling, and dysfunction, which ultimately leads to heart failure. The deleterious remodeling of the LV after MI causes a significant change in its geometry, resulting in less efficient cardiomyocyte contraction and finally, a significant decline in cardiac pump efficiency [4,5].

Understanding the effect of a therapy on the passive mechanical properties of the myocardium is a key component of understanding its efficacy. Angiogenesis is a mechanism by which adverse ventricular remodeling can be limited. It has previously been shown that bone marrow-derived endothelial progenitor cells migrate toward ischemic tissue and incorporate into foci of

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neovascularization [6]. We formerly demonstrated the efficacy of a native protein, stromal cell-derived factor-1 α (SDF), in providing an effective neovasculogenic therapy for ischemic cardiomyopathy [7]. Subsequently, we developed ESA, a computationally designed analog of SDF-1 α , and demonstrated that intramyocardial injection enhances LV function by promoting angiogenesis and retention of the extracellular matrix (ECM) [8]. Angiogenesis in the borderzone reduces collagen deposition, limits infarct spreading, and thereby preserves healthy tissue. The differential collagen content in the borderzone has a significant effect on the tensile modulus of the tissue [9].

There has been significant interest among both the clinical and basic science communities in understanding the regional stresses in the heart. The LV free wall is a thick-walled complex fibrous structure, mainly consisting of myocyte and collagen fibers. The functional and structural complexity of the LV free wall poses a challenge to the application of biaxial biomechanical analysis to the passive tissue state. Myocardium is anisotropic in the fiber and cross-fiber directions, and exhibits both nonlinear elasticity and viscoelasticity, with strain-rate dependence. The fibrous structure also varies throughout the LV, with the direction and degree of anisotropy dependent on the location from which the biopsy was taken. Computing regional stresses in the LV require constitutive mathematical models of both the active and passive tissue states [10].

In this study, we sought to validate an experimental model of testing myocardium that combines viscoelastic properties with previously reported elastic properties, in order to better understand how ESA treatment alters the mechanics of myocardial tissue with a focus on the borderzone. Given our previous results [8,9], we hypothesized that injection of ESA into the borderzone of infarcted cardiac muscle would improve the viscoelastic properties of the ventricle, providing further evidence that ESA effects change at the tissue mechanics level, in addition to globally preserving LV function.

Methods

Peptide Design and Synthesis. We have previously reported on the design, synthesis, and efficacy of ESA [8,11]. Briefly, ESA was synthesized by preserving the N and C termini of SDF-1 α , and deleting the central β -pleated sheet. Native SDF is bulky, and quickly broken down by proteases in the body. ESA was developed by replacing the bulky β -pleated sheet with two proline residues that were computationally predicted to retain the tertiary structure of the native protein as closely as possible.

Animal Care. Male Wistar rats, weighing 250–300 g, were obtained from Charles River Laboratories (Wilmington, MA). The animals received food and water ad libitum. The research was conducted under protocol 803394, which was approved by the Institutional Animal Use and Care committee of the University of Pennsylvania. The experiments we conducted conformed to the *Guide for the Care and Use of Laboratory Animals*, published by the Institute for Laboratory Animal Research, Division on Earth and Life Studies; funded by The National Institutes of Health (8th edition, 2011).

Experimental Model. A total of 54 adult male Wistar rats underwent surgery. A reproducible model of MI was employed in 37 of these animals. Rats were first anesthetized with 3% isoflurane, then were intubated with a 16-gauge angiocatheter and mechanically ventilated (Hallowell EMC, Pittsfield, MA) with 1% isoflurane throughout the procedure. The left ventricle of the heart was exposed via a thoracotomy through the left fourth intercostal space. A 7-0 polypropylene suture was placed around the left anterior descending (LAD) artery 2 mm below the left atrium, and briefly snared to visually confirm the area of ischemia, and then tied into place permanently to produce an anterolateral MI of approximately 30% of the LV. The animals were then randomized to receive either a saline injection (100 μ l, n = 18) or an ESA injection (6 μ g/kg,

n = 19) into four separate peri-infarct locations. An additional group (n = 17), which served as a sham control, also underwent a thoracotomy, but did not receive an LAD ligation, or injection. In all groups, the thoracotomy was closed in multiple layers. All groups received subcutaneous injections of granulocyte macrophage colony-stimulating factor (40 μ g/kg) immediately at the end of the procedure. Buprenorphine was given for pain control (0.5 mg/kg), and animals were allowed to recover for 4 weeks.

Testing Specimen Preparation. Standardized myocardial specimens measuring 20 mm $\times$ 4 mm $\times$ 3 mm were excised from the LV in line from apex to base, generating a composite material comprising a spectrum between normal, borderzone, and infarcted myocardium (Fig. 1(a)). In order to minimize foci of stress at the ends of the sample, polytetrafluoroethylene pledgets were secured to each end with 7-0 polypropylene sutures. A loop of suture was also passed through the pledgets separately, in order to mount the specimens to an Instron testing system. Pledgets measured 4 mm $\times$ 4 mm $\times$ 1.6 mm. All samples were mounted in the same orientation onto the Instron. Strips were marked with two Verhoeff's stain lines 5 mm apart surrounding the borderzone for optical tracking, and imaged in two planes in order to obtain initial cross-sectional area. The samples were processed and tested immediately upon excision (Fig. 1(b)).

Biomechanical Testing. Specimens were placed in a 37 $^{\circ}$ C temperature-regulated bath of phosphate buffered saline and mounted to an Instron 5543 Material Testing System with a 5 N six axis load cell. Specimens were first preloaded at 0.05 N, at which point gauge length was recorded. Twenty cycles of preconditioning were performed (0.05 N–0.1 N at a rate of 0.1 mm/s). After a 60 s hold at 0.05 N, three increments of stress-relaxation were performed with each increment displaced rapidly by an additional 0.5 mm at the rate of 0.5 mm/s. There was a 300 s hold period between each increment, where the extension was held constant. Following a return to gauge length (down 1.5 mm at -0.5 mm/s), and a 60 s hold, a ramp to failure at a rate of 0.1 mm/s was performed [12–14]. The test was stopped as soon as the specimen failed, and lasted a total of 23 min. Load data were captured continuously in Bluehill 2 (Instron, Norwood, Mass) and custom image acquisition software, running in LABVIEW (National Instruments, Austin, TX) was used to acquire optical data at a sampling rate of 1 frame/s.

Statistical Analysis. All analyzed variables approximated a normal distribution, and values for continuous variables were reported as mean $\pm$ SD. A one-way repeated measures analysis of variance with Tukey's HSD correction was used for all pairwise comparisons of continuous variables between groups. Statistical significance was set at p < 0.05. Analyses were performed with GRAPHPAD PRISM (GraphPad Software, San Diego, CA) statistical software package, version 6.01.

Results

Myocardial Material Properties. A custom software package running in MATLAB (Natick, Mass) was utilized to analyze the load data and the optical data. Load data were divided by the cross-sectional area to determine stress, and strain was obtained by selecting two regions of interest (ROIs) for each stain line. The viscoelastic properties were quantified by analyzing the incremental stress-relaxation ramps [15]. Percent relaxation was calculated for each ramp, and then compared as an average for each specimen. The three increments from the sham group had average relaxation rates of 67% $\pm$ 7.0, 75% $\pm$ 6.0, and 77% $\pm$ 5.0, respectively. Infarcted muscle from the saline group had average relaxation rates of 64% $\pm$ 9.0, 74% $\pm$ 6.0, and 77% $\pm$ 5.0. There was no statistically significant difference between the sham and saline groups for any of the ramps (p = 0.338 for the middle ramp). The ESA treatment group had average relaxation rates of 64% $\pm$ 6.0, 74% $\pm$ 4.0, and 77% $\pm$ 5.0, respectively. There was

Fig. 1 (a) Representative image of a sham control heart. Specimens were excised from the LV in line from apex to base (white dashed lines). (b) Ventricular biopsies were mounted to an Instron 5543 material testing system. The purple squares indicate the ROIs used for strain analysis.

no statistically significant difference between the sham and ESA groups for any of the ramps ($p = 0.202$ for the middle ramp).

In order to further elucidate the viscoelastic properties, the rate at which our tissue relaxed during each stress-relaxation ramp was analyzed (Figs. 2(a)–2(c)). Specifically, we determined the relaxation rate of the last 5% of the stress-relaxation cycle [16]. Ventricular biopsies from the sham group had the fastest relaxation rate, with the three increments having an average relaxation rate of $32.8 \mu\text{N/s} \pm 4.0$, $41.6 \mu\text{N/s} \pm 5.3$, and $51.5 \mu\text{N/s} \pm 5.5$, respectively. Infarcted muscle from the saline group had the slowest relaxation rate, with rates of $24.9 \mu\text{N/s} \pm 10.7$, $35.4 \mu\text{N/s} \pm 5.1$, and $46.0 \mu\text{N/s} \pm 5.8$. The first and second ramps demonstrated a significant difference in relaxation rates between the saline and sham groups ($p = 0.0284$ and $p = 0.0065$, respectively). Muscle from animals in the ESA treatment group demonstrated a restoration of normal properties ($29.9 \mu\text{N/s} \pm 5.8$, $38.3 \mu\text{N/s} \pm 3.0$, and $48.3 \mu\text{N/s} \pm 5.2$), which were faster in each ramp compared with the saline group (Fig. 2(d)). Additionally, the bilinear region of the ramp to failure was identified for each test. Inspection of the load versus displacement curve yielded the bilinear region located in the first part of the ramp to failure. This was used to calculate the break point and ultimately the transition strain, which is the point where the two best fit lines meet (Fig. 3(a)). Muscle from animals in the sham group had an average break point of $3.94 \text{ mm} \pm 0.48$, while biopsies from the ESA treatment group had an average break point of $4.03 \text{ mm} \pm 0.43$. Ventricular biopsies from the saline group had an average break point of $4.71 \text{ mm} \pm 0.76$. There was a statistically significant difference in the transition strain between both the saline control and sham groups ($p = 0.0042$) and saline and ESA treatment groups ($p = 0.0110$) (Fig. 3(b)).

Discussion

In this study, we quantified the changes in the viscoelastic material properties of cardiac muscle which occur as a result of a myocardial infarction and how treatment with ESA affects these properties [17]. We previously found significant differences in the elastic properties of infarcted ventricular myocardium, however in this work, we aimed to explore the viscoelastic data in an effort to further examine the mechanics of this unique tissue. We developed a comprehensive loading protocol that tests the viscoelastic properties of our samples, in order to gain a better understanding of the changes brought about by our angiogenic peptide. ESA therapy alters the mechanics of borderzone myocardium, and prior work has demonstrated elevated stress in the borderzone region in

a finite element model [18,19]. Therefore, strain data were captured in the borderzone of the specimens.

Samples were tested on longitudinally oriented ventricular muscle biopsies as opposed to circumferentially in order to minimize the effects of the natural curvature of the sample in the circumferential direction. During preliminary work, we found that mounting the samples circumferentially resulted in a large increase in stress on the endocardial surface compared with the epicardial surface of the sample. Consistently, this was the cause of premature failure of the samples. Furthermore, because of the apical location of the infarct, and since the study was limited to LV mechanics, we developed a way of longitudinally testing the myocardium that was sensitive enough to detect the impact our angiogenic treatment had on the tissue. Excising the entire ventricular strip of myocardium from apex to base gave us the most consistent specimens.

Collagen and elastin are two key components of the ECM which make major contributions to the material properties of cardiac muscle [17,20]. We have shown that our ESA treatment limits expansion of the infarct by neovascularizing the borderzone, thereby preserving the ECM and ultimately giving treated muscle mechanical behavior more similar to that of native tissue. In particular, the restoration of the tensile modulus with ESA therapy demonstrates that reducing the collagen content restores the tissue's elasticity closer to that of its healthy, uninjured state. A reduction in tensile modulus of the tissue correlates to a decrease in LV wall stress, thereby halting the progression toward adverse ventricular remodeling.

Myocardium is characterized as a viscoelastic tissue, and analysis of the relaxation rates and transition strains is widely employed in the literature to study viscoelastic behavior [10,13,14,21–23]. In this study, there was a trend toward increased relaxation rates and decreased transition strains in the ESA group compared with saline control. Infarcted muscle relaxed at a slower rate than noninfarcted muscle, which correlates to the loss of function seen in vivo with LV dilation and diminished contractility secondary to a myocardial infarction. Furthermore, the transition point in a bilinear fit can be used to better understand the dynamic behavior of the collagen fiber network in the tissue [23]. A longer toe region of the tissue, as indicated by the larger transition strain, may indicate more presence and recruitment of collagen fibers, which we see in stiffer ventricles with higher collagen content in the infarcts. The data we present here demonstrate the importance of limiting collagen deposition within the borderzone of an infarct. The slower relaxation rate and the higher transition strain in the saline group demonstrate a decreased response to load, which is indicative of heart tissue that has become stiffer and hypocontractile. These viscoelastic results

Fig. 2 (a) Samples were all preloaded to 0.05 N, at which point gauge length was set. The three incremental stress-relaxation ramps were analyzed to determine the viscoelastic properties of the muscle. (b) The peak and the equilibrium locations of the ramp. (c) The load data for each ramp were normalized using the peak and equilibrium loads in order to calculate the relaxation rate for the last 5% of each cycle. (d) The relaxation rates for each stress-relaxation ramp were analyzed independently for each experimental group. The trend of the ESA treatment group relaxing faster than the saline group is apparent at each increment ($p = 0.2165$ for the first increment and $p = 0.2804$ for the second increment). $*p = 0.0284$ and $**p = 0.0065$.

offer corroboratory data with previously reported tensile modulus data, and add to our understanding of the effectiveness of ESA in limiting diastolic dysfunction, ultimately slowing the progression toward heart failure.

Diastolic dysfunction is commonly seen in cases of ischemic cardiomyopathy, where ventricular compliance is compromised, leading to significant impairment of isovolumetric relaxation [24]. From a mechanical standpoint, diastolic dysfunction will result in an

Fig. 3 (a) The bilinear region of the ramp to failure was identified for each specimen and analyzed separately from the linear elastic region. A bilinear curve was fit to the data and used to calculate the break point and transition strain. (b) The difference in the break point between the two best fit lines in the bilinear region was quantified. * $p = 0.0110$ and ** $p = 0.0042$.

alteration in the stress-relaxation rate [5]. A fast relaxation rate, as seen in healthy tissue, is conducive to rapid diastolic filling. Hearts that have an increased collagen concentration exhibit viscous losses, which is especially evident during diastolic relaxation. This impairs filling of the heart, decreasing the efficiency of pump function.

The primary limitation of the current study is that the samples were tested as ventricular myocardial strips under uniaxial loading, as opposed to as excised sheets of myocardium under biaxial loading. Ventricular myocardium is thick-walled and anisotropic; therefore ongoing work with biaxial testing will be able to further characterize the mechanical response of tissue treated with ESA. In the present study we quantified the viscoelastic response of ESA treated tissue in order to further our understanding of angiogenic therapy for heart failure.

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