Normalization of Postinfarct Biomechanics Using a Novel Tissue-Engineered Angiogenic Construct

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Background—Cell-mediated angiogenic therapy for ischemic heart disease has had disappointing results. The lack of clinical translatability may be secondary to cell death and systemic dispersion with cell injection. We propose a novel tissue-engineered therapy, whereby extracellular matrix scaffold seeded with endothelial progenitor cells (EPCs) can overcome these limitations using an environment in which the cells can thrive, enabling an insult-free myocardial cell delivery to normalize myocardial biomechanics.

Methods and Results—EPCs were isolated from the long bones of Wistar rat bone marrow. The cells were cultured for 7 days in media or seeded at a density of 5×10⁶ cells/cm² on a collagen/vitronectin matrix. Seeded EPCs underwent ex vivo modification with stromal cell–derived factor-1α (100 ng/mL) to potentiate angiogenic properties and enhance paracrine qualities before construct formation. Scanning electron microscopy and confocal imaging confirmed EPC–matrix adhesion. In vitro vasculogenic potential was assessed by quantifying EPC cell migration and vascular differentiation. There was a marked increase in vasculogenesis in vitro as measured by angiogenesis assay (8 versus 0 vessels/hpf; P=0.004). The construct was then implanted onto ischemic myocardium in a rat model of acute myocardial infarction. Confocal microscopy demonstrated a significant migration of EPCs from the construct to the myocardium, suggesting a direct angiogenic effect. Myocardial biomechanical properties were uniaxially quantified by elastic modulus at 5% to 20% strain. Myocardial elasticity normalized after implant of our tissue-engineered construct (239 kPa versus normal=193, P=0.1; versus infarct=304 kPa, P=0.01).

Conclusions—We demonstrate restoration and normalization of post–myocardial infarction ventricular biomechanics after therapy with an angiogenic tissue-engineered EPC construct. (Circulation. 2013;128[suppl 1]:S95-S104.)

Key Words: extracellular matrix ■ progenitor cell ■ revascularization ■ tissue engineering

It is estimated that 16.8 million Americans experience coronary artery disease, and 5.7 million have congestive heart failure.¹ In the present era, we have been effective in acutely treating myocardial infarction and the ensuing negative sequelae, namely cardiogenic shock and immediate death. Unfortunately, complete revascularization with either percutaneous coronary intervention or coronary artery bypass grafting is only possible in 63% to 80% of patients with ischemic heart disease, leaving a large number of patients with nonrevascularizable myocardium.² Immediate postinfarction treatments do not restore microvascular perfusion or address the underlying ventricular cellular pathophysiology, often failing to prevent adverse ventricular remodeling, loss of normal ventricular biomechanics, and heart failure.³–⁵ Although we have been able to diminish mortality from acute myocardial infarction, we have not been able to successfully prevent progression to heart failure or normalize myocardial biomechanics. A justifiably large interest has been devoted to discovery of alternate strategies to restore microcirculation.

Since the discovery of bone marrow–derived endothelial progenitor cells (EPC), the concept of postnatal vasculogenesis as a potential therapy for the sequelae of ischemic heart disease has been intensively investigated.⁶⁷ Many of these therapies have shown a functional benefit following EPC or chemokine therapy in the preclinical setting, but few have been able to demonstrate a long-term clinical benefit.⁸–¹⁴ The lack of clinical translatability may be because of the low circulating EPC number available for cytokine therapy and a high percentage of cell death and systemic dispersion that accompany EPC injection, often with <1% of injected cells remaining.¹⁵–¹⁷ We propose a novel therapy, whereby extracellular matrix (ECM) scaffold seeded with EPCs can overcome these limitations by providing a native environment in which the cells can thrive, enabling an insult-free delivery to the area of interest in high cellular concentration. In this strategy, EPCs are thought to promote neovasculogenesis by 2 separate mechanisms. First, bone marrow–derived EPCs will incorporate themselves into newly formed vessels. Second, EPCs demonstrate paracrine...
capabilities by eluting proangiogenic cytokines that induce vessel growth by promoting the migration and proliferation of circulating endothelial progenitor/precursor cells.\textsuperscript{18–22} The preservation of myocardial perfusion should maintain myocardial integrity and critical cardiomyocyte biomechanics.

Stromal cell–derived factor (SDF)-1α is a potent chemokine that exhibits considerable angiogenic potency.\textsuperscript{23–25} This chemokine interacts with the CXCR4 receptor, which is highly expressed on EPCs, to induce transendothelial migration, recruitment, and vessel formation via a phosphoinositide-3-kinase–mediated pathway. SDF markedly potentiates EPC paracrine activity, vascular engraftment, and neovasculogenic response.\textsuperscript{26–29}

In this study, we sought to develop an easily clinically translatable therapeutic strategy that both provides high concentrations of EPCs in close proximity to ischemic myocardium and amplifies the paracrine effects of EPCs. We hypothesize that surgically implanting an EPC matrix that has been ex vivo supercharged by pretreatment with SDF to serve as a myocardial paracrine factory will induce a robust neovasculogenic response by direct EPC migration, as well as elution of high concentrations of chemokines, thereby maximizing microvascularization. We have previously demonstrated a significant improvement in both perfusion and hemodynamics after this therapeutic strategy.\textsuperscript{30} The aim of this study was to establish molecular mechanisms and tissue-level biomechanical improvements associated with this novel tissue-engineered, cell-mediated therapy. We hypothesized that a tissue-engineered construct that was developed by seeding ECM with SDF-prime EPCs would provide a robust angiogenic response by both direct angiogenic and paracrine effects, leading to a preservation of myocardial biomechanics. Tissue-level myocardial analysis was performed using novel techniques to quantify biomechanical properties.

**Methods**

**Animal Care and Biosafety**

Male adult, Wistar rats (250–300 g) were obtained from Charles River Laboratories (Boston, MA). Food and water were provided ad libitum. This investigation adheres to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (Eight Edition, revised 2011). This study conforms to institutional ethical review and has been approved by the University of Pennsylvania Institutional Animal Care and Use Committee. All measurements were performed by investigators blinded to animal treatment.

**EPC Isolation and Construct Formation**

Bone marrow mononuclear cells were isolated from the long bones of syngeneic adult, male Wistar rats by density-gradient centrifugation (Histopaque 1083; Sigma, St. Louis, MO). After isolation, the cells were seeded on a Food and Drug Administration–approved, commercially available collagen–vitronectin ECM scaffold (ECM, CorMatrix, Alpharetta, GA) at a density of 5 million cells per 1.0 cm² scaffold. The seeded scaffold was then cultured in endothelial basal medium-2 supplemented with endothelial cell growth media-2 singleQuot containing human epidermal growth factor, fetal bovine serum, vascular endothelial growth factor, human fibroblast growth factor-B, R3 insulin–like growth factor I, ascorbic acid, heparin, gentamicin, and amphotericin-B for 6 days (Lonza, Allendale, NJ). The construct was washed every 48 hours, with removal of nonadherent cells. Twenty four hours before implantation, the scaffold was primed with SDF (R & D Systems, Minneapolis, MN) at a concentration of 100 ng/mL to enhance EPC vasculogenic properties for the construct. A subset of EPCs were isolated from transgenic rats ubiquitously expressing enhanced green fluorescent protein (eGFP), with a 2-point mutation to enhance spectral characteristics, fluorescence, and stability, which we made use of for cell fate tracking purposes. The eGFP colony was prepared from eGFP transgene (cDNA fragment of eGFP derived from enhanced green fluorescent protein vector No. 6077-1, Clontech Laboratories, and pCXN2 expression vector containing cytomegalovirus enhancer, chicken β-actin enhancer–promoter, and rabbit β-globin poly(A) signal), obtained from Dr Kobayashi, Jichi Medical School, Tochigi, Japan.\textsuperscript{31}

**EPC Phenotypic Characterization**

Appropriate EPC phenotypic isolation was confirmed using a combination of flow cytometry and immunohistochemical labeling. After 7 days in culture to select for the EPC phenotype, the phenotype was confirmed by flow cytometry on a BD FACSCalibur (BD Biosciences, San Jose, CA). Cells were mechanically isolated, collected, and fixed in 4% formalin for 10 minutes at 37°C. Cells were then stained for vascular endothelial growth factor receptor (VEGFR) 2 (ab2349; Abcam, Cambridge, MA) and CD34 (ab8158; Abcam) using unlabeled primary antibodies. VEGFR2 primary antibody was coupled with Alexa Fluor 488 using a conjugated secondary antibody (ab6798; Abcam), and CD34 was labeled with Alexa Fluor 647 (ab50159; Abcam). Briefly, cells were aliquoted into 1×10⁶ cells per assay tube. After blocking in incubation buffer, primary antibody was added in a 1:500 dilution and incubated for 60 minutes. After rinsing with blocking buffer, cells were incubated with the secondary antibody in a 1:1000 dilution. Flow cytometry was begun by adjusting the photomultiplier tube voltages to place the EPCs on scale for forward and side scatter and to establish the upper right quadrant for determining the double-positive cell population. Data analysis was performed using Kaluza (Beckman Coulter, Brea, CA). A portion of the mononuclear cell fraction was used for Fluorescence Minus One and compensation staining. Fluorescence Minus One was used for identifying negative populations. Cell viability was assessed using 7-Aminoactinomycin D (BD Biosciences) labeling. EPCs were defined as 7-Aminoactinomycin D–VEGFR2–CD34– mononuclear cells.\textsuperscript{32–36} As a corroborative study, we immunocytochemically confirmed EPC phenotype (VEGFR2, isoelectin, and di-iodinated acetyl low-density lipoprotein uptake), as we have previously published.\textsuperscript{30}

**Visualization of ECM–EPC Binding**

To confirm EPC binding to the ECM, samples were visualized with scanning electron microscopy (SEM) and subsequently with confocal microscopy. Samples for SEM were fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 mol/L cacodylate buffer, pH 7.4, overnight at 4°C 0, 2, 5, and 7 days after seeding on ECM. The samples were postfixed in 2.0% osmium tetroxide for 1 hour, washed again in buffer, and dehydrated in a graded ethanol series. Samples were treated with several changes of hexamethyldisilazane and then again in buffer, and dehydrated in a graded ethanol series. Samples were postfixed in 2.0% osmium tetroxide for 1 hour, washed again in buffer, and dehydrated in a graded ethanol series. Samples were treated with several changes of hexamethyldisilazane and then allowed to air dry before mounting and sputter coating with gold/palladium. SEM examinations were performed in a Philips XL20 SEM. Images were acquired with Phillips Microscope Control Software (Philips FEI, Hillsboro, OR). The samples were imaged with scanning laser confocal microscopy (Zeiss LSM 710, Oberkochen, Germany) under ×20 magnification.

**In Vitro Analysis of Vasculogenic Potential of the Engineered Construct: Matrigel Angiogenesis Assay**

To quantify the direct vasculogenic potential of the engineered construct, in vitro angiogenesis was assessed using a Matrigel angiogenesis assay. A 96-well plate coated with growth factor–reduced Matrigel (BD Biosciences) was thawed at 4°C. The Matrigel was allowed to polymerize at 37°C in 5% CO₂ for 30 minutes. Seven-day EPCs cultured in endothelial–specific media on vitronectin-coated plates were trypsinized and brought to a concentration of 40,000 cells/
Chamber was loaded with our desired experimental group: 1000 struct. ECM size was standardized to 0.85 cm². A 560-μL of endothelial basal medium-2, ECM, ECM seeded with EPC, or confluent cell growth media-2 were trypsinized, counted, and brought to a magnification and analyzed via LASAF version 2.0.2 software (Leica). Cells were visualized on a DF5000B Leica Fluorescent scope at ×20 magnification and collected 48 hours after media change. Fifty microliters of undifferentiated media alone, acellular ECM, ECM+EPC, construct were compared with those implanted with acellular ECM alone.

**Quantification of Chemotactic Properties**

SDF1-α and VEGF concentrations were assessed using enzyme-linked immunoassays (ELISA; R & D Systems, Minneapolis, MN). Briefly, culture media was collected from the dish containing the desired samples (media alone, acellular ECM, ECM+EPC, construct). For SDF samples, the media was changed after SDF treatment and collected 48 hours after media change. Fifty microliters of undifferentiated supernatants was added to precoated ELISA plates with 50 μL of assay diluent. The plates were covered and incubated for 2 hours at room temperature. Plates were washed, and conjugated secondary antibody was added to each well. The plates were again incubated for 2 hours at room temperature. After incubation, the plates were washed, and 200 μL of substrate solution was added to each well and the plate was incubated for 30 minutes. The reaction was stopped with 50 μL of stop solution and the optical density read at 450 nm. The average zero standard was subtracted from the average duplicate readings of each sample and standard. The sample concentration was calculated using the average duplicate reading for the standards.

**Induction of Heart Failure**

Male Wistar rats were anesthetized in a 2-L induction chamber (VetEquip, Pleasantville, CA) with 3% isoflurane continuously delivered, endotracheally intubated with a 16-gauge angiocatheter, and mechanically ventilated (Hallowell EMC) with 1.0% isoflurane and 99% O₂. Uniaxial tensile testing was conducted with a NanoTest 5543 Electromechanical Testing Machine (Instron, Norwood, MA) with a 5 N, 6-axis load cell. Bluehill 2 Materials Testing Software was used to control the machine and capture load and displacement data. Custom image acquisition software, running in LabVIEW 2010 (National Instruments, Austin, TX) was used to acquire optical data at a sampling rate of 1/s. Specimens were first preloaded to 0.05 N, at which point the gage length was set. Then (1) 20 cycles of preconditioning were performed (0.05–0.1 N at 0.1

muscle strip and peri-infarct myocardial samples were isolated for biomechanical analysis. In addition, 4-week explanted hearts were stained with Masson trichrome, and total collagen fraction was quantified (ImageJ, National Institutes of Health) to assess differential collagen content with construct treatment.

**Confirmation of EPC Migration Into the Myocardium**

Our first step, in vivo, was to establish that EPCs from the construct had the ability to migrate from the construct into the myocardium. To assess EPC migration, eGFP+ EPCs from transgenic rats were used to create the construct. This construct was then implanted onto the myocardium after left anterior descending ligation. The hearts were explanted 4 weeks after implant, flushed with PBS, and distended with optimum cutting temperature embedding compound (Electron Microscopy Sciences, Hatfield, PA). The hearts were then frozen at −80°C and cryosectioned in 10-μm slices and visualized with scanning laser confocal microscopy (Zeiss LSM 710, Oberkochen, Germany) under x20 magnification. Visualization was performed in the peri-infarct borderzone, which was defined as 1 microscopic field away from the infarct. Hearts treated with construct were compared with those implanted with acellular ECM alone.

**Analysis of Global Ventricular Elasticity and Function**

Before the heart was explanted, transthoracic echocardiography was performed to assess myocardial function using echocardiography (Phillips Sonos 5500 revD system with an S12 probe at 12 MHz and a 3-cm depth of penetration). Ventricular measurements were performed according to the American Society for Echocardiography leading-edge method. Ejection fraction and fractional shortening were quantified. Elastance and σ (the coefficient of relaxation) were quantified using a 2F intraventricular pressure–volume catheter inserted retrograde via the carotid artery (Millar Instruments).

**Analysis of Ventricular Biomechanics**

To analyze ventricular biomechanics, myocardium was harvested from animals 6 weeks after left anterior descending ligation and treatment. A 15-mm by 3-mm strip of myocardium was excised from the left ventricle, taken in line from apex to base. This was determined to generate the most standardized specimens, where anisotropic effects would be minimized. Miniaturized polytetrafluoroethylene pledges were sutured to the ends of the strip. The strips were then placed in a temperature-regulated bath of PBS maintained at 37°C. Uniaxial tensile testing was conducted with an Instron 5543 Electromechanical Testing Machine (Instron, Norwood, MA) with a 5 N, 6-axis load cell. The strips were then placed in a temperature-regulated bath of PBS maintained at 37°C. Uniaxial tensile testing was conducted with an Instron 5543 Electromechanical Testing Machine (Instron, Norwood, MA) with a 5 N, 6-axis load cell. The strips were then placed in a temperature-regulated bath of PBS maintained at 37°C. Uniaxial tensile testing was conducted with an Instron 5543 Electromechanical Testing Machine (Instron, Norwood, MA) with a 5 N, 6-axis load cell. The strips were then placed in a temperature-regulated bath of PBS maintained at 37°C. Uniaxial tensile testing was conducted with an Instron 5543 Electromechanical Testing Machine (Instron, Norwood, MA) with a 5 N, 6-axis load cell. The strips were then placed in a temperature-regulated bath of PBS maintained at 37°C. Uniaxial tensile testing was conducted with an Instron 5543 Electromechanical Testing Machine (Instron, Norwood, MA) with a 5 N, 6-axis load cell.
mm/s); (2) after a 60-second hold at 0.05 N, 3 increments of stress relaxation (SR) were performed, with each increment displaced rapidly by an additional 0.5 mm at the rate of 0.5 mm/s; (3) a 300-second hold period between each increment; (4) return to gage length (down 1.5 mm at −0.5 mm/s); and finally (5) after a 60-second hold, (6) a ramp to failure at a rate of 0.1 mm/s was performed.

Optical strain data were integrated with the Instron load data using Optikos (custom-made software run in MATLAB, Natick, MA), which generated stress–strain curves for each ventricular strip. Strain was obtained by selecting 2 regions of interest for each stain line. The regions of interest were incrementally tracked throughout the ramp to failure. Strain was then mapped in the loading axis (y) and normal to it (x). One sample had to be discarded at this point because it exhibited a large displacement normal to the loading direction. We used these data to corroborate visual findings, where it was noted that this sample was abnormally curved. The tensile moduli ($E$) were calculated for 5% to 20% strain using:

$$E = \frac{\sigma}{\varepsilon}$$

Subsequently, SR was quantified by obtaining the area under the stress–strain curve for each increment. The load function was integrated for each increment of SR between the time at peak force ($t_1$) and the time at equilibrium force ($t_2$). The area was taken from the peak force to the equilibrium force, just before the next incremental ramp, to obtain the SR parameter for the given (ith) increment as given by:

$$SR_i = \int_{t_1}^{t_2} F(t)\, dt$$

The SR increments were performed under low strains to preserve the integrity of the sample and are used to confirm that all the samples had a similar amount of SR.41–43

Statistical Analysis

Quantitative data are expressed as median with first and third quartiles. Statistical significance was evaluated using the Wilcoxon rank-sum test for comparison between 2 samples. For comparison among >2 means, statistical analyses were performed using the Kruskal-Wallis test to analyze for differences among samples. A $P<0.05$ was considered statistically significant.

Results

EPC Phenotypic Characterization

Phenotypic characterization confirmed that we had selected endothelial precursor cell subtypes from the original bone marrow mononuclear cell fraction. Analysis by flow cytometry demonstrated that our cell population had phenotypic characteristics of endothelial precursor cells (CD34+VEGFR2+; Figure 2). Subsequent investigation by immunocytochemical analysis confirmed that at day 7 in culture, isolated mononuclear cells grown on ECM show expression of VEGFR2, isolectin, and di-iodinated acetyl low-density lipoprotein uptake characteristic of EPCs. Phenotypic confirmation of EPC characteristics revealed vasculogenic potential for the cells within the tissue-engineered construct.
Confirmation of Endothelial Precursor Cell Binding to the ECM Scaffold

SEM revealed the presence of densely adherent cells to the ECM scaffold, which was not evident in the acellular scaffold. The cells were evident immediately after seeding and subsequently on days 2, 5, and 7 after implant. To confirm the presence of the desired cell population on the matrix, EPCs were harvested from ubiquitously expressing transgenic eGFP+ rodents and subsequently implanted onto the same ECM scaffold. Imaging by scanning laser confocal microscopy demonstrated the presence of eGFP+ cells on the ECM. These cells were absent in the acellular scaffold. The presence of these cells ≤7 days after binding to the matrix demonstrates viability and adhesion to the construct for 1 week, suggesting that the cells are present at the time of construct implant (Figure 3).

In Vitro Demonstration of Enhanced Vasculogenic Potential

In vitro analysis of vasculogenic potential, by Matrigel assay, revealed a significant induction in vessel formation, length, and branching by our construct compared with the 3 control groups (n=5/group). There was a profound increase in vessel formation (8 vessels/hpf [first quartile-7, third quartile-8]), vessel length (586 µm [first quartile-570, third quartile-587]), and branch formation (11 branches/hpf [first quartile-10, third quartile-12]) with the construct (Figure 4). As expected, there was minimal vasculogenesis evident with either media alone (0 vessels/hpf [first quartile-0, third quartile-0]) or acellular cormatrix (0 vessels/hpf [first quartile-0, third quartile-0]). Priming of the EPCs with SDF seems to significantly induce the direct vasculogenic potential of the construct. A statistically significant increase in vessel formation (8 [first quartile-7, third quartile-8] versus 6 [first quartile-5.5, third quartile-6.25] vessels/hpf; P=0.03), vessel length (586 [first quartile-570, third quartile-587] versus 302 [first quartile-291, third quartile-308] µm; P=0.007), and branch formation (11 [first quartile-10, third quartile-12] versus 5.5 [first quartile-4.75, third quartile-6.25] branches/hpf; P=0.007) was demonstrated with construct compared with ECM+EPC groups. This in vitro assay demonstrates significant direct EPC-mediated angiogenic potential of the engineered construct. These findings help explain the profound increase in perfusion we have demonstrated in previous small animal studies using this construct.30

Increased In Vitro Chemotactic Properties

By using a Boyden chamber with a controlled chemotactic stimulus, we were able to quantify the ability of an individual treatment group to attract EPCs in vitro (n=10/group). Analysis demonstrated a marked increase in EPC migration in the presence of the construct compared with media alone (145 [first quartile-139.75, third quartile-151.25] versus 42 [first quartile-38.25, third quartile-47] cells/hpf; P=0.0004), acellular ECM (43 [first quartile-36, third quartile-56] cells/hpf; P=0.0002), and ECM seeded with nonprimed EPCs (80.5 [first quartile-72.25, third quartile-90.75] cells/hpf; P=0.0002; Figure 5). Interestingly, ECM seeded with EPCs...
was also highly chemotactic for EPCs compared with media control \((P=0.0002)\) and acellular ECM \((P=0.0003)\). This suggests a significant paracrine ability of both construct and ECM+EPCs. Priming the EPCs with SDF significantly enhanced the paracrine properties of the engineered construct. There was no statistical significance between the ECM only and media groups \((P=0.8)\).

### Analysis of EPC Migration From the Construct to the Myocardium

Confocal microscopy of peri-infarct borderzone myocardium after implant of construct seeded with eGFP+ EPCs revealed the presence of a large number of eGFP+ cells within the myocardium, deep from the epicardial surface \((n=7)\). When corresponding regions from hearts implanted with acellular ECM were visualized as a control, there was an absence of fluorescent cells and signal \((16 \text{ [first quartile-9, third quartile-19]} \text{ versus } 0 \text{ [first quartile-0, third quartile-0]} \text{ cells/hpf}; P=0.005; n=5/group; Figure 6)\). This suggests that the cells from the construct have migrated from the ECM to the myocardium. When taken into account the in vitro Matrigel angiogenesis assay results, it seems that the EPCs within the construct have a direct migratory vasculogenic influence on enhancing perfusion.

### Enhanced Paracrine Properties

Analysis of in vitro secretion of VEGF and SDF-1α with ELISA demonstrated a marked increase in VEGF \((439 \text{ [first quartile-254, third quartile-582]} \text{ versus } 36 \text{ [first quartile-22, third quartile-47] pg/mL}; P=0.002)\) and SDF-1α \((1184 \text{ [first quartile-925, third quartile-1741]} \text{ versus } 14 \text{ [first quartile-4, third quartile-27] pg/mL}; P=0.003)\) levels from construct compared with media control \((n=5/group; Table 1)\). There was significant increase in secretion of both VEGF \((439 \text{ [first quartile-254, third quartile-582]} \text{ versus } 266 \text{ [first quartile-249, third quartile-282] pg/mL}; P=0.03)\) and SDF-1α \((1184 \text{ [first quartile-925, third quartile-1741]} \text{ versus } 13 \text{ [first quartile-9, third quartile-18] pg/mL}; P=0.001)\) by the construct after priming compared with the nonprimed ECM+EPC group. Based on these findings, it seems that our construct manifests significant chemokine secretion and likely significant paracrine properties.

### Normalization of Biomechanics After Therapy With the Engineered Construct

Quantification of SR parameters demonstrated no statistical difference among the 4 groups: control (ligation only), acellular ECM, ECM+EPC, and construct (Table 2). The SR parameters were similar for initial, second, and third SR increments.
Therefore, all 4 groups exhibited similar viscoelastic behavior. Subsequent calculation of elastic modulus from the slope of the stress–strain curves demonstrated a normalization of myocardial elasticity with construct compared with normal myocardium (239 [first quartile-186, third quartile-265] kPa; P=0.1) versus infarct (304 [first quartile-272, third quartile-344] kPa; P=0.01; ECM+EPC=240 [first quartile-210, third quartile-250] kPa; P versus construct=0.02; construct=63.1% [first quartile-62.9, third quartile-66.5]). Improved myocardial function was confirmed by fractional shortening (control=17.0% [first quartile-16.4, third quartile-18.2] P versus construct=0.01; ECM+EPC=24.0% [first quartile-21.0, third quartile-25.0] P versus construct=0.02; construct=30.0% [first quartile-25.4, third quartile-34.7]) and ejection fraction (control=36.5% [first quartile-39.0, third quartile-41.9] P versus construct=0.004; ECM+EPC=52.9% [first quartile-51.2, third quartile-54.4] P versus construct=0.003; construct=63.1% [first quartile-62.9, third quartile-66.5]). Intraventricular pressure–volume analysis confirmed global preservation of myocardial elasticity (Table 3), corroborating the findings from the uniaxial testing.

Discussion

In this study, we demonstrate a novel tissue-engineered therapy to enhance myocardial vasculogenesis and improve ventricular biomechanics. Currently, we have investigated the mechanism of therapy. In vitro analysis has demonstrated our construct to have both direct angiogenic properties as manifested by enhanced vasculogenesis with the Matrigel assay and enhanced paracrine properties. Both the Boyden chemotaxis assay and measurement of expressed chemokine concentrations demonstrate a robust secretion of angiogenic factors from our construct. Furthermore, analysis of tagged EPCs from transgenic eGFP+ rats has demonstrated a significant migration of progenitor cells from the epicardial ECM to the deep myocardium, further demonstrating the ability of these cells to move to ischemic myocardium and potentially play a direct vasculogenic role. In combination, it seems that the construct has a direct, migratory angiogenic effect, as well as strong paracrine ability to attract circulating EPCs for subsequent vasculogenesis. The combined mechanism, along with delivery of EPCs long-term in high concentrations, may likely contribute to a significant

Figure 5. Representative images after in vitro Boyden chemotaxis assay. Endothelial progenitor cell (EPC) migration was quantified in relation to media alone, acellular extracellular matrix (ECM), ECM+EPCs, or construct. There was a statistically significant increase in EPC migration in relation to construct compared with 3 control groups (magnification, ×20; dot plot with median±first and third quartile).

Figure 6. Representative images from the peri-infarct border zone 4 weeks after left anterior descending ligation and implant of (A) acellular extracellular matrix (ECM) as control or (B) construct seeded with enhanced green fluorescent protein (eGFP)+ endothelial progenitor cells (EPCs). C, Myocardial vessel with incorporation of eGFP+ EPCs (green=eGFP, red=α-smooth muscle actin, blue=4',6-diamidino-2-phenylindole). There was a significant migration of eGFP+ EPCs noted from the ECM to the deep myocardium in the construct subset that was not present in the control group (magnification, ×20).
potentiation in vasculogenesis compared with traditional cell injection therapies. As we have previously published, in a small animal model of ischemic cardiomyopathy, this vasculogenic therapy translates to significantly enhancing myocardial perfusion and preserving ventricular function.30

EPCs have been shown to deposit collagen IV, fibronec-
tin, and laminin, which assemble into an organized web that allows incorporation into the ECM and subsequent migration in response to adequate chemotactic stimuli.44 Total internal reflection fluorescence microscopy has demonstrated multiple points of contact between EPCs and a fibronectin ECM.45 Adhesion of EPCs to ECM is largely dependent on $\alpha_5\beta_1$ integrin. Biophysical analysis of the EPC–ECM interaction has revealed that increases in shear stress decrease EPC adhesion and interaction with integrin ligands, supporting the hypothesis that seeding EPCs onto an ECM surface that is implanted onto the myocardium will prevent cell dispersion relative to direct injection. Varying integrin subunits, including $\alpha_v$, facilitate subsequent chemotaxis and transendothelial migration.46 Integrins seem to facilitate adherence of the EPCs to the ECM, as we have demonstrated in our article, as well as the ability of the cells to migrate from the ECM to the ischemic myocardium in response to adequate chemotactic stimuli.

Analysis of myocardial biomechanics (Table 2) suggests that this vasculogenic therapy contributes to maintenance of normal myocardial elasticity, therefore reducing the stiffness of the ventricular wall. A loss of elasticity and increased diastolic stiffness have been associated with worse outcomes and mortality in heart failure.47 Measuring the SR parameter by obtaining the area under the load versus time curve is an established way of quantifying viscoelastic behavior in biological tissue.41 It is interesting that we did not see any statistically significant

Table 1. In Vitro Secreted VEGF and SDF Levels as Measured by ELISA

<table>
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<th>VEGF, pg/mL</th>
<th>SDF-1α, pg/mL</th>
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<td>14 (first quartile-4, third quartile-27)</td>
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<td>41 (first quartile-30, third quartile-52)</td>
<td>45 (first quartile-41, third quartile-51)</td>
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<td>ECM+EPCs (n=5)</td>
<td>266 (first quartile-249, third quartile-282)</td>
<td>13 (first quartile-9, third quartile-18)</td>
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<tr>
<td>Construct (n=9)</td>
<td>439 (first quartile-254, third quartile-582)</td>
<td>1184 (first quartile-925, third quartile-1741)</td>
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Samples were collected from the media of the treatment groups to assess elution of these cytokines 48 h after media change. ECM indicates extracellular matrix; EPC, endothelial progenitor cell; and SDF, Stromal cell–derived factor.

Table 2. Quantified Variables of Myocardial Elasticity

<table>
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<th>Cross-Sectional Area, mm²</th>
<th>SR-1 (N×s) (n=4)</th>
<th>SR-2 (N×s) (n=4)</th>
<th>SR-3 (N×s) (n=4)</th>
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<td>33.09</td>
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<td>343.83</td>
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<td>P</td>
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<td>ECM (n=13)</td>
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<tr>
<td>Median</td>
<td>11.21</td>
<td>25.87</td>
<td>33.16</td>
<td>40.64</td>
<td>253.4</td>
</tr>
<tr>
<td>First quartile</td>
<td>9.65</td>
<td>25.64</td>
<td>32.73</td>
<td>38.04</td>
<td>241.74</td>
</tr>
<tr>
<td>Third quartile</td>
<td>12.98</td>
<td>26.37</td>
<td>34.40</td>
<td>43.96</td>
<td>311.48</td>
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<tr>
<td>P</td>
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<tr>
<td>ECM+EPCs (n=12)</td>
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<tr>
<td>Median</td>
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<td>24.51</td>
<td>30.25</td>
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<tr>
<td>First quartile</td>
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<td>29.87</td>
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<tr>
<td>Third quartile</td>
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<td>30.54</td>
<td>36.62</td>
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<td>P</td>
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<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Construct (n=9)</td>
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</tr>
<tr>
<td>Median</td>
<td>12.21</td>
<td>26.02</td>
<td>32.50</td>
<td>39.08</td>
<td>238.0</td>
</tr>
<tr>
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<td>10.50</td>
<td>24.71</td>
<td>29.72</td>
<td>34.73</td>
<td>170.31</td>
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<tr>
<td>Third quartile</td>
<td>13.02</td>
<td>27.18</td>
<td>34.92</td>
<td>43.04</td>
<td>258.09</td>
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<td>Normal myocardium (n=14)</td>
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<td>Median</td>
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<td>157.37</td>
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<tr>
<td>Third quartile</td>
<td>15.22</td>
<td>26.27</td>
<td>33.45</td>
<td>41.44</td>
<td>242.08</td>
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<tr>
<td>P</td>
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<td></td>
<td></td>
<td></td>
<td>0.1</td>
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There was no statistically significant difference in stress–strain relationship between the treatment groups. P values for tensile modulus are in comparison with construct. ECM indicates extracellular matrix; EPC, endothelial progenitor cell; and SR, stress-relaxation relationship.
The enhanced vasculogenic properties correlate with angiogenic benefit. Cell-mediated therapy is likely limited by a limited period of expression and protease degradation which limit long-term angiogenic benefit. Cytokine-mediated therapy may be limited by a limited period of expression provided by our therapy likely enhances perfusion, increases cardiomyocyte viability, limits scar expansion, and ultimately maintains myocardial biomechanics.

Unfortunately, although cytokine and cell therapy initially showed great promise for cardiovascular therapy after myocardial infarction, it has not demonstrated clinical translatability. Cytokine-mediated therapy may be limited by a limited period of expression and protease degradation which limit long-term angiogenic benefit. Cell-mediated therapy is likely limited by a lack of effective cell delivery and poor cell engraftment. The therapy outlined in this study was designed to provide sustained cell delivery to the ischemic myocardium, enhance local cytokine expression, and provide rapid clinical translatability. The components in our construct are all either endogenously present or Food and Drug Administration approved and commercially available. This not only makes it easy to create, but also limits its future regulatory concerns. As the ECM has demonstrated clinical safety, there are limited concerns of safety of the ECM within the construct. This therapy may be rapidly delivered either thoracoscopically or robotically with a very limited left thoracotomy, much like modern transapical transcatheter valve therapy. This therapy may be combined with either percutaneous coronary intervention or coronary artery bypass grafting or performed as a stand-alone treatment to completely revascularize ischemic myocardium. The precise timing of delivery remains to be clearly elucidated. Early after infarction, there is marked inflammation as well as elevations of inhibitory proteins, such as CD26 (inhibitor of SDF), that will limit cell engraftment and chemotaxis to proteins, such as SDF. In summary, we demonstrate direct angiogenic and parenchyma properties of our tissue-engineered EPC-mediated construct. The enhanced vasculogenic properties correlate with normalization of myocardial biomechanics as measured by tensile modulus. Preclinical large animal studies will allow us to determine the clinical translatability of this novel therapy.

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This project was supported by the National Institutes of Health, National Heart, Lung, Blood Institute grant 1R01 HL089315 (Dr Woo); The American Association for Thoracic Surgery, David C. Sabiston Research Scholarship (Dr Atluri); and the Thomas B. McCabe and Jeanette Laws McCabe Fund Fellowship, Perelman School of Medicine, University of Pennsylvania (Dr Atluri).

Disclosures
None.

References

Table 3. Global Measures of Elasticity as Measured by an Intraventricular Pressure–Volume Analysis

<table>
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<th>Control</th>
<th>Control–ECM Alone</th>
<th>ECM+EPC</th>
<th>Construct</th>
</tr>
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<tbody>
<tr>
<td>Elastance, mmHg/µL</td>
<td>0.80 (first quartile-0.79, third quartile 0.81)</td>
<td>0.74 (first quartile-0.67, third quartile 0.76)</td>
<td>1.37 (first quartile-1.16, third quartile 1.64)</td>
<td>1.69 (first quartile-1.41, third quartile 2.03)</td>
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<td>τ, ms</td>
<td>20.8 (first quartile-20.8, third quartile 20.9)</td>
<td>22.2 (first quartile-20.5, third quartile 23.0)</td>
<td>18.8 (first quartile-18.2, third quartile 19.5)</td>
<td>15.26 (first quartile-14.7, third quartile 18.1)</td>
</tr>
</tbody>
</table>

P values as compared with construct. ECM indicates extracellular matrix; and EPC, endothelial progenitor cell.
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