Tissue Engineered, Hydrogel-Based Endothelial Progenitor Cell Therapy Robustly Revascularizes Ischemic Myocardium and Preserves Ventricular Function

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Abstract

Objective—Cell based angiogenic therapy for ischemic heart failure has had limited clinical impact, likely related to very low cell retention (<1%) and dispersion. We developed a novel, tissue engineered, hydrogel based cell delivery strategy to overcome these limitations and provide prolonged regional retention of myocardial endothelial progenitor cells (EPC) at high cell dosage.

Methods—EPCs were isolated from Wistar Rats and encapsulated in fibrin gels. In vitro viability was quantified using a fluorescent live-dead stain of transgenic eGFP+ EPCs. EPC-laden constructs were implanted onto ischemic rat myocardium in a model of acute myocardial infarction (LAD ligation) for 4 weeks. Intramyocardial cell injection (IC, 2×10^6 EPCs), empty fibrin, and isolated LAD ligation groups served as controls. Hemodynamics were quantified using echocardiography, Doppler flow analysis, and intraventricular pressure-volume analysis. Vasculogenesis and ventricular geometry were quantified. EPC migration was analyzed by utilizing EPCs from transgenic eGFP+ rodents.

Results—EPCs demonstrated an overall 88.7% viability for all matrix and cell conditions investigated after 48 hours. Histologic assessment of 1-wk implants demonstrated significant migration of transgenic eGFP+ EPCs from the fibrin matrix to the infarcted myocardium as...
compared to IC (28±12.3 vs. 2.4±2.1 cells/hpf, p=0.0001). We also observed a marked increase in vasculogenesis at the implant site. Significant improvements in ventricular hemodynamics and geometry were present following EPC-hydrogel therapy as compared to control.

**Conclusion**—We present a tissue engineered hydrogel-based EPC mediated therapy to enhance cell delivery, cell retention, vasculogenesis, and preservation of myocardial structure and function.

**Keywords**

Bioengineering; Hydrogel; Endothelial Progenitor Cell; Vasculogenesis; Ischemic cardiomyopathy

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**INTRODUCTION**

Ischemic heart failure is a major global health concern. Medical and surgical therapies for heart failure have largely been palliative. Though we have been able to successfully reduce mortality from acute myocardial infarctions, the progression to ischemic heart failure has not been ameliorated (1–3). The concept of post-natal vasculogenesis has been widely investigated for molecular therapy to revascularize ischemic myocardium. Endothelial progenitor cells (EPCs) are pluripotent, bone marrow derived stem cells with the ability to differentiate into de novo vasculature. Initial enthusiasm was focused on genetic and cytokine therapy to locally recruit EPCs to ischemic myocardium. Subsequent studies have attempted to utilize cell therapy to directly deliver EPCs locally to the myocardium. Though significant neovasculogenic responses have been seen in small and large animal trials, this benefit has not been translatable to the clinical setting.

Much concern has been raised about cell delivery, dispersal, and engraftment. Cell tracking has demonstrated that less than 1% of cells remain following direct myocardial injection (4–7). We propose a novel therapy, whereby engineered fibrin gel constructs encapsulating EPCs can overcome these limitations by providing an environment in which the cells can thrive, enabling an insult-free delivery to ischemic myocardium in high cellular concentration for a prolonged period. In this strategy EPCs are thought to promote neovasculogenesis by primarily forming de novo vasculature. Additionally, EPCs demonstrate paracrine capabilities by eluting pro-angiogenic cytokines that induce vessel growth by promoting the migration and proliferation of circulating endothelial progenitor/precursor cells. (8, 9) Hydrogels are biocompatible materials capable of providing the microenvironment necessary to allow cell viability and migration to ischemic myocardium following delivery. Fibrin, once polymerized by thrombin, is a particularly attractive biogel that is part of the normal hemostatic pathway and hence is entirely biocompatible. It is already approved for clinical utilization, thereby facilitating rapid clinical translatability of this therapy. We hypothesize that engineered gels seeded with endothelial progenitor cells at high cellular density will allow very high cellular retention rates with direct migration of a large proportion of stem cells to ischemic myocardium.
METHODS

Animal Care and Biosafety

Male adult, Wistar rats (250–300 grams) 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.

Endothelial Progenitor Cell Isolation

Bone marrow mononuclear cells were isolated from the long bones of syngeneic adult, male Wistar rats by density-gradient centrifugation (Histopaque 1083, Sigma). Following isolation, the cells were seeded on a vitronectin coated dish. The cells were cultured in endothelial basal medium-2 supplemented with EGM-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 six days (Lonza). 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 pEFP vector No. 6077-1, Clontech Laboratories, and pCXN2 expression vector containing cytomegarovirus enhancer, chicken b-actin enhancer–promoter and rabbit b-globin poly(A) signal], obtained from Dr. Kobayashi, Jichi Medical School, Tochigi, Japan(10). EPC phenotype (DiDL+VEGFR2+CD34+) was confirmed as previously published by our group(11, 12).

EPC Fibrin Hydrogel Construct Creation

To form the 20 mg/mL Fibrin gels, 5.32 mg of Fibrinogen (Sigma) was added to PBS, and added to the appropriate quantity of EPCs for a final volume of 266 μl. To manufacture the 10 mg/mL Fibrin gels, 2.66 mg of Fibrinogen (Sigma) was added to PBS, and added to the appropriate quantity of EPCs for a final volume of 266 μl. After preparation of the Fibrinogen and EPC mixture in PBS, this 266 μl mixture was mixed into 0.333 Units of thrombin (Sigma). 100 μl of this liquid mixture was pipetted directly onto the center of a rectangular piece of vicryl mesh (approximately 15×12 mm in size) to provide integrity for implant.

In Vitro Analysis of EPC Migration and Viability Within the Construct

In order to assess the viability of EPCs embedded into Fibrin-derived gels, EPCs were embedded into Fibrin-derived gels as described above. After gel preparation the LIVE/DEAD Viability/Cytotoxicity Kit (Invitrogen) was used to determine the percentage of viable EPCs, according to manufacturer instructions. Samples were visualized on a DFS000B Leica fluorescent microscope and analyzed using LASAF version 2.0.2 software (Leica).
Induction of Heart Failure

Male Wistar rats were anesthetized in an induction chamber (VetEquip) with 3% isofluorane, endotracheally intubated, and mechanically ventilated (Hallowell EMC) with 1.0% isoflurane. A left 4th interspace thoracotomy was performed and the left anterior descending (LAD) coronary artery was ligated with a 7-0 Prolene suture at the level of the left atrial appendage. This induced a consistent and reproducible anterolateral infarction of 35–40% of the left ventricle(12–17). Following LAD ligation the animals were randomized to one of eight groups: Control (coronary ligation alone), implant of a fibrin patch without cells (10 mg/ml – FIB 10 or 20 mg/ml – FIB 20), injection of EPCs (IC, 2 million cells/in 250μl PBS), implant of EPC-Fibrin hydrogel (10 mg/ml Fibrin 7 × 10^6 EPCs/ml, 10 mg/ml Fibrin 17 × 10^6 EPCs/ml, 20mg/ml Fibrin 7 × 10^6 EPCs/ml, 20 mg/ml Fibrin 17 × 10^6 EPCs/ml). The Constructs were sutured in place with a 7-0 prolene suture over the area of myocardial injury with the cellular surface facing the myocardium. The thoracotomy was closed in 3 layers and the animals were allowed to recover. Four weeks following myocardial infarction the animals were sacrificed following hemodynamic analysis for histologic analysis.

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. In order to assess EPC migration, eGFP+ EPCs from transgenic rats were utilized to create the Construct (20 mg/ml Fibrin, 17 × 10^6 EPCs/ml). This Construct was then implanted onto ischemic myocardium following LAD ligation. The hearts were explanted 1 week following implant, flushed with PBS, and distended with OCT embedding compound (Electron Microscopy Sciences). Visualization was performed in the peri-infarct borderzone, which was defined as one microscopic field from the infarct. Hearts treated with Construct were compared to IC. Sections were briefly washed, then fixed in 4% paraformaldehyde and then stained for anti-GFP and anti-α smooth muscle actin (SMA, pericytes). Primary antibodies used for indirect immunofluorescence were goat anti-GFP (1:200, Rockland) and rabbit anti-SMA (1:150, Abcam). Secondary antibodies included donkey anti-goat conjugated to FITC (1:200, Abcam), and donkey anti-rabbit conjugated to Alexa Fluor 594 (1:200, Abcam). Nuclei were counterstained with DAPI (Vector Laboratories). Construct was compared to Control (isolated LAD ligation) and IC.

Quantification of Myocardial Vasculogenesis

Sections were briefly washed three times in PBS, fixed in 4% paraformaldehyde for 10 minutes at room temperature, and blocked in 10% Fetal Bovine Serum (FBS) (Gibco) for 1 hour at 37°C. Primary antibodies were diluted 1:150 in PBS and incubated for 2 hours at 37°C. Secondary antibodies were diluted in PBS and incubated for 1 hour at 37°C. Primary antibodies included sheep anti-vWF (endothelial cells of vasculature) conjugated to FITC (Abcam) and rabbit anti-SMA (Abcam). Secondary antibodies (1:200) were donkey anti-goat conjugated to FITC (Abcam), and donkey anti-rabbit conjugated to Alexa Fluor 594 (Abcam). Nuclei were stained with DAPI (Vector Laboratories). Vasculature was quantified
using ImageJ (NIH). Measurements were made 1 high power field from the infarct at 20× magnification.

**Analysis of Myocardial Fibrosis**

Hearts were explanted from animals, flushed with PBS, and distended with Tissue Tek optimum cutting temperature (OCT) compound. Hearts were then submerged in OCT, and frozen at −80°C. 10-μm-thick sections were prepared from each heart using a cryostat at the level of the mid-papillary muscles. Masson’s Trichrome stain was performed on the sections using the Accustain Trichrome Stains Kit (Sigma) according to the manufacturer’s instructions. Stained slides were scanned and images were analyzed in ImageJ (NIH) to assess scar fraction.

**Analysis of Global Ventricular Function**

Prior to explanting the heart, transthoracic echocardiography was performed to assess myocardial function (Phillips Sonos 5500 revD system with an S12 probe at 12MHz and a 3cm depth of penetration). Ventricular measurements were performed according to the American Society for Echocardiography leading-edge method. Subsequently, A 2Fr pressure-volume catheter (Millar Instruments) was inserted into the left ventricle retrograde via the right common carotid artery for analysis of left ventricular function. In addition to steady-state hemodynamic parameters, contractility was determined from pressure-volume relationships obtained by reducing pre-load via occlusion of the inferior vena cava. Following echocardiographic and pressure volume analysis, a midline sternotomy was performed and a 2.5mm ascending aortic flow probe (Transonic Systems) was placed for doppler analysis of cardiac output.

**Statistical Analysis**

Quantitative data are expressed as means ± standard error of the mean (SEM). Statistical significance was evaluated using the unpaired Student’s *t* test for comparison between two means. A *p*-value of less than 0.05 was considered statistically significant.

**RESULTS**

**Endothelial Progenitor Cells Remain Viable Within the Engineered Fibrin Construct**

In order to ensure viability of EPCs within the Fibrin Hydrogel Construct, *in vitro*, live-dead stain was analyzed. Quantification of viability revealed significant viability of EPCs within the construct at 2 days [TABLE 1]. Lower viability was noted with prolonged time. Excellent viability was noted to 10 days post-implant in the Construct, *in vitro*. Confocal imaging demonstrated characteristic EPC spreading and migration by 2 days within the Construct [Figure 1].

**Robust Endothelial Progenitor Cell Migration from the Construct to Ischemic Myocardium**

Establishing migratory potential of eGFP+ EPCs from the engineered EPC Fibrin construct to the ischemic myocardium helps to determine the vasculogenic potential of this therapy. Immunofluorescent microscopy demonstrated a marked number of EPCs within the deep
myocardium, indicating significant migration of EPCs from the construct. When compared to Control a statistically significant number of EPCs were noted within the deep myocardium (28.5±12.3 vs. 0 cells/hpf, n=5/group, p<0.00001). Additionally, when compared to IC there was a markedly increased number of delivered EPCs within the myocardium (28±12.3 vs. 2.4±2.1 cells/hpf (n=6), p=0.0001), denoting enhanced delivery efficiency with the Construct as compared to IC. [FIGURE 2]

**Tissue Engineered EPC-Fibrin Hydrogel Construct Implant Enhances Myocardial Vasculogenesis**

Quantification of myocardial vasculogenesis demonstrated a statistically significant increase in neovasculogenesis within the ischemic myocardium following treatment with all 4 EPC Fibrin Constructs when compared to Control [Figure 3]. Furthermore, all 4 Construct treatment groups had a statistically significant increase in neovasculogenesis when compared to IC. This demonstrates that enhanced cell retention may contribute to enhanced vasculogenesis. Interestingly, the isolated, empty Fibrin gels (FIB 10 and FIB 20) also demonstrated increased vascular density when compared to control. The Construct with the high cell and high density (20 mg/ml Fibrin 17×10⁶ EPCs/ml) demonstrated a statistically significant increase in vasculogenesis when compared to the 20 mg/ml Fibrin 7×10⁶ EPC/ml (p=0.02), 10mg/ml Fibrin 7×10⁶ EPC/ml (p=0.001) and 10mg/ml Fibrin 17×10⁶ EPC/ml (p=0.04). Both 20 mg/ml Fibrin 7×10⁶ EPC/ml and 10mg/ml Fibrin 7×10⁶ EPC/ml hydrogels demonstrated a significantly increased vasculogenic potential when compared to the low density, low cell Construct (10mg/ml Fibrin 7×10⁶ EPC/ml), p=0.02 and 0.05 respectively. There was no statistically significant difference in vasculogenesis between 20 mg/ml Fibrin 7×10⁶ EPC/ml and 10mg/ml Fibrin 7×10⁶ EPC/ml Constructs (p=0.9). [FIGURE 4]

**EPC Fibrin Construct Implants Minimize Myocardial Fibrosis**

A marked reduction in myocardial fibrosis and ventricular scar was demonstrated with treatment with all 4 Construct groups when compared to Control [Table 2]. Moreover, IC demonstrated a significant reduction in scar fraction when compared to Control as well (p=0.0009). There was no significant difference in scar formation between isolated fibrin gel implant (FIB 10 or FIB 20) and Control. All 4 Construct groups demonstrated a statistically significant reduction in scar formation when compared to IC. There was not a significant difference in scar fraction between the 4 EPC-Fibrin hydrogel Construct groups. [FIGURE 5]

**EPC Fibrin Construct Implant Markedly Improves Myocardial Function**

Hemodynamic analysis demonstrated a statistically significant improvement in cardiac output, ejection fraction, contractility, dP/dT max, and maximum generated pressure for all EPC-Fibrin Constructs when compared to IC and Control [Table 3]. There was no significant difference in hemodynamic function as measured by these parameters between the treatment groups. Interestingly, maximum generated pressure was significantly higher for both FIB 10 and FIB 20 groups when compared to IC.

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Discussion

In this study, we demonstrate a very robust vasculogenic response to therapy with Construct encapsulating endothelial progenitor cells. We witnessed a very high rate of cell migration from the Construct to the underlying ischemic myocardium. Moreover, there was a marked increase in vasculogenesis with prolonged exposure of EPCs to ischemic myocardium at a very high concentration. This enhanced vasculogenesis appears to have contributed to reduced myocardial scar and likely subsequent preservation of myocardial structure and function. Over the past decade, numerous clinical trials have attempted to utilized cell injection techniques, whether it be direct myocardial injection, endocardial catheter based injection, or intracoronary delivery. None of these techniques have demonstrated a clinically significant benefit that would successfully impact long-term survival(18–26). Novel tissue engineered therapies as outlined in this manuscript may provide ability to overcome the limitations of cell dispersal and engraftment that have thus far limited clinical trials exploring cell therapy.

Hydrogels have the unique ability to slowly degrade, in a very controlled fashion, and therefore provide a very continuous and long-term exposure of cytokines or cell therapy to the myocardium(27, 28). It is important to ensure that the desired hydrogel has biologic compatibility and will allow for appropriate cell migration and differentiation(29). Cell density, adhesive ligands, and gel stiffness intrinsic to the gels can dramatically alter stem cell fate, hence it is vital to carefully control these factors to maximize stem cell potential(30). Based upon our in vitro analysis, we found 10mg/ml and 20mg/ml gels to have desirable stiffness to allow cell sprouting and spreading. Additionally, this density seemed to allow maximum cell migration. As was evident in this manuscript, no significant differences in vasculogenesis or hemodynamics were present between 7 and 17 million EPCs/ml cell densities.

Fibrin is very desirable for cell delivery applications given its inherent biocompatibility and ability to maintain encapsulated cell viability(31, 32). Additionally, tissue engineered EPC Fibrin Constructs as utilized in this study can easily be translated to the clinical arena, given preexistent FDA approval. In fact, fibrin sealant and glues are widely used in cardiac surgery and other surgical specialties for hemostatic applications. Moreover, Fibrin has been studied for its intrinsic angiogenic abilities(33). Previous studies have demonstrated a statistically significant increase in myocardial angiogenesis and endothelialization(34–36). Similarly, we have demonstrated the intrinsic angiogenic capabilities of Fibrin in our studies when compared to control. This intrinsic angiogenic potential may explain the enhanced vasculogenesis and diminished fibrosis that we witnessed with both FIB10 and FIB 20 groups in this study. This angiogenic capability likely further complements the vasculogenesis and improved function we have noticed in this study with EPC Fibrin Constructs. Thereby, maximizing vasculogenic response beyond that of the delivered EPCs with the Constructs.

Tissue engineering, as utilized in this study, may be able to provide the ability to overcome the limitations that we have witnessed over the past decade with cell based therapies. These novel approaches may allow investigators to enhance the delivery of desired cells, utilize
multiple cell types, incorporate concomitant cytokine therapy, and provide gel mediated myocardial stabilization. Intricate tissue engineered cell delivery strategies, as exemplified in this study, may provide the robust clinical response needed to normalize myocardial function.

CONCLUSION

We present a novel tissue engineered hydrogel-based EPC mediated therapy to enhance cell delivery, cell retention, vasculogenesis, and preservation of myocardial structure and function. This therapeutic strategy may provide the ability to overcome the limitations of cell dispersal and engraftment associated with prior cell based clinical therapies.

Acknowledgments

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References


Figure 1.
Endothelial progenitor cells demonstrate characteristic spreading, in vitro, within the EPC Fibrin Hydrogel construct 2 days following Construct (10mg/ml Fibrin, 17×10⁶ EPCs/ml) creation (eGFP+ EPCs, confocal microscopy, 20× magnification).
Figure 2.
Immunofluorescent imaging of eGFP+ transgenic EPCs migration from the EPC-Fibrin Construct to the myocardium. Sections were triple stained for nuclei (DAPI, blue), eGFP (green), α-smooth muscle actin (red). 20× magnification, scale bar= 75µm.
Figure 3.
Quantification of myocardial neovasculogenesis within the ischemic peri-infarct myocardium.
Figure 4.
Representative immunofluorescent images demonstrating vascular density in peri-infarct ischemic myocardium following therapy. (20x magnification; green = vWF, blue = DAPI, red= αSMA)
Figure 5.
Representative myocardial cross sectional images following Masson’s Trichrome staining to delineate myocardial scar. (scar=blue, myocardium=red)
Table 1

In Vitro EPC viability within EPC-Fibrin Hydrogel Constructs. Viability is demonstrated as percent live cells as compared to overall cell density (n=6/group).

<table>
<thead>
<tr>
<th>Construct Composition</th>
<th>Day 2</th>
<th>Day 6</th>
<th>Day 10</th>
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<tbody>
<tr>
<td>5mg/ml Fibrin 7 × 10^6 cells/ml</td>
<td>85.9±3.2%</td>
<td>65.2±8.5%</td>
<td>48.7±1.8%</td>
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<tr>
<td>5mg/ml Fibrin 17 × 10^6 cells/ml</td>
<td>80.8±4.7%</td>
<td>71.9±6.1%</td>
<td>56.9±3.5%</td>
</tr>
<tr>
<td>10mg/ml Fibrin 7 × 10^6 cells/ml</td>
<td>89.1±2.3%</td>
<td>61.3±5.6%</td>
<td>44.8±5.3%</td>
</tr>
<tr>
<td>10mg/ml Fibrin 17 × 10^6 cells/ml</td>
<td>85.2±3.6%</td>
<td>59.5±3.7%</td>
<td>66.0±5.8%</td>
</tr>
<tr>
<td>20mg/ml Fibrin 7 × 10^6 cells/ml</td>
<td>90.3±2.9%</td>
<td>63.7±5.4%</td>
<td>45.4±4.8%</td>
</tr>
<tr>
<td>20mg/ml Fibrin 17 × 10^6 cells/ml</td>
<td>88.3±3.7%</td>
<td>73.5±2.8%</td>
<td>55.0±4.2%</td>
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Table 2

Left ventricular myocardial scar fraction 4 weeks following left anterior descending coronary artery ligation and treatment.

<table>
<thead>
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<th>Left Ventricular Infarct</th>
<th>p vs. control</th>
<th>p vs. cell injection</th>
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<tr>
<td>Control (n=7)</td>
<td>13.0±5.5%</td>
<td>0.0009</td>
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<tr>
<td>IC (n=6)</td>
<td>8.2±3.0%</td>
<td>0.0009</td>
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<tr>
<td>FIB 10 (n=13)</td>
<td>11.7±5.6%</td>
<td>0.6</td>
<td>0.00006</td>
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<tr>
<td>FIB 20 (n=16)</td>
<td>12.3±11.8%</td>
<td>0.5</td>
<td>0.0002</td>
</tr>
<tr>
<td>10 mg/ml Fibrin with 7×10⁶ EPCs/ml (n=11)</td>
<td>6.2±3.7%</td>
<td>0.02</td>
<td>0.002</td>
</tr>
<tr>
<td>10 mg/ml Fibrin with 17×10⁶ EPCs/ml (n=8)</td>
<td>5.0±2.9%</td>
<td>0.009</td>
<td>0.002</td>
</tr>
<tr>
<td>20 mg/ml Fibrin with 7×10⁶ EPCs/ml (n=14)</td>
<td>5.0±3.6%</td>
<td>0.008</td>
<td>0.0002</td>
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<tr>
<td>20 mg/ml Fibrin with 17×10⁶ EPCs/ml (n=10)</td>
<td>4.0±3.3%</td>
<td>0.004</td>
<td>0.02</td>
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Left ventricular function assessed 4 weeks following LAD ligation and treatment. Cardiac output was quantified utilizing an ascending aortic flow probe, ejection fraction was measured with transthoracic echocardiography, and maximum pressure, contractility, and dP/dt were measured with an intraventricular pressure-volume catheter. p-values are in relation to IC.

<table>
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<tr>
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<th>Control (n=10)</th>
<th>IC (n=6)</th>
<th>FIB 10 (n=9)</th>
<th>FIB 20 (n=11)</th>
<th>10 mg/ml Fibrin with 7 × 10^6 EPCs/ml (n=9)</th>
<th>10 mg/ml Fibrin with 17 × 10^6 EPCs/ml (n=7)</th>
<th>20 mg/ml Fibrin with 7 × 10^6 EPCs/ml (n=10)</th>
<th>20 mg/ml Fibrin with 17 × 10^6 EPCs/ml (n=12)</th>
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<tr>
<td><strong>Cardiac Output (ml/min)</strong></td>
<td>24.5±5.4</td>
<td>22.6±4.7</td>
<td>25.4±10.8 (p=0.6)</td>
<td>21.0±13.1 (p=0.8)</td>
<td>30.5±2.0 (p=0.006)</td>
<td>33.7±3.5 (p=0.001)</td>
<td>33.7±6.8 (p=0.01)</td>
<td>32.5±4.9 (p=0.006)</td>
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<td><strong>Ejection Fraction (%)</strong></td>
<td>39.7±14.2</td>
<td>39.8±12.2</td>
<td>44.8±15.6 (p=0.6)</td>
<td>41.4±21.8 (p=0.9)</td>
<td>66.3±8.2 (p=0.0006)</td>
<td>68.8±10.7 (p=0.007)</td>
<td>69.7±6.4 (p=0.00002)</td>
<td>61.7±13.8 (p=0.0009)</td>
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<tr>
<td><strong>Maximum Pressure (mmHg)</strong></td>
<td>62.7±14.5</td>
<td>63.6±9.4</td>
<td>88.2±8.7 (p=0.001)</td>
<td>84.2±16.8 (p=0.02)</td>
<td>106.2±15.4 (p=0.0006)</td>
<td>109.1±23.3 (p=0.007)</td>
<td>101.8±13.7 (p=0.0001)</td>
<td>107.9±20.4 (p=0.002)</td>
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<td><strong>Contractility (μl/mmHg)</strong></td>
<td>0.57±0.14</td>
<td>0.88±0.21</td>
<td>1.01±0.24 (p=0.5)</td>
<td>1.08±0.48 (p=0.5)</td>
<td>2.23±1.05 (p=0.03)</td>
<td>1.77±0.24 (p=0.008)</td>
<td>2.32±0.67 (p=0.005)</td>
<td>2.50±0.82 (p=0.005)</td>
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<td><strong>dP/dt max (mmHg/sec)</strong></td>
<td>2156±677</td>
<td>2691±554</td>
<td>3880±758 (p=0.01)</td>
<td>3728±1185 (p=0.06)</td>
<td>5011±1281 (p=0.008)</td>
<td>5090±1257 (p=0.002)</td>
<td>6686±2587 (p=0.03)</td>
<td>5970±1661 (p=0.004)</td>
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