



Adipose-Derived Cell Construct Stabilizes Heart Function and Increases Microvascular Perfusion in an Established Infarct

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ABSTRACT

We have previously shown that myocardial infarction (MI) immediately treated with an epicardial construct containing stromal vascular fraction (SVF) from adipose tissue preserved microvascular function and left ventricle contractile mechanisms. In order to evaluate a more clinically relevant condition, we investigated the cardiac recovery potential of an SVF construct implanted onto an established infarct. SVF cells were isolated from rat adipose tissue, plated on Vicryl, and cultured for 14 days. Fischer-344 rats were separated into MI groups: (a) 6-week MI (MI), (b) 6-week MI treated with an SVF construct at 2 weeks (MI SVF), (c) 6-week MI with Vicryl construct at 2 weeks (MI Vicryl), and (d) MI 2wk (time point of intervention). E_{max} , an indicator of systolic performance and contractile function, was lower in the MI and MI Vicryl versus MI SVF. Positron emission tomography imaging (¹⁸F-fluorodeoxyglucose) revealed a decreased percentage of relative infarct volume in the MI SVF versus MI and MI Vicryl. Total vessel count and percentage of perfusion assessed via immunohistochemistry were both increased in the infarct region of MI SVF versus MI and MI Vicryl. Overall cardiac function, percentage of relative infarct, and percentage of perfusion were similar between MI SVF and MI 2wk; however, total vessel count increased after SVF treatment. These data suggest that SVF treatment of an established infarct stabilizes the heart at the time point of intervention by preventing a worsening of cardiac performance and infarcted volume, and is associated with increased microvessel perfusion in the area of established infarct. *STEM CELLS TRANSLATIONAL MEDICINE 2013;2:000–000*

INTRODUCTION

Cardiac disease, including coronary heart disease (CHD) and associated heart failure following myocardial infarction (MI), accounts for most of the direct and indirect costs of major cardiovascular diseases [1]. Since the innate ability of the heart to repair itself following an MI is largely limited, regenerative therapies for patients with acute MI are being investigated as a means to reduce the extent of ischemic damage, whether as a novel treatment or a supplement to current therapies, such as primary percutaneous coronary intervention and thrombolytic therapy [2]. To successfully intervene in the heart's progressive functional decline with CHD, and specifically MI, there has been a large growth in clinical trials devoted to treating acute and chronic CHD. Multiple phase II clinical trials, such as the TIME (ClinicalTrials.gov identifier NCT00684021) and LateTIME (ClinicalTrials.gov identifier NCT00684060) trials, have recently completed randomization and will help determine the appropriate timing of cell-based therapy to improve ventricular function and structure post-MI [3]. Specifically, the LateTIME trial is evaluating the efficacy of cell

treatment 2–3 weeks following initial MI in an effort to address high-risk patients with persistent left ventricle (LV) dysfunction [3]. This has prompted the need to evaluate the efficacy of successful acute MI cellular therapies in a more clinically challenging model of established or chronic ventricular dysfunction.

Currently, there are many options as to which cell populations are under consideration for treatment of CHD. Of interest and as the focus of this study, adipose tissue houses an easily isolatable, regenerative, and multipotent cell population defined as the stromal vascular fraction (SVF), consisting of endothelial cells, smooth muscle cells, blood cells, and mesenchymal cells containing perivascular and adventitial cells [4, 5]. The broad clinical potential of SVF has significantly boosted the number of ongoing clinical trials using adipose-derived cells as a cell therapy [6]. It is worth noting, though, that most cell-based therapies are limited by the lack of retention of cells into the target tissue. In fact, one study found that only ~1.3%–2.6% of transplanted cells could be detected in the infarcted myocardium in as little as 50–75 minutes after

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intracoronary injection [7]. However, by changing the delivery method to a cell-laden sheet (implanted onto the epicardium), greater postinfarct survival and greater cell engraftment have resulted compared with intramyocardial injections of the same cells [8]. Furthermore, others have shown that an epicardial cell sheet [8] can be implanted as long as 30 days post-MI and still result in improved angiogenesis and cell survival [9]. These outcomes led us to use an SVF-laden epicardial construct in the treatment of an established MI as an efficient method to retain implanted regenerative cells in the area of infarct.

Our laboratory has previously shown that implantation of an epicardial SVF construct preserved coronary blood flow following MI compared with untreated MI hearts in rats [10]. Although it was implanted immediately after MI, the SVF construct intervention led to favorable structural and functional outcomes. In light of the aforementioned clinical trials focused on therapy timing and the relative explosion in adipose-derived regenerative cell use, we sought to implant the SVF construct after the MI was more established in order to evaluate potential therapeutic effects. This approach allowed us to study the progressive and sometimes irreversible pathological changes that occur weeks to months following an MI, such as cellular necrosis, the beginning of scar tissue formation, and thinning of outer LV wall [11]. The major hypothesis tested was that a cell-based intervention onto an established infarct would halt further deterioration of LV function compared with untreated hearts, and that this is accomplished through an increase in coronary neovascularization and perfusion. Therefore, our objective was to compare treated versus untreated hearts by assessing cardiac function through pressure-volume (PV) relationships and viability through positron emission tomography (PET) imaging, with particular emphasis on assessing functional microvessels in the area of ischemia.

MATERIALS AND METHODS

All animal surgeries were performed in accordance with protocols approved by the University of Louisville animal review committee and the NIH *Guide for the Care and Use of Laboratory Animals* (9th ed., 2011). In all animal surgeries, adequate anesthesia was maintained by monitoring lack of tactile toe pinch reflex, heart rate, and/or respiration.

Stromal Vascular Fraction Isolation

Green fluorescent protein (GFP)-tagged adult male Fischer-344 rats (Rat Research and Resource Center, University of Missouri, Columbia, MO) were put under anesthesia (40–80 mg/kg ketamine and 5–10 mg/kg xylazine). GFP⁺ SVF cells were isolated from epididymal fat pads. Cervical dislocation was used to euthanize the rats. Harvested fat pads were washed in 0.1% bovine serum albumin in phosphate-buffered saline (BSA-PBS), finely minced, and digested in 2 mg/ml type I collagenase solution for 40 minutes at 37°C with vigorous shaking. Buoyant adipocytes were removed by centrifugation, and the entire cell pellet suspended in 0.1% BSA-PBS. Cells were immediately plated (1×10^6 cells per cm²) onto a piece of Vicryl (Ethicon/Johnson & Johnson, Somerville, NJ, <http://www.ethicon.com>) 1×1.5 cm and cultured for 14 days in Dulbecco's modified Eagle's medium with 10% fetal bovine serum.

Myocardial Infarction

After anesthesia was introduced (1%–3% isoflurane-O₂ balance), the MI was performed as described previously [10]. Briefly, in-

Table 1. Study design of all experimental groups

Groups	0 Weeks	2 Weeks	6 Weeks
MI 2wk	LAD artery ligation	Explant MI 2wk	
MI	LAD artery ligation		Explant MI
MI SVF	LAD artery ligation	Implant SVF	Explant MI SVF
MI Vicryl	LAD artery ligation	Implant Vicryl	Explant MI Vicryl

Abbreviations: LAD, left anterior descending; MI, 6-week myocardial infarction; MI 2wk, 2-week myocardial infarction; MI SVF, 6-week myocardial infarction treated with a stromal vascular fraction construct at 2 weeks; MI Vicryl, 6-week myocardial infarction with Vicryl construct at 2 weeks; SVF, stromal vascular fraction.

farcts were performed by left anterior descending (LAD) artery ligation with a 7-0 silk suture (Ethicon) and confirmed via visualization of blanched tissue distal to the ligation site. The rat was allowed to recover and given buprenorphine (0.05 mg/kg, s.c.) every 8–12 hours for the next 48 hours.

Study Design and Construct Implantation

Table 1 illustrates the study design of the four experimental groups used in the study. Infarcted animals were immediately randomized into one of four groups ($n = 9$ per group). Two groups were left untreated until explant, 2-week MI (MI 2wk) and 6-week MI (MI). Two weeks following the initial MI surgery, rats in the treated groups were assigned to either the SVF (MI SVF) or Vicryl (MI Vicryl) group. Rats were surgically prepared as described above, and the procedure was performed as described previously [10]. Briefly, the SVF construct or Vicryl mesh without cells was laid onto the epicardium, covering the area of infarct and overlapping slightly into the proximal noninfarct region. A single 7-0 silk suture was sewn immediately distal to the LAD artery occlusion to secure the construct to the epicardial surface, and rats were allowed to recover as described above for 4 weeks.

The MI 2wk group was included to compare the SVF-treated group to the time point of intervention. The Vicryl-alone implant group was included as an epicardial implant control. All endpoint parameters were assessed at 2 or 6 weeks following initial MI. Rats were euthanized by removal of the heart.

Left Ventricular Function Assessment

The PV loop relationships of the LV used the Millar conductance system and were performed according to our published methods [12]. Briefly, after receiving an injection of anesthesia (40–80 mg/kg ketamine and 5–10 mg/kg xylazine), a substernal transverse incision to expose the inferior vena cava (IVC) was performed. An apical stab was used for catheter insertion, and the catheter was positioned along the cardiac longitudinal axis with the distal electrode in the aortic root and the proximal electrode in the LV apex. Placement of the catheter was monitored directly. Overall LV function was assessed under baseline conditions, following transient IVC occlusion (to assess contractility and LV stiffness), and after intravenous administration of 20–40 μ l of 30% saline (for conductance volume calibration). Parameters assessed included heart rate (HR), cardiac output (CO), end systolic pressure (ESP), end diastolic pressure (EDP), end systolic volume (ESV), end diastolic volume (EDV), stroke volume (SV), ejection fraction (EF), and maximal slope of systolic pressure increment (+dP/dt) and diastolic pressure decrement (–dP/dt). E_{max} (end systolic pressure-volume relationship [ESPVR]) and end diastolic pressure-volume relationship (EDPVR) slopes were calculated by linear regression of the loops during IVC occlusion.

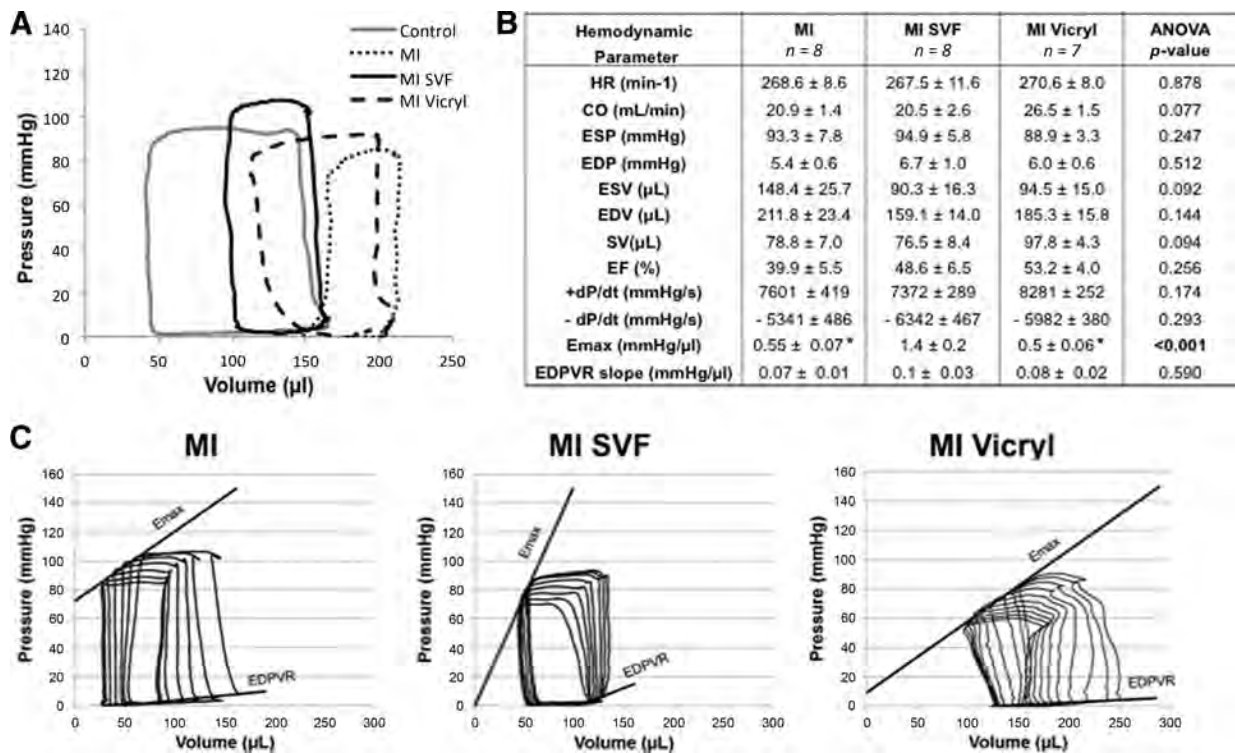


Figure 1. Cardiac function as assessed through pressure-volume (PV) loop recordings. **(A):** Representative single PV loop recordings during baseline. All MI hearts displayed a rightward shift in PV relationship compared with the representative control PV loop. **(B):** Table summarizing cardiac functional parameters during PV loop recordings and respective analysis of variance *p* values. E_{max} was significantly higher for MI SVF than either the MI or MI Vicryl groups. *, Significantly different from MI SVF. **(C):** Representative PV loops obtained at different preloads, showing differences in the end-systolic PV relationship (ESPVR, or E_{max}) between MI (left), MI SVF (center), and MI Vicryl (right). The less steep E_{max} in the MI and MI Vicryl loops indicate decreased systolic performance. Abbreviations: CO, cardiac output; dP/dt, maximal slope of systolic pressure increment; EDP, end diastolic pressure; EDPVR, end diastolic pressure-volume relationship; EDV, end diastolic volume; EF, ejection fraction; ESP, end systolic pressure; ESV, end systolic volume; HR, heart rate; MI, 6-week myocardial infarction; MI SVF, 6-week myocardial infarction treated with a stromal vascular fraction construct at 2 weeks; MI Vicryl, 6-week myocardial infarction with Vicryl construct at 2 weeks; SV, stroke volume.

PET Imaging

The most widely used and validated PET radiotracer for the assessment of myocardial glucose use and metabolically active tissue is ¹⁸F-fluorodeoxyglucose (FDG) [13]. Briefly, each rat was injected with a dose of ¹⁸F-FDG (28–44 MBq × 0.6 ml of saline) via the lateral tail veins (see supplemental online data). Thirty to forty-five minutes after the ¹⁸F-FDG i.v. injection, each rat was anesthetized with isoflurane (1%–3%) and securely taped onto the imaging bed of a Siemens R4 MicroPET (Knoxville, TN, <http://www.medical.siemens.com>). A 17-partitioned polar cardiac map was produced for each rat. Imaging data were analyzed with the Siemens Inveon Imaging system. Reduced uptake regions (<70% of the maximum uptake) were calculated by summing the individual volumes (in mm³) from each reduced ¹⁸F-FDG uptake region. Total LV volume was calculated by summing the individual volumes from all short axis slices. The extension of infarction was compared between animals using the following equation: Percent relative infarcted volume = (Infarcted myocardial volume/Total LV volume) × 100.

Histology

Rats received an injection of anesthesia (40–80 mg/kg ketamine and 5–10 mg/kg xylazine) and then were perfused with dextran (tetramethylrhodamine; Molecular Probes, Eugene, OR, <http://probes.invitrogen.com>) at 2 mg/ml via the jugular vein (circulat-

ing 15 minutes) before hearts were explanted. The general histological structure of the heart was determined using an ultramicrotome (Leica, Heerbrugg, Switzerland, <http://www.leica.com>) approximately 2 mm proximal to the apex, taking ~15-μm-thick sections. A subset of sections was stained using Masson's Trichrome protocol, and infarct regions were manually traced as previously described [10]. Vascular characteristics were identified from infarcted regions (approximately 1 mm proximal to the apex on the free wall of the LV). Vascular endothelial cells (indicative of total vessel density) were identified using a rodent-specific lectin, *Griffonia simplicifolia* I (GS-1) (1:250). On separate slides, perfused vessels were identified by counting dextran⁺ vessels, and vascular smooth muscle cells were identified using α-smooth muscle actin (αSMC-actin)⁺ staining (1:100). The total number of vessels in these slides was calculated by counting positive dextran or αSMC-actin staining and then subtracting vessels positive for both markers (overlap). The percentage of perfusion was calculated by calculating [Dextran⁺/(Total vessels)] × 100. In each animal, five discrete, random images (magnification, ×20) from the infarct region or area at risk were analyzed for total vessel density and percentage of perfused vessels (see supplemental online data).

Statistical Analysis

The mean differences between groups in all PV loop parameters, PET infarct volume calculations, infarct size, and vessel counts

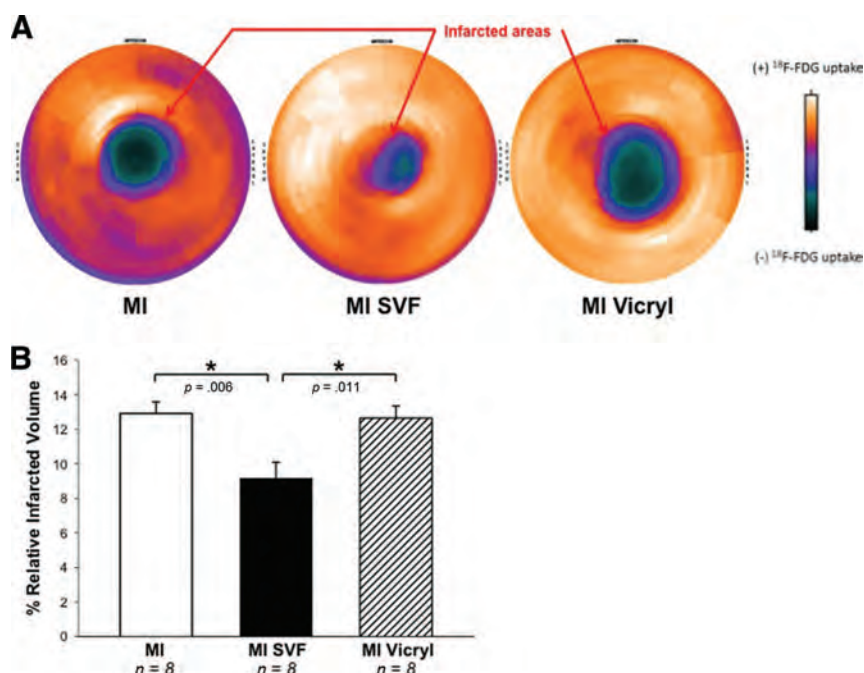


Figure 2. Cardiac metabolism as evaluated through the use of ^{18}F -FDG positron emission tomography. **(A):** ^{18}F -FDG positron emission tomography shows an uptake defect in partitioned polar maps in all groups (anterior [top], lateral [right], and apex [center]). **(B):** The percentage of relative infarcted volume, calculated as a ratio of infarcted volume (regions with ^{18}F -FDG uptake <70%) to total left ventricle volume, was significantly higher in the MI and MI Vicryl groups compared with MI SVF. Abbreviations: FDG, fluorodeoxyglucose; MI, 6-week myocardial infarction; MI SVF, 6-week myocardial infarction treated with a stromal vascular fraction construct at 2 weeks; MI Vicryl, 6-week myocardial infarction with Vicryl construct at 2 weeks.

were determined by one-way analysis of variance (ANOVA) calculation, with post hoc analysis determined by Tukey's test, $p \leq .05$.

RESULTS

Impact of Construct Implantation on Overall Heart Function

To determine whether the SVF construct impacted overall LV function compared with other MI groups, PV relationships were acquired. The PV relationship was shifted to the right in MI and MI Vicryl animals, mainly because of an increase in both ESV and EDV compared with MI SVF (although these failed to reach significance: ANOVA ESV $p = .09$, EDV $p = .14$; Fig. 1A, 1B). A representative sham "control" PV loop is included to illustrate the rightward shift of all MI groups. There were no significant differences between groups in hemodynamic parameters at rest, such as HR, CO, ESP, EDP, EF, SV, $\pm dP/dt$, and EDPVR slope (Fig. 1B).

Figure 1C displays representative PV loops obtained during IVC occlusion in MI, MI SVF, and MI Vicryl hearts. Indices of contractility and LV stiffness were calculated (slope of ESPVR [E_{\max}] and EDPVR) and are displayed in Figure 1B and 1C. The E_{\max} slopes during IVC occlusion were significantly less steep in the MI and MI Vicryl groups compared with MI SVF (MI vs. MI SVF $p = .005$, MI SVF vs. MI Vicryl $p = .002$), indicating decreased systolic performance and contractile function. Chronic changes in E_{\max} from CHD can also give us insight into changes in cardiac morphometry, such as hypertrophy and fibrosis, and are considered a more complex indicator of "contractility" [14]. Pacher et al. reported E_{\max} slope values of 2.6 ± 0.2 for normal male Fischer-344 rats [15], so it is important to note that treatment with the

SVF construct 2 weeks post-MI did not reverse cardiac dysfunction back to normal/sham levels.

Myocardial Viability

^{18}F -FDG PET is considered the most sensitive means to assess myocardial viability compared with any other imaging modality [16]. Myocardial glucose use (indicative of metabolism) measured by ^{18}F -FDG uptake demonstrated the ischemic injury in the anterior wall of infarcted rats in the representative polar maps (Fig. 2A). To compare the relative myocardial volume that was defined as infarcted (<70% maximum ^{18}F -FDG uptake), percentage of relative infarcted volume was calculated in each group. Percentage of relative infarcted volume was significantly increased in MI and MI Vicryl compared with MI SVF hearts (MI: 12.9 ± 0.7 , MI Vicryl: 12.6 ± 0.7 , MI SVF: 9.1 ± 0.9 ; Fig. 2B).

Heart Remodeling and Fibrosis Following Post-MI Intervention

To corroborate infarct volume analysis by in vivo PET imaging, histologic evaluation of hearts using Masson's Trichrome analysis was performed. As expected, the MI and MI Vicryl groups exhibited more percentage of collagen and fibrosis in the LV than the MI SVF group (MI: 47 ± 5 , MI Vicryl: 38 ± 4 , MI SVF: 24 ± 3 ; Fig. 3C), although the post hoc analyses between MI Vicryl and MI SVF failed to reach significance ($p = .074$).

Vessel Characteristics in the Area of Infarct

Total vessel count in the area of infarct was determined through GS-1⁺ staining. Both MI and MI Vicryl exhibited less vascular density in the infarct region compared with the MI SVF (Fig. 4). Since previous data indicate that vascular density does not imply

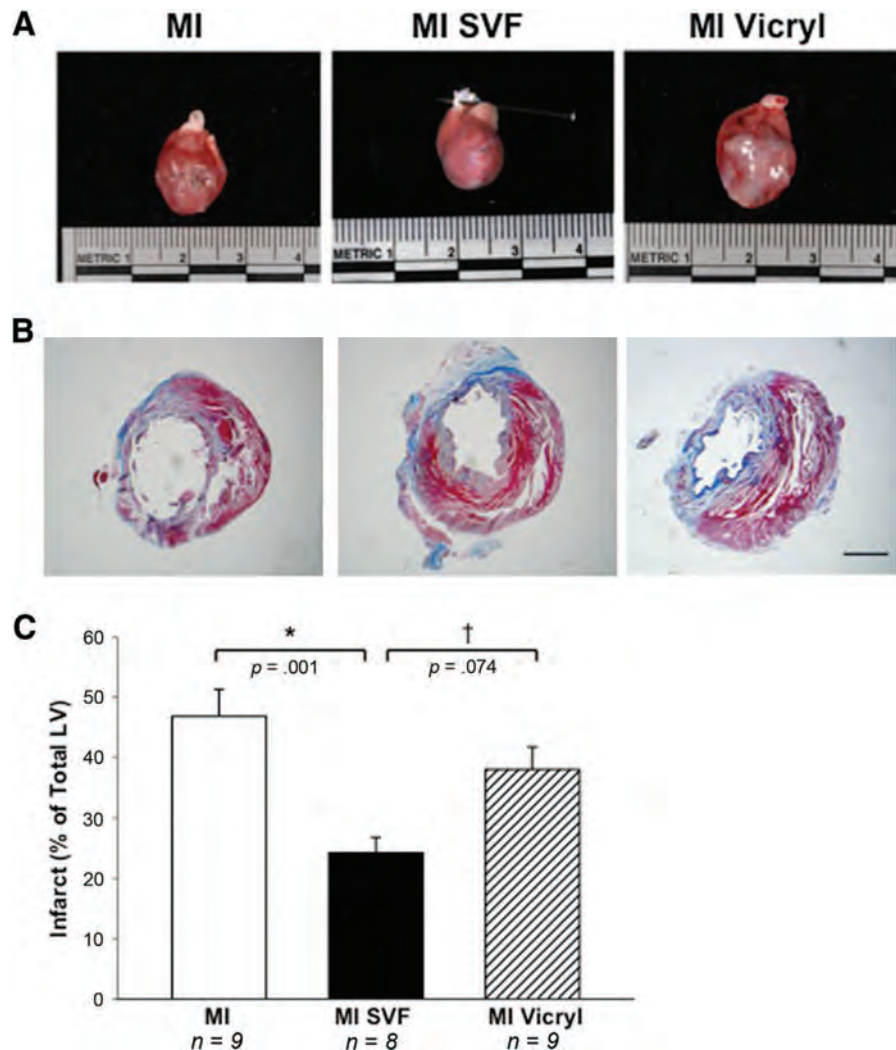


Figure 3. Gross pathology and histology of MI, MI SVF, and MI Vicryl hearts. Representative images of explanted hearts (**A**) and trichrome-stained sections (**B**). Scale bar = 1 mm. (**C**): Infarct size as a percentage of total LV per experimental group by trichrome analysis. Abbreviations: LV, left ventricle; MI, 6-week myocardial infarction; MI SVF, 6-week myocardial infarction treated with a stromal vascular fraction construct at 2 weeks; MI Vicryl, 6-week myocardial infarction with Vicryl construct at 2 weeks.

vascular perfusion [10], we also evaluated vessel perfusion through dextran perfusion prior to explant. When treated with the SVF construct at 2 weeks post-MI, there were significantly more perfused vessels in the area of infarct compared with both MI and MI Vicryl hearts (MI SVF: 59 ± 5 , MI: 43 ± 4 , MI Vicryl: 41 ± 7 ; Fig. 5).

SVF Construct and Time Point of Intervention

The current results support the hypothesis that the SVF construct acts to preserve or halt ventricular dysfunction at the time of implant, as there were no significant differences in hemodynamic parameters assessed via PV loops between the time point of intervention, MI 2wk, and MI SVF (Fig. 6A, 6B). There were no significant differences in ^{18}F -FDG percentage of relative infarcted volume between MI SVF and MI 2wk (Fig. 6C, 6D) or in Masson's Trichrome percentage of infarct (data not shown). Compared with the time point of intervention (MI 2wk), the infarct area in MI SVF exhibited greater vascular density (Fig. 6E, 6F), but there was no difference in the percentage of perfused vessels between these groups (Fig. 6E, 6G).

SVF Cell Engraftment

Four weeks after SVF construct implantation, immunohistochemistry (IHC) staining did not reveal the presence of engrafted GFP⁺ SVF elements in the area of infarct (data not shown; see supplemental online data). In order to further evaluate the possibility of GFP⁺ cells that may not be identified through IHC, polymerase chain reaction amplification of the GFP gene was performed on LV tissue from MI SVF. Confirming IHC results, the GFP gene was not amplified in samples from MI SVF (data not shown; see supplemental online data).

Vascular Endothelial Growth Factor Production

To determine the amount of vascular endothelial growth factor (VEGF) produced by the SVF construct, medium was sampled from wells of individual SVF constructs following 4 days of culture (see supplemental online data). Using the ELISA Kit for VEGF (Invitrogen, Carlsbad, CA, <http://www.invitrogen.com>), medium without an SVF construct was 5 pg/ml. The average VEGF from $n = 7$ SVF constructs was $8,434 \pm 454$ pg/ml (data not shown).

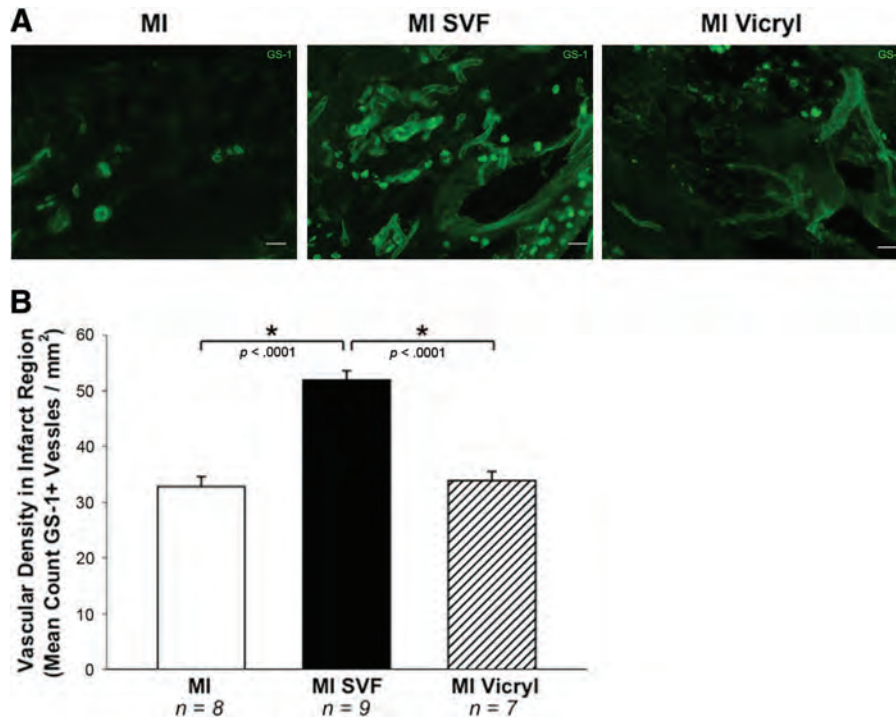


Figure 4. Vascular density as evaluated through immunohistochemistry in MI, MI SVF, and MI Vicryl hearts. **(A):** Representative GS-1⁺ staining indicating coronary vascular density in the infarct and peri-infarct region. **(B):** The MI SVF group exhibited significantly more GS-1⁺ vessels in the area of infarct compared with both the MI and MI Vicryl groups. Scale bars = 20 μ m. Abbreviations: GS-1, *Griffonia simplicifolia* I; MI, 6-week myocardial infarction; MI SVF, 6-week myocardial infarction treated with a stromal vascular fraction construct at 2 weeks; MI Vicryl, 6-week myocardial infarction with Vicryl construct at 2 weeks.

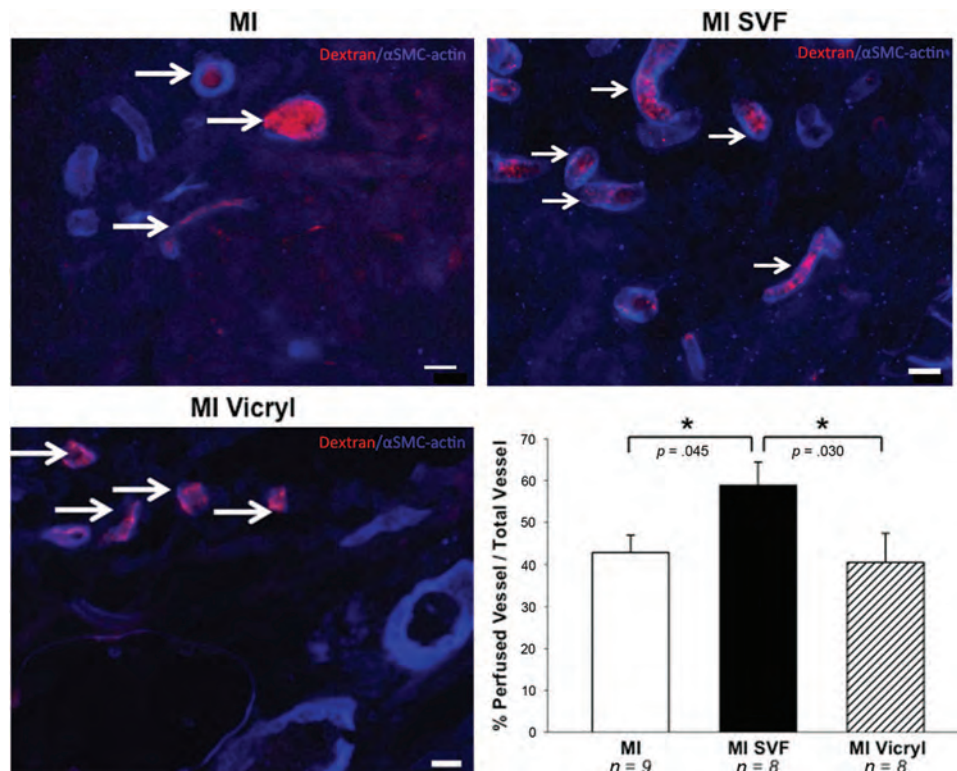


Figure 5. Representative merged images of immunohistochemistry staining for dextran⁺ (red) and α SMC-actin⁺ (blue) in MI (top left), MI SVF (top right), and MI Vicryl (bottom left). MI SVF showed more perfused vessels (white arrows) compared with both MI and MI Vicryl hearts. Scale bars = 20 μ m. Abbreviations: MI, 6-week myocardial infarction; MI SVF, 6-week myocardial infarction treated with a stromal vascular fraction construct at 2 weeks; MI Vicryl, 6-week myocardial infarction with Vicryl construct at 2 weeks; α SMC, α -smooth muscle cell.

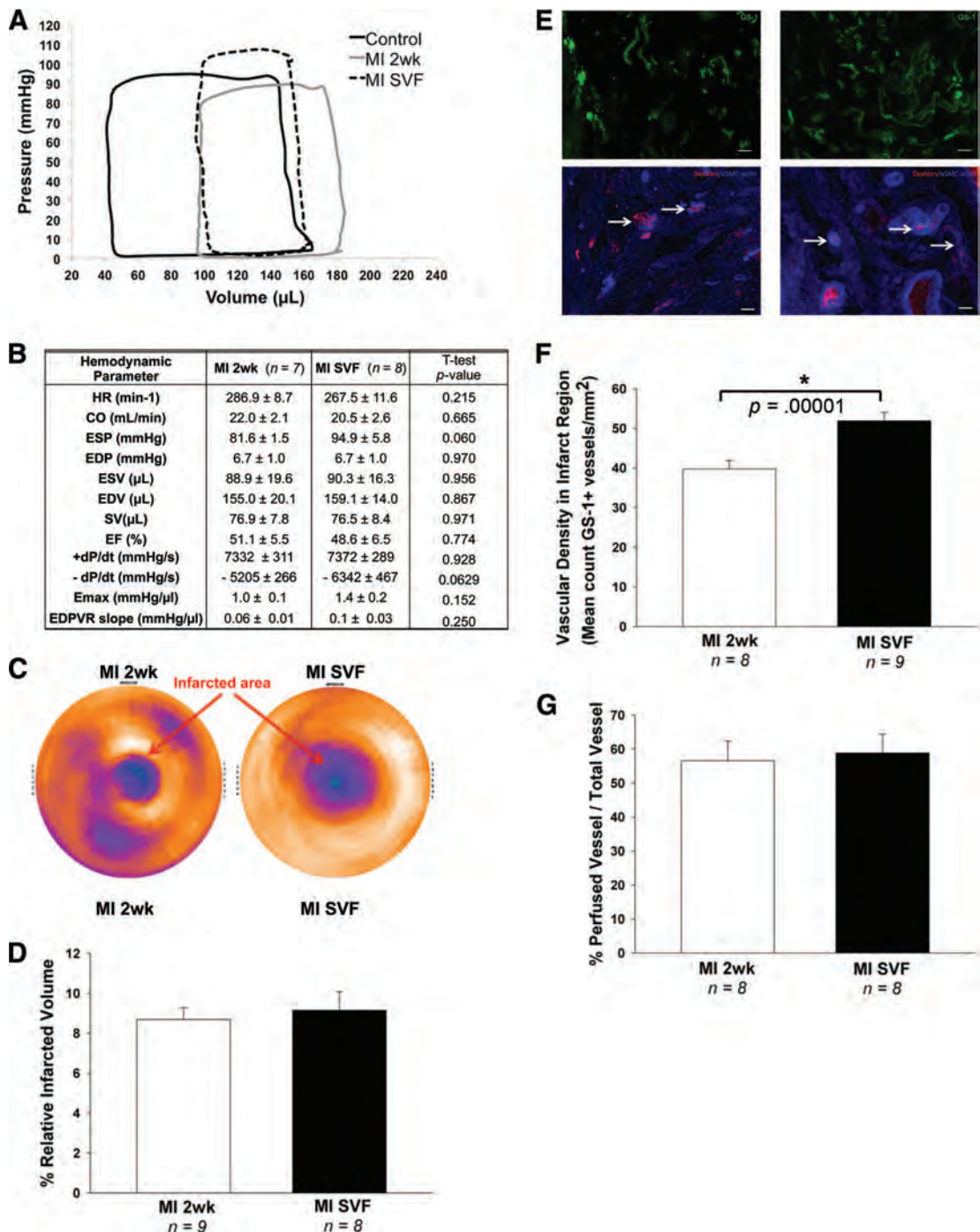


Figure 6. Cumulative data comparing MI SVF with the time point of intervention, MI 2wk. On several parameters, MI SVF hearts maintained cardiac function and vascular dynamics similar to those of the MI 2wk group. **(A):** Representative PV loops showing both the lack of rightward shift (vs. MI 2wk) or reversal of dysfunction (vs. control) for MI SVF. **(B):** Summary of cardiac functional parameters as assessed by PV loops. **(C):** ¹⁸F-Fluorodeoxyglucose uptake positron emission tomography (PET) polar map views. **(D):** Percentage of relative infarct volume from PET data. **(E):** GS-1⁺ vessel density (top) and immunohistochemistry staining showing perfused vessels (bottom). Scale bars = 20 µm. **(F):** MI SVF had a significantly higher count of GS-1⁺ vessel density in the area of infarct compared with MI 2wk. **(G):** Graph displaying the percentage of perfused vessels in the area of infarct. Abbreviations: αSMC, α-smooth muscle cell; CO, cardiac output; dP/dt, maximal slope of systolic pressure increase; EDP, end diastolic pressure; EDPVR, end diastolic pressure-volume relationship; EDV, end diastolic volume; EF, ejection fraction; ESP, end systolic pressure; ESV, end systolic volume; HR, heart rate; GS-1, *Griffonia simplicifolia* I; MI 2wk, 2-week myocardial infarction; MI SVF, 6-week myocardial infarction treated with a stromal vascular fraction construct at 2 weeks; SV, stroke volume.

DISCUSSION

Our goal was to determine whether regenerative medicine therapy previously shown to preserve myocardial function following

MI could restore or maintain function if applied at a later time point. The primary findings from this study are that (a) clinical indices of heart function, such as E_{max} (indicative of systolic performance and contractile function) and PET imaging of cardiac

viability, established that treatment with the SVF construct at 2 weeks post-MI halted the progressive worsening of LV function displayed by untreated and control MI hearts (MI and MI Vicryl), and (b) hearts treated with an SVF construct exhibited an increase in both total and perfused vessels in the infarcted area compared with MI and MI Vicryl. These data, coupled with the similarities between the time of intervention and the endpoint of the treated group (MI 2wk and MI SVF; Fig. 6), indicate that the SVF construct implanted onto an established infarct prevented or halted worsening of cardiac function and targeted coronary vascular perfusion in the infarct region, allowing sustained coronary viability.

A major barrier to the efficacy of regenerative medicine in a given tissue is the method of delivery of the selected cell population. The volume of cells that remain in the heart, delivered either by direct injection into the myocardium or through intracoronary infusion, can be <10% hours after transplantation [7, 17]. Catheter-based injectable materials offer an alternative solution to delivering cells directly to the infarcted area while simultaneously facing the need for a longer-lasting cell-matrix platform and interaction within the myocardium; however, it is less clear whether injectable gels laden with cells would be able to withstand (and survive) the mechanical load of a contracting myocardium after injection [18]. Fortunately, tissue engineers have propelled the efficacy of cell retention by developing cell sheets that can be implanted into/onto a particular tissue [8, 19]. Direct comparisons between direct injections (in-scar myocardial) and an epicardial trilayered cell-sheet implant resulted in higher rates of postinfarction survival in rats and greater numbers of engrafted cells in the hearts treated with a cell sheet [8]. These studies, along with our previous results [10, 12, 20], certainly suggest that a cell-laden patch is a very effective delivery method to improve cell retention postimplant.

Although transplanted cells have multiple modes of action in damaged myocardial tissue, the leading mechanism of action put forth is by increasing myocardial perfusion, followed by enhancing endogenous cell survival, progenitor homing, and decreasing fibrosis [21]. Central to all of these mechanisms is the potential for SVF-induced paracrine activity. Contrary to our previous results, GFP⁺ cells were not incorporated into the coronary microvasculature upon explant, yet improvements in functional parameters of the microcirculation were realized through the increase in both total and perfused vessels in hearts treated with the SVF construct. Adipose-derived microvessels [22] and cells [23] have the capability of forming a de novo microvasculature and migrating into the vessel wall of existing neovessel segments to assemble parts of the vasculature; however, this study suggests a likelier mechanism where implantation of the SVF construct following MI promotes a positive neovascularizing environment. In fact, medium collected from the SVF construct prior to implant exhibited high amounts of VEGF ($8,434 \pm 454$ pg/ml), which is approximately 7 times the amount of VEGF produced by similar three-dimensional (3D) constructs laden with human dermal fibroblasts (~1,000 pg/ml [24] and 1,600 pg/ml [25]). Cultured SVF isolated from human lipoaspirate has previously been shown to produce high levels of VEGF [26]. In addition to VEGF, adipose-derived stem cells can also produce large amounts of transforming growth factor β_1 (TGF β_1), placental growth factor, and basic fibroblast growth (FGF) factor [27], all of which support the impressive angiogenic effects reported previously [10, 27, 28]. Culturing cells on a 3D construct may alter the relative

expression and production of these growth factors and cytokines secreted by SVF, but this is unlikely in our hands. When examining a variety of angiogenic growth factor gene expressions, including VEGF, FGF, hepatocyte growth factor, angiopoietins, platelet-derived growth factor receptor β , transforming growth factors, and Tnf α , we have shown gene expression between flask-cultured SVF and the SVF cultured onto Vicryl similar to that used in the present study (data not shown). How long these proangiogenic cytokines remain following implant and which, if not all, are necessary for scar neovascularization and vessel maturation remain open for future exploration.

Although PET imaging is a well-established modality by which to evaluate myocardial perfusion, metabolism, and viability in the clinical population, it is used significantly less often in rodent models of MI [29]. ¹⁸F-FDG is considered the most sensitive means to assess myocardial viability. Following ischemia, the uptake of glucose by the myocardium is increased acutely, but it is decreased in areas of very severe ischemia. Clinical trials using bone marrow-derived stem cell (BMSC) intracoronary injection therapy for both acute [30, 31] and chronic [32] MI have shown an increased ¹⁸F-FDG uptake in the infarct zone of patients. Direct comparison of ¹⁸F-FDG uptake between hearts treated with either adipose-derived stem cells (ADSCs) or BMSCs after MI showed that ADSC injections resulted in greater sum uptake of ¹⁸F-FDG in all 17 segments of the polar map [33]. The present results support these studies, as the amount of myocardial area with reduced ¹⁸F-FDG uptake was larger in MI and MI Vicryl hearts compared with those treated with the SVF construct (Fig. 3B). Furthermore, we believe this is the first study to use PET imaging, and specifically ¹⁸F-FDG, as a means to evaluate the functional changes in myocardial viability after MI and adipose cell construct therapy in rats. However, more studies are needed to determine how SVF could potentially contribute to more viable tissue and subsequently higher metabolic activity in the myocardium, and whether that contribution is necessary for the realization of successful postinfarct repair.

Auspicious microcirculatory function is a critical factor in the post-MI repair process and is linked with increased viable myocardium following acute MI [34, 35]. The current results support this association, as MI SVF hearts exhibited preserved myocardial viability (Fig. 2) and increased vessel count and perfusion in the at-risk area compared with MI and MI Vicryl hearts (Figs. 4, 5). Multiple studies have shown a similar increase in vessel density (through IHC) in chronic MI hearts after treatment with either BMSCs [36] or ADSCs [37, 38]. Furthermore, a large animal study by Valina et al. described an increase in both neovascularization and coronary perfusion (through single photon emission computed tomography) in the infarct area after acute MI treatment with either BMSCs or ADSCs [39]. This concurrent increase in vascular density and perfusion is important to note, because our previous study demonstrated that neovascularization in infarcted regions of the heart does not necessarily translate into improved coronary blood flow (BF), as only hearts treated with an SVF construct at the time of infarct maintained functional BF reserve in the infarct region compared with untreated hearts [10].

We recently demonstrated that the SVF construct correlated with preserved heart function and microvascular blood flow in

the infarct area when implanted immediately following infarction [10], but it was unknown whether this therapy could impart similar beneficial effects if it was implanted on an established and/or remodeling infarct. In 2006, Miyahara et al. used a cultured monolayer of adipose-derived mesenchymal stem cells (MSCs) and implanted 4 weeks after coronary ligation [19]. The MSC sheet, created following three to five passages, showed engraftment into the epicardium and progressively thickened the outer LV wall in situ for 28 days until explant [19]. Hamdi et al. also used a 4-week delayed implant of a GFP⁺ progenitor cell sheet following MI and successfully identified implanted cells in the infarct region 2 months following implant [8]. However, one major protocol difference that these studies incorporated was a daily immunosuppression injection to suppress host-versus-graft reaction mediated by T cells, which can be triggered by GFP [40]. Conversely, we were unable to detect GFP⁺ cells upon explant in the present study, either through immunohistochemistry or through RNA analysis (data not shown). It should be noted that the present study did not include an immunosuppressive therapy, which may explain why GFP⁺ cells were not detected 28 days following implant. However, why we were previously able to detect GFP⁺ SVF cells in acute MI without immunosuppressive therapy is unknown [10], but we hypothesize that it could be attributed to a time-dependent stage of post-MI inflammation that is particularly specific to transplanted GFP⁺ cells. Furthermore, at the time point of intervention (MI 2wk), there are significantly fewer GS-1⁺ vessels in the area of infarct than compared with explant after SVF treatment (Fig. 6F). It is possible that in addition to the lack of immunosuppressive therapy in the present study, the acute reduction in coronary vessels at the time point of implant influences the ability of the GFP⁺ cells to survive and remain in the infarcted area.

CONCLUSION

We have shown that therapeutic treatment with the SVF construct on an established infarct results in preserved myocardial viability and function, in addition to increased microvascular perfusion in the infarcted area compared with untreated MI hearts in rats. This tissue engineering approach of creating an SVF-laden construct increases the cell quantity that can be implanted into an ischemic area without massive rates of acute cell

death and also improves cell retention over time [18]. The present results, combined with our most recent study [10], indicate that treatment with an SVF construct, either immediately or during the active remodeling phase of scar formation post-MI, halts deteriorating cardiac function and maintains LV viability and microcirculatory perfusion. With advances in future construct delivery, the clinical potential of an autologous construct made from adipose-derived SVF is high, as the SVF construct may be used in conjunction with existing MI therapies to promote microvessel survival and/or growth of new vessels following coronary infarct.

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AUTHOR CONTRIBUTIONS

A.J.L.: conception and design, financial support, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; Q.T.N.: collection and/or assembly of data, manuscript writing; J.S.T. and A.L.A.: collection and/or assembly of data; R.C.C. and C.K.N.: collection and/or assembly of data, data analysis and interpretation; J.B.H.: conception and design, final approval of manuscript; S.K.W.: conception and design, financial support, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

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