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Cardiac Patch Constructed from Human Fibroblasts Attenuates Reduction in Cardiac Function after Acute Infarct

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ABSTRACT

The current experiments used a scaffold-based, three-dimensional, human dermal fibroblast culture (3DFC) as a cardiac patch to stimulate revascularization and preserve left ventricular (LV) function of the infarcted LV in severe combined immunodeficient (SCID) mice. The 3DFC contains viable cells that secrete angiogenic growth factors and has been previously shown to stimulate angiogenesis. The hypothesis tested was that a 3DFC cardiac patch would attenuate a reduction in LV function of infarcted hearts. Five groups of mice were studied, including normal SCID mice ($n = 13$), normal SCID mice with 3DFC ($n = 6$), infarcted SCID mice ($n = 6$), infarcted mice with nonviable 3DFC ($n = 6$), and infarcted SCID mice with 3DFC ($n = 6$). An occlusion of a branch of the left anterior descending (LAD) coronary artery was performed by thermal ligation, and 3DFC was sized to the damaged area and implanted onto the epicardium at the site of tissue injury. Fourteen days postsurgery, LV mechanics were characterized with the Millar conductance catheter system (CCS). The data demonstrated that 3DFC-treated infarcted myocardium had significantly higher ejection fractions (EFs) compared with infarct-only mice (58.9 ± 10.8 versus 31.0 ± 5.8%, respectively; $p < 0.05$). Preload recruitable stroke work (PRSW) parameters were significantly higher in 3DFC-treated mice compared with infarct-only mice (64.6 ± 11.9 versus 36.8 ± 6.4 mmHg, respectively; $p < 0.05$). These results show that the 3DFC as a cardiac patch functioned to attenuate further loss of LV function accompanying acute myocardial infarct and that this may be related in part to myocardial revascularization.

INTRODUCTION

REVASCULARIZATION OF DAMAGED MYOCARDIUM represents an important therapeutic goal that targets patients with coronary artery disease. Many of these patients are treated conventionally through endovascular procedures or by coronary artery bypass surgery. However, patient populations that cannot completely revascularize through these conventional treatments may benefit from the development of safe and effective angiogenic therapies. Several investigators evaluating the use of pharmacologically induced revascularization (e.g., vascular endothelial growth factor [VEGF] and basic fibroblast growth factor [bFGF])[1–3] have been successful at simulating new microvessel growth or an increase in myocardial collateral blood flow. In addition, clinical trials evaluating the safety and efficacy of therapeutic angiogenic technologies in patients who suffer severe myo-

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cardial ischemia and who are not candidates for percutaneous or surgical revascularization procedures have demonstrated safety dose ranges for intracoronary delivery of recombinant human VEGF (rhVEGF) and limited myocardial perfusion benefits.[4] Furthermore, clinical data from patients receiving intracoronary injection(s) of granulocyte-macrophage colony-stimulating factor (GM-CSF) have indicated angiogenic benefits.[5]

Previously, angiogenic benefits have been reported through the use of a tissue-engineered, three-dimensional, human dermal fibroblast culture (3DFC) as a cardiac patch.[6] In those studies, 3DFC was implanted as a cardiac patch over a region of acute infarct in a severe combined immunodeficient (SCID) mouse model. The 3DFC stimulated the formation of a significant mature microvascular network (including arterioles, capillaries, and venules) at both 14 and 30 days compared with untreated infarcted control mice. The 3DFC contains structural extracellular matrix proteins and viable cells that synthesize a number of angiogenic growth factors (including VEGF, bFGF, and hepatocyte growth factor [HGF]) that have been previously shown to stimulate angiogenic activity.[7] However, demonstrating the ability to stimulate angiogenesis may not correlate with a therapeutic benefit. Therefore, the purpose of the current study was to evaluate global left ventricular (LV) function of acutely infarcted hearts after treatment with a 3DFC cardiac patch.

The technique described in the current study involved the implantation of a 3DFC cardiac patch over a region of infarcted cardiac tissue to stimulate revascularization of the damaged tissue and the evaluation of cardiac function with an LV conductance catheter. The current study tested the hypothesis that 3DFC used as a cardiac patch would attenuate a reduction in cardiac function after acute infarction.

MATERIALS AND METHODS

3DFC

Dermagraft (Smith&Nephew, London, UK),[8] a human tissue composed of human dermal fibroblasts cultured on 5×7 cm pieces of knitted Vicryl mesh [90:10 poly(glycolide:lactide)], was used throughout this study as the viable 3DFC. The cryopreserved tissue was stored at $-70°C$ until needed and then rapidly thawed, rinsed, and used immediately. Cell viability for the 3DFC was confirmed via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reductase activity (MTT assay).[7]

Nonviable 3DFC

Nonviable 3DFC was created by exposing Dermagraft to a subsequent freeze–thaw cycle without cryoprotectant to lethally injure the cells (confirmed via MTT assay).

Study design

All animal studies were approved by the University of Arizona (Tucson, AZ) animal review committee. Animals were housed in facilities in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care International (Rockville, MD). All procedures were performed according to the National Institutes of Health (NIH, Bethesda, MD) *Guide for the Care and Use of Laboratory Animals* (NIH publication 85-23, revised 1985). Female SCID mice (21–29 g) were induced with acute cardiac infarction. The 3DFC, as a single epicardial patch, was positioned on the anterior surface of the LV approximating the infarcted site. Five groups of mice were studied, including normal SCID mice ($n = 13$), normal SCID mice with 3DFC ($n = 6$), infarcted SCID mice ($n = 6$), infarcted mice with nonviable 3DFC ($n = 6$), and infarcted SCID mice with 3DFC ($n = 6$). At 14 days postinfarction, LV hemodynamics were determined and tissue samples were collected from all treatment groups. One-way analysis of variance (ANOVA) was used to determine a significant difference ($\alpha = 0.05$) between treatment groups, followed by post hoc testing using Duncan's multiple comparison test for pairwise comparisons between treatment groups.

Coronary occlusion and implant surgery

Initially, animals were randomly assigned to each treatment arm. General anesthesia was induced and maintained by an intraperitoneal injection of 2.5% Avertin (2.5% 2,2,2-tribromoethanol, 2.5% *tert*-amyl alcohol in normal saline; Sigma-Aldrich, St. Louis, MO). Sterility was maintained throughout the procedure. Hair was removed from the animal's neck and chest areas, using a depilatory agent. A tracheotomy was performed and a 24-gauge gel catheter was inserted into the trachea to allow for artificial respiration (rodent ventilator model 683 [Harvard, Holliston, MA], set to deliver 0.9 ml of air at 120 breaths/min). Epicardial access was accomplished by performing a left thoracotomy. Occlusion of the first branch of the left anterior descending (LAD) coronary artery for the infarct treatment groups was performed by thermocoagulation according to the procedures of Kumashiro *et al*.[9] Vessel occlusion was confirmed by a blanching in surface color. 3DFC patches (4 mm in diameter) were sutured onto the epicardial surface over the ischemic region, using 10-0 Prolene (Ethicon, Somerville, NJ). Mice in the infarct-only treatment group had a coronary occlusion with only suture implanted. Mice in the normal with 3DFC treatment group and mice in the nonviable 3DFC treatment group had 3DFC or nonviable 3DFC implanted, respectively, in a similar anatomical position. The chest was closed in three layers and syringe-evacuated with a 30-gauge needle. Mice were removed from ventilation and allowed to recover under a warming lamp.

FIG. 1. Gross image illustrating a 3DFC-treated infarcted heart during pressure–volume loop data acquisition.

LV function before explant

The functional analysis of each animal was completed in a blinded fashion. Functional data collected on the scheduled takedown day received a recorded file number, which was used on a separate day of analysis so as to blind the investigator to the identity of each animal. On completion of data analysis, the identity of each animal was revealed to categorize it into its respective treatment arm. The identities of animals that died prematurely were revealed so that replacement animals could be re-enrolled into the study. Survivability of each animal was documented at the 2-week time point. LV functional analysis was performed according to the procedures of Yang *et al.*[10,11] At 14 days, mice were anesthetized with an intraperitoneal injection of urethane (1000 mg/kg) and α-chloralose (50 mg/kg), selected for its reported minimal effects on cardiovascular reflexes and tolerance in mice.[12,13] Respiration was controlled through a tracheostomy cannula connected to the ventilator. The external jugular vein was cannulated for volume administration, which was limited to 200 μL of saline–albumin (50:50). A substernal transverse incision was made to expose the apical portion of the heart and the inferior vena cava (IVC). Electrical cautery was used to ensure minimal blood loss. A Millar conductance catheter (1.4F) was inserted through a 30-gauge needle apical stab wound into the LV (Fig. 1). 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. Real-time pressure–volume (P–V) data were collected for approximately 10 s for both baseline and IVC-occlusion time points. Data collection was done with the ARIA-1 system (Millar Instruments, Houston, TX) with National Instruments (Austin, TX) BioBench software (version 1.0). Subsequent data analysis was performed with PVAN cardiac pressure–volume analysis software (version 2.8; Millar Instruments).

After P–V loop analysis, heart samples were either immediately immersed into HistoChoice fixative (AMRESCO, Solon, OH) or quick frozen in liquid nitrogen and kept at -70°C. Sections (6 μm) of fixed samples were subsequently processed for hematoxylin and eosin (H&E) and cytochemical evaluation. Sections for cytochemistry were reacted with *Griffonia simplicifolia* lectin (peroxidase-conjugated Gs-1 lectin [EY Laboratories, San Mateo, CA]; used at a final dilution of 1:100), which binds carbohydrate domains on endothelial cells, and visualized with a peroxidase recognition system (universal mouse kit; DakoCytomation, Carpinteria, CA) to identify vascular elements.

RESULTS

Survival evaluation

The total number of animals that survived the 2-week study period are reported in Fig. 2. All of the normal mice and the normal mice with viable 3DFC survived the 2-week time point to be successfully evaluated for P–V loop analysis. In the infarct with viable 3DFC group, 100% of the animals survived the 2-week infarct time. In

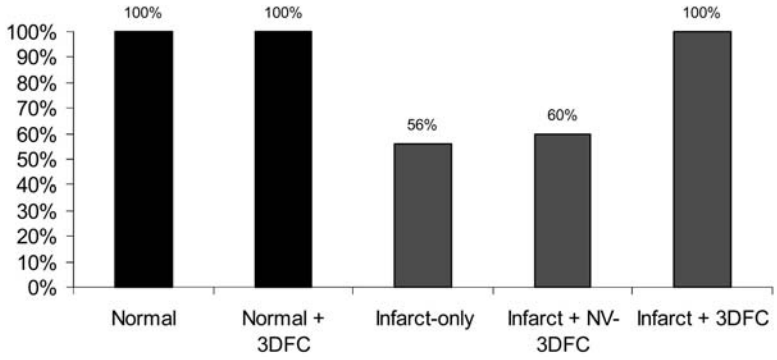

FIG. 2. Two-week survival percentages.

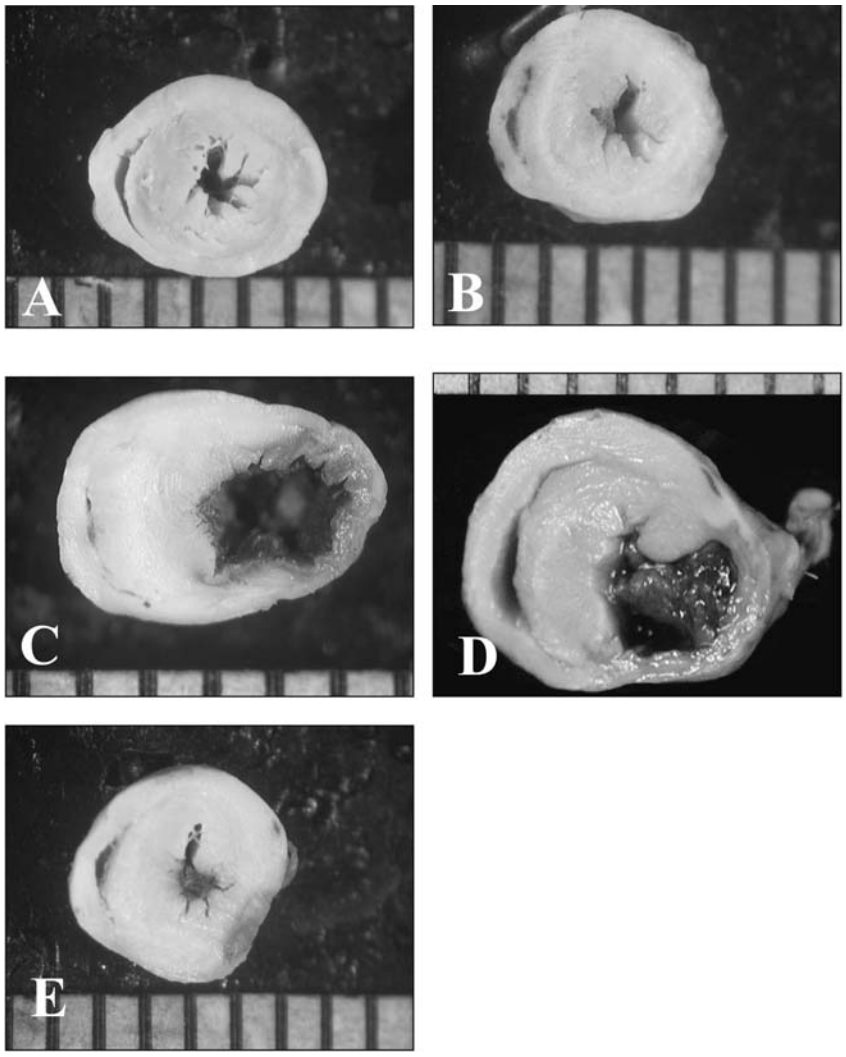

FIG. 3. Representative gross images in cross-sectional view after explant of (**A**) normal SCID heart, (**B**) normal SCID with 3DFC treatment, (**C**) infarct-only SCID heart, (**D**) infarcted SCID heart with nonviable 3DFC treatment, and (**E**) infarcted SCID heart with 3DFC treatment. Scale markings are in millimeters.

the infarct with nonviable 3DFC group, 60% of the animals survived the 2-week infarct period. Similarly, 56% of the infarct-only mice survived the 2-week infarct period.

Heart pathology

Gross photography. At explant, both infarct-only mice and infarcted mice treated with nonviable 3DFC revealed a thinned myocardium with a large aneurysmal section of the LV in comparison with the other treatment groups (Fig. 3). In contrast, infarcted mice receiving 3DFC treatment had a smaller infarct area, less ventricular wall thinning, and no aneurysm formation (Fig. 3). There were no gross differences between normal SCID hearts and normal SCID hearts with 3DFC treatment (Fig. 3). All mouse hearts that experienced the first primary surgery revealed minimal thoracic adhesions. No recognizable pattern of the adhesive tissue present in infarct-only or 3DFC-treated animals was noted.

Histology and cytochemistry. Figure 4 illustrates representative light micrographs of H&E-stained sections and serial sections reacted with Gs-1 lectin. No significant differences were seen between normal SCID mice (Fig. 4A and B) and normal SCID mice with 3DFC treatment (Fig. 4C and D). Infarcted mice with 3DFC treatment (Fig. 4I and J) revealed a greater angiogenic response (increased microvessel formation) within the infarcted myocardium when compared with infarct-only mice (Fig. 4E and F) and infarcted mice that received nonviable 3DFC (Fig. 4G and H). These results are consistent with our previous findings[6] in which quantitative

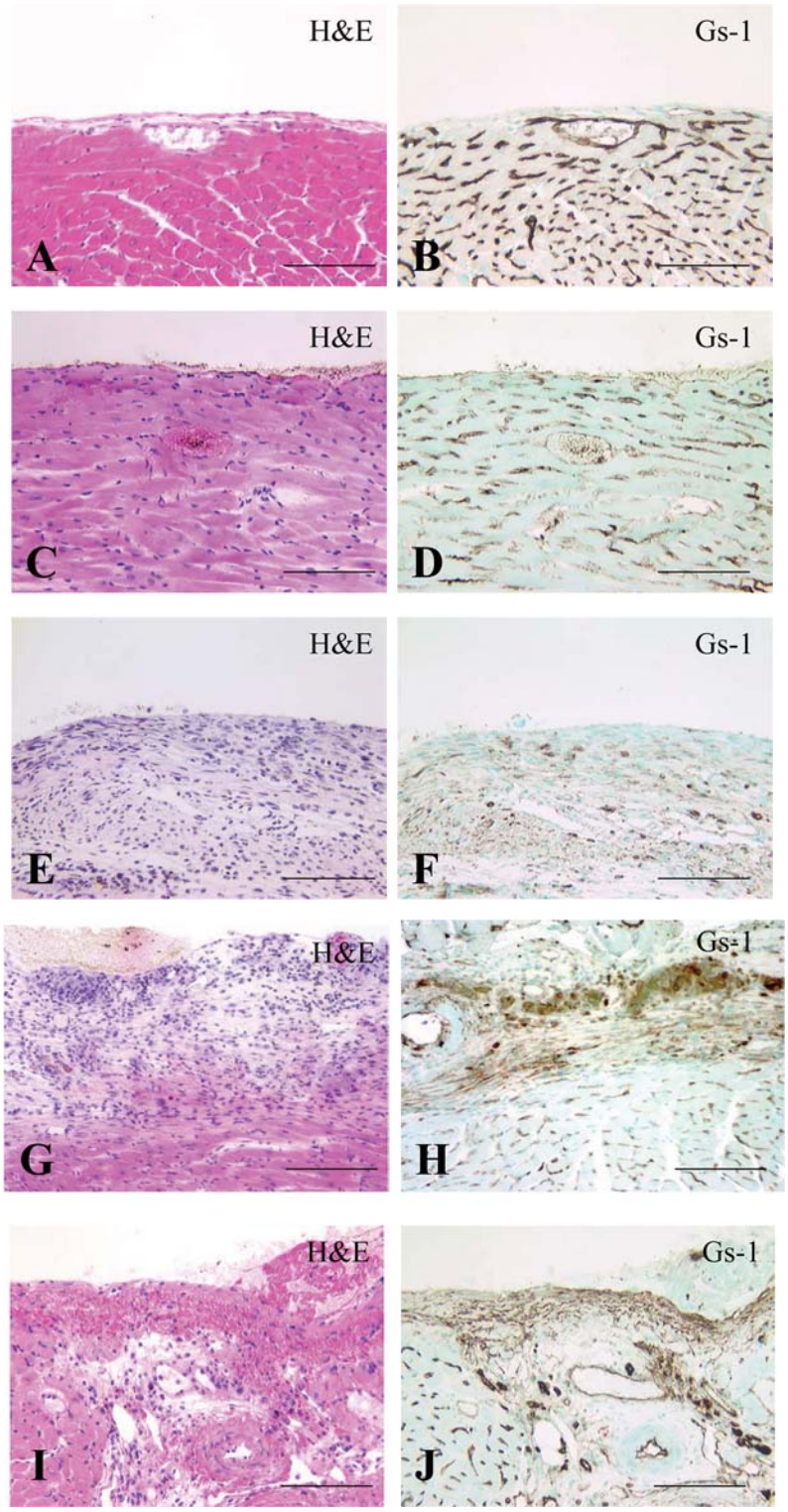

FIG. 4. Light micrographs of H&E-stained sections and Gs-1 lectin-reacted serial sections (14-day explants) in (**A** and **B**) normal SCID mice, (**C** and **D**) normal SCID mice treated with 3DFC, (**E** and **F**) infarct-only mice, (**G** and **H**) infarcted mice treated with nonviable 3DFC, and (**I** and **J**) infarcted mice treated with 3DFC. Scale bars: 100 μm.

Table 1. Hemodynamic Measurements of SCID Mice from Pressure–Volume Loop Analysis[a]

Parameter	*Normal*	*Normal + 3DFC*	*Infarct only*	*Infarct + NV 3DFC*	*Infarct + 3DFC*
HR (bpm)	519 ± 127	456 ± 64	477 ± 46	546 ± 49	494 ± 58
V_{ed} (μL)	14.26 ± 6.4	13.7 ± 3.5	22.3 ± 8.7	12.7 ± 9.0	20.2 ± 6.9
V_{es} (μL)	4.38 ± 2.4	3.13 ± 1.6	16.8 ± 6.3	8.6 ± 6.4	9.5 ± 4.9
SV (μL)	11.22 ± 4.9	11.3 ± 2.3	6.9 ± 2.5	6.1 ± 5.5	11.7 ± 2.8
CO (μL/min)	5644 ± 2856	5150 ± 1338	3351 ± 1521	3577 ± 3405	5722 ± 1230
SW (mmHg × μL)	678 ± 329	693 ± 190	315 ± 104	360 ± 351	542 ± 180
EF (%)	74.99 ± 8.6	81.2 ± 6.1	31.0 ± 5.8	43.2 ± 20.9	58.9 ± 10.8
P_{es} (mmHg)	70.69 ± 10.4	77.9 ± 25.1	72.6 ± 13.3	84.85 ± 40.4	65.0 ± 13.9
P_{ed} (mmHg)	8.11 ± 6.7	6.2 ± 1.7	5.4 ± 4.4	3.3 ± 1.4	4.9 ± 0.6
PRSW (slope)	88.0 ± 21.7	96.0 ± 22.0	36.8 ± 6.4	44.6 ± 15.5	64.6 ± 11.9
E_a (mmHg/μL)	8.10 ± 4.9	6.96 ± 1.7	12.3 ± 5.9	29.1 ± 27.4	5.9 ± 2.0
E_{es} (mmHg/μL)	6.42 ± 36.1	24.5 ± 23.0	5.1 ± 13.7	3.64 ± 37.3	14.2 ± 10.5
τ, Weiss method (ms)	9.11 ± 3.6	8.5 ± 1.1	9.6 ± 3.8	7.1 ± 1.4	7.4 ± 1.1
β (slope of EDPVR)	0.18 ± 0.277	0.18 ± 0.13	0.03 ± 0.15	0.13 ± 0.56	0.18 ± 0.21

Abbreviations: 3DFC, three-dimensional, human dermal fibroblast culture; τ, relaxation time constant [Weiss method: regression of log(pressure) versus time]; CO, cardiac output; E_a, elastance of artery; EDPVR, end-diastolic pressure–volume relationship; E_{es}, end-systolic volume elastance; EF, ejection fraction; HR, heart rate; NV, nonviable; P_{ed}, end-diastolic pressure; P_{es}, end-systolic pressure; PRSW, preload recruitable stroke work; SCID, severe combined immmunodeficiency; SV, stroke volume; SW, stroke work; V_{ed}, end-diastolic volume; V_{es}, end-systolic volume.

[a]Data are reported as means ± SD ($n = 13$ in normal treatment group; ($n = 6$ for all other treatment groups).

evaluations of microvessel density and an evaluation of the type of microvessels were performed.

Hemodynamics

Table 1 compares the hemodynamic functional parameters of normal SCID mice with those of normal SCID mice with 3DFC treatment, infarcted mice with 3DFC treatment, infarct-only mice, and infarcted mice with nonviable 3DFC ($n = 13$ for normal SCID mice and $n = 6$ for all other treatment groups). There were no significant differences found between normal SCID mice and normal SCID mice with 3DFC treatment in functional parameters, such as heart rate (HR), end-systolic volume (V_{es}), end-diastolic volume (V_{ed}), stroke volume (SV), cardiac output (CO), ejection fraction (EF), end-systolic pressure (P_{es}), end-diastolic pressure (P_{ed}), preload recruitable stroke work (PRSW), elastance of artery (E_a), end-systolic volume elastance (E_{es}), τ (Weiss method), and slope of the end-diastolic P–V relationship (β).

In addition, infarct-only mice had significantly lower EF and PRSW values in comparison with normal SCID mice or normal SCID mice with 3DFC treatment (Fig. 5A and B). Infarct-only mice were also found to have significantly lower SW values (Fig. 5F) and significantly higher V_{es} values in comparison with normal SCID mice (Fig. 5C). Infarcted mice that received 3DFC treatment had significantly higher EF and PRSW values when compared with infarct-only mice (Fig. 5A and B). Furthermore, infarcted mice that received 3DFC treatment had significantly lower V_{es} values than infarct-only mice (Fig. 5C).

Infarcted hearts treated with nonviable 3DFC had similar EF and PRSW values compared with infarct-only mice. In addition, these hearts demonstrated significantly reduced LV function when evaluating EF and PRSW values compared with infarcted hearts treated with viable 3DFC (Fig. 5A and B).

DISCUSSION

Previously, we have shown that treatment with viable 3DFC stimulates a significant angiogenic response in infarcted cardiac tissues that leads to the development of arterioles, capillaries, and venules.[6] This angiogenic response was found to be a result of treating damaged cardiac tissue with a viable 3DFC patch. In those experiments, the nonviable 3DFC patch did not differ from the infarct-only treatment group in its ability to stimulate new microvessel formation. The current study evaluated the ability of 3DFC as a cardiac patch to attenuate a reduction in LV function after acute infarction in SCID mice. Although the cardiac function of infarcted mice treated with viable 3DFC was not that of normal mice, these mice demonstrated significantly better cardiac function parameters compared with infarct-only mice. Specifically, with respect to EF and PRSW, infarcted mice treated with 3DFC had significantly higher values than infarct-only mice. In addition, 3DFC treatment of infarcted mice did

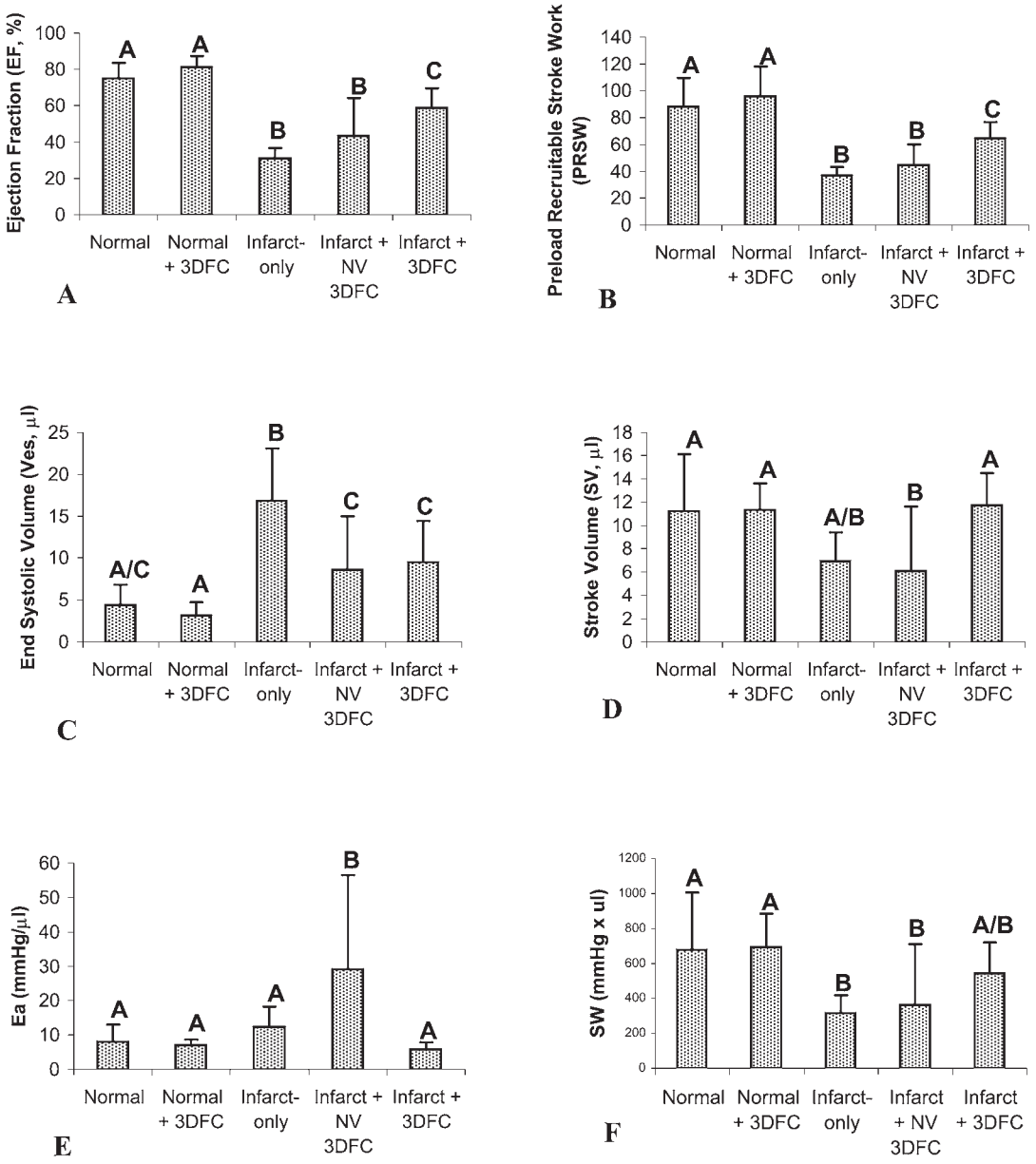

FIG. 5. (**A**) Ejection fraction, (**B**) preload recruitable stroke work, (**C**) end-systolic volume, (**D**) stroke volume, (**E**) elastance of artery, and (**F**) stroke work. Treatment groups with different letter designations (*A*, *B*, or *C*) are significantly different ($p < 0.05$).

not reveal as great an aneurysmal LV wall in comparison with infarct-only mice as evidenced by explant observations and end-systolic volume measurements. Furthermore, 3DFC treatment of normal SCID mice did not significantly alter (either positively or negatively) cardiac function measurements, which suggests that this specific treatment does not raise safety concerns when used on normal cardiac tissues.

These data help to continue efforts exploring the use of tissue-engineered constructs as inducers of therapeutic angiogenesis. Simply demonstrating the ability to stimulate angiogenesis may not correlate with therapeutic benefit. Therefore, the current study evaluated the LV performance benefits from a tissue-engineered cardiac patch that has previously been demonstrated to stimulate the formation of a new microvessel supply into damaged cardiac tissues.[6] Data from the current study indicate that the therapeutic benefit of this technology requires the viable cells that make up the cardiac patch. Previously, nonviable 3DFC constructs have been shown to make limited angiogenic contributions in damaged cardiac tissues.[6] Therefore, the findings in the current study, in

which infarcted hearts treated with nonviable 3DFC had no improvement in EF or PRSW values relative to infarct-only mice, were somewhat expected. Furthermore, EF and PRSW values were significantly higher in infarcted mice treated with viable 3DFC compared with either infarct-only or nonviable 3DFC-treated mice.

The viable 3DFC construct has previously been shown to produce a heterogeneous population of cytokines and growth factors including, but not limited to, VEGF, hepatocyte growth factor, platelet-derived growth factor A chain, keratinocyte growth factor, interleukin-6, interleukin-8, transforming growth factor-β, and angiopoietin-1 (Ang1).[7] In addition, the angiogenic activities of the 3DFC construct have been previously demonstrated in a variety of *in vitro* assays such as tests of endothelial cell proliferation and $\alpha_v\beta_3$ integrin induction[14] and new vessel outgrowth and human vascular endothelial cell motility in rat aortic ring assays,[15] and in *in vivo* tests of the chick chorioallantoic membrane.[14] Furthermore, the 3DFC construct has been shown to stimulate the development of a mature microvasculature with the presence of arterioles, capillaries, and venules within infarcted myocardial tissues.[6]

The current data indicate that the viable 3DFC patch helps to benefit global LV function in infarcted hearts. Although the exact mechanisms of action are not known, it is clear that viable 3DFC is a tissue-engineered construct that is capable of inducing angiogenic processes (evidenced through the previous *in vitro* studies)[14,15] and stimulating a new microvascular network within damaged cardiac tissues.[6] These angiogenic properties may be one mechanism by which the viable 3DFC patch helps to benefit global left ventricular function in infarcted or ischemic hearts. For example, when coronary vessels are narrowed and regions of myocardial perfusion are diminished, procedures are focused on preventing a loss in myocardial perfusion.[3,16–20] When infarcted tissue is already present in a diseased heart, increasing myocardial perfusion into this damaged myocardium may help to prevent continued progression of the infarct condition by revascularizing ischemic (reversible or hibernating) myocardium that borders the infarct.[20–22] This ischemic or hibernating myocardium represents an underperfused tissue that can be revascularized to achieve functional recovery.[22] Hibernating myocardium may be present in up to 50% of patients with significantly impaired LV function and evidence of heart failure.[23] In addition, data indicate that hibernating myocardium is present in about 78% of patients after acute myocardial infarction.[23] Importantly, these conditions of reduced cardiac function can be prevented or reversed by lessening the ischemic burden through reestablishing myocardial perfusion into the hibernating region.[22]

A second possible mechanism by which 3DFC may influence acutely infarcted myocardium lies in the extracellular matrix remodeling that is induced by the 3DFC material. 3DFC constructs have been well described to contain a number of different matrix proteins including collagen types I and III, fibronectin, and tenascin, and to produce a number of matrix-responsive factors.[8] The collagen proteins represent major structural proteins of myocardium whereas fibronectin serves a primary role in cell adhesion, spreading, and migration through the extracellular matrix. From the gross photographs (Fig. 3), it was apparent that the infarct-only mice experienced dramatic matrix remodeling in which structural integrity was compromised under the volumetric changes throughout the cardiac cycle. This resulted in an aneurysm of the myocardium. However, in infarcted mice treated with 3DFC, the matrix remodeling may have been influenced by the cytokines, growth factors, and matrix proteins produced by the 3DFC material, resulting in a more structurally intact myocardium that did not experience an aneurysm defect as a result of volumetric loading. The nonviable 3DFC group also experienced an aneurysm of the left ventricular wall, suggesting that the matrix alone does not have the same benefits as the matrix with viable cells. These observations are supported by the trends in the measured parameter of β. A decreased β parameter indicates that the heart is more distensible compared with normal heart β values. Infarct-only hearts demonstrated decreased β values, suggesting that these hearts had compromised structural integrity and were more distensible with increasing pressures. This measured β parameter helps to explain the observed aneurysm defect in infarct-only hearts. In contrast, 3DFC-treated hearts exhibited β values similar to those of normal SCID mice.

The possibility exists that 3DFC treatment may utilize a number of mechanisms to achieve overall improvements in left ventricular function. The significant improvements in LV function may collectively be a result of the following mechanisms: (1) an increase in new mature microvessel formation that increases local perfusion into the myocardium, thus reversing hibernating myocardium and preventing expansion of the infarct; and (2) matrix remodeling of the infarcted myocardium that results in a more compliant tissue that can adapt to changing volumes throughout the cardiac cycle.

Other investigators have evaluated a variety of therapeutic angiogenic modalities in efforts to stimulate "expansion of a collateral network that leads to enhanced myocardial perfusion, and ultimately, improved left ventricular function."[24] Specifically, single growth factors such as VEGF[1,18] or bFGF[2,25] have been injected into the coronary vasculature to stimulate myocardial collateral flow. In those studies, angiogenic therapies have been successful at stimulating new microvessel growth or an increase in myocardial collateral blood flow. However, in separate VEGF studies the use of VEGF treatment alone supported a leaky, immature, and hemorrhagic cap-

illary vasculature.[26] In addition, the combined use of VEGF and Ang1 was reported to stimulate the formation of a leakage-resistant vasculature that led to the development of a more mature microvasculature.[26] Nevertheless, other investigators have begun to observe positive results in clinical trials using single growth factor therapies. For example, intracoronary delivery of recombinant human vascular endothelial growth factor (rhVEGF) has produced limited myocardial perfusion benefits in patients.[4] Furthermore, clinical trials in which patients who have received intracoronary injection of granulocyte-macrophage colony-stimulating factor (GM-CSF) have reported some angiogenic benefits.[5] In light of these findings, future angiogenic therapies may need to appropriately select the correct single growth factor to be delivered in the appropriate dose; or these therapies may need to evaluate the use of multiple angiogenic factors to achieve a desired angiogenic benefit.

One advantage that tissue-engineered cardiac patches may have over single or multiple growth factor therapies is the ability to create a local angiogenic environment. For example, the viable 3DFC produces an angiogenic milieu that contains a variety of angiogenic growth factors, cytokines, and extracellular matrix proteins. This angiogenic milieu may serve to provide the local tissue needs with a biologic strategy that provides a complete environment for stimulation of new vessel formation and matrix remodeling. While we have not been able to clearly identify which component of the viable 3DFC construct is primarily contributing to the observed angiogenic response that positively influences global LV function, we speculate that this angiogenic milieu is responsible for the observed results.

Future angiogenic growth factor therapies, specifically tissue-engineered constructs that have the ability to produce local concentrations of angiogenic growth factors combined with a beneficial extracellular matrix environment, offer promising treatments for patient populations suffering from diffuse coronary disease. This patient population represents approximately 37% of patients who have undergone coronary artery bypass grafting (CABG).[16] In these patients, the myocardium that suffers diffuse disease remains ischemic because of the inability to perform a bypass procedure to reestablish local perfusion. Therefore, adjunct angiogenic therapies may serve an important role when used in combination with conventional treatments such as CABG.

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