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A Tissue Engineering Approach to Progenitor Cell Delivery Results in Significant Cell Engraftment and Improved Myocardial Remodeling

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ABSTRACT

Cell replacement therapy has become an attractive solution for myocardial repair. Typical cell delivery techniques, however, suffer from poor cell engraftment and inhomogeneous cell distributions. Therefore, we assessed the hypothesis that an epicardially applied, tissue-engineered cardiac patch containing progenitor cells would result in enhanced exogenous cell engraftment. Human mesenchymal stem cells (hMSCs) were embedded into a rat tail type I collagen matrix to form the cardiac patch. Myocardial infarction was induced by left anterior descending coronary artery ligation in immunocompetent male cesarean-derived fischer rats, and patches with or without cells were secured to hearts with fibrin sealant. After patch formation, hMSCs retained a viability of >90% over 5 days in culture. In addition, >75% of hMSCs maintained a high degree of potency prior to patch implantation. After 4 days in culture, patches were applied to the epicardial surface of the infarct area and resulted in 23% ± 4% engraftment of hMSCs at 1 week ($n = 6$). Patch application resulted in a reduction in left ventricle interior diameter at systole, increased anterior wall thickness, and a 30% increase in fractional shortening. Despite this improvement in myocardial remodeling, hMSCs were not detectable at 4 weeks after patch application, implying that improvement did not require long-term cell engraftment. Patches devoid of progenitor cells showed no improvement in remodeling. In conclusion, pluripotent hMSCs can be efficiently delivered to a site of myocardial injury using an epicardial cardiac patch, and such delivery results in improved myocardial remodeling after infarction. STEM CELLS *2007;25:2350–2357*

Disclosure of potential conflicts of interest is found at the end of this article.

INTRODUCTION

Myocardial infarction is the term for heart muscle death, either apoptotic or necrotic, resulting from an impaired myocardial blood supply. Repair of infarcted myocardium is mediated largely by fibroblast proliferation, collagen deposition, and scar formation [1, 2]. The myocardium does not regenerate appreciably because of the limited pool of cardiac specific progenitor cells present and the inability of adult cardiomyocytes to proliferate [3]. Recently, cellular cardiomyoplasty has been proposed as a strategy to repair myocardial damage after injury. This strategy involves encouraging replacement of lost myocardium with new cells having desirable properties. To date, addition of skeletal myoblasts [4–6], smooth muscle cells [7], fibroblasts [8], hematopoietic stem cells [9], cardiomyocytes [10, 11], umbilical cord blood-derived cells [12], embryonic stem cells [13, 14], and mesenchymal stem cells [15, 16] have shown improvement in cardiac function after myocardial infarction in animal models. Despite some disappointing results, several clinical trials suggest efficacy of cellular cardiomyoplasty in the treatment of human heart disease [17–20].

One factor likely to influence the success of cellular cardiomyoplasty is the number of cells delivered to the area of damage. Typical approaches to deliver cells to infarcted myocardium include i.v. injection, intracoronary (IC) injection, retrograde venous intracoronary (RIC) infusion, and intramyocardial (IM) injection. Although i.v. injection offers the advantage of being minimally invasive, it suffers from low cell engraftment (<1%) into the injury area [21, 22]. IC injection and RIC infusion provide somewhat more localized delivery of cells, resulting in improved but still limited cell engraftment (3%–6%) [21, 23]. IM injection offers direct localization of cells to the injured area, but engraftment (6%–12%) is limited by leakage out of the injection sites and cellular washout into the native venous shunts [22]. Moreover, this technique results in inhomogeneous cell delivery, with cell "islands" within the myocardial scar [18]. Therefore, we tested the possibility that a biodegradable, cellularized construct applied directly on the epicardial surface of the infarction could result in uniform delivery of cells with better engraftment efficacy than the more traditional cell delivery techniques. We refer to this construct as a "cardiac patch."

Using the cardiac patch as a delivery vehicle, we were able to achieve higher levels of cell engraftment in the infarct zone than have been demonstrated with more conventional delivery techniques. Moreover, this level of engraftment resulted in favorable indices of myocardial repair, suggesting

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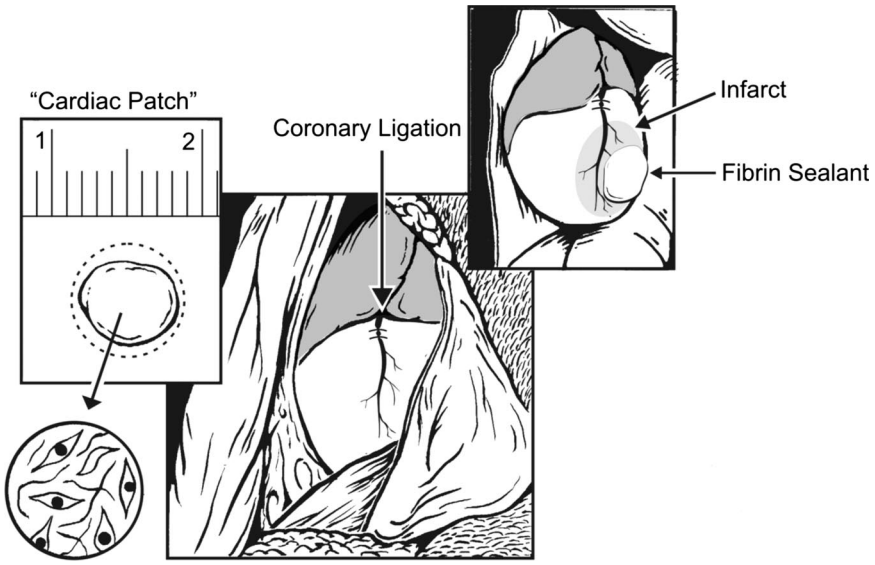

Figure 1. Schematic of infarct generation and epicardial patch placement. Collagen patches seeded with human mesenchymal stem cells are cultured for 4 days before placement. A permanent ligation of the left anterior descending coronary artery is used to induce infarction. Afterward, the patch is placed on the epicardial surface of the infarcted region and held in place with fibrin sealant.

that a tissue-engineered approach to progenitor cell delivery is feasible and may prove a desirable methodology to enhance cell engraftment.

MATERIALS AND METHODS

Animal Handling

Male CDF rats obtained from Charles River Laboratories (Wilmington, MA, http://www.criver.com) were allowed to acclimate to housing conditions for 1 week before use. All animals received care in compliance with federal and institutional guidelines with approval from the Institutional Animal Care and Use Committee.

Production of Cardiac Patches

We used patches containing bone marrow-derived human mesenchymal stem cells (hMSCs) because several studies have shown benefits with the use of these cells and because they are currently being used in several human clinical trials [17–19, 24]. CD34-negative hMSCs obtained from Cambrex (Walkersville, MD, http://www.cambrex.com) were expanded to passage (P)3–P6 before being embedded into a rat tail type I collagen matrix (BD Biosciences, San Jose, CA, http://www.bdbiosciences.com). hMSCs were cultured in complete medium consisting of Dulbecco's modified Eagle's medium (DMEM) containing 10% MSC qualified serum, L-glutamine, and penicillin/streptomycin at 37°C in 5% CO_2 (Cambrex). To produce cardiac patches for progenitor cell delivery, 1 million hMSCs were resuspended in a solution of rat tail collagen type I (BD Biosciences), 10% fetal bovine serum (FBS), and 0.1 M NaOH and adjusted with 5× DMEM (Gibco, Carlsbad, CA, http://www.invitrogen.com) such that the final collagen concentration was 2 mg/ml and the initial volume was 200 μl. Then, the solution was placed in individual wells of a tissue culture-treated 48-well plate to create a patch that was between 0.3 and 0.7 cm in diameter. Patches were cultured at 37°C in 5% CO_2 for 4–7 days before use. For the control experiments, nonviable cardiac patches were prepared by freezing 4-day-old patches overnight in phosphate-buffered saline at −80°C. The patches were thawed at room temperature and used for subsequent experiments.

Compaction of the patch, a property thought to describe cell interaction with the collagen, was determined by measuring the change in area of the patch over 5 days. The change in cross-sectional area was measured by taking images of the construct every 24 hours and measuring the diameter along at least three different dimensions using Matrox Inspector 3.0 software (Matrox Imaging, Dorval, QC, Canada, http://www.matrox.com/imaging/). Diameters were converted into areas, and change was represented as the percentage of reduction in area over 4 days.

Characterization of hMSCs in the Cardiac Patch

Viability. To assess cell viability within the construct, patches were digested in type I collagenase (650 U/ml; Worthington Biochemical Corp., Lakewood, NJ, http://www.worthington-biochem.com) for 45 minutes at 37°C. Collagenase activity was inhibited by the addition of FBS and complete hMSC media. Viability was measured using trypan blue with a hemocytometer. In addition, viability was assessed via fluorescence microscopy. Briefly, constructs were washed three times in phosphate-buffered saline (PBS) to remove serum. Fluorescent EthD-1 (4 μM, red) and Calcein AM (4 μM, green) (Molecular Probes Inc., Eugene, OR, http://probes.invitrogen.com) were then added for 45 minutes. Afterward, constructs were washed three times in PBS and viewed with a confocal microscope.

Differentiation. Differentiation of hMSCs within the patch was measured by monitoring the expression of CD73 (SH3) and CD105 (SH2) over 7 days. Cells were isolated from the patch by treatment in collagenase (650 U/ml) for 30 minutes at 37°C. Collagenase activity was inhibited by the addition of FBS and complete medium, and cells were washed in complete medium. Cells were stained with anti-CD73 and anti-CD105 diluted in 0.3% bovine serum albumin at 2°C–4°C for 30 minutes. Cells were than washed with PBS and analyzed by flow cytometry (BD Biosciences).

Infarct Model and Patch Application

Myocardial infarction (MI) was induced by permanent ligation of the left anterior descending (LAD) coronary artery in immunocompetent male CDF rats. Briefly, rats were anesthetized with 1.5% isoflurane. After endotracheal intubation and initiation of ventilation, the heart was exposed via a left thoracotomy, and the proximal LAD was ligated. Ten minutes after ligation, patches were applied onto the anterior wall of infarct site and secured with fibrin glue (Baxter, Deerfield, IL, http://www.baxter.com; Fig. 1). Rats with induced infarction and without construct application or with an acellular construct served as controls. Buprenorphine (0.03 mg/kg) was injected subcutaneously after surgery (and as necessary), and rats were allowed to recover under close supervision.

Echocardiography

Transthoracic echocardiograms were performed on rats using a SONOS 5500 ultrasound unit (Philips Medical Systems, Bothell, WA, http://www.medical.philips.com) equipped with a 15-MHz linear-array transducer and a 12-MHz phase-array transducer. The animals were maintained lightly anesthetized during the procedure

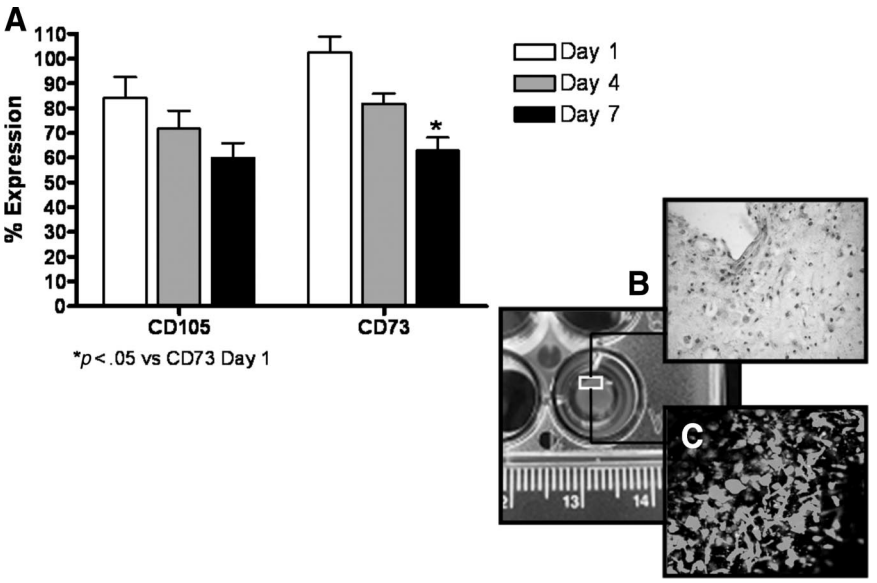

Figure 2. In vitro characterization of the cardiac patch. **(A):** Human mesenchymal stem cell (hMSC) potency as a function of time measured by monitoring the expression of CD105 and CD73. **(B):** A patch with representative sections showing hMSC incorporation using hematoxylin and eosin staining. **(C):** hMSC viability using live/dead staining (magnification, ×20).

with 1% isoflurane delivered through a face mask at a rate of 5 l/minute. The animals were kept warm on a heating pad. The body temperature was continuously monitored using a rectal thermometer probe and maintained between 36°C and 37°C by adjusting the distance of a ceramic heating lamp. Under these conditions, the animal's heart rate could be maintained above 300 beats per minute. Two-dimensional and M-mode echocardiography were used to assess wall motion, chamber dimensions, wall thickness, and fractional shortening. Color flow Doppler was used to assess valve function. Images were obtained from the parasternal long axis, parasternal short axis at the midpapillary level, apical four-chamber, apical two-chamber, and apical three-chamber views.

Baseline echocardiograms were acquired at 2–3 days post-MI with additional echocardiograms acquired at 4 weeks post-MI. The baseline post-MI echocardiograms served two purposes: (*a*) they allowed us to determine whether there were initial differences in infarct size between the MI control group and the patch-treated groups, and (*b*) they allowed us to select only animals with sufficiently large MI. We have prospectively established that an animal must have sustained a sizable anterior MI to be included in subsequent studies. We defined a sizable anterior MI as wall motion abnormalities involving at least two of the three anterior myocardial segments. Using this pre-established criterion, we excluded a total of two animals from our study: one from the MI control group and one from the MI + patch group.

Cardiac Hemodynamics

Cardiac hemodynamics were measured after the final echocardiographic examination. Rats were anesthetized with 1% isoflurane, and a 1.4F Millar Mikro-Tip catheter (SPR-671; Millar Instruments, Houston, TX, http://www.millarinstruments.com) was inserted into the right carotid artery and advanced into left ventricle. Aortic and left ventricular pressures were recorded on a PowerLab system and analyzed using Chart v4.2.4 software (ADinstruments, Colorado Springs, CO, http://www.adinstruments.com).

Myocardial Histology

After the hemodynamics study, hearts were removed, perfused with 4% paraformaldehyde, and then cryoprotected by immersion in 30% sucrose for 48–96 hours. Isopentane cooled in liquid nitrogen was used to freeze hearts immersed in optimal cutting temperature (OCT) medium. Sections were cut to 7 μm using a commercial cryostat and used for either immunohistochemistry or staining with hematoxylin and eosin or Masson's Trichrome. To assess engraftment efficiency, serial sections were taken at 1-mm intervals along the axis of the heart from the apex to the base. Total cell number was interpolated using a physical dissector methodology for stereology [25]. Using fluorescence microscopy, the numbers for human and 4′,6-diamidino-2-phenylindole (DAPI)-positive cells were counted within the midinfarct and peri-infarct region, and serial sections were compared to exclude points of cell intersection. Human cells were detected using a fluorescein isothiocyanate (FITC)-conjugated anti-human IgG (1:20; Sigma-Aldrich, St. Louis, http://www.sigmaaldrich.com). Equivalent results were obtained using anti-human human leukocyte antigen (1:20; Sigma-Aldrich), and a lack of fluorescence in the hearts without applied cells confirmed the antibody specificity. Engraftment was calculated as the number of FITC-positive cells within the native tissue divided by the number of intact cells initially delivered within the patch. Immunohistochemical staining with antibodies against α-smooth muscle actin (α-SMA; 1:200; Sigma-Aldrich) and von Willebrand factor (vWF; 1:500; Sigma-Aldrich) with appropriate secondary antibodies (anti-mouse IgG [Jackson Immunoresearch Laboratories, West Grove, PA, http://www.jacksonimmuno.com], anti-goat IgG, anti-rabbit IgG) were used to show blood vessel (calculated as vessels per field) and myofibroblast locations, respectively, within and around the infarct zone. Frozen sections were air-dried, and OCT was removed by rinsing slides in PBS. Nonspecific binding was blocked by incubating slides with 5% donkey or goat serum (Sigma-Aldrich) for 1 hour. The primary antibody was then added for 2 hours at room temperature or overnight at 4°C. After rinsing in 0.45% fish skin gelatin oil (Sigma-Aldrich), the secondary antibody was added for 1 hour and then counterstained with DAPI and mounted with DAKO anti-fade aqueous mounting medium (DAKO, Carpinteria, CA, http://www.dako.com).

RESULTS

In Vitro Characterization of the Cardiac Patch

To determine the suitability of the cardiac patch for transplantation of progenitor cells, we performed a series of experiments to assess hMSC differentiation, collagen compaction, and hMSC viability in vitro after casting cells in the collagen hydrogel. Progenitor cell potency is thought to be an important factor determining the degree of cardiac repair with cell replacement therapy. On days 4 and 7 of culture, the extent of hMSC differentiation was measured by monitoring the expression of two markers of hMSC potency, CD105 and CD73 [26]. As shown in Figure 2A, there was no significant decrease in either marker after 4 days, the day patches were applied in our experiments. Longer incubation resulted in a modest decrease in CD73 but not CD105 expression at 7 days. Therefore, hMSCs

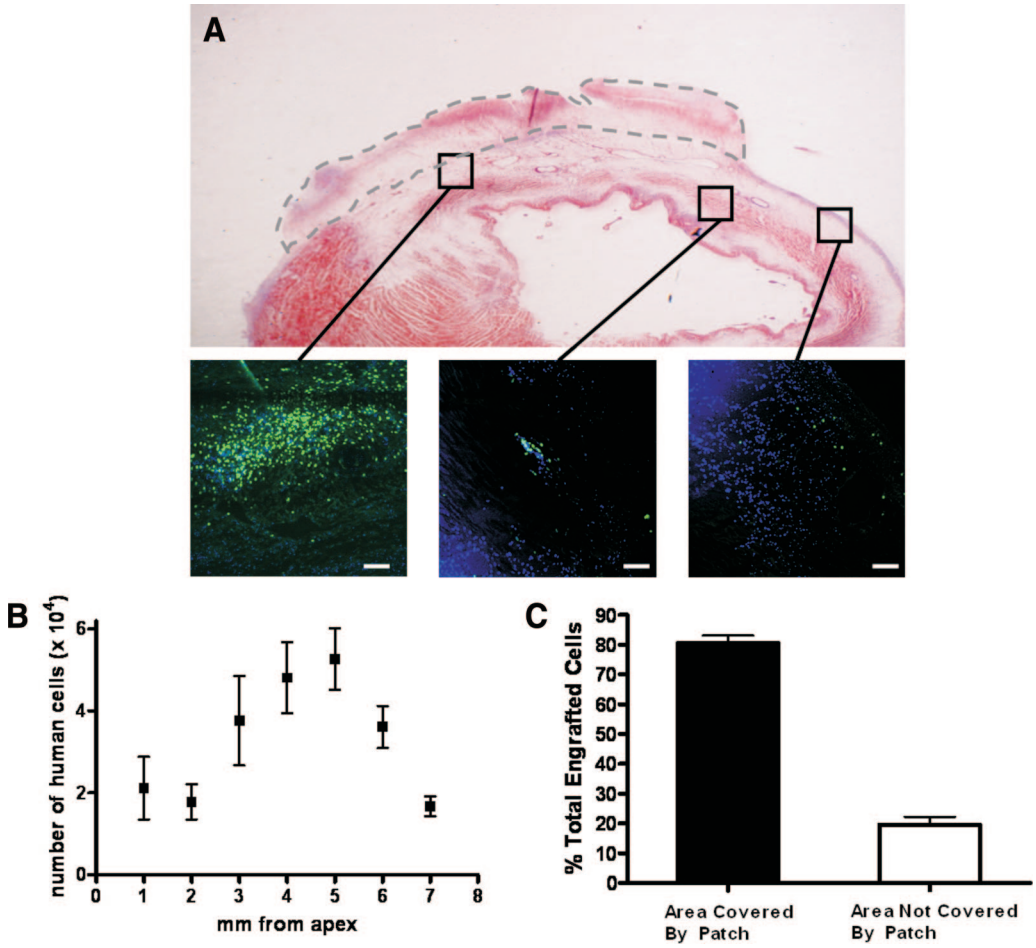

Figure 3. Engraftment of human mesenchymal stem cells (hMSCs) in the infarcted rat heart at 1 week. **(A):** Histological sections stained with Masson's trichrome showing myocardial engraftment of hMSCs beneath and remote from the cardiac patch, outlined by the dotted line, at 1 week (magnification, ×20; scale bars = 100 μm). **(B):** Engraftment distribution as a function of distance from the cardiac apex. **(C):** Engraftment distribution beneath and remote from the cardiac patch.

retained their original potency on the day the patch constructs were used.

During the in vitro culture period, there was evidence of cell-matrix interactions. Compaction is a measure of the interaction of cells and matrix. Compaction of the collagen construct imparted increased mechanical strength to the patch, providing for easier manipulation during transplantation. Compaction was quantified by the measurement of the maximum construct diameter on days 0 and 4. After 24 hours, constructs compacted maximally to 38% ± 2% (n = 5) of their original area, to a diameter of 3.7 ± 0.4 mm (Fig. 2B). This degree of compaction is similar to that seen using other cell types embedded in a collagen matrix [27]. Also, for successful delivery to the infarct, cells must remain viable in the patch. hMSC viability was found to be 94% ± 2% over 4 days in culture (Fig. 2C). Therefore, patches developed suitable mechanical properties, and embedded cells retained appropriate characteristics for in vivo application.

Progenitor Cell Engraftment and Distribution with Cardiac Patch Application

Cardiac patches were fixed in place on the epicardial surface of the heart, and cell engraftment was determined at 1 week. After the removal of rat hearts, they were fixed, cryopreserved, and embedded in OCT medium. Serial sections at 1-mm intervals were cut and stained for human antigen. Engraftment was analyzed across the infarcted region with fluorescence microscopy. Only cells that were positive for human antigen and DAPI and located within the myocardium were counted as being engrafted (Fig. 3). We found that 23% ± 4% of the applied cells engrafted at this time (i.e., 1 week after patch application; n = 6). Most engraftment occurred directly underneath the applied patch, with 80% ± 3% of the hMSCs found in this area of the myocardial anterior wall. Of these engrafted cells, 1.0% ± 0.2%, 14% ± 3%, and 85% ± 3% were found in the endocardial, midmyocardial, and epicardial regions, respectively. Nevertheless, some cell migration away from the patch occurred. Of the engrafted hMSCs, 20% ± 3% were found in the infracted region not covered by the patch. These cells showed a similar proclivity to engraft in the epicardial region. Of the cells engrafted away from the patch, 3% ± 1%, 39% ± 4%, and 58% ± 4% were found in the endocardium, midmyocardium, and epicardium, respectively. Cells within the midmyocardium were typically found among necrotic myocardium. Occasionally, we observed hMSC engraftment in regions as far as 1 mm from the apex of the heart.

Efficacy of the Cardiac Patch in Postinfarct Remodeling

To assess the efficacy of this cell delivery method, postinfarct remodeling was studied using echocardiography and invasive hemodynamics. Baseline echocardiograms at days

Table 1. Echocardiographic measures of myocardial remodeling and function

	Sham control ($n = 4$)	MI control ($n = 10$)	MI + patch ($n = 11$)	MI + NV patch ($n = 7$)
LVIDd (mm)	7.5 ± 0.2	9.5 ± 0.1[a]	8.9 ± 0.1[a,b,c]	9.7 ± 0.1[a]
LVIDs (mm)	3.9 ± 0.2	7.8 ± 0.1[a]	6.6 ± 0.3[a,b,c]	8.0 ± 0.2[a]
FS (%)	48 ± 2	19 ± 1[a]	25 ± 2[a,b,c]	18 ± 1[a]
AWTh (mm)	1.01 ± 0.01	0.34 ± 0.03[a]	0.59 ± 0.03[a,b,c]	0.37 ± 0.02[a]
L/S	1.55 ± 0.04	1.29 ± 0.02[a]	1.40 ± 0.02[a,b,c]	1.26 ± 0.03[a]

[a] $p < .05$ vs. sham.
[b] $p < .05$ vs. MI.
[c] $p < .05$ vs. MI + NV patch.
Abbreviations: AWTh, anterior wall thickness; FS, fractional shortening; L/S, end-diastolic major axis/minor axis ratio; LVIDd, left ventricular internal diameter at end diastole; LVIDs, left ventricular internal diameter at end systole; MI, myocardial infarction; NV, nonviable.

Table 2. Hemodynamic measures of myocardial function

	Sham ($n = 5$)	MI ($n = 10$)	MI + patch ($n = 13$)	MI + NV patch ($n = 7$)
SBP (mmHg)	132 ± 3	126 ± 3	131 ± 2	123 ± 2
DBP (mmHg)	98 ± 1	96 ± 3	99 ± 2	93 ± 2
LVESP (mmHg)	136 ± 4	126 ± 4	128 ± 3	124 ± 1
LVEDP (mmHg)	5.1 ± 0.3	8.9 ± 1.2	9.1 ± 1.2	7.6 ± 1.6
+dp/dt (mmHg/s)	10,080 ± 567	8,320 ± 213[a]	8,774 ± 228[a]	8,518 ± 229[a]
−dp/dt (mmHg/s)	−9,476 ± 463	−6,960 ± 245[a]	−7,065 ± 168[a]	−6,396 ± 202[a]

[a] $p < .05$ vs. sham.
Abbreviations: −dp/dt, maximum rate of decrease in left ventricular pressure during diastole; +dp/dt, maximum rate of rise in left ventricular pressure during systole; DBP, diastolic blood pressure; LVEDP, left ventricular end-diastolic pressure; LVESP, left ventricular end-systolic pressure; MI, myocardial infarction; SBP, systolic blood pressure.

2–3, presumably before any substantial effects of the applied progenitor cells, showed no difference in the initial infarct size between the MI control group and patch-treated animals, 39% ± 1% and 38% ± 2% of the left ventricle (LV), respectively. At 4 weeks, MI resulted in statistically significant adverse remodeling in all five parameters measured, as shown in Table 1. Application of patches containing hMSCs statistically significantly improved all five parameters as compared with MI-only animals. At 4 weeks after infarction, the hearts of patch-treated animals showed less dilatation in left ventricular internal dimensions and better preserved anterior wall thickness (Table 1). These findings were consistent with a 11.5% reduction in internal left ventricular diameter and a 29.8% increase in wall thickness in the MI + patch group compared with the MI-only group as assessed by histological morphometry. Fractional shortening was also better preserved in patch-treated hearts as compared with MI control hearts (25% ± 2% vs. 19% ± 1%; $p < .05$), and the end-diastolic major axis/minor axis ratios suggested a less spherical LV in the patch-treated animals as compared with the MI control group.

To determine whether the improvements were mediated by hMSCs or by cardiac patch placement itself, we used patches without viable cells (i.e., nonviable patches). A freeze/thaw cycle was used to eliminate hMSCs within 4-day old patches, and these nonviable patches with size and mechanical properties equivalent to those of the cellular patches were transplanted onto infarcted myocardium in an identical manner. As shown in Table 1, all remodeling parameters were statistically unchanged between MI control animals and nonviable-patch-treated animals. Also, animals treated with the cardiac patch showed improvements in all five parameters compared with animals treated with nonviable patches, suggesting that hMSCs, and not the collagen alone, were responsible for favorable remodeling.

In general, hemodynamic measures were less sensitive to infarction and subsequent patch application. Despite creating relatively uniform, large infarcts, only +dp/dt, the rate of rise of left ventricular pressure, and −dp/dt, a measure of diastolic relaxation, were statistically different between the control and MI groups, and despite the improvements in postinfarct structural remodeling as assessed by echocardiography, there was no statistical differences in hemodynamic parameters between the MI and MI + patch groups (Table 2).

Increased Myofibroblast Expression with Patch Application

The mechanism whereby bone marrow-derived progenitor cell replacement therapy improves myocardial function is unknown. Possibilities include proliferation and differentiation of exogenously applied MSCs or paracrine effects on native cells [28, 29]. To investigate these two possibilities further in our system, we replicated the histological analysis for MSCs at 4 weeks. This analysis showed that despite high initial engraftment rates at 1 week, no hMSCs or residual patch were detectable at 4 weeks. This suggested that the advantageous effect of patches containing MSCs was not the result of long-term MSC proliferation and differentiation.

Since MSC application has been reported to increase angiogenesis [30], we performed additional histological analysis for this possibility. Immunohistochemical staining for vWF at 4 weeks after MI showed only a trend toward an increase in the number of blood vessels throughout the peri-infarct and infarcted regions of patch-treated animals (7.2 ± 2.1 vs. 10.3 ± 1.3 vessels per field for control and patch-treated animals, respectively; Fig. 4A, 4B). On the other hand, we found a marked increase in the number of cells in the infarct area expressing α-SMA. α-SMA-positive cells were increased in patch-treated animals (1.5% ± 0.5% vs. 4.6% ± 1.1%; $p < .01$;

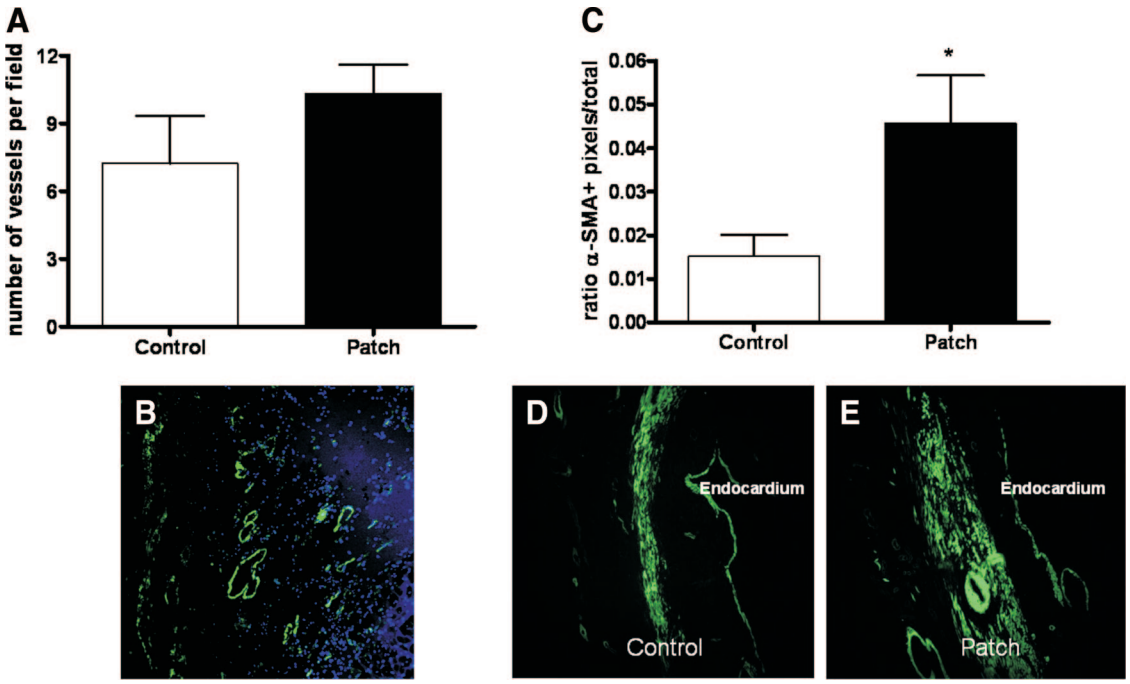

Figure 4. Increased number of α-SMA-positive cells at 4 weeks with patch placement. **(A):** At 4 weeks, the number of blood vessels was not increased by patch placement. **(B):** Representative image from patch-treated heart stained for vessels with anti-von Willebrand factor. **(C):** α-SMA expression was significantly increased with the application of patches compared with controls ($n = 4$; $*$, $p < .05$ vs. controls). Representative fluorescence microscopy using anti-αSMA for control **(D)** and patch-treated **(E)** hearts (magnification, $\times 20$). Abbreviation: α-SMA, α-smooth muscle actin.

Fig. 4C). Most of these α-SMA-expressing cells were located in the midmyocardium and away from blood vessels (Fig. 4D, 4E). Since α-SMA expression is a marker for vascular smooth muscle cells and myofibroblast and most cells expressing the marker were not associated with blood vessels, these cells likely represent an increase in myofibroblasts within the infarct region in response to patch application. Finally, these α-SMA-positive cells did not stain for human antigen, suggesting that patch application encouraged recruitment, differentiation, or both of native cells.

DISCUSSION

Reconstituting infarcted tissue with cells capable of performing the functions of the heart or providing beneficial trophic factors for native cells is an attractive solution for myocardial repair [31]. To reconstitute myocardium, a large number of cells will need to be delivered efficiently. In this study, we tested the feasibility of a tissue-engineered approach to stem cell delivery by delivering hMSCs embedded in a biodegradable collagen matrix to the site of an infarct. Using this approach, we were able to achieve high levels of cell engraftment and show improvements in postinfarct remodeling, despite the relatively modest number of cells initially delivered (nominally, 1×10^6 cells).

Several tissue engineering approaches have been undertaken for cardiac cell replacement therapy [6, 14, 32–37]. These include the use of biomaterial-based cellular patches to restore myocardial function. Additional studies have attempted to optimize different cardiac constructs for myocardial repair in vitro [38–40]. Our approach, however, differs in several regards. First, previous studies using a type I collagen hydrogel were conducted in immunosuppressed models, making comparison with our results difficult [33, 35]. Second, to date, no study has investigated the use of hMSCs embedded in a collagen matrix as a repair tool in vivo. Finally, we have attempted to quantify the extent of early cell engraftment using the cardiac patch as a delivery vehicle.

This method of cell delivery leads to engraftment that exceeds reported rates for other delivery techniques. In a study comparing the relative efficiency of cell delivery by IM, IC, and interstitial retrograde coronary venous (IRV) delivery, it was found that these injection procedures resulted in only modest engraftment [23]. Specifically, IM injection resulted in 11% engraftment, whereas IC and IRV injections resulted in 2.6% and 3.2% engraftment, respectively. Similar studies performed by Freyman et al. [21] showed that 14 days after IC infusion, engraftment was 6%, and this delivery procedure was also accompanied by reduced coronary blood flow and subsequent myocardial injury. In our study, patch delivery achieved an initial cell engraftment of 23%. Although this is an improvement over the other techniques, calculating the exact percentage of cells delivered is complicated by an unknown rate of hMSC proliferation and loss. Nevertheless, these rates are likely to be similar between delivery techniques. Possible explanations for this increased delivery include that exogenously applied cells remained fixed in proximity to the infarct area, increasing the opportunities for engraftment. The technique had the additional advantage of delivering cells in a relatively homogenous manner as compared with the next most efficacious delivery technique, direct injection.

Although our purpose in these experiments was to demonstrate the feasibility of the patch delivery system, applied MSCs showed favorable effects on postinfarct myocardial remodeling. This outcome is similar to that described by others using MSCs. For example, Uemura et al. found that with a similar number of applied bone marrow stem cells delivered via intraventricular injection, there were significant improvements in left ventricular ejection fraction and left ventricular internal diameter at systole

[29] Other measures of remodeling and function in their study, however, were unchanged, including wall thickness and infarct size [29].

Despite the improvements in echocardiographic measures of remodeling, we were not able to show significant changes in hemodynamic parameters at 4 weeks after patch placement. This result is consistent with reports using a similar number of MSC [30, 41]. At higher doses of 5 million or more cells, however, there is evidence of improvements in hemodynamic measures, as well as structural remodeling [28, 42]. This observation reinforces the need for high engraftment rates to maximally affect myocardial remodeling. Nevertheless, the fact that echocardiographic parameters showed improvements, whereas hemodynamic parameters showed only trends toward improvement in our experiments, suggests that pressure measurements in rats are a relatively insensitive measure of myocardial function. Alternatively, it is possible that hemodynamic improvements might have been noted if remodeling had been allowed to continue longer.

Based on the experiments with nonviable patches, it seems likely that the improvement in remodeling seen was mediated by the applied cells rather than some effect of the biodegradable matrix. Despite this conclusion, it is possible that future permutations with different matrices or different configurations of the same matrix will allow improvements in the results with the patch approach. For example, Gaballa et al. found that acellular three-dimensional collagen foam scaffolds can reduce cardiac remodeling and induce angiogenesis, reporting that these scaffolds can lead to reduced left ventricular dilation and scar area [43].

Although the mechanism of any benefit of MSC application in humans is likely multifactorial, our experiments imply that favorable remodeling can be seen in the absence of long-term cell engraftment. Probably related to the xenograft nature of our experiments and the application of cells into immunocompetent animals, we observed no retained hMSCs 4 weeks after patch application. Our results are similar to those of Leor et al. [12], where the injection of activated human macrophages into immunocompetent male Sprague-Dawley rats resulted in improved myocardial healing and function. In these experiments, human macrophages survived only 4–7 days, but their presence during early healing led to significant improvements in left ventricular dimensions and fractional shortening at 5 weeks. Alternatively, we observed more cells in the infarct region that expressed α-SMA, presumably mostly myofibroblasts, since most of the additional cells were not associated with blood vessels. Myofibroblasts have previously been shown to assist in favorable postinfarct remodeling [44] and therefore may represent a novel mechanism for the observed remodeling effects of the patch. In any event, our experiments confirm that some improvement in myocardial repair with MSCs is mediated through a paracrine effect.

CONCLUSION

Our experiments suggest that it is possible to deliver progenitor cells to injured myocardium using a collagen hydrogel. Moreover, this approach appears to result in higher initial engraftment rates than conventional approaches. Refinements of this approach, such as using matrices with desirable effects on cell differentiation or maintenance, may serve to enhance any benefits gained by cellular cardiomyoplasty.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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