Functional Consequences of a Tissue-Engineered Myocardial Patch for Cardiac Repair in a Rat Infarct Model

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Cell therapies have emerged as a promising treatment for the prevention of heart failure after myocardial infarction (MI). This study evaluated the capacity of an aligned, fibrin-based, stretch-conditioned cardiac patch consisting of either the native population or a cardiomyocyte (CM)-depleted population (i.e., CM+ or CM- patches) of neonatal rat heart cells to ameliorate left ventricular (LV) remodeling in the acute-phase post-infarction in syngeneic, immunocompetent rats. Patches were exposed to 7 days of static culture and 7 days of cyclic stretching prior to implantation. Within 1 week of implantation, both patches became vascularized, and non-CMs began migrating from CM+ patches. By week 4, patches had been remodeled into collagenous tissue, and live, elongated, donor CMs were found within grafted CM+ patches. Significant improvement in cardiac contractile function was seen with the administration of the CM+ patch (ejection fraction increased from $35.1\% \pm 4.0\%$ for MI only to $58.8\% \pm 7.3\%$ with a CM+ patch, $p < 0.05$) associated with a 77% reduction in infarct size ($61.3\% \pm 7.9\%$ for MI only, $13.9\% \pm 10.8\%$ for CM+ patch, $p < 0.05$), and the elimination of LV free-wall thinning. Decreased infarct size and reduced wall thinning also occurred with the administration of the CM- patch (infarct size $36.9\% \pm 10.2\%$, LV wall thickness: $1058.2 \pm 135.4 \mu\text{m}$ for CM- patch, $661.3 \pm 37.4 \mu\text{m}$ for MI only, $p < 0.05$), but without improvements in cardiac function. Approximately 36.5% of the transplanted CMs survived at 4 weeks; however, they remained separated and electrically uncoupled from the host myocardium by a layer of CM-free tissue, which suggests that the benefits of CM+ patch transplantation resulted from paracrine mechanisms originating from CMs. Collectively, these observations suggest that the transplantation of CM-containing engineered heart tissue patches can lead to dramatic improvements in cardiac function and remodeling after acute MI.

Introduction

HEART FAILURE often develops after acute myocardial infarction (MI) because the injured myocardial tissue fails to recover or regenerate.¹ Therapies designed to increase the rate of repair by injecting cells of various lineages, including progenitor cells, directly into injured myocardial tissue have produced some evidence of benefit in both preclinical and clinical studies,^{2–6} but the engraftment and survival rates of the transplanted cells are low, and the differentiation of engrafted cells into cardiomyocytes (CMs) occurs even less frequently.^{2–8} Thus, the effectiveness of cell therapy for myocardial regeneration remains limited,^{9–12} and engineered cardiac tissue patches have emerged as an attractive alternative. Cardiac patch implantation enables a large number of cells to be administered and

retained near the site of infarction, and if the patch is composed of actively contracting cells, then it may restore function and prevent bulging in the scarred region, reduce wall stress, and improve myocardial bioenergetics.^{5,13}

The mechanisms responsible for the improvements associated with cell therapy post-MI are largely unknown. The transplanted cells may limit left ventricular (LV) remodeling and infarct expansion by fusing directly with native tissues, stimulating the growth of new blood vessels that subsequently increase perfusion to the infarcted area,² releasing cytokines that reduce CM apoptosis,³ to improve the passive mechanical properties of the ventricle.⁶ However, very few transplanted cells are retained in the infarcted region, perhaps because they fail to create focal adhesions with neighboring cells, or because the inflammatory response to myocardial injury and the hypoxic environment of the infarct zone

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reduce cell survival.¹⁴ Cell retention increases when the transplanted cells are administered in a three-dimensional scaffold,^{9,13} which isolates the cells from the inflammatory environment of the infarct and provides a niche for the cells to reside in after implantation. Tissue engineering can refine this approach by manipulating the scaffold to improve cellular organization, intercellular communication, and extracellular matrix (ECM) deposition.

A number of different scaffolds have been used for myocardial tissue engineering, including synthetic polymers^{15–17} and biological materials, such as collagen and fibrin with or without matrigel,^{18–23} and a variety of methods have been developed for conditioning the engineered tissues before implantation.^{15,17,18,24–26} For CM-containing engineered heart tissues, cyclical stretching stimulates gap-junction formation (including expression of the gap-junction protein connexin 43^{20,27}), and CM hypertrophy,²⁴ which collectively increase the contractile force generated by the engineered tissue.^{20,24} While some studies have assessed the benefits of a cardiac patch in the acute phase using non-cardiac cells²⁸ or when administered 14 days postinfarction,²⁹ an engineered cardiac patch using a fibrin scaffold in the acute phase, postinfarction has yet to be investigated. Thus, for the experiments described in this report, we created engineered heart tissue patches by seeding a fibrin gel with either the complete native population or a CM-depleted population of neonatal rat cardiac cells, and then stimulated cellular alignment and maturation by cyclically stretching the patches for 7 days. A CM-depleted population was used to evaluate whether or not any benefits obtained from the patch originated from CMs or from non-CM paracrine effects. The patches were evaluated in a rat acute myocardial injury model to determine whether fibrin-based engineered tissues could improve cardiac function and limit adverse cardiac remodeling 1 and 4 weeks after experimentally induced MI.

Materials and Methods

Construction and culture of myocardial (CM+) patches

Patches containing CMs (CM+) were constructed by combining a mixed population of neonatal rat cardiac cells from 48- to 72-h-old neonatal syngeneic Fisher F344 rats (Harlan Sprague-Dawley), isolated via a serial collagenase digestion, as previously described,²⁰ at a final concentration of 4×10^6 cells/mL with a fibrin-forming solution consisting of bovine fibrinogen (5 mg/mL; Sigma), bovine thrombin (2 U/mL; Sigma), and 2 μ M CaCl₂ to create a final fibrin concentration of 3.3 mg/mL. The fibrin-forming solution containing cells was injected into 13-mm-long, 1.25-mL tubular molds consisting of an 8-mm-OD Teflon mandrel coated in 2% agarose to allow for cell-induced gel compaction and alignment in the circumferential direction as previously described.²⁰ The cell-containing solutions were incubated for 15 min at 37°C to allow the formation of fibrin gel. Once formed, the gels were placed into culture medium [Dulbecco's modified Eagle's medium, 10% heat-inactivated horse serum (to limit fibroblast proliferation), 1% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 2 mg/mL aminocaproic acid (to limit fibrinolysis), with 2 μ g/mL insulin and 50 μ g/mL ascorbic acid (to promote ECM production)], and cultured statically for 7 days.

Non-CM (CM-) patch construction

Non-CMs were selected through preplating of the cardiac cell isolate for 45 min in complete medium (10% FBS) to remove slow-adhering CMs. Cells were cultured in growth medium (DMEM high glucose with 15% FBS and 1% penicillin/streptomycin) until confluent, before harvesting for patch construction. Immunostaining for cardiac troponin T (cTnT) revealed the initial cell population had been reduced to $6.0\% \pm 2\%$ CMs at the time of harvest. Non-CM (CM-) patches were fabricated at a cell concentration of 2.4×10^6 cells/mL in order to match the approximate number of non-CMs contained in CM+ patches made from the entire isolate, as the freshly isolated cardiac cell population was found to contain ~40–45% CMs and 55–60% non-CMs. The CM- patches were cultured otherwise identical to CM+ patches.

Cyclic stretching of patches

After 7 days of static culture, samples were transferred onto inflatable latex tubes mounted in a cyclic distension bioreactor.³⁰ Two different parameters were investigated: stretch amplitude and duration of stretching, and a regimen of 5% circumferential stretch ratio and 1 Hz stretch frequency was determined to be optimal based on the magnitude of patch contractile response. Patches were cyclically stretched for 7 days before implantation, for a total culture time of 14 days, with medium change every other day. Further methods and results on cyclic stretching can be found in Supplementary Data (Supplementary Data are available online at www.liebertpub.com/tea).

Implantation of patches into an acute rat infarct model

Procedures used in this study were reviewed and approved by the University of Minnesota-Twin Cities Institutional Animal Care and Use Committee (IACUC) and the Research Animal Resources (RAR) Committee.

Female Fisher F344 syngeneic immune-competent rats, weighing 175–199 g (Harlan Sprague-Dawley), were used in these studies. Rats were anesthetized and intubated, and the heart was exposed through a limited left lateral thoracotomy. To induce an MI, the pericardium was opened and the left anterior descending (LAD) coronary artery was permanently ligated with a 6-0 polypropylene suture. After an MI had been established, ~5–10 min postligation, a single patch was applied across the epicardial surface of the infarcted area of the left ventricle, approximately parallel with surface myocardial alignment. Patches, some of which were incubated with 1 μ g/mL DAPI overnight prior to implantation to label donor cells, were removed from their mandrels, cut into three individual 8-mm strips, and sutured parallel to each other on the epicardial surface of the LV over the infarct area immediately below the ligation using resorbable sutures. The epicardium was scratched in this process to allow for the patch to bond with the host myocardium. After patch placement, the chest was closed and the animal was allowed to recover.

Functional analysis

Cardiac function was evaluated via echocardiograph assessments of fractional shortening (FS) and left-ventricular

ejection fraction (LVEF), as previously described,³¹ both 1 and 4 weeks postimplant. Hearts were harvested at either the 1- or 4-week time point for histological assessment.

Histology and immunohistochemistry

Nonimplanted patches were fixed in 4% paraformaldehyde, frozen in embedding medium (Tissue-Tek OCT), cut with a cryostat into 5–9- μ m sections at -18°C , and placed onto Superfrost Plus Microscope slides (ThermoFisher Scientific) for histological analysis.

After harvest, hearts were sliced circumferentially into 2–4-mm slices and slices were either fresh frozen in embedding medium, cut into 5- μ m slices at -18°C , and fixed in 4% paraformaldehyde, or fixed overnight in a 10% formalin solution, paraffin embedded, and cut into 4- μ m slices and placed onto slides for histological analysis.

Cyrosections were labeled with fluorescent antibodies and subsequently imaged to assess the cellularity and cell composition of the patch prior to implantation, and the patch-grafted area on the heart. Cell density and cellular composition of the patches were determined using a custom MATLAB program. Live cell percentage was determined as the percentage of cells staining positive for both DAPI and f-actin.

Infarct size and wall thicknesses are reported as the average of measurements performed on Masson's trichrome-stained sections from each of three locations in the heart: immediately below the ligation suture, mid-way between the ligation suture and the apex, and near the apex. Infarct sizes were calculated as the percentage of LV free-wall surface area that was occupied by scar tissue, and wall thicknesses were measured across the infarcted portion of the wall.

Twitch force measurements

Nonimplanted CM+ patches were subjected to twitch force assessment before fixation and histological analysis. Twitch force measurements were recorded on a custom-built testing system as previously described²⁰ both spontaneously and in response to various pacing frequencies. Briefly, a CM+ patch was placed in medium containing 2 mM CaCl_2 , looped over a fixed post and one adjustable post coupled to a force transducer, pretensioned to 1 g, and subjected to electrical field stimulation as previously described. Contractile force generation both spontaneously and in response to stimulation was recorded using a custom LabVIEW algorithm, and data were analyzed using Matlab. Twitch force was characterized as peak amplitude of the contractile response minus baseline force.

Experimental design and statistical analysis

Four groups were investigated for this study: (1) sham ($n=5$): no ligation, the chest and pericardium were opened and then closed; (2) MI only ($n=6$): ligation of the LAD coronary artery and no treatment; (3) CM– patches ($n=5$): patches made with nonmyocyte cardiac cells; and (4) CM+ patches (1 week, $n=3$; 4 weeks, $n=7$): patches made with CMs+ nonmyocyte cardiac cells.

Statistical analysis

Data are presented as means $\pm$ standard deviations. All results were compared using MiniTab statistical software.

One-way analysis of variance (ANOVA) in conjunction with Tukey HSD *post hoc* testing was used to compare all groups. p -Values <0.05 were considered significant.

Results

In vitro characterization of CM+ and CM– myocardial patches

Figure 1A shows stretch-conditioned patches prior to implantation. Both CM+ and CM– patches had compacted into short tubes, or rings, and were dimensionally similar with thicknesses of $657 \pm 41.3 \mu\text{m}$ and $694 \pm 70.9 \mu\text{m}$, and axial lengths of $3.15 \pm 0.29 \text{ mm}$ and $2.72 \pm 0.3 \text{ mm}$, respectively. Both samples had a circumferential length of 25.1 mm, based on the mandrel outer diameter. Both patches had similar moduli (CM+: $86.0 \pm 3.8 \text{ kPa}$; CM–: $135.5 \pm 46.7 \text{ kPa}$) and ultimate tensile strength (UTS; CM+: $75.7 \pm 11.5 \text{ kPa}$, CM–: $47.2 \pm 12.1 \text{ kPa}$) in the aligned (circumferential) direction in the rings, values near to what has been reported for myocardium (50–100 kPa for both modulus and UTS).^{16,32,33}

CM+ patches exhibited visible, measurable contractions both spontaneously and synchronously in response to electrical pacing from 1 to 4 Hz frequency (Fig. 1B, C). The maximum pacing frequency in which a twitch force could be measured occurring synchronous with stimulation averaged between 5 and 7 Hz, comparable to the heart rates of the rats used in these studies. The twitch force ranged from 1.3 to 2.7 mN over 1–4 Hz in a negative force–frequency relation, which has been reported previously,³⁴ values that were over twofold higher than samples cultured statically for 14 days (see Supplementary Data).

To assess the quality and consistency of implanted patches, a subset of samples intended for implantation were histologically and functionally evaluated after stretch conditioning to quantify their cellular composition and contractile response. These patches had approximate cell densities of $1540 \pm 631 \text{ cells/mm}^2$ for CM+ patches and $1030 \pm 219 \text{ cells/mm}^2$ for CM– patches based on immunofluorescent staining of 5- μ m sample cross-sections (Table 1). However, cellularity was not homogenous throughout the patches. α -Smooth muscle actin–positive (SMA+) cells being located primarily on the abluminal surface of CM– patches (Fig. 1I) and higher densities of CMs were located near the outer surface and edges of the ring-shaped patches (Fig. 1E). SMA+ cells within the CM+ patches were primarily found in areas containing CMs (Fig. 1F). Cells throughout the patches exhibited elongated morphologies aligned in the circumferential direction (Fig. 1D, G) and adjacent CMs within the CM+ patches exhibited gap junctions as well (Fig. 1D). CM+ patches were found to contain $90.1 \pm 7.1\%$ live cells (f-actin+/DAPI+), $39.1 \pm 16.5\%$ of which were CMs (f-actin+/DAPI+/cTnT+) and $15.2 \pm 3.7\%$ were positive for α -smooth muscle actin (f-actin+/DAPI+/SMA+), indicating they were either smooth muscle cells or myofibroblasts. The final percentage of CMs was similar to that of the initial cell population, which can be attributed to the use of horse serum, which limits fibroblast proliferation, in the culture medium. Only a few cells were found to be CD31+ endothelial cells in either group. Cells positive for neither of the CM markers, SMA or CD31, were assumed to be

FIG. 1. *In vitro* characterization of cardiac patches: twitch force and cellularity. Ring-shaped patches prior to cutting into strips for implantation were characterized. (A) Two patches on a distensible latex mandrel in the cyclic distension bioreactor. Scale bar = 5 mm. (B, C) Twitch force generation of cardiomyocyte-positive (CM+) patches in response to electrical pacing of variable frequencies. Circumferential (D, G) and cross-sectional (E, F), (H–I) views of CM+ and CM– patches, showing elongated, aligned cells in each patch (D, G) and cell distribution of both cardiac troponin T-positive (cTnT+) and α -smooth muscle actin-positive (SMA+) cells in each patch. Few CMs were found in the CM– patch, and SMA+ cells were located close to the abluminal surface in both patches. Color images available online at www.liebertpub.com/tea

quiescent cardiac fibroblasts, which constituted $45.7\% \pm 20.2\%$ of the final cell population. CM– patches were found to contain $92.7\% \pm 3.8\%$ live cells, $1.8\% \pm 1.0\%$ CMs (as shown in Fig. 1H), $11.5\% \pm 14.1\%$ SMA+ cells, and $80.4\% \pm 15.2\%$ quiescent fibroblasts.

In addition to histological interrogation of the patches to assess cellularity, extracellular matrix production by the entrapped cells was also evaluated. Trichrome staining (Fig. 2B) revealed that after 14 days of *in vitro* culture, the content of the patch ECM remained primarily fibrin. However, collagen I and the basement membrane proteins laminin and collagen IV were deposited in the extracellular space surrounding all cells within the CM+ patches, and

significantly less of collagens I and IV were produced in the CM– patches (Fig. 2A, C) based on quantitative image analysis of staining intensity. Small amounts of collagen III and fibronectin were also present in the ECM of both patches (not shown).

Functional consequences of patch implantation

Echocardiography was conducted both 1 and 4 weeks postoperatively. One week postimplant, neither treatment group displayed a reduction in EF or FS of the LV wall compared with the sham surgery, but there was a reduction in cardiac function in the animals receiving MI only (data

TABLE 1. CELL COMPOSITION IN PATCHES PREIMPLANTATION

Patch	Cell density (cells/mm ²)	% Live	% CMs	SMA+	CD31 ⁺
CM+	1540 $\pm$ 631	90.1% $\pm$ 7.1%	39.1% $\pm$ 16.5%	15.2% $\pm$ 3.7%	<0.5%
CM–	1030 $\pm$ 219	92.7% $\pm$ 3.8%	1.8% $\pm$ 1.0%	11.5% $\pm$ 14.1%	<0.5%

CM, cardiomyocyte; SMA+, α -smooth muscle actin-positive.

not shown). Four weeks postimplantation, both EF and FS were further reduced in animals receiving MI only as compared with values 1 week postligation (Fig. 3A, B). Animals that received the CM- patches showed no improvement in either EF or FS over MI only. Hearts that received CM+ patches had EFs and FSs substantially higher than both MI-only and CM- patch groups, values that were not different

than the sham-operated group (Fig. 3B). This indicates that a patch containing the full complement of cardiac cells, CMs included, yielded the best functional outcome.

Histological assessment of LV remodeling

Animals that received MI only had large infarcts (Fig. 4A), comprising on average $61.3\% \pm 7.9\%$ (range 53.7–69.5%) of

FIG. 4. Infarct size and LV free-wall thickness from coronal sections. Masson's trichrome image of an MI-only heart (A), and hearts that received a CM-patch (B) and CM+ patch (C). The engrafted patch can be seen as the thin blue layer on the outside of the heart in (C). CM-patches were not easily distinguishable using Trichrome due to large infarct scars. Color images available online at www.liebertpub.com/tea

the LV free-wall surface area, with significant wall thinning to $661.3 \pm 37.4 \mu\text{m}$, or $\sim 25\%$ of the thickness of the non-infarcted myocardium (Table 2). CM- patches resulted in significant reduction of infarct size (Fig. 4B) to $36.9\% \pm 10.1\%$ (range 30.9–48.6%). Thinning of the LV free wall was also substantially reduced to $1058.2 \pm 136.4 \mu\text{m}$, or $\sim 40\%$ of the thickness of the noninfarcted myocardium. Concurring with the functional data, animals that received the CM+ patch had very small infarcts (Fig. 4C) of $13.9\% \pm 10.8\%$ (range 5.4–29.1%) of the LV free wall. Wall thinning was also substantially reduced over both MI-only and CM- patch groups, to $2274.6 \pm 111.5 \mu\text{m}$, a value not significantly different than that of the sham-operated myocardium (Table 2).

Engraftment of Patches

At 1 week postimplant, CM+ patch recipients were found to have a large amount of adhesions and scar tissue surrounding the heart, rendering it difficult to remove the heart with the patch intact. After 4 weeks *in vivo*, the presence of adhesions was reduced, and hearts that received either CM+ or CM- patches had a thin, pale film covering the epicardial surface of the LV anterior wall, consistent with a collagenous tissue film revealed with trichrome staining not seen on a sham-operated myocardium (Fig. 5A–C) extending across both the infarcted and noninfarcted areas of the LV epicardial surface. This film was not seen in sham-operated hearts (Fig. 5A) or those receiving ligation only (not shown) and had an average thickness of $372.6 \pm 55.39 \mu\text{m}$ and $131.0 \pm 39.0 \mu\text{m}$ for CM+ patch and CM- patch recipients, respectively. The engrafted patches underwent near-complete remodeling *in vivo*, with the ECM being converted from being primarily fibrin at implantation (Fig. 2B) to highly collagenous at explantation (Fig. 5B, C).

TABLE 2. INFARCT SIZE AND LEFT VENTRICULAR THICKNESS OF 4-WEEK IMPLANTS

Group	Infarct size	LV anterior wall thickness
Sham (no ligation)	—	$2618.9 \pm 221.4 \mu\text{m}^{\text{a,b}}$
MI only	$61.3\% \pm 7.9\%$	$661.3 \pm 37.4 \mu\text{m}$
MI+CM- patch	$36.9\% \pm 10.2\%^{\text{a}}$	$1058.2 \pm 135.4 \mu\text{m}^{\text{a}}$
MI+ CM+ patch	$13.9\% \pm 10.8\%^{\text{a,b}}$	$2274.6 \pm 37.4 \mu\text{m}^{\text{a,b}}$

^a $p < 0.05$ versus MI only.

^b $p < 0.05$ versus CM- patch.

LV, left ventricular; MI, myocardial infarction.

Elongated CMs were found within the patches of animals that received CM+ patches (Fig. 5E, F), but not CM- patches (Fig. 5D).

Cryosections of these hearts revealed that the tissue film contained large numbers of DAPI+ cells (Fig. 6), indicating that these cells were donor cells and that the film seen was the remodeled patch. DAPI+ cells were also found in the host myocardium in hearts that received CM+ patches, indicating that there was cell migration from the engrafted patch into the myocardium. Invading donor cells were found both 1 and 4 weeks postimplantation (Fig. 6A, B), and donor cells were associated with vascular structures within the host myocardium and interspersed with host cells (Fig. 6B). Invading donor cells located outside of vascular structures, presumably cardiac fibroblasts (cells both SMA- and CD31-), were found inside the host myocardium and in neotissue identifiable as the zone devoid of CMs between the host myocardium and patch. At 1 week postimplant, bright DAPI+ cells were widely spread throughout the patch and the interface, and cells were seen in the myocardium as well. At 4 weeks postimplant, DAPI+ cells were much dimmer. This dimming may be attributed to dilution of the DAPI label through cell division.

Both the CM+ and CM- patches were vascularized *in vivo*, with ingrowth of host endothelial cells seen both 1 and 4 weeks postimplantation (Fig. 7C, D). Vascular structures that contain red blood cells were found throughout the entire engrafted areas of both CM+ (Fig. 7A, B) and CM- (not shown) patches, with no difference in the vascular density within the patches between the two groups (not shown). The presence of red blood cells in these vessels indicates that the patches were perfused through angiogenesis from the host myocardium. In the engrafted CM+ patch, the percentage of CMs present was reduced to $11.0\% \pm 10\%$ after 4 weeks *in vivo*, with a very heterogeneous distribution. There was over an 18-fold increase in the cell density in the engrafted patch compared with patches prior to implantation. This increase in cell density and reduction in CM percentage could be attributed to cell death due to ischemia postimplantation, proliferation of donor non-CMs *in vivo*, and the infiltration of host cells into the patch. Factoring in the increase in cell density and the percentage of CMs within the patches, it can be estimated that 36.5% of implanted CMs survived transplantation. Gap junctions between donor CMs were found within the patch (Fig. 5F); however, electrical coupling of donor CMs to the host myocardium was not possible due to the presence of a $101 \pm 40 \mu\text{m}$ non-CM neotissue zone between the CM+

FIG. 5. Patch engraftment 4 weeks postimplant. Masson's trichrome-stained sections of a sham-operated (A), CM- patch (B), and CM+ patch (C) recipient heart, indicating the presence of a remodeled patch affixed to the epicardium. (D-F) Immunohistochemistry of patches. CMs (cTnT+ cells) are present in the engrafted CM+ patch (E), and an absence of cTnT+ cells is visible in the CM- patch (D), along with substantial thinning of the LV free wall. (F) Sarcomeric organization is visible in CMs within the engrafted CM+ patch, along with evidence of gap junctions between adjacent CMs within the patch. Color images available online at www.liebertpub.com/tea

patch and the host myocardium. This area consisted primarily of fibroblasts of both donor (DAPI+) and host origin, but also contained numerous microvessels spanning the zone. Additionally, alignment of the patch with the host myocardium was not completely maintained. Regional alignment of donor CMs within the CM+ patches was seen, but this alignment did not consistently match the alignment of the host CMs along the epicardial surface of the heart. This misalignment may be attributed to movement or misplacement of the patch during or shortly after implantation, but before patch attachment to the host engraftment.

Discussion

The major new findings of this study are that engraftment of the CM+ patch occurred by 4 weeks after implantation, accompanied by a remarkable amelioration of LV re-

modeling underneath the patch (Figs. 4 and 5) in the form of a reduction in both LV scar size and wall thinning accompanied by a substantial improvement of LV contractile function (Fig. 3). The administration of a CM- patch also resulted in a reduction of LV scar formation and thinning of the LV free wall, but to a lesser extent than the CM+ patch (Fig. 4 and Table 2) and without improvements in contractile function, suggesting that the cytokine benefits observed with this treatment require communication between CMs and nonmyocyte cardiac cells.

Fibrin-based cardiac patch

The creation of an *in vitro* myocardial patch is an area that has seen significant and increasing interest for many years, leading to a number of studies that utilize a variety of different cell sources and fabrication methods. The objective of restoring LV contraction by transplantation of a patch that

FIG. 6. Invasion of DAPI-pre-labeled cells into host myocardium. Blue = DAPI-pre-labeled donor cells. Cell invasion occurred for CM+ patches at 1 week (A) and 4 weeks (B) postimplantation. Some of the invading cells were associated with vascular structures within the host myocardium (white arrowheads). Color images available online at www.liebertpub.com/tea

FIG. 7. Vascularization of the CM+ patch. (A, B) H&E of an engrafted CM+ patch after 4 weeks *in vivo*. Red blood cells, noted by black arrowheads and indicating perfusion, are visible in the patch–host interface (A) and in the patch itself far (B) from the host myocardium. SMA/CD31 staining of engrafted patches both 1 week (C) and 4 (D) weeks after implantation, showing both host endothelial cell migration into the patch and the establishment of vessels in the patch. Color images available online at www.liebertpub.com/tea

generates physiological contractile stress to the injury site of the LV has been largely unsuccessful.^{4–6,8–12} Rather than trying to replace the damaged myocardium, the present study evaluated the capacity of a patch, with or without donor CMs present, to restore LV contraction. Fibrin was chosen as the scaffold due to its biocompatibility and capacity to be remodeled both *in vitro*^{35,36} and *in vivo*.³⁷ Fibrin possesses many of the properties of collagen that are advantageous for tissue engineering,³⁸ such as being a native substrate for cell adhesion and the capacity for the protein fibrils to become aligned when cell-induced gel compaction is mechanically constrained.³⁹ This cell-induced alignment has been shown to be associated with an increase in the contractile force generation of engineered myocardial tissues.²⁰ Fibrin gels have also been seeded with human embryonic stem cell–derived CMs,⁴⁰ murine cardiovascular progenitor cells, or with both mESC-derived CMs and nonmyocyte-supporting cells to generate engineered cardiac tissues. These tissues contained aligned, highly differentiated, and electromechanically coupled CMs that exhibited rapid-conduction velocities (22–25 cm/s) and contractile forces of up to 2 mN in static culture.²¹

Cardiac-patch-induced myocardial protection

One of the major objectives of the present study was to evaluate the efficacy by which a fibrin-based, stretch-conditioned myocardial patch can limit LV remodeling and infarct expansion when administered in the acute-phase postinfarction. It was a surprise finding that the patch implantation caused a dramatic reduction of infarct size (Fig. 7).^{13,41} In addition, the beneficial effects were remarkably more prominent in hearts that received a CM+ patch compared with a CM– patch (51% reduction in CM– and 77% reduction for CM+, $p < 0.05$, Fig. 4 and Table 2).

Although the transplantation of a non-CM patch was associated with reduction in infarct size and LV wall thinning, these beneficial effects were improved and cardiac function was completely restored in hearts that received a patch containing both CMs and non-CMs (Figs. 3 and 4). Although a large fraction of the donor cardiomyocytes (36%) were estimated to survive 4 weeks of implantation within the CM+ patches, these remarkable effects were not caused by their electrical coupling to the host myocardium as the presence of a physical barrier in the form of a non-CM neotissue zone separates donor CMs from the host myocardium and prevents electrical coupling between them. This zone could be scar formation as a result of the patch implantation or the patch remodeling and engraftment process itself. Due to the thin size, low stiffness properties, and modest twitch force generation of the CM+ patch when implanted, these effects were also not likely a result of passive or active force inhibition of LV scar formation and dilation, but rather through cytokine support provided by donor CMs. The affixed patch on the surface of the infarcted region prevented host CM death immediately after infarction. Two physical effects occur as a consequence of this cytokine support. First, a reduction in host CM death in the infarct zone prevents overstretching of neighboring host ventricular CMs, thus blocking the detrimental signaling pathways initiated by CM overstretch.⁴² Second, the regional LV wall stress, and therefore the energy demand of the host myocardium is decreased which in turn prevents LV bulging.^{43,44} Consequently, ischemic CMs that would otherwise progress toward apoptosis are spared until perfusion can be re-established. The end result of these two events is a significant reduction in LV scar size and a subsequent reduction in LV dilation.

Since we employed a permanent LAD occlusion model, the level of reduction of coronary flow at initiation of

occlusion was identical among the hearts with or without patch implantation. Therefore, the reduction of infarct size that resulted from the patches was a result of either sparing the ischemia-threatened CMs that otherwise would go on to the apoptotic pathways, as noted earlier, or from the mobilization of the endogenous cardiac progenitors to the injury site to facilitate regeneration. Survival of donor CMs within the patch (Fig. 6C, D, F) was not surprising, as neonatal rat CMs have been shown to be hypoxia resistant due to their ability to rely on glycolysis⁴⁵ until a vascular network has been established.

Cytokine effects

Invasion of donor cells from the CM+ patch, but not from the CM- patch, into host myocardium was seen both 1 and 4 weeks postligation (Fig. 6), suggesting that the important paracrine effect of the patch requires communication between CM and non-CM cardiac cells. For this reason, a CM-enriched patch might not be as effective as a CM+ patch. Cytokine-driven cardiac repair has been hypothesized to be mediated primarily by non-CMs,^{46,47} and yet apparent cytokine benefits in this study were seen to a much higher degree in the CM+ patch compared with the CM- patch. Thus, CMs played an indirect yet crucial role in this system. While there are a wealth of studies that investigate the role non-CMs have on CMs in culture and during development,^{18,21,46–49} the influence CMs exert on non-CMs has been less thoroughly investigated. When exposed to stretch, cardiac fibroblasts have been shown to increase ECM production, but only in the presence of CMs or CM-conditioned media through CM-produced TGF- β 1.^{50,51} This result is consistent with the CM+ patches used in this study, with an increase in both collagen I and collagen IV compared with CM- patches (Fig. 2C).

Previous work has shown that cell-based therapies can improve cardiac function postinfarction through paracrine factors or neovascularization.^{43,44} It was also shown that stretch-conditioned engineered patches constructed from neonatal rat cells entrapped in a collagen gel limit further LV remodeling and electrically couple to the host myocardium when implanted 14 days postinfarction, with improvements in cardiac function in cases of severe infarctions.²⁹ However, these functional improvements were limited, and no invasion of donor cells into the host myocardium was reported. The patch-to-heart size ratio was larger compared to this study, perhaps resulting in benefits through passive or active mechanical effects not seen in this study. Interestingly, no beneficial effects were reported with the administration of collagen-based non-CM patch, whereas a decreased size of infarct and a reduction in LV free-wall thinning occurred in this study for the fibrin-based CM- patches. These differences may be attributed to either or both the difference in scaffold (collagen/matrigel) of the patch and the timing of treatment.

Conclusions and Future Work

This study demonstrated the capacity of a fibrin-based heart patch containing neonatal rat heart cells to engraft 4 weeks after transplantation, accompanied by a remarkable reduction of infarct size and the elimination of LV thinning, and the restoration of cardiac function. The mechanisms for

these effects are not clear, but are likely related to a cytokine effect in which CMs play an indirect yet crucial role. Thus, future studies that investigate the signaling pathways of this cytokine effect initiated by this myocardial patch are warranted.

Acknowledgments

The authors would like to thank M. Tradewell for Matlab and cell culture contributions. This work was funded in part by a grant from the Minnesota Partnership for Biotechnology and Medical Genomics, and by an NIH NHLBI grant (HL095077, HL67828, HL114120, HL100407). J.S. Wendel was supported in part by a GAAN assistantship from the Department of Education.

Disclosure Statement

The authors declare that no competing financial interests exist.

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Received: May 24, 2013

Accepted: November 13, 2013

Online Publication Date: February 3, 2014