Functional Effects of a Tissue-Engineered Cardiac Patch From Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes in a Rat Infarct Model

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Abstract

A tissue-engineered cardiac patch provides a method to deliver cardiomyocytes to the injured myocardium with high cell retention and large, controlled infarct coverage, enhancing the ability of cells to limit remodeling after infarction. The patch environment can also yield increased survival. In the present study, we sought to assess the efficacy of a cardiac patch made from human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to engraft and limit left ventricular (LV) remodeling acutely after infarction. Cardiac patches were created from hiPSC-CMs and human pericytes (PCs) entrapped in a fibrin gel and implanted acutely onto athymic rat hearts. hiPSC-CMs not only remained viable after in vivo culture, but also increased in number by as much as twofold, consistent with colocalization of human nuclear antigen, cardiac troponin T, and Ki-67 staining. CM+PC patches led to reduced infarct sizes compared with myocardial infarction-only controls at week 4, and CM+PC patch recipient hearts exhibited greater fractional shortening for all groups over 4 weeks, and LV thinning was not mitigated. CM+PC patches became vascularized in vivo, and microvessels were more abundant in the host myocardium border zone, suggesting a paracrine mechanism for the improved cardiac function. PCs in a PC-only control patch did not survive 4 weeks in vivo. Our results indicate that cardiac patches containing hiPSC-CMs engraft onto acute infarcts, and the hiPSC-CMs survive, proliferate, and contribute to a reduction in infarct size and improvements in cardiac function.

Significance

In the present study, a cardiac patch was created from human induced pluripotent stem cell-derived cardiomyocytes and human pericytes entrapped in a fibrin gel, and it was transplanted onto infarcted rat myocardium. It was found that a patch that contained both cardiomyocytes and pericytes survived transplantation and resulted in improved cardiac function and a reduced infarct size compared with controls.

Introduction

Cardiovascular diseases remain a leading cause of death in the developed world [1]. Despite advances in interventional and pharmacological therapies, these treatments remain largely palliative. In light of these shortcomings, cell-based therapies have become an attractive approach to prevent heart failure after infarction. Cardiac tissue engineering, which aims to generate functional myocardium in vitro, provides a method to deliver cells to the injured myocardium with enhanced cell survival and large graft sizes, enhancing the ability of cells to regenerate myocardium in vivo and limit left ventricular (LV) remodeling after infarction. With the discovery of induced pluripotent stem cells (iPSCs) [2] and the ability to differentiate human iPSCs into functional cardiomyocytes (hiPSC-CMs) [3, 4], these cells have become a prime candidate for use in cardiac tissue engineering. First differentiated to cardiomyocytes using the embryoid body method [3], hiPSCs are now differentiated using a matrix sandwich method [5] or small molecule methods modulating the Wnt/β-catenin signaling pathway [6] to provide the high yields of hiPSC-CMs needed for use in engineered cardiac tissues (ECTs). These methods have been able to achieve highly variable CM purities; however, for enhanced therapeutic effect or to study the interactions of CMs with a defined population of non-CM cells, further purification could be necessary. Purification of CMs has been achieved through genetic methods using puromycin resistance [7] and non-genetic...
methods such as the use of CM-specific cell surface markers for fluorescence [8–10] and magnetic-activated cell sorting [11], microdissection [12], and targeting metabolic pathways, such as used in lactate-based purification [13]. Human pluripotent stem cell–derived CMs have been used for myocardial repair by direct myocardial injection; however, the engraftment rates have been very low a few weeks after transplantation [14, 15], making engineered tissues an attractive method to deliver CMs to the myocardium. Although numerous studies have been reported for ECTs constructed using neonatal rat cells [16–18], far fewer have been made from human pluripotent stem cells. Human embryonic stem cell–derived CMs (hESC-CMs) have been entrapped in fibrin-Matrigel [19], collagen-Matrigel [20] (BD Biosciences, San Diego, CA, http://www.bdbiosciences.com), and Gelfoam scaffolds (Pfizer, New York, NY, http://www.pfizer.com) [21]. These studies have shown that the addition of non-CM cell types not only facilitates the compaction of biopolymer hydrogels, but also results in improved contractile force generation and survival of entrapped cardiomyocytes. Only a few reports have been published of an ECT prepared from hiPSC-CMs, entrapped in fibrin-Matrigel [19], collagen-Matrigel [20] or fibrin-Matrigel [23] scaffolds, and these were not implanted onto infarcted myocardium. Thus, it is as yet unknown whether ECTs made from clinically relevant hiPSC-CMs will yield similar effects as ECTs made from hESC-CMs in an infarct model.

The present study reports ECTs made from hiPSC-CMs entrapped in a fibrin gel and used pericytes (PCs) as a non-CM cell population. PCs are vascular support cells that in the context of microvascular tissue engineering have been shown to regulate capillary stability and diameter [24–27]. PCs were chosen for the present study to induce compaction of the fibrin gel [24], which is essential to concentrate and align CMs [17] and to support the ingrowth of host microvessels into the ECT [28]. In addition, future strategies that include preformed microvessels in fibrin gel, together with CMs, can use a mixture of hiPSC-CMs, PCs, and endothelial cells. Our results demonstrate that PCs can be coentrapped with hiPSC-CMs as a support cell type, eliminating the need for additional cell types in future studies.

Expanding on a previous study that reported on ECTs fabricated with neonatal rat cardiac cells, the present study investigated the use of an aligned ECT, referred to as a patch, consisting of lactate-purified hiPSC-CMs and human PCs entrapped in a sacrificial fibrin gel to engraft into a nude rat acute infarct model and limit LV remodeling in vivo. The efficacy of the patch was assessed 4 weeks after transplantation via echocardiography and histological characterization of the scar size and patch engraftment and compared with the effects of an engineered cardiac tissue created using only PCs.

**MATERIALS AND METHODS**

**Culture and Purification of Human iPSC-Derived Cardiomyocytes and Human Brain Pericytes**

Human iPSC-derived cardiomyocytes were prepared in the laboratory of Dr. Timothy Kamp at the University of Wisconsin-Madison. The hiPSC line DF19-9-11T [29] was used in the present study for cardiomyocyte differentiation. The cells were differentiated via the small molecule Wnt/GSK3 inhibition protocol [6], and the purity of the cardiomyocytes was measured by flow cytometry for cardiac troponin T-positive (cTnT+) cells at 15 days into differentiation. hiPSC-CMs with a purity of 70%–95% cTnT+ cells were frozen into cryovials in 90% fetal bovine serum (FBS), 10% dimethyl sulfoxide at 10 × 10⁶ cells per vial before shipping. The cells were thawed into T75 flasks coated with 2.5 µg/cm² fibrinogen (Sigma-Aldrich, St. Louis, MO, http://www.sigmaaldrich.com), 0.1 mM non-essential amino acids (NEAA; Gibco), 0.1 mM β-mercaptoethanol (Gibco) in Dulbecco’s modified Eagle’s medium (DMEM)/F12 basal media (Corning, Acton, MA, http://www.corning.com/lifesciences) for 48 hours and reduced to 2% serum media (EB2) for an additional 24 hours before beginning the purification process on day 4 of culture. The cells were purified using medium composed of glucose-free DMEM (Invitrogen, Carlsbad, CA, http://www.invitrogen.com) supplemented with 4 mM lactate acid (Sigma-Aldrich; stock diluted to 1 M in HEPES), NEAA, β-mercaptoethanol, penicillin/streptomycin, and 2 mM l-glutamine (Sigma-Aldrich) [13]. The cells were treated with lactate media for 4 days, changing the lactate media twice on days 4 and 6, before being returned to EB2 on day 8 for an additional 2 days, before being harvested for construct preparation on day 10 of culture. The final cardiomyocyte purity was 94.2% ± 2.9% cTnT+ cells. hiPSC-CMs were harvested for construct preparation by exposing the cells to a solution of 0.25% Trypsin (HyClone, GE Healthcare Life Sciences, South Logan, UT, http://www.promo.gelifesciences.com) with 2% chicken serum (HyClone) for 5–10 minutes before quenching with EB20 medium. Green fluorescent protein (GFP)-labeled human brain pericytes were obtained from the laboratory of Dr. George Davis (University of Missouri-Columbia, Columbia, MO) and had been previously expanded, characterized, and transfected to express nuclear GFP [30]. Pericytes were cultured for 10 days on gelatin-coated flasks in culture media consisting of low-glucose DMEM (Gibco), supplemented with 10% FBS, 1% penicillin/streptomycin, and 0.01 mg/ml gentamycin (Gibco). Pericytes were harvested by exposing cells to a solution of 0.05% Trypsin EDTA (HyClone) for 5 minutes before quenching with FBS.

**Construction and Culture of Patches**

Aligned patches containing both cardiomyocytes and pericytes (CM+PC) were constructed by mixing 2.2 × 10⁶ purified CMs and 3.4 × 10⁶ PCs with a fibrin-forming solution and injecting it into a 8-mm-ID, 1.25-mm tubular mold, as previously described [17], to create a final fibrin concentration of 3.3 mg/mL. The cell-containing, fibrin-forming solutions were incubated at 37°C for 15 minutes to allow formation of a fibrin gel. The gels were then removed from their casings and placed in EB20 culture medium supplemented with 2 mg/ml aminocaproic acid (to limit fibrinolysis; Acros Organics, Geel, Belgium, http://www.acros.com) for the first 48 hours of culture, followed by culture in 2% serum medium for the remaining 12 days of culture and 14 days of total static culture. Pericyte-only (PC) patches were constructed identical to CM+PC patches, containing 3.4 × 10⁶ PCs in a 1.25-mL, 3.3 mg/mL fibrin gel. hiPSC-CM-only patches were not possible to construct because the cell-induced fibrin gel compaction required to convert soft isotropic fibrin gel into a patch with functional CMs that can be sutured does not occur without the presence of non-CMs, which has also been reported by others [31, 32].
Implantation of Patches into an Acute Nude Rat Infarct Model

The procedures used in the present study were reviewed and approved by the Institutional Animal Care and Use Committee and Research Animal Resources at the University of Minnesota.

Female Foxn1nu nude rats (Harlan Sprague-Dawley) weighing 135–190 g were used in these studies. The rats were anesthetized using isoflurane, intubated, and received a limited left lateral thoracotomy to expose the heart. The pericardium was opened, and myocardial infarction (MI) was achieved by permanently ligation of the left anterior descending coronary artery with a 7-0 polypropylene suture. After MI was established, a single patch was applied by using isoflurane, intubated, and received a limited left lateral thoracotomy to expose the heart. The pericardium was opened, and myocardial infarction (MI) was achieved by permanently ligation of the left anterior descending coronary artery with a 7-0 polypropylene suture. After MI was established, a single patch was applied by removing one ring from its mandrel (Fig. 1A) and cutting it into 3 strips and sputuring them parallel to each other over the epicardial surface of the left ventricle below the ligation suture, approximately parallel to the alignment of the surface myocardium (Fig. 1B). After patch placement, the chest was closed, and the rat was allowed to recover. The rats were administered 2.5 mg/kg ketoprofen and 15 mg/kg enrofloxacin (Baytril; Bayer, Leverkusen, North Rhine-Westphalia, Germany) immediately postoperatively.

Cardiac Functional Analysis

Cardiac function was evaluated via echocardiographic assessments (Veo2100; VisualSonics, Toronto, CA, http://www.visualsonics.com) of left ventricular ejection fraction and fractional shortening before ligation (baseline) and at both 1 and 4 weeks after implantation. The hearts were harvested after 4 weeks in vivo for histological assessment.

Characterization of Patches

A subset of patches from the same batches was not implanted, and instead these patches were subjected to twitch force assessment to evaluate their contractile force generation. Twitch force measurements were recorded, as previously described [16], using a custom-built testing system both spontaneously (when applicable) and in response to pacing. In brief, CM-containing patches were placed in a media bath containing DMEM/F12 basal medium supplemented with 2 mM CaCl2, pretensioned between two posts, and subjected to field stimulation from two carbon electrodes. Data were recorded using LabVIEW and analyzed using a MATLAB script.

Histology and Immunohistochemistry

Nonimplanted patches and explanted hearts were fixed in 4% paraformaldehyde, frozen in embedding medium (Tissue-Tek O.C.T. compound; Sakura Finetek USA, Torrance, CA, http://www.sakura.com), and cryosectioned into 5-μm sections for immunohistochemistry and histological characterization. For immunofluorescence staining, the sections were permeabilized with 0.01% Triton-X100 (Sigma-Aldrich), blocked in 5% normal donkey serum (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, http://www.jacksonimmuno.com), incubated for 10 minutes at room temperature. After washing, the sections were incubated overnight at 4°C in primary antibody, followed by a 60-minute incubation in secondary antibody at room temperature and a 10-minute incubation in Hoescht 33342 (Life Technologies, Carlsbad, CA, http://www.lifetech.com). Slides were mounted in fluorescent mounting medium (Dako, Glostrup, Denmark, http://www.dako.com) and imaged within 24 hours. The sections labeled for isolecitin B4 (IB4) were incubated for 10 minutes at room temperature. Additional information on immunofluorescence antibodies can be found in supplemental online Table 1.

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The infarct size was measured from Masson’s trichrome-stained sections as the percentage of the surface area of the left ventricular anterior wall occupied by scar. For each heart, the infarct size was averaged from three areas of the heart: just below the ligation suture, midway between the ligation suture and the apex, and a section close to the apex. The left ventricular anterior wall thickness was measured across the infarcted region of the heart wall.

Statistical Analysis and Experimental Design

Three groups were investigated for the present study: group 1, MI only (n = 5): ligation only, no treatment; group 2, CM+PC patch (n = 6): ligation plus patch constructed with cardiomyocytes and pericytes; and group 3, PC patch (n = 4): ligation plus patch constructed with PCs only.

The data are presented as the mean ± SD. All results were analyzed using MiniTab statistical software. Student’s t tests and one-way analysis of variance, in conjunction with Tukey honest significant difference post hoc testing, were used to compare all groups. p values < .05 were considered significant.

RESULTS

In Vitro Characterization of Patches

To establish that hiPSC-CMs can be coentrapped with PCs to create an aligned, beating patch, patches were created and characterized in vitro before implantation. Both CM+PC and PC patches compacted into short tubes of comparable length from an initial length of 13 mm to less than 3 mm (Fig. 1A). CM+PC patches had compacted down from an initial thickness of 2.83 mm to less than 0.5 mm (406 ± 1.2 m). PC patches had a thickness of 403.8 ± 2.8 m, and PC patches in both patches were well distributed across the patch thickness (Fig. 2A, 2D), elongated and
aligned in the circumferential direction (Fig. 2B, 2C), and elongated CMs displayed organized sarcomeres (Fig. 2C).

CM+PC patches generated measurable forces in response to multiple pacing frequencies (Fig. 2E, 2F). The average twitch force generation ranged from 0.35 to 0.46 mN (0.15–0.20 mN/input CM) over 0.5–1.5 Hz stimulation frequency. The maximum frequency in which a patch beat synchronously with pacing ranged from 1.5 to 2.0 Hz for each patch.

Cells within both CM+PC and PC patches had begun to deposit extracellular matrix (ECM) throughout the scaffold during in vitro culture (Fig. 3), despite the overall ECM remaining primarily fibrin at the time of implant (Fig. 3G, 3H). Collagen I (Fig. 3A, 3D) and basement membrane proteins collagen IV and laminin were present in both patches in the pericellular space and in the surrounding ECM (Fig. 3B, 3C, 3E, 3F). Cell-deposited fibronectin was not distinguishable histologically owing to the presence of fibronectin from serum in the fibrin ECM (data not shown). An increase occurred in the amount of non-CM-deposited collagen IV in the CM+PC patches compared with the PC-only patches (Fig. 3I).

### Functional Consequences of Patch Implantation

Echocardiography was conducted before infarction and patch implantation and at 1 and 4 weeks postoperatively. At 1 week postoperatively, all the groups displayed a reduction in both the left ventricular ejection fraction (LVEF) and fractional shortening (LVFS) relative to baseline (Fig. 4A; supplemental online Fig. 1). However, the rats that received CM+PC patches had a higher LVEF and LVFS (Fig. 4B, 4C) and a smaller left ventricular inner diameter (LVID) in systole compared with both MI-only controls and the rats that received a PC patch and a smaller diastolic LVID compared with the MI-only controls (supplemental online Table 3).

After 4 weeks postoperatively, the LVEF and LVFS remained lower than the baseline values; however, the CM+PC patch group had a higher LVFS than both the MI-only controls and PC patch group and higher LVEF (Fig. 4B), systolic LVID, stroke volume, and cardiac output (supplemental online Table 3) than MI-only controls. No improvements compared with MI-only controls were seen with the administration of a PC patch at any time point.

### Left Ventricular Remodeling

The rats received no patch (MI only) had infarct scars that covered 47.9% ± 14.7% (range, 30.7%–71.5%; median, 45.4%) of the left ventricular free wall (Fig. 5). The CM+PC patch rats had an infarct size of 30.7% ± 14.6% (range, 15.9%–50.0%; median, 27.9%), smaller than that of the noninfarcted controls. The patch group had an infarct size of 35.8% ± 7.8% (range, 27.3%–46.1%; median, 34.9%), not different from that of the MI-only or CM+PC patch groups. Wall thinning of the infarct area was seen in all groups compared with the noninfarcted rats, with no reduction in wall thinning seen with the application of either CM+PC or PC patches. Patches are not easily visualized in Figure 5. Additional measurements can be found in supplemental online Table 4.

### Patch Engraftment

After 4 weeks postoperatively, the hearts that received CM+PC patches were observed to have a pale, thin, tissue layer covering much of the left ventricular epicardial surface. This layer was found to be composed of both scar tissue from the initial surgery and a thin, 81.3 ± 33.5-μm layer of densely packed CMs located on the left ventricular epicardial surface (Fig. 6A–6C). The patch contained interspersed PCs (Fig. 6B, 6E, 6F) and covered both infarcted and intact myocardium, separated from the host myocardium by a 103.3 ± 33.1-μm nonmyocyte interface zone (Fig. 6A–6D). Imaging of trichrome-stained sections revealed the ECM of the engrafted patch to be primarily collagenous (Fig. 6A). Elongation and circumferential alignment was observed in most engrafted CMs (Fig. 6C, 6E, 6F), but the cells were very small in size, indicating immaturity of the CMs. Gap junction protein connexin 43 was found between adjacent CMs within the engrafted patches (Fig. 6E), which was only sparse after in vitro culture (supplemental online Fig. 3), although not concentrated at cell-cell junctions as observed in adult myocardium. These CMs were verified to be transplanted cells through human nuclear antigen (HNA) staining (Fig. 6D). Also, 10.9% ± 1.4% of imaged donor CMs stained positive for Ki-67 (Fig. 6F) at the 4-week time point, consistent with some level of CM proliferation over
Figure 3. Cell-deposited ECM in CM+PC and PC patches. Collagen I deposition in CM+PC (A) and PC (D) patches in vitro. Basement membrane proteins collagen IV (B, E) and laminin (C, F) were also present in both CM+PC and PC patches. Overall, ECM of patches remained primarily fibrin at implantation as seen in Masson’s trichrome-stained sections (G, H), in which blue staining indicates the presence of collagen and red staining labels noncollagenous proteins. More collagen IV was produced by noncardiomyocytes in the CM+PC patch than in the PC patch (I). Red indicates matrix protein; blue, nuclei. Scale bars = 100 μm. Abbreviations: CM, cardiomyocyte; Col, collagen; ECM, extracellular matrix; PC, pericyte.

Figure 4. Echocardiography results at 1 and 4 weeks after infarction and transplantation. (A): Representative M-mode echocardiographic images of CM+PC patch recipient, PC patch recipient, and MI-only hearts at each study time point. At the 1-week time point, rats receiving a CM+PC patch had improved left ventricular ejection fraction (LVEF) and left ventricular fractional shortening (LVFS) compared with both MI-only controls and rats receiving PC patches (B, C). At 4 weeks after infarction, rats receiving CM+PC patches had improved LVFS over both groups and improved LVEF compared with infarct-only controls (B, C). p < .05 was considered significant. Abbreviations: CM, cardiomyocyte; MI, myocardial infarction; PC, pericyte.
4 weeks in vivo. Assuming no lateral contraction of the patches, after 4 weeks in vivo, the number of cTnT\(^+\) nuclei in the engrafted patches had increased twofold (102.9% increase). Few to no Ki-67\(^+\) cells were found in the patches at the end of in vitro culture (supplemental online Fig. 3).

Pericytes entrapped in PC patches did not survive 4 weeks in vivo, with few to no GFP\(^+\) and HNA\(^+\) cells found within the host myocardium or along the epicardial surface of the heart. The ECM of the patches was converted from being primarily fibrin at implantation to collagenous after 4 weeks in vivo, similar to that of the infarct scar or adhesions from the chest wall, making it difficult to confidently identify the presence of a patch at this time point without the presence of HNA\(^+\) donor cells.

Two rats that had received CM+PC patches died at the 1-week time point due to anesthesia. The patch on these rats was visible in trichrome staining and contained somewhat sparsely distributed CMs and PCs, with a few CMs Ki-67\(^+\) (supplemental online Fig. 2). All other rats that survived the initial surgery lived to the 4-week time point.

CM+PC patches became vascularized via angiogenesis from the host based on IB4 staining, with small diameter vessels present in the patch at 4 weeks postoperatively (Fig. 7A). Active
perfusion of the vessels was verified by the presence of red blood cells in H&E-stained images of the engrafted patches (Fig. 7B). In the host myocardium, no differences were found in the density of IB4+ microvessels in infarcted myocardium among the groups after 4 weeks, but an increased density occurred in the area of myocardium bordering the infarct zone with the administration of a CM+PC patch (Fig. 7C, 7D).

**DISCUSSION**

The present study has demonstrated that the coentrapment of hiPSC-CMs and human PCs in a fibrin gel results in a compacted, aligned, and beating cardiac patch (engineered cardiac tissue). After 4 weeks in vivo, the patch remained viable and resulted in a reduced infarct size (Fig. 5) and improved cardiac function compared with infarct-only controls (Fig. 4). In contrast, a patch containing only PCs did not survive 4 weeks in vivo and resulted in no improvements in contractile function or reduction in infarct size. These results suggest that, similar to our recent report of an ECT similarly made from a neonatal rat heart isolate and also implanted acutely [17], the improvements seen with patch administration originate from cardiomyocytes. To our knowledge, this is the first demonstration of a cardiac patch using hiPSC-CMs that not only remains viable, with CM proliferation after 4 weeks in vivo, but also covers large portions of the left ventricular epicardial surface and contributes to the limitation of left ventricular remodeling after infarction.

The creation of an ECT in vitro is a subject that has received increasing interest as a method to deliver cells to the injured myocardium, supplement the contractility of the left ventricular wall, and ultimately contribute to and facilitate cardiac regeneration. The present study focused on limiting the loss of left ventricular contraction and infarct expansion by acute placement of the patch on the infarcted myocardium (rather than replacing infarct scar tissue with the patch in a second surgery) and compared the use of hiPSC-CMs in a patch to a previous study using neonatal rat cardiomyocytes and noncardiomyocytes [17]. The same gel formulation was used in both studies, 1.25 ml of a 3.3 mg/ml fibrin gel in a tubular mold, and cultured for a total of 14 days. The concentration of cardiomyocytes was kept the same, with the neonatal rat study containing ~60% (3.3 × 10^6) of a heterogeneous population of noncardiomyocytes and the present study containing 15% (3.4 × 10^5) human PCs and 5% (1.13 × 10^5) noncardiomyocytes that survived the purification process. However, the neonatal rat CM patches were stretch conditioned before implantation, and these CM+PC patches were cultured statically for the entirety of culture. Early-stage differentiated hiPSC-CMs have shown a relatively high proliferation ability compared with late-stage mature hiPSC-CMs [6]. We used the day 15-differentiated hiPSC-CMs in the present in an effort to maintain the hiPSC-CM proliferation after patch transplantation. Furthermore, immature CMs are more tolerant of perturbation and are more likely to survive transplantation than more mature CMs [33]. Both patches exhibited measurable twitch forces, although lower for the hiPSC-CM patches (0.2 nN per input CM) compared with the neonatal rat cell patches (1.2 nN per input CM) [17].

In both the study using neonatal rat CMs and the present study using hiPSC-CMs, a reduction in infarct size and improvements in cardiac function occurred with the implantation of a patch that contains both CMs and non-CMs. The implantation of patches containing non-CMs resulted in minimal (neonatal rat) or no (hiPSC-CM) improvement. However, near complete rescue of the myocardium resulted from the implantation of a neonatal rat cell patch [17], and moderate infarct sizes of 30.7% ± 14.6% (p < .05; Fig. 5B) resulted from the implantation of a CM+PC patch in our study. These larger infarct sizes were associated with reduced improvements in cardiac function and thinning of the left ventricular wall, which were not...
observed with the application of the neonatal rat CM patch. Additionally, invasion of noncardiomyocyte cells into the host was seen with the neonatal rat CM patch but was not observed in the present study. The reduced improvement found with the administration of a CM+PC patch compared with our previous neonatal rat patch could have resulted from several reasons. Different strains of rats (Fisher rats vs. athymic nude rats), a species mismatch of the transplanted cells (rat-rat vs. human-rat), and a different initial maturation state of the transplanted cardiomyocytes (neonatal vs. embryonic/fetal) were used. Additionally, fewer non-CM cells were transplanted in the CM+PC patches in the present study (0.45 × 10⁵ cells vs. 2.75 × 10⁵ cells), and the population of non-CM cells in the neonatal rat cell patches was a heterogeneous population consisting of cardiac fibroblasts and other noncharacterized cells from the myocardial interstitium, including PCs. Pericytes are normally found in the myocardium as microvascular support cells; however, the results from the present study indicate that pericytes are likely not the migratory cell type seen in our neonatal rat patch study. Pericytes have been shown to minimally improve cardiac function and suppress fibrosis when transplanted into mouse myocardium [34], but these effects did not occur in our study, because of the different source tissue, method of delivery, or culture differences before implantation. Although the PCs did not survive 4 weeks in vivo when transplanted as a PC-only patch, they did survive when transplanted in the CM+PC patches, suggesting that not only are PCs required for the compaction and alignment of fibrin gels for patches containing CMs, but also that CMs are required for PC survival in vivo. In contrast, the hiPSC-CMs not only survived but also appeared to proliferate based on colocalization of cTnT and Ki-67 staining, a common marker for proliferation of stem cell-derived CMs [35, 36] but could also indicate multinucleation of CMs [37, 38]. It can be also be noted that although the noncardiomyocytes that survived purification composed only 5% of the total cell number at the time of ECT fabrication, they expanded over the 14 days of in vitro culture to constitute 12% of the final cell population at implantation, higher numbers than the PCs. These results indicate that future studies will have to further optimize the CM purification process and further characterize these non-CM cell types to elucidate any potential contribution these cells contribute to myocardial protection after infarction. In both studies, the ECM of the patch was converted from fibrin to primarily collagen (Fig. 6A), and a collagenous interface zone developed separating the host myocardium from the patch, suggesting the beneficial effects of the patches were not due to electrical integration of the CMs. The thin size of the engrafted patches in both studies makes any patch beating, if it occurred, an unlikely mechanism of functional improvement. In all, the mechanism by which the benefits afforded by CM+PC patch administration occurred are not clear; however, the increase in microvessel density in the myocardium bordering the infarct could implicate paracrine mechanisms that stabilize the infarct scar and prevent further expansion. Although cell migration from the CM+PC patch into the host was not seen in the present study, the increase in collagen IV deposition in vitro in the CM+PC patch compared with PC-only patches after in vitro culture indicate an altered behavior of pericytes in the presence of cardiomyocytes. Some of the ECM deposition could also be attributed to the entrapped non-CM cells remaining after the CM purification process.

No other reports thus far have been published of ECTs made from hiPSC-CMs that have been implanted for comparison with the present study. Improvement in cardiac function of nude rats was recently reported with the delayed administration of a scaffold-free cell sheet consisting of hiPSC-CMs cocultured with endothelial and vascular support cells [31]. However, the cell sheets engraved with limited success and did not substantially limit infarct expansion or left ventricular thinning [31]. In combination, the findings from the cell sheet study and the present study indicate a definite benefit from hiPSC-CMs administered to nude rat infarcts. However, optimization of this hiPSC-CM patch will be needed to achieve the near complete rescue of the myocardium we achieved with the neonatal rat patch.

The present study provides preliminary data demonstrating not only the ability of an ECT containing hiPSC-CMs to remain viable after transplantation, but also the potential for cell expansion. However, the present study was limited by the acute infarct model used, small sample size, and 4-week endpoint. For clinical applicability, an alternative, potentially autologous source of pericytes will need to be used, such as dermal pericytes [39]. Longer term studies with delayed implantation will also be needed to assess the long-term fate of the graft and any potential for teratoma formation of these hiPSC-CMs. Larger animal models will be additionally required to fully characterize the engraftment of immature, donor hiPSC-CMs with the adult, recipient myocardium, because rodent and human hearts have different intrinsic heart rates, which might hamper accurate assessment of electrophysiological integration.

CONCLUSION

We created an aligned, force-generating, engineered cardiac patch using hiPSC-CMs and PCs entrapped in a sacrificial fibrin gel and found that when transplanted onto acutely infarcted rat myocardium, the patch remained viable and the CMs proliferated. This patch resulted in improved cardiac function and a reduced infarct size 4 weeks after transplantation, along with increased microvessel density in the host border zone myocardium, implicating potential paracrine-based, infarct-stabilizing mechanisms originating from the transplanted CMs.

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AUTHOR CONTRIBUTIONS

J.S.W.: conception/design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; L.Y.: collection and/or assembly of data, final approval of manuscript; R.T.: provision of study material or patients; Jianyi Zhang: final approval of manuscript; Jianhua Zhang: provision of study material or patients, manuscript writing, final approval of manuscript; Jianyi Zhang: provision of study material or patients; Jianhua Zhang: provision of study material or patients, manuscript writing, final approval of manuscript; T.J.K.: conception/design, provision of study material or patients, manuscript writing, final approval of manuscript; R.T.T.: conception/design, data analysis and interpretation, financial support, manuscript writing, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

T.J.K. is a compensated consultant for and has compensated stock options in Cellular Dynamics International. The other authors indicated no potential conflicts of interest.
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