Collagen Matrices Enhance Survival of Transplanted Cardiomyoblasts and Contribute to Functional Improvement of Ischemic Rat Hearts

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Background—Cardiac cell transplantation is limited by poor graft viability. We aimed to enhance the survival of transplanted cardiomyoblasts using growth factor-supplemented collagen matrices.

Methods and Results—H9c2 cardiomyoblasts were lentivirally transduced to express firefly luciferase and green fluorescent protein (GFP). Lewis rats underwent ligation of the left anterior descending artery (LAD) ligation to induce an anterior wall myocardial infarction. Hearts (n=9/group) were harvested and restored ex vivo with 1×10^6 genetically labeled H9c2 cells either in (1) saline-suspension, or seeded onto (2) collagen-matrix (Gelfoam [GF];), (3) GF/Matrigel (GF/MG), (4) GF/MG/VEGF (10 μg/mL), or (5) GF/MG/FGF (10 μg/mL). Hearts were then abdominally transplanted into syngeneic recipients (working heart model). Controls (n=6/group) underwent infarction followed by GF implantation or saline injection. Cell survival was evaluated using optical bioluminescence on days 1, 5, 8, 14, and 28 postoperatively. At 4 weeks, fractional shortening and ejection fraction were determined using echocardiography and magnetic resonance imaging, respectively. Graft characteristics were assessed by immunohistology. Bioluminescence signals on days 5, 8, and 14 were higher for GF-based grafts compared with plain H9c2 injections (P<0.03). Signals were higher for GF/MG grafts compared with GF alone (P<0.02). GFP-positive, spindle-shaped H9c2 cells were found integrated in the infarct border zones at day 28. Left ventricular (LV) function of hearts implanted with collagen-based grafts was better compared with controls (P<0.05). Vascular endothelial growth factor or fibroblast growth factor did not further improve graft survival or heart function.

Conclusions—Collagen matrices enhance early survival of H9c2 cardiomyoblasts after transplantation into ischemic hearts and lead to improved LV function. Further optimization of the graft design should make restoration of large myocardial infarctions by tissue engineering approaches effective. (Circulation. 2006;114[suppl 1]:I-167–I-173.)

Key Words: cells ■ collagen ■ imaging ■ myocardial infarction ■ transplantation

Increasing evidence suggests that cell transplantation into infarcted myocardium may lead to improvement of left heart function.1–3 The exact mechanisms by which cell implants contribute to gross cardiac function, however, have not been completely elucidated. Potential mechanisms include stem cell differentiation into cardiomyocytes, induction of angiogenesis, or influences on ventricular remodeling processes. Several recent studies have observed significant early cell death attributable to oxygen deprivation and inflammatory response, questioning the usefulness of implanting cells alone.4–6 However, the role of the micro-structural graft environment, in terms of its ability to provide space and guidance for cell homing, growth, and differentiation, has not been thoroughly investigated.

Collagen as a natural extracellular matrix (ECM) component has been shown to support cell growth both in cell culture and in vivo.7–9 Several attempts have been made to construct 3-dimensional collagen-based tissue grafts for surgical restoration of myocardial infarction.9,10 The advantages of collagen matrices are their large surface area for cell seeding, porosity for capillary ingrowth, stability for mechanical support, biodegradability, and minimal immunogenicity.7–9 Collagen matrices supplemented with growth factors or other ECM components should therefore provide a favorable environment for cells transplanted into ischemic myocardium.

In the present study, we explored the use of collagen matrix (Gelfoam [GF]; Upjohn) in conjunction with growth-factor-
reduced Matrigel (MG) (ie, extracellular protein extracts containing laminin, collagen type IV; BD Biosciences) as both a scaffold for structural support of implanted cells and a reservoir for growth factor delivery. We genetically modified rat H9c2 cardiomyoblasts with optical and fluorescent reporter genes and seeded them onto collagen matrices in the presence of MG and selected growth factors (rat vascular endothelial growth factor [VEGF]; human basic fibroblast growth factor [FGF]) to produce a robust, imageable bioartificial graft for myocardial restoration. Using multimodal imaging techniques including optical bioluminescence, echocardiography, and small animal magnetic resonance imaging (MRI), we were able to perform a thorough, systemic assessment of the graft survival and its therapeutic efficacy in living rats.

**Methods**

**Animal Care**

Surgery and animal care were provided in accordance with Guide for the Care and Use of Laboratory Animals (National Institutes of Health, volume 25, no 28, revised 1996).

**Optically Bioluminescent H9c2 Cell Line**

H9c2 rat cardiomyoblasts (American Type Culture Collection) were cultured and maintained as previously described. The cells were transduced with 1.10^7 pfu of a lentiviral vector carrying a cytomegalovirus promoter driving the expression of firefly luciferase reporter gene (fluc) upstream and green fluorescent protein (GFP) gene downstream of an internal ribosomal entry site element. The genetically modified cells (mH9c2) underwent FACS sorting followed by single clone selection. The brightest clone was used for further experiments.

**In Vitro Optical Bioluminescence of Collagen/H9c2 Grafts**

GF (purified pork skin gelatin) blocks (3×3×1-mm) pre-wetted with culture medium were placed in a 96-well plate under sterile conditions. Experiments were performed in triplicates. 1×10^6 mH9c2 cells were seeded onto the GF matrix and incubated for 24 hours (37°C). Control wells contained medium and cells only or GF blocks without cells.

To determine the appropriate number of cells, mH9c2 cells (0.5×10^6, 1×10^6, and 2×10^6) were seeded onto the foam blocks and incubated for 24 hours (37°C). GF blocks without cells served as controls.

At 24 hours, 1 μg/mL of D-Luciferin (Biosynth) was added to each well and the plate was scanned for bioluminescence signal (photons/second) using the IVIS 50 imaging system (Xenogen).

**Heterotopic Transplantation and Cell Grafting**

Donor animals (male Lewis rats, 240 to 280 grams, 8 to 10 weeks old, Harlan Sprague-Dawley, Inc; Indianapolis, Ind) were anesthetized with isoflurane (2%) and ketamine (25 mg/kg), and ventilated after oral intubation. The proximal left anterior descending artery
was ligated to induce a sizable left ventricular (LV) infarct. After cold perfusion, the hearts were harvested for further transplantation. An end-to-end anastomosis of the left atrium to the pulmonary artery (Figure 1A) was performed, and an intramyocardial pouch was created to allow implantation of a 3 × 3 × 1-mm sterile block GF (Figure 1B). The hearts were then transplanted into the abdomen of syngeneic rats to create a “working heart” model (anastomoses; Figure 1A). Before reperfusion, 50 μL of mH9c2 cell suspension (1 × 10⁶ cells) was injected into the GF scaffolds or the infarcted areas, and the flap was closed using a 9-0 nonabsorbable suture. The ischemia time for the hearts ranged from 60 to 70 minutes and was kept constant for all of the following groups (n = 9 animals/group): (1) GF/mH9c2/2% isoflurane and saline (PBS); (2) GF/mH9c2/MG; (3) GF/mH9c2/MG/VEGF; and (4) GF/mH9c2/MG/FGF. Control animals (n = 6 per group) underwent myocardial infarction only, infarction followed by saline injection, or infarction followed by GF implantation and saline injection. For GF/mH9c2/MG grafts, cells were suspended in liquid prechilled growth factor-reduced MG (BD Biosciences). VEGF or FGF supplementation (R&D Systems) was given at 10 μg/mL cell suspension (liquid MG). Before wound closure an intraperitoneal port catheter with a subcutaneous injection site was implanted to allow error-free delivery of substrate (D-Luciferin). Animals received cyclosporine daily (7.5 mg/kg per day orally) to avoid cell rejection.

**Magnetic Resonance Imaging**

Four weeks postoperatively, ECG-gated MRI was performed using a 4.7-Tesla small animal scanner built of a Varian console (Varian, Inc), a 15-cm horizontal bore magnet (Oxford Instruments, Ltd) with GE Technom Gradients (12 G/cm), and a Varian volume coil with an inner diameter of 5 cm. Rodents (n = 3/group) were anesthetized with 2% isoflurane. Two subcutaneous precordial ECG leads (SA Instruments, Inc) were placed for heart rate monitoring and ECG gating. LV function was evaluated using an ECG-triggered cine sequence (echo time 2.8-ms, repetition time 160-ms, flip angle 60°, field of view 3 × 3-cm², matrix 128 × 128, slice gap 0 mm, slice thickness 1 mm, number of excitations: 8, and 12 cardiac phases). The imaging plane was localized using scout images in a coronal plane followed by double-oblique acquisition. The data were analyzed using the MR Vision software (Winchester). LV ejection fraction (LVEF) was calculated by tracing the endocardial and epicardial borders in end-systole and end-diastole. Measurements were performed by an independent blinded investigator (T.A.).

**Histology and Histochemistry**

At 2 (n = 3/group, no controls) and 4 weeks (n = 6/group) postoperatively, hearts were perfused with saline, explanted, and fixed for 2 hours in paraformaldehyde before overnight cryopreservation in 30% sucrose. The tissue was embedded in OCT and stored at −70°C. Immunohistochemical staining was performed on 6-μm sections using following primary antibodies: polyclonal Alexa-Fluor-488–conjugated anti-GFP antibody (Molecular Probes), rabbit anti-α-sarcromeric-actin antibody (Sigma-Aldrich), and rabbit anti-connexin 43 antibody (Sigma-Aldrich). Alexa 546-conjugated or Alexa 647-conjugated secondary antibodies (Molecular Probes) were used. Sections were counterstained with Hoechst (Sigma-Aldrich) and analyzed using a Zeiss Axioplan fluorescent microscope. Additional cryosections were stained for endothelial cells using anti-platelet endothelial cell adhesion molecule/CD31 antibodies (Chemicon) in combination with Hoechst nuclear stain (n = 3, collagen-based grafts only). The slice showing the largest area of implanted matrix was subsequently imaged with low-magnification fluorescence microscopy. The area of vascular in-growth was measured and expressed as a percentage of the total area of implanted matrix using calibrated software (Openlab; Improvision). For determining infarct size and evaluating cell morphology, additional sections were stained with hematoxylin and eosin (HE) and Masson’s Trichrome (Figure 2). Four level cuts of the infarcted area were evaluated using computer-assisted planimetry. The infarct size was quantified as the infarcted area divided by the total left ventricular area. The anterior LV wall thickness (AWT) and the posterior LV wall thickness (PWT) were also measured.

**In Vivo Optical Biomimlessness Imaging**

Optical biomimlessness imaging was performed using an ultrasensitive charged coupled device camera (IVIS 50; Xenogen). Rats were anesthetized with 2% isoflurane and placed in the imaging chamber. The animals were rotated to the left with the heterotopic heart pointing to the camera. After acquisition of a baseline image, rats were injected with D-Luciferin, 400 mg/kg intraperitoneally, via the port catheter system. Rats were imaged on days 1, 5, 8, 14, and 28 after reperfusion using an ultrasensitive charged coupled device camera (IVIS 50; Xenogen). Rats were kept constant for all of the following groups (n = 6/group): (1) GF/mH9c2; (2) GF/mH9c2/MG; (3) GF/mH9c2/MG/VEGF; and (4) GF/mH9c2/MG/FGF. Control animals (n = 6 per group) underwent myocardial infarction only, infarction followed by saline injection, or infarction followed by GF implantation and saline injection. For GF/mH9c2/MG grafts, cells were suspended in liquid prechilled growth factor-reduced MG (BD Biosciences). VEGF or FGF supplementation (R&D Systems) was given at 10 μg/mL cell suspension (liquid MG). Before wound closure an intraperitoneal port catheter with a subcutaneous injection site was implanted to allow error-free delivery of substrate (D-Luciferin). Animals received cyclosporine daily (7.5 mg/kg per day orally) to avoid cell rejection.

**Echocardiography**

On days 14 (n = 9/group) and 28 (n = 6/group) echocardiography of the abdominal hearts was performed (15.8 MHz, Sequoia 256; Acuson). Rats were anesthetized with isoflurane. Standardized views of the heterotopic heart were obtained at the papillary muscle level. Fractional shortening was determined from m-mode images. The thicknesses of both LV anterior wall (AWT) and interventricular septum (SWT) were measured. Examinations were evaluated by an independent experienced investigator (R.S.).
Statistics
Data are given as mean ± standard deviation. Optical imaging data were displayed as mean ± standard error of mean. Comparisons of cell survival and ventricular function were made using the Mann-Whitney U test (SPSS 11.0 for Windows). Differences were considered significant at *P* < 0.05.

Authorship Responsibility
The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results

In Vitro Optical Bioluminescence of Collagen/H9c2 Grafts
After 24 hours of incubation, mH9c2 cells (1×10⁶) seeded onto GF blocks showed significantly greater bioluminescence signals (1577±250×10³ p/s) than cells plated in plain culture medium (625±67×10³ p/s; *P*<0.03). A seeding density of 1×10⁶ cells per GF block led to a significantly (*P*<0.05) greater luminescence signal (1484±352×10³ p/s) than cell densities of 0.5×10⁶ cells (472±163×10³ p/s) or 2×10⁶ cells (861±115×10³ p/s), respectively, and was used for further experiments.

In Vivo Optical Bioluminescence Imaging of Cardiac Cell Grafts
Bioluminescence signals among groups did not differ significantly at day 1 (*P*>0.3), suggesting standardized delivery of cells. Signals for the different groups decreased markedly during the first 8 days, indicating early cell death. At days 5 and 8, GF/mH9c2 grafts and GF/mH9c2/MG composite grafts showed significantly (*P*<0.01) greater signal intensities than mH9c2 injections alone (Figures 3, 4). Supplementation of GF/mH9c2/MG grafts with VEGF or FGF did not increase cell survival further. GF/mH9c2 grafts and mH9c2 controls reached background signals at day 14, whereas MG supported grafts (±VEGF or FGF) were detectable in vivo up to day 28 (Figure 3).

Histology and Histochemistry
The overall size of the infarcted myocardium was similar among all groups at day 28 after surgery (Table 1). The
AWT/PWT ratio was significantly greater in hearts having received GF/mH9c2/MG grafts, compared with either infarcted heart and infarct/GF/no cells) compared with normal heterotopic hearts at 4 weeks. mH9c2 cell injections and GF/H9c2 implantations alone did not increase LVEF of infarcted hearts. GF/mH9c2/MG grafts improved LVEF compared with sham operations \( (P=0.03) \). VEGF or FGF supplementation did not further improve LVEF compared with GF/mH9c2/MG alone (Table 3). The best LV function was obtained for hearts having received GF/mH9c2/MG grafts. Administration of VEGF or FGF did not further increase FS (Table 2). At 4 weeks, the AWT was significantly greater in groups having received mH9c2/collagen grafts compared with infarct controls \( (P<0.05; \text{Figure } 1) \). The SWT did not differ between groups (data not shown).

**Magnetic Resonance Imaging**

LVEF was significantly \( (P=0.03) \) decreased in sham-operated rats (infarcted heart and infarct/GF/no cells) compared with normal heterotopic hearts at 4 weeks. mH9c2 cell injections and GF/H9c2 implantations alone did not increase LVEF of infarcted hearts. GF/mH9c2/MG grafts improved LVEF compared with sham operations \( (P=0.03) \). VEGF or FGF supplementation did not further improve LVEF compared with GF/mH9c2/MG alone (Table 3).

**Discussion**

To our knowledge, this is the first study using in vivo optical bioluminescence imaging to investigate the modulation of myocardial graft survival by various therapeutic agents. As a non-invasive, high-throughput technique, that can be used to assess graft survival longitudinally, optical bioluminescence offers a more objective comparison of graft viability at different time points compared with histological analysis. It maximizes diagnostic information while minimizing the number of animals required for a statistically meaningful evaluation.4,14

We demonstrated that mH9c2 cardiomyoblasts undergo a massive cell death within the first week after injection into the ischemic heart, resulting in a marked reduction of the bioluminescence signal intensity to nearly 30% of the baseline level. This finding is consistent with previously reported survival data of H9c2 cardiomyoblasts injected into healthy rat myocardium.4 With collagen as a support material, however, we were able to improve the survival of composite collagen/MG grafts, leading to 3-fold enhancement of imaging signals at day 8 compared with plain cell injections. This confirms other studies describing favorable cell survival using MG suspensions for cell grafting.15

**TABLE 1. Histomorphometry**

<table>
<thead>
<tr>
<th>Histomorphometry</th>
<th>Infarcted Area</th>
<th>( P )</th>
<th>AWT/PWT</th>
<th>( P )</th>
</tr>
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<tr>
<td>Infarct</td>
<td>0.34±0.04*</td>
<td>0.47±0.17*</td>
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<td></td>
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<tr>
<td>Infarct/mH9c2/Inj</td>
<td>0.35±0.03 NS*</td>
<td>0.54±0.19*</td>
<td>NS +</td>
<td></td>
</tr>
<tr>
<td>GF/no cells</td>
<td>0.37±0.03 NS*</td>
<td>0.62±0.19</td>
<td>NS +</td>
<td>0.04 NS*</td>
</tr>
<tr>
<td>GF/mH9c2</td>
<td>0.35±0.03 NS*</td>
<td>0.62±0.10</td>
<td>NS +</td>
<td>0.02 NS*</td>
</tr>
<tr>
<td>GF/mH9c2/MG</td>
<td>0.39±0.07 NS*</td>
<td>0.75±0.14</td>
<td>&lt;0.05*</td>
<td>0.02 NS*</td>
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<tr>
<td>GF/mH9c2/MG/VEGF</td>
<td>0.34±0.05 NS*</td>
<td>0.79±0.08</td>
<td>&lt;0.02*</td>
<td></td>
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<tr>
<td>GF/mH9c2/MG/FGF</td>
<td>0.40±0.06 NS*</td>
<td>0.76±0.07</td>
<td>&lt;0.02*</td>
<td></td>
</tr>
</tbody>
</table>

Computer-assisted planimetry of H&E samples. Infarct size is shown as the ratio of infarcted area to total LV area. LV wall thickness is displayed as the ratio of anterior wall thickness (AWT) to posterior wall thickness (PWT).

FGF indicates human basic fibroblast growth factor; GF, Gelfoam; Inj, injection; MG, Matrigel; NS, not significant; PBS, phosphate-buffered saline; VEGF, rat vascular endothelial growth factor.

**Echocardiography**

Fractional shortening (FS) of the heterotopic hearts was significantly \( (P<0.01) \) decreased in control animals (infarcted heart and infarct/GF/no cells) compared with normal heterotopic hearts 2 and 4 weeks postoperatively. Cell transplantation using mH9c2 injections or implantation of mH9c2/collagen grafts improved FS markedly after 2 and 4 weeks.

**Figure 5.** GFP \(^{+}\)-mH9c2 cells (green) 4 weeks after cell transplantation showed an elongated shape and were generally aligned with the host myocardium in the peri-infarct region. A, Host myocardium expresses connexin 43 in the typical punctuated pattern along cell boundaries (red, left side). B and C, A single spindle-shaped GFP \(^{+}\) cell, not expressing connexin 43. D, Host myocardium expressing \( \alpha \)-sarcomeric-actin (red). E and F, GFP \(^{+}\) cells co-stain for \( \alpha \)-sarcomeric-actin. Nuclei are stained with Hoechst (blue).
The increased survival could be partially attributed to the ability of collagen matrices to serve as a guide structure for vessel ingrowth into the porous graft.10,16 (Figure 2). We found significant capillary in-growth into the collagen matrices, especially in those that have been supplemented with VEGF or FGF. Nevertheless, the impact of additional VEGF and FGF administration on cell survival appeared to be marginal, as shown by our optical bioluminescence data. It is possible that a single shot of VEGF and FGF is not sufficient to further improve cell survival. Repeated administration of growth factors or sustained angiogenic expression may be more efficient and should be further investigated.

As expected from the bioluminescence data, only a few cells survived 4 weeks after surgery. GFP-positive, spindle-shaped mononuclear cells were located in the peri-infarct region and showed interaction with host cardiomyocytes. The GFP-positive mH9c2 cells expressed \( \alpha \)-sarcomeric-actin, which stains myotubes of skeletal and cardiac muscle. mH9c2 cells did not stain for connexin 43, indicating that they do not electromechanically couple with the host myocardium to actively contribute to the heart’s contractile function.

Nevertheless, using MRI and echocardiography, we found an improvement in LV function after cardiac cell/tissue grafting, a finding that is consistent with other studies using different cell types.12,13,15 Interestingly, we found the thickness of the infarcted anterolateral wall to be significantly greater in the GF/mH9c2/MG groups than in controls. Such finding suggests that collagen/myoblast grafts may improve cardiac function by minimizing adverse ventricular remodeling or wall thinning.16,17 Recently published data also support such notion by showing that collagen injection alone could improve heart function.18 Whether transplanted myoblasts themselves contribute to functional improvement through paracrine effects remains unclear and needs to be addressed in ongoing studies. Our data suggest that mH9c2 cell-seeded grafts are superior to plain collagen implantation in terms of their impact on left ventricular function. Because we found a correlation between heart function and early myoblast survival, we assume that the viability of collagen-based grafts, even during the first few days, plays a crucial role in the restoration process of the infarcted myocardium. Importantly, this does not include differentiation of H9c2 myoblasts into cardiomyocytes, as confirmed by our histological analysis.

We used a “working heart” heterotopic transplant model,13 which provided several advantages for our purposes. Although in situ models may be ideal for cell injections, the transplant model offers the opportunity to restore a large portion of the left ventricle using solid tissue grafts. The ex situ restoration of the infarcted heart facilitated a standardized matrix implantation and the “working heart” transplant technique allowed for functional evaluation.13 In terms of cell transfer, we decided for a formerly described two step in situ restoration of the infarcted heart facilitated a standardized matrix implantation and the “working heart” transplant technique allowed for functional evaluation.13 In terms of cell transfer, we decided for a formerly described two step in situ technique.16 Whereas the collagen scaffold was implanted ex situ, cells were injected into the matrix before reperfusion of the arrested heart. This technique reduced warm ischemia for the cells, minimized the risk of graft contamination and resulted in adequate cell detection up to 28 days. As a further advantage of the heterotopic model the hearts were localized in a superficial position below the abdominal skin, which minimized light attenuation for optical imaging and resulted in adequate cell detection up to 28 days.

The functional data of this “working heart” transplant model need to be interpreted cautiously. Surgical variability of anasto-

### TABLE 2. Echocardiography

<table>
<thead>
<tr>
<th></th>
<th>2 Weeks</th>
<th>4 Weeks</th>
<th>AWT</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>FS</td>
<td></td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>No infarct</td>
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<td>0.29±0.09</td>
<td>2.2±0.5</td>
<td></td>
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<tr>
<td>Infarct</td>
<td>0.11±0.01*</td>
<td>0.14±0.05**</td>
<td>1.6±0.4</td>
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<tr>
<td>Infarct/mH9c2/inj</td>
<td>0.17±0.02</td>
<td>&lt;0.04*</td>
<td>1.9±0.4</td>
<td>NS*</td>
</tr>
<tr>
<td>GF/no cells</td>
<td>0.14±0.03*</td>
<td>0.15±0.04**</td>
<td>2.0±0.3</td>
<td>0.05*</td>
</tr>
<tr>
<td>GF/mH9c2</td>
<td>0.23±0.06</td>
<td>&lt;0.03*</td>
<td>0.06**</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>GF/mH9c2/MG</td>
<td>0.27±0.10</td>
<td>&lt;0.01*</td>
<td>0.03**</td>
<td>2.5±0.2</td>
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<tr>
<td>GF/mH9c2/MG/VEGF</td>
<td>0.25±0.11</td>
<td>&lt;0.01*</td>
<td>0.03**</td>
<td>2.2±0.3</td>
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<tr>
<td>GF/mH9c2/MG/FGF</td>
<td>0.18±0.06</td>
<td>0.06*</td>
<td>0.05**</td>
<td>2.4±0.2</td>
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</table>

Fractional shortening (FS) at 2 and 4 weeks. The infarct/mH9c2/injection (Inj) group was compared to the Infarct control group (+/+ +). The GF/mH9c2 groups were individually compared to the GF/no cells group (*). Measurements of anterior LV wall thickness (AWT, 4 weeks) are in millimeters. Groups are compared to infarcted hearts (#).

### TABLE 3. MRI

<table>
<thead>
<tr>
<th></th>
<th>LVEF (%)</th>
<th>P</th>
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<tr>
<td>No infarct</td>
<td>57.3±9.0</td>
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<tr>
<td>Infarct</td>
<td>33.0±3.6*</td>
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<tr>
<td>Infarct/mH9c2/inj</td>
<td>33.0±15.6</td>
<td>NS*</td>
</tr>
<tr>
<td>GF/no cells</td>
<td>34.0±6.0*</td>
<td></td>
</tr>
<tr>
<td>GF/mH9c2</td>
<td>42.0±10.4</td>
<td>0.07*</td>
</tr>
<tr>
<td>GF/mH9c2/MG</td>
<td>48.7±7.9</td>
<td>0.03*</td>
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<td>GF/mH9c2/MG/VEGF</td>
<td>40.1±7.5</td>
<td>0.09*</td>
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<tr>
<td>GF/mH9c2/MG/FGF</td>
<td>48.0±8.0</td>
<td>0.03*</td>
</tr>
</tbody>
</table>

MRI was performed 4 weeks after cell transplantation. Left ventricular ejection fraction (LVEF) is displayed as a percentage. The infarct/mH9c2/ injection (Inj) group was compared to the infarct control group (+). The GF/mH9c2 groups were individually compared to the GF/no cells group (*).
moses, cardioprotection, ischemia/reperfusion injury, and synchronization of heterotopic and native hearts might limit heart performance. Nevertheless, using echocardiography and MRI, we found antegrade filling and ejection of the hearts and observed significant differences between control hearts, infarcted hearts, and hearts after cell/tissue grafting.

In conclusion, this study demonstrates the beneficial effects of collagen support on cell graft survival after cardiac cell transplantation. Collagen matrices can reduce ventricular remodeling and help myoblast grafts to survive the critical early time period after transplantation. The molecular mechanisms by which collagen-based grafts improve left ventricular function are not yet known and will be an important subject for ensuing studies.

Acknowledgments
We thank Pauline Chu and Milton J. Merchant for tissue processing and staining. We also thank V. Mariano for animal care.

Sources of Funding
This work was supported by the Falk Cardiovascular research fund (R.C.R.), NHLBI 5R01HL078632 (S.S.G.), NCI ICMIC P50 CA114747 (S.S.G.) and NCI SAIRP. I.K. was supported by the annual Bayer Research Grant of the German Society of Cardiology and staining. We also thank V. Mariano for animal care.

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Circulation. 2006;114:I-167-I-173
doi: 10.1161/CIRCULATIONAHA.105.001297

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