Cell Therapy Attenuates Deleterious Ventricular Remodeling and Improves Cardiac Performance After Myocardial Infarction

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Background—Myocardial infarction (MI) promotes deleterious remodeling of the myocardium, resulting in ventricular dilation and pump dysfunction. We examined whether supplementing infarcted myocardium with skeletal myoblasts would (1) result in viable myoblast implants, (2) attenuate deleterious remodeling, and (3) enhance in vivo and ex vivo contractile performance.

Methods and Results—Experimental MI was induced by 1-hour coronary ligation followed by reperfusion in adult male Lewis rats. One week after MI, 10^6 myoblasts were injected directly into the infarct region. Three groups of animals were studied at 3 and 6 weeks after cell therapy: noninfarcted control (control), MI plus sham injection (MI), and MI plus cell injection (MI+cell). In vivo cardiac function was assessed by maximum exercise capacity testing and ex vivo function was determined by pressure-volume curves obtained from isolated, red cell-perfused, balloon-in-left ventricle (LV) hearts. MI and MI+cell hearts had indistinguishable infarct sizes of ~30% of the LV. At 3 and 6 weeks after cell therapy, 92% (13 of 14) of MI+cell hearts showed evidence of myoblast graft survival. MI+cell hearts exhibited attenuation of global ventricular dilation and reduced septum-to-free wall diameter compared with MI hearts not receiving cell therapy. Furthermore, cell therapy improved both post-MI in vivo exercise capacity and ex vivo LV systolic pressures.

Conclusions—Implanted skeletal myoblasts form viable grafts in infarcted myocardium, resulting in enhanced post-MI exercise capacity and contractile function and attenuated ventricular dilation. These data illustrate that syngeneic myoblast implantation after MI improves both in vivo and ex vivo indexes of global ventricular dysfunction and deleterious remodeling and suggests that cellular implantation may be beneficial after MI. (Circulation. 2001;103:1920-1927.)

Key Words myocardial infarction n remodeling n exercise n myocardial contraction

Despite advances in the treatment of myocardial infarction (MI), congestive heart failure secondary to infarction continues to be a major complication. MI promotes acute and chronic transformation of both the necrotic infarct zone and the nonnecrotic, peri-infarct tissue, leading to global alterations that have collectively been termed "ventricular remodeling." The cardiac myocytes lost during an MI cannot be regenerated, and the extent of the loss is inversely related to cardiac output, pressure-generating capacity, and, ultimately, survival. Cell therapy, or the supplementation of tissue with exogenous cells, has previously been used in the treatment of disease in which terminally differentiated cells are irreparably damaged. Recently, it has been suggested that cell therapy with skeletal myoblasts may be effective in the treatment of MI.

Myoblasts maintain the regenerative potential of skeletal muscle and, during periods of stress, proliferate and differentiate into myotubes, eventually forming new muscle fibers capable of contraction. Previous studies have shown that myoblasts implanted into myocardium undergo myotube formation, withdraw from the cell cycle, and remain viable. Furthermore, myoblasts implanted into cryoinfarcted myocardium have yielded similar results, with differentiation into slow-twitch skeletal myocytes expressing β-MHC and capable of contraction on stimulation. Functional studies have also shown an improvement in regional contractility and compliance in cryoinfarcted myocardium after myoblast implantation. We therefore hypothesized that supplementing infarcted myocardium with syngeneic skeletal myoblasts would result in the formation of viable muscle grafts capable

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of attenuating deleterious post-MI remodeling and improving global cardiac performance. With the use of a rat coronary ligation model of MI,1,2,13 we demonstrate the physiological efficacy of myoblast implantation on both in vivo and ex vivo indexes of global cardiac remodeling and contractile failure.

**Methods**

**Animal Model**

Male inbred adult Lewis rats were obtained from Charles River Laboratories at 8 weeks of age, placed on a rat chow diet and water ad libitum, and housed under an alternating 12-hour light-dark cycle. Experimental MI was induced by coronary ligation of the main branch of the left marginal artery, as previously described.14 After 1 hour of coronary occlusion, the suture was removed, the myocardium reperfused, and the chest closed. Noninfarcted control animals received an identical procedure with the exception of tying the coronary suture. All animal handling and procedures strictly adhered to the regulations of Boston University Animal Care and the National Society for Medical Research.

**Myoblast Generation and Cell Implantation**

Myoblasts were isolated from skeletal hind leg muscle of neonatal Lewis rats. Neonatal tissue allowed for generation of a greater number of myoblast cells, with less fibroblast contamination, in a shorter time frame. We have previously isolated competent skeletal myoblasts from adult animals and humans with similar results. Neonatal tissue was minced and digested (incubated at 37°C for 10 minutes) with a mixture of trypsin (0.5 mg/mL; GibcoBRL) and collagenase (0.5 mg/mL; GibcoBRL) to release satellite cells. Cell release was repeated 10 times for a given tissue isolation to maximize satellite cell recovery relative to contaminating fibroblasts. Cells from each isolation were seeded on poly-L-lysine/laminin (Sigma)–coated plates for expansion in myoblast growth basal medium.

**TABLE 1. Animal Characteristics**

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Heart Weight, g</th>
<th>Heart Weight/Body Weight</th>
<th>Lung Wet/Dry</th>
<th>Liver Wet/Dry</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Wk after Control</td>
<td>9</td>
<td>358±7</td>
<td>1.16±0.06</td>
<td>3.23±0.15</td>
<td>4.88±0.05</td>
<td>3.31±0.01</td>
<td></td>
</tr>
<tr>
<td>therapy</td>
<td>MI</td>
<td>6</td>
<td>347±5</td>
<td>1.44±0.03*</td>
<td>4.15±0.12*</td>
<td>4.83±0.08</td>
<td>3.28±0.07</td>
</tr>
<tr>
<td>MI+cell</td>
<td>7</td>
<td>340±4</td>
<td>1.43±0.06*</td>
<td>4.11±0.08*</td>
<td>4.86±0.04</td>
<td>3.24±0.04</td>
<td></td>
</tr>
<tr>
<td>6 Wk after Control</td>
<td>9</td>
<td>391±7</td>
<td>1.18±0.06</td>
<td>3.01±0.13</td>
<td>4.94±0.04</td>
<td>3.26±0.01</td>
<td></td>
</tr>
<tr>
<td>therapy</td>
<td>MI</td>
<td>7</td>
<td>398±8</td>
<td>1.54±0.05*</td>
<td>3.83±0.11*</td>
<td>4.97±0.02</td>
<td>3.25±0.01</td>
</tr>
<tr>
<td>MI+cell</td>
<td>7</td>
<td>383±7</td>
<td>1.42±0.04*</td>
<td>3.70±0.17*</td>
<td>4.88±0.07</td>
<td>3.27±0.01</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 vs control.

Figure 1. Myoblast survival in infarcted myocardium at 9 days after implantation. Infarcted LV free wall of rat is shown under increasing magnification with trichrome staining (A, B, and C) and immunohistochemical staining for myogenin (nuclear transcription factor unique to skeletal myoblasts) (D, E, and F). Under trichrome staining, replacement fibrosis appears blue and remaining viable myocardium red. Myogenin staining demonstrates presence of myoblasts within infarct region. Encircled area (A and D) identifies one area of graft survival within infarct region. Areas marked by arrows in B and E are shown under higher magnification in C and F. Bars represent distances of 1 mm in A and D; 200 μm in B and E; and 100 μm in C and F.
medium (SkBM; Clonetics) containing 20% fetal bovine serum (Hyclone), recombinant human epidermal growth factor (rhEGF: 10 ng/mL), and dexamethasone (0.39 μg/mL). Myoblast-enriched plates were identified after 48 hours of expansion and harvested with 0.05% trypsin-EDTA (GibcoBRL). For a given experiment, ~10⁷ cells were harvested from 6 to 10 plates, seeded by satellite cells isolated from the limb muscle of 2 neonate equivalents. Similar results have been reported with adult skeletal muscle.¹¹,¹² Cells were washed and suspended in cold HBSS at 10⁷ cells/mL and kept up to 4 hours on ice before injection. A total of 100 μL (10⁶ cells) was injected into each animal. Under these conditions, cells were determined to be ~50% myoblasts by flow cytometry with the monoclonal antibody H36 (anti-rat α-7 integrin).¹⁵ The remaining cells were fibroblast-like, as determined by cell morphology. The ability for myoblasts to fuse into multinucleated myotubes in vitro was also confirmed.

Seven days after MI, infarcted animals were randomized to receive cell or sham implantation. Rats underwent a second thoracotomy, and the left ventricle was visualized. Each rat received 6 to 10 injections (total of 10⁶ cells/heart) of 10 to 16 μL of myoblast suspension in HBSS directly into the infarct and peri-infarct regions, ~1 to 2 mm apart, with a 30-gauge Hamilton needle. Infarcted animals not receiving cells underwent an identical injection procedure with HBSS alone. Noninfarcted control animals were subjected to the same surgical procedures without injection.

Animal Groups
Three groups of animals were studied: control animals receiving neither infarction nor implantation (control), infarcted animals without cell therapy (MI), and infarcted animals receiving myoblast cell therapy (MI+cell). In vivo and ex vivo cardiac physiology and myoblast cell survival were studied at 3 and 6 weeks after cell therapy. Cell grafts were also examined at 9 days and 12 weeks after implantation in several animals to determine the time course of survival of implanted cells.

In Vivo Maximum Exercise Capacity
Maximum exercise capacity is often used as a measure of in vivo ventricular function and overall cardiac performance and recently has been demonstrated to be a valuable tool in the assessment of cardiac performance in smaller animal models.¹⁶,¹⁷ Maximum exercise capacity was assessed before implantation (1 week after MI) as well as at 3 and 6 weeks after implantation. Maximum exercise capacity was measured as the distance run on a modified rodent treadmill (Columbus Instruments) until exhaustion.¹⁸ Exhaustion
was defined as the inability to run for 15 consecutive seconds despite minor electric shock. Initial treadmill speed was set at 15 m/min at a 15 degree grade and increased by 1-m/min increments every minute.

Ex Vivo Ventricular Function

To characterize myocardial remodeling and ex vivo cardiac function at 3 and 6 weeks after implantation, whole-heart Langendorff perfusion studies were performed in isolated isovolumically beating (balloon–in–left ventricle [LV]) hearts as previously described.\textsuperscript{19,20} Briefly, isolated hearts were retrogradely perfused with a perfusate consisting of bovine red blood cells suspended in modified Krebs-Henseleit buffer at a hematocrit of 40%. A fluid-filled cling-film balloon connected to a Statham P23Db pressure transducer (Statham Instruments) was placed into the left ventricle to monitor ventricular pressures. Coronary perfusion pressure was set to 80 mm Hg, and active pressure-volume relations were then generated. From a balloon volume of zero, the balloon was filled in increments of 0.05 mL, and subsequent peak systolic and end-diastolic pressures were recorded. Systolic and diastolic pressure-volume relations were derived as previously described.\textsuperscript{19,20}

Tissue Histology and Morphometry

After pressure-volume experiments, hearts were arrested in diastole and fixed with 4% buffered paraformaldehyde at a final ventricular distending pressure of 5 mm Hg. Hearts were then weighed, paraffin-embedded, and sectioned (5 to 7 μm thick) from each of 4 equally spread levels (atrium through apex).

Six-micron-thick sections were cut, mounted, and stained with trichrome. Myogenin immunohistochemistry was performed for identification of implanted skeletal myoblasts, whereas skeletal-specific myosin heavy chain immunohistochemistry was performed for identification of differentiated myotubes.\textsuperscript{9} For detection of myogenin, deparaffinized sections were blocked for endogenous peroxidase activity and subjected to antigen retrieval by boiling for 10 minutes in citrate buffer. Sections were blocked for endogenous biotin before adding primary polyclonal rabbit anti-rat myogenin antibody (Santa Cruz Biotechnology) followed by biotinylated goat anti-rabbit secondary antibody. For detection of myosin heavy chain, deparaffinized sections were incubated directly with alkaline phosphatase–conjugated MY-32 mAb (Sigma), specific for skeletal muscle myosin heavy chain, overnight at 4°C. Sections were developed with dianinobenzidine (DAB Substrate Kit; Vector) for myogenin or with BCIP-NBT (Zymed) for MY-32 mAb and counterstained with nuclear red or fast green.

In addition, trichrome sections were used for morphometric analysis. Stained sections were digitally imaged, and infarct size was determined as the mean percentage of epicardial and endocardial circumference occupied by scar tissue.\textsuperscript{20} LV endocardial chamber diameter, septal wall thickness, and infarct wall thickness were derived from an average of 5 measurements taken throughout the respective regions.

Statistics

Data were analyzed by 1-factor ANOVA or paired / test where appropriate. Pressure-volume relations were analyzed by a 2-factor repeated-measures ANOVA and a least-significant-difference post hoc test. All data are presented as mean±SEM. A value of \( P<0.05 \) was considered statistically significant.

Results

Animal Characteristics

Table 1 outlines the animal characteristics of control, MI, and MI+cell animals at both 3 and 6 weeks after therapy. All groups had a comparable increase in body weight over time. In addition, at all time points, hearts from MI and MI+cell groups displayed comparable degrees of LV hypertrophy of \( \approx 20\% \) relative to control animals, as indicated by increased heart weights and heart-to–body weight ratios. Neither MI nor cell therapy altered lung or liver wet-to-dry ratios, suggesting the absence of pulmonary or hepatic congestion.

Animal Survival, Infarct Size, and Myoblast Implantation

Experimental MI resulted in \( \approx 15\% \) acute mortality rate within 24 hours of operation, whereas the cell implantation procedure caused no additional animal deaths. Comparable infarct sizes of 31±1% and 32±1% of the LV were observed in MI and MI+cell hearts, respectively. Hearts receiving

![Image A](https://via.placeholder.com/150)

![Image B](https://via.placeholder.com/150)

![Image C](https://via.placeholder.com/150)

Figure 3. Myoblast survival in infarcted myocardium at 12 weeks after implantation. Infarcted LV free wall is shown with trichrome staining (A) and immunohistochemical staining for myogenin (B) or skeletal-specific myosin heavy chain (C). Myogenin and skeletal myosin heavy chain expression demonstrates presence of viable myoblasts and myotubes at 12 weeks after implantation. Bar represents distance of 200 μm in A, B, and C.
vascular structures were present within or adjacent to the myocardium in similar alignment to cardiomyocytes. Furthermore, appeared to orient parallel to the endocardium and epicardium in similar alignment to cardiomyocytes. Implanted cells occasionally developed the elongated morphology characteristic of fused polynucleated myotubes. Implanted cells occasionally developed the elongated morphology characteristic of fused polynucleated myotubes. Implanted cells occasionally appeared to orient parallel to the endocardium and epicardium in similar alignment to cardiomyocytes. Furthermore, vascular structures were present within or adjacent to the engrafted areas, suggesting that blood supply was available for implanted cells. In the nonnecrotic, peri-infarct region, implanted cells formed regions of myoblasts and myotubes surrounded by fibrosis (Figure 2, C and D).

Maximum Exercise Capacity
As seen in Figure 4, at baseline, before implantation, both MI and MI+cell animals exhibited comparable reductions in exercise capacity of ≈10% relative to control animals, again suggesting similar degrees of myocardial damage before cell therapy. Control animals maintained a stable exercise capacity over the observation period. In contrast, MI animals exhibited a gradual decline in exercise performance with time, with a >30% reduction in exercise capacity relative to control animals at 6 weeks. Cell therapy, however, prevented the continued decline of post-MI exercise capacity, suggesting a protection against the progressive deterioration of in vivo cardiac function.

Ex Vivo Contractile Function
Cardiac contractile function was further investigated in isolated hearts through generation of systolic pressure-volume curves (Figure 5). Noninfarcted control hearts exhibited a typical rise in systolic pressure with increasing ventricular volume. Three weeks after implantation (4 weeks after MI), MI hearts displayed a rightward shift in the systolic pressure-volume curve (Figure 5A). Cell implantation prevented this shift in MI+cell hearts, resulting in greater systolic pressure generation at any given preload (ventricular volume). There was, however, no significant difference in the peak systolic pressure generated at maximum ventricular volume (at an end-diastolic pressure of 40 mm Hg) among groups. The beneficial effects of cell therapy were also seen at 6 weeks after therapy (Figure 5B), suggesting an improvement of ex vivo cardiac function with myoblast implantation.

Ventricular Dilation
In addition to pump dysfunction, ventricular remodeling characteristically results in progressive global cavity enlargement. Ventricular dilation was assessed with diastolic pressure-volume relations, established in isolated hearts through monitoring of distending pressures over a range of diastolic volumes (Figure 6). At all time points during the observation period, MI hearts exhibited substantially enlarged LVs relative to noninfarcted control hearts at any given distending pressure, demonstrated by a rightward repositioning of the pressure-volume curve. Cell therapy, however, caused a significant reduction in ventricular cavity dilation, placing hearts from the MI+cell group significantly leftward of the MI group at both 3 weeks (Figure 6A) and 6 weeks (Figure 6B) after implantation, thereby suggesting an attenuation of deleterious post-MI ventricular remodeling with cell implantation.

Ventricular Morphometry
Ventricular remodeling was further investigated through morphometric analysis of tissue sections (Table 2). At all time points, MI and MI+cell hearts exhibited enlarged chamber diameters compared with noninfarcted control hearts. Six
weeks after cell therapy, hearts from the MI+cell group had a reduced endocardial cavity diameter relative to MI hearts, suggesting an attenuation of ventricular dilation, similar as seen with diastolic pressure-volume curves in Figure 6B. In addition, MI hearts exhibited a decrease in infarct wall thickness at both 3 and 6 weeks after therapy, suggesting characteristic post-MI scar thinning and infarct expansion. MI hearts receiving cell therapy, however, had similar infarct wall thickness relative to both noninfarcted control and infarcted MI hearts. Septal wall thickness was comparable among all groups at both 3 and 6 weeks after therapy.

Discussion

Previous studies have suggested that implanted myoblasts form viable grafts with the potential to improve regional cardiac function in infarcted myocardium. Our experiments build on these initial studies and demonstrate the in vivo and ex vivo therapeutic benefits of myoblast implantation on global post-MI ventricular remodeling and cardiac function.

In this model of experimental MI, coronary occlusion release resulted in typical histological and physiological changes characteristic of deleterious post-MI ventricular remodeling, including an infarct region deficient in endogenous myocytes, infarct wall thinning, ventricular dilation,

**TABLE 2. Ventricular Morphometry**

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>LV Cavity Diameter, mm</th>
<th>Septum Thickness, mm</th>
<th>Infarct Wall Thickness, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Wk after</td>
<td>Control</td>
<td>5.49±0.34</td>
<td>1.78±0.11</td>
<td>2.15±0.13</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>7.65±0.33</td>
<td>1.68±0.19</td>
<td>1.44±0.24†</td>
</tr>
<tr>
<td></td>
<td>MI+cell</td>
<td>6.77±0.13†</td>
<td>1.50±0.07</td>
<td>1.78±0.11</td>
</tr>
<tr>
<td>6 Wk after</td>
<td>Control</td>
<td>5.93±0.24</td>
<td>1.75±0.07</td>
<td>2.01±0.06</td>
</tr>
<tr>
<td></td>
<td>MI</td>
<td>8.11±0.43†</td>
<td>1.53±0.12</td>
<td>1.67±0.16*</td>
</tr>
<tr>
<td></td>
<td>MI+cell</td>
<td>6.79±0.30*‡</td>
<td>1.63±0.07</td>
<td>1.77±0.19</td>
</tr>
</tbody>
</table>

*P<0.05 vs control, †P<0.01 vs control.  
‡P<0.05 vs MI.
decreased ventricular function, and impaired exercise tolerance similar to previous reports. In addition, MI and MI+ cell animals exhibited comparable infarct sizes and similar decreases in maximum exercise capacity before cell implantation, suggesting that differences in cardiac remodeling or function resulting from cell therapy were not due to disparities in initial ischemic injury. Cell implantation at 7 days after MI was selected on the basis of preliminary experiments indicating the greatest degree of cell survival relative to both longer and shorter post-MI time periods. Myoblast cell implantation resulted in focal areas of significant cell graft formation in >90% of animals tested.

LV cavity dilation and exercise intolerance are predictors of cardiovascular morbidity and are often used to gauge efficacy of experimental treatments and to guide therapy. Infarcted hearts receiving cell therapy had an attenuation of ventricular dilation assessed both in isolated hearts and LV cross sections. Cell implantation also improved ex vivo contractile function at 6 weeks after implantation and augmented in vivo maximum exercise capacity after MI. No apparent correlation, however, was observed between graft size and cardiac function.

Although global contractile function was increased after cellular implantation, it remains uncertain if implanted myoblasts are actively responsible for force generation during the cardiac cycle. Although several mechanisms may be responsible for the improved cardiac function, our data suggest that enhanced in vivo and ex vivo generated pressures are more likely to be a result of overall attenuation of deleterious ventricular remodeling within the infarcted and viable myocardium rather than an active force generation by myoblasts. In both animals and humans, myocardial pressure—generating capacity and corresponding exercise capacity decline gradually after MI as the infarcted and viable myocardium undergo progressive dilation and remodeling. Prevention of this dilation, even by physical restraint, results in increased cardiac performance. Therefore, implanted myoblasts may be responsible for augmented ventricular function through a mechanism involving attenuation of dilation of the viable myocardium and prevention of scar thinning, potentially through an increase in myocardial fibrosis. In addition, it is possible that growth factors, released by implanted cells, may exert a protective effect through stimulation of angiogenesis within the infarct and noninfarct regions.

Cell therapy has been used effectively in the treatment of a variety of human disorders, from Parkinson’s disease to diabetes, and holds promise in the therapy of many diseases in which nonregenerative cell death or abnormal cellular function plays a role. As with organ transplantation, the limitation of cell therapy revolves around both the availability of human cells and the possibility of immune rejection. It is for these reasons that skeletal myoblast implantation remains highly attractive as a potential medical treatment. Skeletal myoblasts are readily available and ensure immunological compatibility of myoblast cells cultured from a skeletal muscle biopsy of the recipient. In addition, myoblasts have been shown to have increased tolerance to ischemia and can survive in regions of reduced coronary perfusion, as is often present in patients with coronary artery disease. These data illustrate the therapeutic benefits of syngeneic myoblast implantation after MI on both in vivo and ex vivo indexes of global ventricular dysfunction and deleterious remodeling and suggest that cell therapy may be beneficial after MI.

Acknowledgments

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References


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