Improved Left Ventricular Aneurysm Repair With Bioengineered Vascular Smooth Muscle Grafts

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Background—Recurrent ventricular dilatation can occur after surgical repair of a left ventricular (LV) aneurysm. Use of an autologous bioengineered muscle graft to replace resected scar tissue may prevent recurrent dilatation and improve cardiac function.

Methods—Vascular smooth muscle cells (SMCs, 5×10⁶ cells) from rat aortas were seeded onto synthetic PCLA (sponge polymer of ε-caprolactone-co-L-lactide reinforced with knitted poly-L-lactide fabric) patches and cultured for 2 weeks to allow tissue formation. Syngenic rats underwent proximal left coronary artery ligation to create a transmural myocardial scar. Four weeks after coronary ligation, cell-seeded patches (n=15) or unseeded patches (n=12) were used for a modified endoventricular circular patch plasty (EVCPP) repair of the infarct area. Ligated controls (n=14) and nonligated normal rats (n=10) had sham surgeries without EVCPP. Cardiac function was assessed by echocardiography and isolated Langendorff heart perfusion. Graft histology and morphology was also assessed.

Results—After 8 weeks in vivo, seeded patches were thicker (P<0.05) and smaller in area (P<0.003) than unseeded patches. Only seeded patches had prominent elastic tissue formation (P<0.001) in association with SMCs. LV systolic function by echocardiography was improved in the seeded group compared with both unseeded (P<0.002) and control groups (P<0.0001). LV volumes in both patch repair groups were comparable but were significantly smaller (P<0.05) than controls. LV distensibility tended toward improvement in the seeded group as compared with unseeded hearts, but the difference did not achieve statistical significance (P=0.06).

Conclusions—Surgical repair with muscle-cell seeded grafts reduced abnormal chamber distensibility and improved LV function after myocardial infarction as compared with unseeded grafts. Bioengineered muscle grafts may be superior to synthetic materials for the surgical repair of LV scar. (Circulation. 2003;108[suppl II]:II-219-II-225.)

Key Words: left ventricular aneurysm — remodeling surgery — tissue engineering — smooth muscle cells — biomaterials

Cell transplantation is a promising new therapy for patients with ischemia and left ventricular (LV) dysfunction. We previously reported that muscle cell transplantation into myocardial infarct scar tissue improves heart function and prevents LV dilatation.¹⁻⁴ However, the engraftment process is limited and a large mature scar does not benefit from cell transplantation alone. Resection of the aneurysm and surgical remodeling of the ventricle to restore chamber size and shape may improve cardiac function under ideal circumstances. For surgical repair of LV aneurysm, the endoventricular circular patch plasty technique (EVCPP) has been proposed. Many clinical studies using EVCPP have reported acceptable early and midterm results.⁶⁻⁸ EVCPP increases ventricular systolic function by normalizing LV chamber size and shape. However, in the long-term, chamber re-dilatation and decompensation is a concern with both the EVCPP and traditional linear closure techniques.⁵⁻⁹

The benefits of surgical scar resection and ventricular remodeling procedures may be limited by the use of non-viable synthetic patch materials. EVCPP using autologous muscle cell-seeded bioengineered grafts may prevent recurrent dilatation and improve cardiac function. The present study compared EVCPP repair using a vascular smooth muscle bioengineered graft as compared with a similar but acellular graft in a rat model of myocardial infarction and LV dysfunction.

Methods

Experimental Animals
The Animal Care Committee of the University Health Network approved all experimental procedures, which were performed according to the “Guide to the Care and Use of Experimental Animals” of the Canadian Council on Animal Care and the “Guide for the Care and Use of Laboratory Animals” (the National Academy Press, revised 1996). Adult male syngenic Lewis rats were obtained from...
Isolation and Culture of Smooth Muscle Cells (SMCs)
Vascular SMCs were isolated from rat aortas as described previously.2 The purity of the cultured SMCs was evaluated with a monclonal antibody against a-smooth muscle actin (SMA, Sigma-Aldrich Corp, St. Louis, MO) and endothelial cells were identified with factor VIII antibody (1:2000, Polyclonal, DAKO Diagnostics Canada, Inc, Mississauga, ON, Canada) as described previously.3 Five fields of each culture (from 5 individual animals) were randomly selected and the number of positive and negative cells was counted. The percentage of each cell type was expressed as mean±SE.

In Vitro Graft Preparation
The patch of copolymer sponge made of ε-caprolactone-co-L-lactide reinforced with knitted poly-L-lactide fabric (PCLA) (GUNZE Ltd., Kyoto, Japan) was used as the biodegradable scaffold in this study. SMCs were harvested from culture dishes using 0.05% trypsin solution and resuspended to a concentration of 5×10⁶ cells in 80 μL of culture medium, and seeded onto the PCLA patches (8 mm, 1 mm thickness) as previously described.4 After incubation for 1 day, 7 mL of culture medium was added to culture dish. The patches with SMCs were then cultured for 2 weeks with media changes every 2 days to allow tissue formation. To facilitate the identification of the seeded cells after patch implantation, 40 μL of 0.4% 5-bromo-2′-deoxyuridine (BrdU; Sigma-Aldrich Corp) solution was added to 10 mL of the culture medium and incubated with cell-seeded PCLA for 3 days before implantation.10

Experimental Myocardial Infarction
Rats were anesthetized with ketamine hydrochloride (22 mg/kg) and sodium pentobarbital (30 mg/kg). The animal was intubated and ventilated at a rate of 60 cycles/min with a tidal volume of 5 mL under room air supplemented with oxygen (2 L/min) and 1 to 2.5% isoflurane. Through a left lateral thoracotomy, the proximal left anterior descending coronary artery (LAD) was circumferentially ligated with a 6-0 polypropylene suture. Myocardial ischemia was confirmed by regional myocardial color changes and ST segment elevation by electrocardiography. The incision was closed in layers with 3-0 silk continuous sutures. Penlong XL (Penicillin G benzathine, 150,000 U/mL, and penicillin G procaine, 150,000 U/mL) was injected intramuscularly (0.4 mL per rat). Buprenorphine hydrochloride (0.01 mg/kg) was given subcutaneously.

Scar Resection and Graft Implantation Surgery
Four weeks after LAD ligation, animals were screened by echocardiography for infarct size as estimated by the percentage of scar area (akinetic or dyskinetic regions) to LV free wall (LVFW) area. Animals with infarcts greater than 25% of the LVFW area were used as experimental animals. Animals weighing 200 to 250 g served as cell donors and those weighing 300 to 350 g were used as experimental animals.

Assessment of Cardiac Function
Echocardiography
Echocardiography was performed before patch implantation and at 1, 3, 5, and 8 weeks after patch implantation. The left parasternal images were taken in the right lateral decubitus position using a 1.3-MHz transducer (SonoAce C256 and 15L8, respectively, Acuson, Mountain View, CA). Short-axis 2-dimensional images at the mid-ventricular level of left ventricle were stored as digital loops and the end-systolic (ESA) and end-diastolic (EDA) cavity areas were determined by tracing endocardial borders. The fractional area change (FAC) was calculated as [(EDA–ESA)/EDA×100]. For each measurement, 3 consecutive cardiac cycles were traced and averaged by an experienced examiner in a blind fashion, according to the American Society for Echocardiography’s Leading Edge Method.11

Isolated Langendorff Heart Perfusion
Eight weeks after patch implantation, global heart function was evaluated using a non-working constant pressure Langendorff perfusion as described previously.1,12 The rats were anesthetized, and sodium heparin (100 U) was administered intravenously. The heart was quickly isolated and perfused in a Langendorff apparatus with filtered Krebs-Henseleit buffer (mM: NaCl, 118; KCl, 4.7; KH2PO4, 1.2; CaCl2, 2.5; MgSO4, 1.2; NaHCO3, 25; and glucose, 11; pH 7.4) equilibrated with 5% carbon dioxide and 95% oxygen. A latex balloon was passed into the left ventricle through the mitral valve and connected to pressure transducer (model p10EZ, Viggo-Spectramed, Oxnard, CA) and differentiator amplifier (model 11-G4113–01; Gould Instrument System Inc, Valley View, OH). After 30 minutes of stabilization, the balloon size was increased by 0.02 mL increments from 0.02 mL by the stepwise addition of saline. LV peak systolic and end-diastolic pressures were measured at ventricular volumes from 0.04 mL and in 0.02-mL increments until the end-diastolic pressure was over 30 mm Hg. Developed pressure was calculated as the difference between the peak systolic and end-diastolic pressures at each ventricular volume by an automated real-time computer software program (Ponemah physiology platform, Gould Instrument System Inc). Hearts were then arrested with 10 mL of KCl solution (20 mmol). Passive diastolic pressures were recorded at each balloon volume in 0.04-mL increments until the diastolic pressure was over 60 mm Hg. The heart was then fixed at a ventricular pressure of 30 mm Hg with 10% phosphate-buffered formalin solution for 48 hours.

Morphological and Histological Studies
The patch area (PA) at before implantation (pre-PA) and 8 weeks postimplantation (post-PA) was measured and graft expansion expressed as post-PA/pre-PA×100. Formalin-fixed hearts were cut into 3-mm-thick sections and both apical and basal sections were digitally photographed (Coolpix, Nikon, Tokyo, Japan) and quantified using the public domain NIH image program (National Institutes of Health, Springfield, VA). The thickness of LVFW, implanted patches and scar region were measured as previously described.1 The LV chamber volume was calculated from planimetric measures. Staining with hematoxylin and eosin and elastica Masson trichrome (EMT) was used to assess tissue formation and extracellular matrix composition.13 Immunohistochemical staining with a monoclonal antibody against smooth muscle myosin heavy chain (SM1) (1:3000, Yamasa Corp, Tokyo, Japan) was used to assess muscle tissue formation and an antibody against factor VIII (1:2000, Polyclonal, DAKO Diagnostics Canada, Inc) was used to identify endothelial cells on the endocardial surfaces of patches and blood vessels. Cells seeded on the grafts were identified with a monoclonal antibody against BrdU (Zymed Laboratory, Inc, South San Francisco, CA). Five different microscopic fields (200x by ECLIPSE-TE200; Nikon, Tokyo, Japan) of each patch portion were randomly selected and digitally photographed. Muscle tissue area and extracellular matrix area in the grafts were analyzed using the NIH image program and size of the patch. The sternotomy was closed and the rat was recovered as described in the previous section.
The cultured cells used to seed the patches were 94% Histology died of congestive heart failure 10 weeks after LAD ligation. no cases of patch dehiscence or rupture. One control animal from excessive blood loss during the procedure. There were surgery (3 seeded patches, 5 unseeded patches), presumably patch-implanted animals, 8 animals died within 24 hours after patch implantation and 15 animals for controls. Of the remaining 50 rats were randomly divided into 35 animals for because their infarcts were less than 25% of the LVFW. The four weeks after LAD ligation, 6 of 56 rats were excluded Mortality.

Results

Histology

The cultured cells used to seed the patches were 94±1% SMC and 6±1% endothelial cells. Eight weeks after patch implantation, the cell-seeded patch was integrated with the LVFW (Figure 1). In both patch groups, the endocardial surface of the patch was lined with endocardial cells (Figure 2). The density of the capillaries was similar in both patch groups. The spongy scaffold matrix had degraded. The poly-lactide fibers of the PCLA scaffolds were present in both the cell-seeded and unseeded PCLA patches. No immunonecrosis was evident in any of the patches (Figure 2). SM1 staining was increased in the cell-seeded patches as compared with unseeded patches (6.7±1% versus 1.8±0.2%; P<0.002) and was co-localized with BrdU staining and extracellular elastin deposition (Figure 3). These changes were predominately localized in the subendocardial portion of the patches where the cells were initially seeded ex vivo. Interestingly, some BrdU-labeled cells were present in the scar rim outside of the patch indicating migration. At the border zone between the host myocardium and patch, elastin formation was evident and did not differ in amount among the cell-seeded, unseeded and control hearts. As compared with the unseeded patches, cell-seeded patches contained more extracellular elastin (6.6±0.9% versus 0.68±0.1%; P<0.001) and collagen (32.4±2.3% versus 25.9±1.5%; P<0.05) matrix components.

LV Remodeling

Eight weeks after implantation, LV chamber volumes were beneficially reduced in the cell-seeded and unseeded patch groups as compared with unrepaired controls although they were not reduced to normal values. LV chamber size was similar between the cell-seeded and unseeded groups (Figure 4 upper). The normal LVFW was thicker than that of the ventricular wall of the patched hearts. However, the LVFW of the normal and cell-seeded patched hearts were thicker than that of the control free wall. The thickness of the scar rim between the patch and myocardium of the cell-seeded patched hearts was thicker than the scar thickness of the unseeded patched hearts and control scars. The control scar thickness was thinner than the scar rim thickness of the unseeded patched hearts. The cell-seeded patch was thicker than the unseeded patch (Figure 4 middle). The cell-seeded patch increased in thickness from its pretransplant thickness of 1.0 mm to 1.32±0.07 mm, while the acellular patch thickness of 0.99±0.04 mm did not differ from its pretransplant thickness of 1 mm. The area of the unseeded patch was larger than that of the cell-seeded patch (Figure 4 lower).

Statistical Analysis

All data are expressed as the mean±SE. The StatView and the Statistical Analysis System software (SAS Institute Inc, Cary, NC) were used for analysis. Comparisons between 2 groups were performed by unpaired t test. Comparisons of continuous variables among the 3 and 4 groups were performed by a one-way analysis of variance (ANOVA). Scheffe’s test was used to specify differences among groups. Comparisons of echocardiography data were performed by a two-way repeated measures ANOVA with group, time and the interaction between group and time as the variables. Langendorff function data were evaluated by an analysis of covariance (ANCOVA). Main effects were group, volume and the interaction between group and time as the variables. Langendorff perfusion data were fit by a two-way repeated measures ANOVA with group, time and the interaction between group and volume for Langendorff perfusion data. Variance values less than 0.05 were considered statistically significant.

Mortality

Four weeks after LAD ligation, 6 of 56 rats were excluded because their infarcts were less than 25% of the LVFW. The remaining 50 rats were randomly divided into 35 animals for patch implantation and 15 animals for controls. Of the patch-implanted animals, 8 animals died within 24 hours after surgery (3 seeded patches, 5 unseeded patches), presumably from excessive blood loss during the procedure. There were no cases of patch dehiscence or rupture. One control animal died of congestive heart failure 10 weeks after LAD ligation.
LV Function

Echocardiography indicated that both cell-seeded and unseeded PCLA patches were akinetic over the 8-week period after implantation. EDAs were equal between the two patch groups but FAC, an index of systolic function, was significantly improved in the cell-seeded patch compared with unseeded patches (Figure 5). Both patch groups had significantly improved FAC measures as compared with controls, although they did not reach the normal values of noninfarcted hearts. Cardiac function as assessed by Langendorff perfusion determined that cell-seeded patch hearts were less distensible than unseeded patched hearts, although the difference was not statistically different ($P=0.06$). These measures were confined to the physiological range of 0 to 30 mm Hg.

Discussion

For patients with an extensive myocardial infarction, surgical remodeling procedures such as EVCPP can improve heart function.9 However, even after successful repair the LV can dilate again.8 Accordingly, mitral regurgitation has also been reported as a late complication of the procedure in association with recurrent dilatation.7 Nishina and coworkers14 reported that improvements in LV size and function after plication in a rat ischemic cardiomyopathic model was transient because of residual scar remodeling and loss of normal LV shape. The same investigators (Sakakibara and coworkers15 and Nomoto and coworkers16) recently reported that LV surgical repair combined with fetal cardiomyocyte transplantation in the border zone or postoperative ACE inhibitor therapy improved LV function and prevented recurrent dilatation. However, the scar area was not completely removed and the loss of viable myocardium was not addressed. In a sheep model of a myocardial infarction, suturing a polypropylene mesh over the infarcted area prevented progressive LV expansion. This technique maintained normal ventricular geometry and heart function for 8 weeks despite substantial tissue losses.17 Similarly, Leor and colleagues showed that implantation of a bioengineered fetal rat cardiomyocyte graft onto the surface of a myocardial infarct attenuated ventricular dilatation.18 The beneficial effects of this technique may be explained by the elastic properties of the bioengineered muscle graft. We believe that a complete resection of myocardial scar tissue and total replacement of the infarct with an elastic bioengineered muscle graft may avoid long-term recurrent dilatation and decompensation.
In the present study, we selected a PCLA biodegradable scaffold to create our grafts because of our earlier successes with this polymer in repairing the right ventricular outflow tract. In the manufacturing process, the biodegradable spongy matrix (50% ε-caprolactone and 50% L-lactide) is reinforced with a knitted poly-L-lactide fabric that degrades more slowly as compared with the spongy matrix. The porous PCLA patch enhances cell colonization while the outer poly-L-lactide fabric layers maintain patch structure and dimensions until the seeded cells develop into a tissue with their own extracellular matrix. In contrast, non-biodegradable patches such as Dacron (polyethylene terephthalate) and Gore-Tex (polytetrafluoroethylene) are nonviable, will not grow, and are incapable of significant remodeling and self-repair. Eight weeks after implantation, the PCLA spongy matrix fibrous scaffold was only partially dissolved. However, our analysis of the PCLA after implantation was complicated by nonspecific damage during the histological processing. Despite the chemical damage to the scaffold, it appeared that much of the spongy layer was biodegraded and that the outer fibrous layers were still intact.

To enhance the elasticity of the bioengineered muscle graft, we used vascular SMCs to seed our biodegradable synthetic PCLA scaffolding. Vascular SMCs are easy to harvest, expand rapidly in culture, and are capable of significant elastic tissue formation. SMCs will respond to external stresses by hypertrophy, hyperplasia, and migration in vivo, properties that may enhance tissue formation after LV implantation. We seeded our patches ex vivo and allowed 2 weeks in culture for tissue formation before implantation. This approach may control host fibroblast cell infiltration of the patch that may produce excessive non-elastic fibrous tissue. After 8 weeks in vivo, the SMC-seeded patched hearts showed prominent elastic tissue formation in the subendocardial layer where the seeded cells were co-localized. Interestingly, BrdU positive SMCs migrated out of the patch into the residual scar rim suggesting active remodeling after implantation. However, a modest area of the graft was devoid of the seeded cells. Better in vitro cellular distribution of the seeded cells within the patch might have been possible if a
Mechanical stretch regimen had been applied or techniques of dynamic cell seeding with spinner flasks or orbital shakers were used. In contrast, tissue formation in the unseeded patches was minimal and appeared rich in acellular fibrous matrix, similar to the scar that was resected. Accordingly, the unseeded patch had a decreased thickness and larger surface area suggesting reduced structural support.

EVCPP, either with or without cell seeding the PCLA scaffold, had beneficial effects on limiting LV dilatation and preserving systolic function as compared with non-repaired but infarcted control hearts. However, while EVCPP did restore ventricular size and shape toward normal, cardiac function was reduced as compared with noninfarcted hearts. Importantly, 8 weeks after implantation, SMC-seeded PCLA graft hearts demonstrated improved systolic function by echocardiography and reduced LV distensibility as compared with unseeded graft repairs. Although not directly measured, we believe that the improved elasticity of our cell-seeded patches mediated these beneficial effects. In contrast to a stiff synthetic material, we speculate that our bioengineered smooth muscle graft absorbs wall stress (potential energy) during isovolumic contraction and when the aortic valve opens transmits the energy into the ejection thus aiding in contraction. It is also possible that the elastic muscle graft attenuates the sharp increases in wall stress within the adjacent myocardium during the cardiac cycle, similar to the aorta, which may limit recurrent chamber dilatation. The outer fibrous layer of the PCLA scaffold would have prevented major structural changes in the patch and may explain why the LV chamber volumes were similar for both the seeded and unseeded patched hearts. We believe that our data indicate that LV aneurysm repair may be facilitated by a dynamic cell-seeded smooth muscle graft to restore LV size and function.

Limitations
A limitation of the current study was that it was a short-term proof of concept study using a small animal. A larger animal model would have better simulated the human condition and improved measurements of cardiac size and function. Adding a non-biodegradable patch group, such as Dacron, would have further increased the clinical relevance of the study. Although stress-strain analysis could have been performed if we had possessed the equipment, the outer knitted poly-L-lactide fabric was still intact at 8 weeks after patch implantation and would have limited the usefulness of the measurements. We have shown that cell-seeded biodegradable Gelfoam patches were more elastic than non-cell seeded patches. With complete biodegradation of the PCLA material, stress-strain analysis would have been very informative. At least two years would be required for the PCLA scaffold to completely dissolve. Since the left ventricular chamber volumes in Figure 4 were calculated from computerized planimetric measurements, the volumes could have been overestimated compared with the physiological volumes. Measurement of left ventricular volumes by passive pressure in arrested hearts (Figure 6) might have underestimated the ventricular chamber volume because the balloon and part of the tube connecting the balloon occupied part of the LV chamber. Although there was a difference in chamber volumes between Figures 4 and 6, the trend of the volume measurements was similar.

Conclusions
Surgical repair with muscle-cell seeded grafts reduced abnormal chamber distensibility and improved LV function after myocardial infarction as compared with unseeded grafts. Bioengineered muscle grafts may be superior to synthetic materials for surgical repair of LV scar.

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