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Circulation 2002;106;I-176-I-182
DOI: 10.1161/01.cir.0000032901.55215.cc
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0009-7322. Online ISSN: 1524-4539

Optimal Biomaterial for Creation of Autologous Cardiac Grafts

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Background—The optimal cardiac graft for the repair of congenital heart defects will be composed of autologous cells and will grow with the child. The biodegradable material should permit rapid cellular growth and delayed degradation with minimal inflammation. We compared a new material, ϵ-caprolactone-co-L-lactide sponge reinforced with knitted poly-L-lactide fabric (PCLA), to gelatin (GEL) and polyglycolic acid (PGA), which are previously evaluated materials.

Methods—Syngenic rat aortic smooth muscle cells (SMCs, 2×10^6) were seeded onto GEL, PGA, and PCLA patches and cultured (n=11 per group). The DNA content in each patch was measured at 1, 2, and 3 weeks after seeding. Histological examination was performed 2 weeks after seeding. Cell-seeded patches were employed to replace a surgically created defect in the right ventricular outflow tract (RVOT) of rats (n=5 per group). Histology was studied at 8 weeks following implantation.

Results—In vitro studies showed that the DNA content increased significantly ($P<0.05$) in all patches between 1 and 3 weeks after seeding. Histology and staining SMCs for anti-α-smooth muscle actin (αSMA) revealed better growth of cells in the interstices of the grafts with GEL and PCLA than the PGA graft. In vivo studies demonstrated that seeded SMCs survived at least 8 weeks after the patch implantation in all groups. PCLA scaffolds were replaced by more cells with larger αSMA-positive areas and by more extracellular matrix with larger elastin-positive areas than with GEL and PGA. The patch did not thin and expanded significantly. The GEL and PGA patches thinned and expanded. All grafts had complete endothelialization on the endocardial surface.

Conclusions—SMC-seeded biodegradable materials can be employed to repair the RVOT. The novel PCLA patches permitted better cellular penetration in vitro and did not thin or dilate in vivo and did not produce an inflammatory response. The cell-seeded PCLA patch may permit the construction of an autologous patch to repair congenital heart defects. **(*Circulation*. 2002;106[suppl I]:I-176-I-182.)**

Key Words: congenital cardiac defect ■ myocardium ■ tissue engineering ■ smooth muscle cells ■ biomaterials

Children with truncus arteriosus require a conduit from the right ventricle to the pulmonary artery and patients with tetralogy of Fallot may require a transannular patch. Currently, the conduits or patches are usually created from Dacron (polyethylene terephthalate), Gore-Tex (polytetrafluoroethylene), glutaraldehyde-treated bovine pericardium, or glutaraldehyde-treated homografts. These materials are not viable, do not grow with the children, and do not provide pulsatile pulmonary flow. Lack of growth of the biomaterial may necessitate reoperations to replace the conduit or patch.[1] The synthetic material is a foreign body and may become thrombogenic or infected.[2] The long-term results of congenital heart surgery have been compromised by the biomaterials employed in the repair.[3,4]

The ideal cardiac constructs will consist of a biodegradable material which is sufficiently strong to resist damage from the contracting myocardium. The material should permit diffusion of nutrients and metabolic waste necessary for cell growth; enable cell adhesion, migration, proliferation, and differentiation; facilitate extracellular matrix formation; and permit endothelialization of the endocardial surface.

The material should also be biocompatible and bioabsorbable at a rate compatible with the repair process so that the patch ultimately becomes a tissue supplied by blood vessels from the host myocardium. The patch of copolymer sponge made of ϵ-caprolactone-co-L-lactide reinforced with knitted poly-L-lactide fabric (PCLA) is a newly invented material for cardiac tissue engineering. The PCLA patch consists of a knitted fibrous component made of poly-L-lactic acid (PLLA) and a spongy component made of 50% ϵ-caprolactone and 50% L-lactic acid (P(CL+LLA)). The exciting feature of this new biomaterial is that the spongy portion is absorbed within 2 months and the fibrous portion persists for 1 to 2 years. Because polyglycolic acid (PGA) is one of the most common synthetic bioabsorbable polymers used in tissue engineering[5,6] it was included in the study. Gelatin sponge (Gelfoam; GEL) was also chosen because of its protein composition and honeycomb structure, which allows cell growth. We have

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Circulation **is available at http://www.circulationaha.org** **DOI: 10.1161/01.cir.0000032901.55215.cc**

previously reported the results of in vitro and in vivo studies using this gelatin sponge.[7–9]

We elected to seed the patches with cultured vascular smooth muscle cells (SMCs) obtained from a syngenic adult rat aorta. We chose SMCs because they can be harvested from a number of sources in the donor and returned to the donor as an autologous cell patch. The SMCs are easily cultured, should induce angiogenesis in the graft,[10] and can respond to in vivo mechanical stress by hyperplasia and hypertrophy to prevent patch dilatation and thinning.

In this paper we studied the in vitro and in vivo characteristics of 3 biodegradable cell-seeded patches used to repair a surgically created myocardial defect in the right ventricle of the rat. We compared the new PCLA bioabsorbable material to the PGA mesh and to GEL.

Methods

Experimental Animals

All animal procedures were carried out in compliance with 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" prepared by the Institute of Laboratory Animal Resource, National Research Council, and published by the National Academy Press, revised 1996. Aortas used to harvest smooth muscle cells were obtained from male syngenic Lewis rats (Charles River Canada Inc, Quebec, PQ, Canada) weighing 200 to 300 g. Male syngenic Lewis rats weighing 300 to 350 g were used for the right ventricular outflow tract (RVOT) replacement procedure.

Biomaterials

Three materials were used in the in vivo studies, GEL (Pharmacia Co., Kalamazoo, MI), PGA (JMS Inc., Hiroshima, Japan), and PCLA (GUNZE Ltd., Kyoto, Japan). Rectangular pieces (7×7 mm) were used for the ventricular wall replacement.

Isolation and Culture of Vascular Smooth Muscle Cells (SMCs)

The rats were anesthetized with an intramuscular injection of Ketamine hydrochloride (22 mg/kg), followed by an intraperitoneal injection of sodium pentobarbital (30 mg/kg). After heparin was administered intravenously, rats were euthanized with an intraperitoneal injection of Euthanyl (2 mL/4.25 kg body weight) (MTC Pharmaceuticals, Cambridge, ON, Canada). The rat aorta was harvested and SMCs were isolated as previously described using protease solution containing 0.4% trypsin, 0.2% collagenase, and 0.02% glucose in phosphate-buffered saline (PBS).[10] The cells were cultured in culture medium (Iscove's modified Dulbecco medium) (GIBCO Laboratory, Life Technologies, Grand Island, NY) containing 10% fetal bovine serum, 0.1 mmol/L beta-mercaptoethanol, penicillin G (100 U/mL), and streptomycin (100 μg/mL) at 37°C in 5% carbon dioxide and 95% air. When the cells reached to confluence, the cells were detached from the dish with 1 mL of 0.05% trypsin in PBS solution with 0.02% glucose and subcultured. The passage 2 SMCs were detached and cell suspension was centrifuged. The cell pellet was washed and 2×10^6 SMCs were resuspended in 30 to 50 μL of culture medium for seeding onto each patch.

Before cell seeding, the culture dishes (N=6) were stained with a monoclonal antibody against α-smooth muscle actin (αSMA; 1:3000, Sigma-Aldrich Corp., St. Louis, MO) as we previously reported.[11] The cells were observed under microscope and positive and negative cells were counted in 5 randomly selected areas of each culture dish. The percentage of positive cells was calculated.

Cell Seeding Onto Patches

The sheets of GEL, PGA, and PCLA were immersed in culture medium. After the excessive culture medium was removed from the patches by gentle compression, each patch was placed into a 10-cm culture dish. The SMC suspension (2×10^6 cells/patch) was transferred onto the center of each patch. Twenty minutes after incubation, a drop of culture medium was added at each corner of the patch. After another 60 minutes of incubation, 20 mL of culture medium were added to the culture dishes. The patches with SMCs were then incubated. The culture medium was changed every 2 days.

DNA Measurement for Cell Growth Curve

To confirm the linear relation between the SMC numbers and DNA contents, 0.15, 0.3, 0.6, 1.0, 2.0, 4.0×10^6 cells were homogenized and total DNA was measured using DNeasy^M Tissue Kits (Catalog No. 69504, QIAGEN Inc., Mississauga, ON, Canada). SMC-seeded (N=11) and non cell-seeded (N=9) patches in each of GEL, PGA, and PCLA were used for DNA measurement to quantify cell number at 1, 2, and 3 weeks after the cells were seeded onto the biomaterials. Briefly, each patch was gently rinsed with PBS twice and was then cut into small pieces. The cells in the samples were lysed using Proteinase K (40 mAU/mg protein) at 55°C for 12 hours in a shaking water bath. The sample lysate was loaded onto the mini column and centrifuged at 6000*g* for 1 minute. DNA, bound to the membrane of the column, was then eluted in buffer after washing. The DNA yield was determined by measuring the concentration of DNA in the eluate by its absorbance at 260 nm.

Histological Study

At 2 weeks after cell seeding onto patches, cell-seeded patches were fixed in 10% phosphate-buffered formalin solution for 2 days. The fixed patches were cut vertically through the center. The slices were fixed in 5% glacial acetic acid and methanol, embedded in paraffin, and sectioned to 3-μm-thick specimens. The sections were stained with hematoxylin and eosin (H&E), as described in the manufacture's specifications (Sigma-Aldrich Corp., St. Louis, MO), and stained with αSMA. The percentage of αSMA positive cells in the scaffolds was calculated.

Right Ventricular Outflow Tract Free Wall Resection and Replacement With Patches

The rats were anesthetized as described in *Isolation and Culture of SMCs* section. The rats were endotracheally intubated and ventilated at a rate of 60 cycles/min with a tidal volume of 3 mL under room air supplemented with oxygen (2 L/min) and 1 to 2.5% Isoflurane.

The rat heart was exposed through a median sternotomy. A purse-string stitch (5 to 6 mm in diameter) was placed in the free wall of the right ventricular outflow tract (RVOT). Both ends of the suture were passed through a 22-gauge plastic vascular cannula (Angiocath; Becton Dickinson and Company, Franklin Lakes, NJ), which was used as a tourniquet. The tourniquet was tightened and the bulging part of the RVOT wall inside the purse-string stitch was lopped off. The tourniquet was briefly loosened to absolutely confirm whether massive bleeding occurred or not, and because we needed to make a sufficiently large transmural defect in each heart. A cell-seeded GEL, PGA, or PCLA patch (N=5 per group) 2 weeks after cell seeding, or a non cell-seeded patch (N=5 per group) was sutured along the margin of the purse-string stitch with over-and-over method with 7–0 polypropylene (Prolene; Ethicon, Inc, Somerville, NJ) to cover the defect in the RVOT. The cell-seeded surface of the patch was on the endocardial side of the ventricular wall. After completion of suturing, the tourniquet was released and the purse-string stitch was removed. The chest incision was closed in layers with running sutures of 3–0 silk.

Penlong XL (Penicillin G benzathine, 150,000 U/mL, and penicillin G procaine, 150,000 U/mL) was injected intramuscularly (0.3 mL per rat). Buprenorphine hydrochloride (0.01 mg/kg) was given subcutaneously every 8 hours for the first 48 hours after the operation. After the operation, rats were monitored in a warmed environment until they recovered from the anesthesia and were then returned to their cages.

Morphological Evaluations of the In Vivo Patches

Eight weeks after implantation, the rats were euthanized after heparin injection. Each heart was excised and fixed with 10% phosphate-buffered formalin solution for 2 days. The patch area, used to repair the RVOT, was measured with a scale by using a digital videocamera (DCR-PC110, SONY, Tokyo, Japan). The ratio

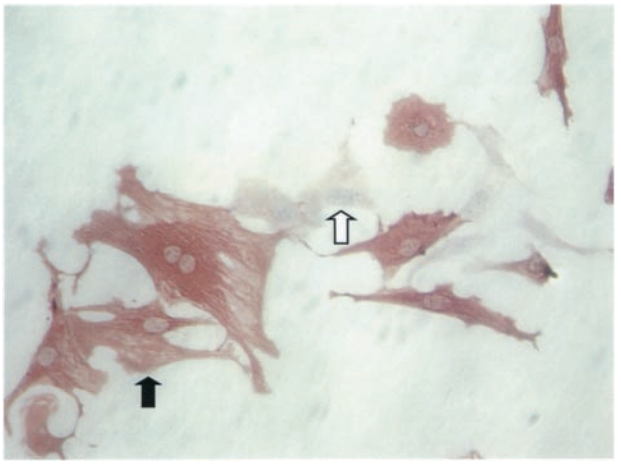

Figure 1. Cultured aortic smooth muscle cells (SMCs, black arrow) stained with a monoclonal antibody against α-smooth muscle actin before seeding the scaffolds (200×). Non SMCs were also observed in culture (white arrow).

of pre- and postoperative patch areas was calculated and compared among all groups.

The patches were then cut across the center. The thickness of the center of the patch replacing the defect in the RVOT was measured. The ratios of the pre- and postoperative patch thickness were calculated and compared among groups.

Histologic Studies for In Vivo Patches

The patch sections were stained with H&E, as described in the previous section, with elastica van Gieson (EVG) to assess the extracellular matrix in the patches,[12,13] with a monoclonal antibody against αSMA (1:3000, Sigma-Aldrich Corp., St. Louis, MO) to assess muscle tissue formation and with an antibody against factor VIII (1:2000, Polyclonal, DAKO Diagnostics Canada, Inc. Mississauga, ON, Canada) to identify endothelial cells on the endocardial surfaces of patches.

Grafted Cell Identification in the Patches

The SMCs within the patches were labeled in vitro with the thymidine analog 5-bromo-2′-deoxyuridine (BrdU; Sigma-Aldrich Corp., St. Louis, MO). Three days before the patch implantation, 90 μL of 0.4% BrdU solution was added to the dishes containing cell-seeded patches and incubated for 3 days. At 8 weeks post implantation, histological sections of the patches were stained with a monoclonal antibody against BrdU (Zymed Laboratory. Inc. South San Francisco, CA) as we previously described.[11]

Histology of the Implanted Patches

Five different microscopic fields (x400 by ECLIPSE-TE200; Nikon, Tokyo, Japan) of each patch portion of the RVOT were randomly selected and images were taken by a digital camera (Coolpix, Nikon, Tokyo, Japan). All the digital images of morphology and histology of the patch portions in RVOT were analyzed using the public domain NIH image program (National Institutes of Health, Springfield, VA) and Adobe Photoshop (Adobe Systems Incorporated, San Jose, CA). Briefly, after tracing the outlines of the target areas and subtracting the backgrounds, the color images were converted to gray images. A fixed threshold (90 in 255 grades) of gray color scale determined automatically the number of nuclei staining positively for BrdU and the percentage of each field staining positively for elastin or αSMA.

Data Analysis

All data are expressed as the mean±standard error. The StatView (SAS Institute Inc., Cary, NC) was used to analyze statistical data. Comparisons between 2 groups were performed by unpaired *t* test. Comparisons of continuous variables among the 3 groups were performed by one-way analysis of variance (ANOVA). The Scheffe's test was used to specify differences among groups. A probability value of less than 0.05 was considered statistically significant.

Results

In Vitro Evaluation of SMC Growth in Biomaterials

Before seeding to scaffold, 89±6% of cultured aortic SMCs stained positively for α−smooth muscle actin (Figure 1).

To quantify cell number in the biomaterial during culture, cell number and DNA content correlation was studied. There was a significant linear relation between DNA contents and the SMC numbers (R=0.965, P<0.0001). Cell number in the biomaterials increased significantly during the 3 weeks of culture. The DNA contents in cell-seeded patches were greater at 3 weeks than at 1 week (GEL: 7.6±1.1 and 22.3±2.6 μg at 1 and 3 weeks, respectively; PGA: 8.9±1.6 and 16.8±0.7 μg at 1 and 3 weeks, respectively; PCLA: 9.0±1.6 and 19.9±0.7 μg at 1 and 3 weeks, respectively; P=0.003, P=0.005, and P=0.002, respectively). At 2 weeks, the DNA contents of the GEL (16.2±1.2 μg) and PCLA (17.0±1.2 μg) patches were greater (P=0.011 and P=0.006, respectively) than that of the PGA patch (9.4±0.6 μg).

Before implantation into RVOT, there was no difference in the percentage of cells stained positively for αSMA in the scaffolds (GEL: 82.8±3.5; PGA: 84.0±4.2; PCLA: 84.3±3.7%). However the cells were localized primarily in the surface layer of PGA patches (Figure 2). In contrast more cells penetrated and grew into the deeper layer of the scaffolds of PCLA and GEL patches.

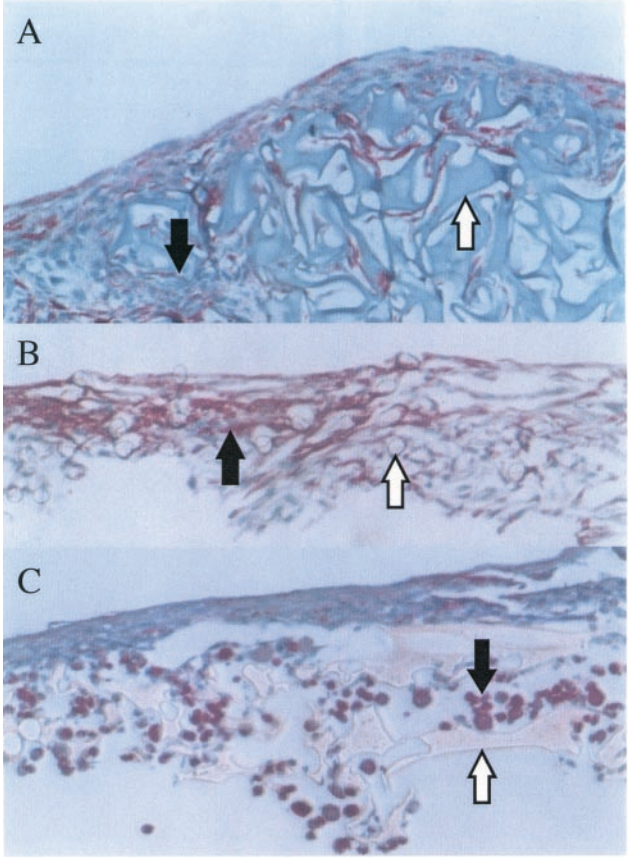

Figure 2. In vitro cell-seeded patches stained for αSMA at 2 weeks after culture (200×). A: Gelatin sponge (GEL). B: Polyglycolic acid mesh (PGA). C: Knitted poly-L-lactic acid fabric with copolymer sponge (PCLA). Seeded SMCs (black arrows) grew in the biomaterials (white arrows).

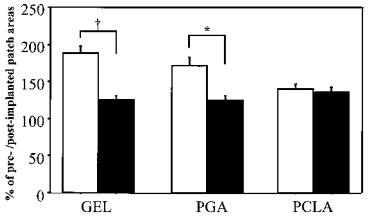

Figure 3. Percentage of the pre- to postimplanted patch sizes of SMC-seeded (■) and non-seeded (□) GEL, PGA, and PCLA patches. *$P<0.01$, †$P<0.001$. N=5, 5 for each biomaterial.

Biomaterial Expansion In Vivo

At 8 weeks after implantation, unseeded GEL, PGA, and PCLA increased in size 188%, 171%, and 140% compared with their preoperative sizes (Figure 3). The SMC-seeded GEL and PGA patches were smaller ($P<0.001$, <0.01) than their non-seeded control patches. The SMC-seeded PCLA patch sizes were unchanged from that of their unseeded patch sizes.

The thickness of SMC-seeded and unseeded GEL, PGA, and PCLA patches are shown in Figure 4. At 8 weeks following implantation, the thicknesses of the unseeded and seeded GEL patches decreased. The thickness of cell-seeded GEL patch was greater ($P<0.001$) than its unseeded control. The cell-seeded PGA patch slightly increased in thickness and the unseeded PGA patch decreased in thickness. The difference in thickness between the seeded and unseeded PGA patches was significant ($P=0.002$). The cell-seeded PCLA patches did not differ in thickness from their non-seeded control patch.

Cell Survival, Extracellular Matrix Secretion, and Muscle Tissue Formation in the Scaffolds

At 8 weeks after implantation, the number of BrdU-positive SMCs in the GEL patches (825±288 cells/mm^2) was significantly greater ($P=0.05$) than in the PGA patches (125±42 cells/mm^2). The PCLA patches contained more ($P=0.03$, $P=0.02$) BrdU-positive cells (3615±590 cells/mm^2) than the GEL and PGA patches.

In agreement with BrdU-positive cell number, αSMA-positive area differed among the 3 biomaterials (Figures 5 and 6). There were greater ($P<0.01$, <0.01) αSMA-positive areas in the cell-seeded GEL and PCLA groups than their unseeded patches. αSMA-positive cells were predominantly in the endocardial layer of the PGA scaffold and few in numbers were found in the middle layers of PGA patches. Many αSMA-positive cells were found in the PCLA patches.

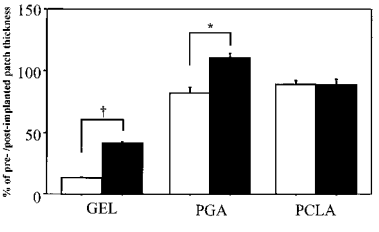

Figure 4. Percentage of the pre- to postimplanted patch thickness of SMC-seeded (■) and non-seeded (□) GEL, PGA, and PCLA patches. *$P<0.01$, †$P<0.001$. N=5, 5 for each biomaterial.

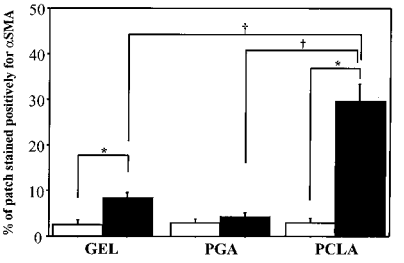

Figure 5. Percentage of the αSMA positive areas in the cell-seeded (■) and non-seeded (□) GEL, PGA, and PCLA biomaterials being used to repair the right ventricular outflow tract for 8 weeks. *$P<0.01$, †$P<0.001$ among the 3 cell-seeded groups. N=5, 5 for each biomaterial.

The cells were observed in all layers of the scaffold and occupied 30% of the total area of the PCLA patch.

Elastin was present in the cell-seeded scaffold but considerably less elastin was present in the non-cell-seeded control patches (Figures 7 and 8). The amount of elastin in the cell-seeded GEL and PGA patches was similar. The elastin content in the SMC-seeded PCLA patch was 32% of the total scaffold area and was more ($P<0.001$, <0.001) than the amounts of elastin present in the cell-seeded GEL and PGA patches. The distribution and localization of BrdU-labeled cells corresponded to the location of smooth muscle identified by staining with anti-αSMA and with the occurrence of elastin fibers in the cell-seeded patches (Figure 9). Although collagen was present in both cell-seeded and unseeded patches of GEL, PGA, and PCLA, it was more prevalent in their respective unseeded patches.

Patch Degradation and Extracellular Matrix

At 8 weeks each polymeric scaffold was partially absorbed and replaced with cells and extracellular matrix. In both the cell-seeded and control patches, only the fibrous part of the PCLA patch remained. Some fibers of the PGA and the spongy gelatin matrix of the GEL patch were present in the cell-seeded and control patches. No obvious differences in the residual scaffold were observed except that the density of the PGA matrices appeared to be decreased.

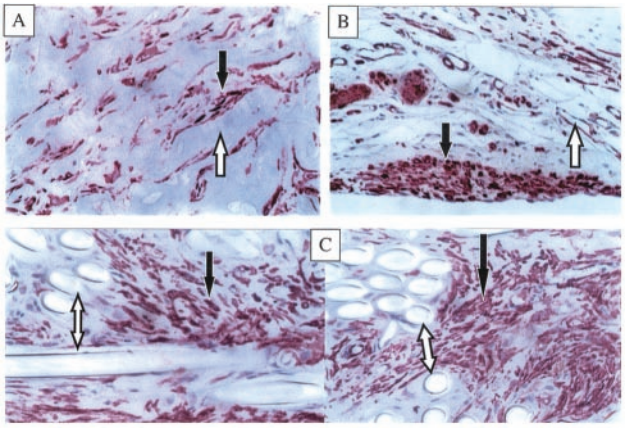

Figure 6. Immunohistochemical staining of SMC-seeded GEL (A), PGA (B), and PCLA (C) patches after being used to repair the right ventricular outflow tract for 8 weeks. SMCs stained positively (brown in color, black arrows). Biomaterials were not stained (white arrows) (400×).

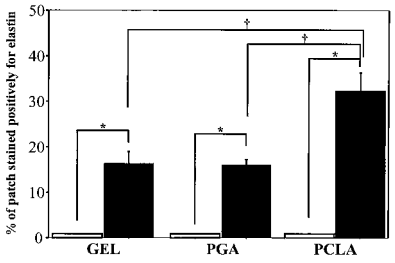

Figure 7. Percentage of the elastin-positive area in the patch section of cell-seeded (■) and non-seeded (□) GEL, PGA, and PCLA after being used to repair the right ventricular outflow tract for 8 weeks. *$P<0.01$, †$P<0.001$ among the 3 cell seeded groups.

Angiogenesis

There was a nonstatistically significant tendency for the SMC-seeded patches to have more angiogenesis than their respective control patches. The angiogenesis was primarily a capillary angiogenesis located along the borders of the patch.

Endothelialization

Factor VIII staining showed complete endothelialization on the endocardial surfaces in the GEL, PGA, and PCLA patches. No clot was observed on the surface of the biomaterial.

Discussion

In vitro autologous muscle cell seeding of a biodegradable scaffold before implantation provides a muscle graft, which can maintain the elasticity and structure of the myocardium by establishing an organized muscular tissue with its extracellular matrix. Without in vitro seeding, host fibroblasts will colonize the biodegradable graft. After bioabsorption of the scaffold, only a scarred patch will replace the myocardial wall. Fibrous tissue lacks elasticity and can thin and dilate because of the intraventricular pressures. Ventricular dilatation can induce heart failure. An advantage of the nonbioabsorbable polyethylene terephthalate (Dacron) and polytetrafluoroethylene (Gore-Tex) scaffolds is that they do not thin

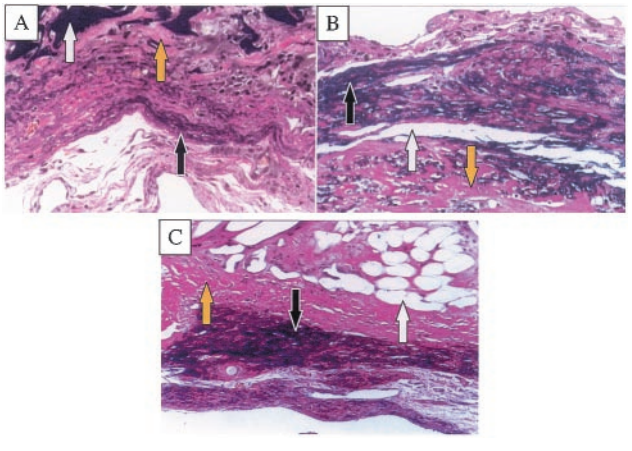

Figure 8. SMC-seeded GEL (A), PGA (B), and PCLA (C) stained with elastica van Gieson (200×). Collagen (pink in color, yellow arrows) and elastin (black in color, black arrows) were found in the biomaterial (black in color for Gelform (A) and white in color for PGA (B) and PCLA (C), white arrows).

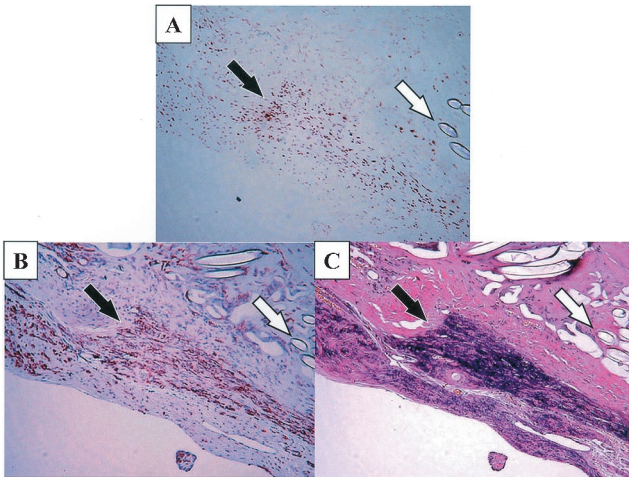

Figure 9. The distribution and localization of BrdU-labeled cells (A, brown in color; black arrow, 100×) corresponded to the location of smooth muscle identified by staining with anti-αSMA (B, brown in color; black arrow, 100×) and with the location of elastin fibers (C, black in color; black arrow, 100×) in the cell-seeded patches. White arrow indicates the remaining polymer fibers.

and dilate in response to the intraventricular pressures. We have shown in our myocardial cell transplantation research that transplantation of myogenic cells will prevent ventricular remodeling in both the infarcted and cardiomyopathic myocardium.[11,14,15] We attribute the success of the cell transplantation to the establishment around the implanted muscle cells of an extracellular matrix, which maintained the structure, thickness, and elasticity of the ventricular wall. An autologous cell-seeded biodegradable patch may preserve the structure and elasticity of the ventricular wall while providing the potential for growth and contractility.

We chose SMCs for this study because they were effective in preventing ventricular remodeling when injected into an injured myocardial region and because they can form an extensive elastic extracellular matrix, that could maintain the elasticity of the ventricular wall.[10,11] SMCs also stimulate angiogenesis, that is necessary for the long-term survival of the graft. Finally, SMCs proliferate and hypertrophy in response to stress and the muscle cell-seeded patch should be able to adapt to changing intraventricular pressures. Because an occluded pulmonary artery in a patient was reconstructed with an autologous tissue-engineered vessel graft using the patient's vein,[16] building an autologous tissue engineered cardiac graft may be possible with SMCs. In addition, SMCs are readily obtainable from patients and easily cultured.

In this study, the purity of the SMC culture before cell-seeding was 89%, The remaining 11% of the cells consisted of endothelial cells and fibroblasts. To obtain a high purity of SMCs, the tunica adventitia of the aorta was peeled off to minimize fibroblast contamination and the aortic lumen was flushed and immersed with the protease solution to remove endothelial cells. The SMCs were then isolated from the tunica media. Approximately 83% of cultured aortic cells stained positively for αSMA before implantation in all the seeded groups. The percentage of SMCs decreased minimally during the in vitro patch incubation. In contrast to *in vitro* cell

distribution in the scaffold, the in vivo results showed that a considerable number of host cells, that stained negatively for αSMA, migrated, and proliferated into all the scaffolds. Although the differences in quantity of muscle formation with αSMA between the cell-seeded and non-seeded patches were evident, a small number of αSMA-positive cells were observed in the non-seeded patches. All the endocardial surfaces in the seeded and non-seeded patches were endothelialized. The findings indicate that host progenitor cells from the systemic circulation or from the surrounding tissue may be relevant to the endothelialization and the presence of SMC-like cells in the patches. The findings are consistent with other studies.[17–20]

We implanted 2-week-old cell-seeded patches into the defect of RVOT. Before implantation, the cell numbers of the PCLA and GEL patches were greater than that of the PGA patch. The distribution of the cells in the PGA patch was localized predominantly in the surface layer. In contrast, the PCLA and GEL patches permitted more cell penetration and growth into the deeper layer of their scaffolds. These in vitro results are consistent to some extent with the in vivo results. The in vivo numbers of BrdU-positive SMCs in the PCLA and GEL patches were significantly greater than the number in the PGA patch. Significantly greater αSMA-positive areas were present in the cell-seeded PCLA and GEL patches than in their corresponding non-seeded patches. No statistical difference in αSMA-positive areas was found between the cell-seeded and non-seeded PGA patches. The distribution of the SMC in the PGA patches was predominantly in the endocardial layer of the scaffold. The SMC distribution in the PGA patch differed from the uniform cell localization of PCLA. The in vivo localization of the BrdU-labeled cells agreed with the distribution of SMC muscle and elastin fibers in the cell-seeded patches (Figure 9). The cell-seeded PCLA patches had the most SMC and elastin formation among the 3 patches studied. All the cell-seeded patches were sutured so that the cell-seeded surface of the patch was on the endocardial surface. The non-seeded patches contained more fibroblasts and collagen than their respective seeded patches. The findings are consistent with in vitro cell proliferation and distribution in the scaffolds before implantation affecting the in vivo cell distribution outcomes. More efficient in vitro cell seeding and cell-scaffold cultivation may be important in building a successfully tissue-engineered graft for in vivo use because the non-seeded grafts contained mostly fibroblasts and collagen. Kim et al[21] and Carrier et al[22] have tested techniques of the dynamic cell seeding that result in tissue engineered grafts with increased cellularity, more uniform cell distribution, more aerobic cell metabolism, more physiological, elongated cell shape, and greater elastin deposition compared with the static cell seeding method. They used spinner flasks to stir polymer matrices and a cell suspension, rotating vessels with laminar flow, or orbital shakers to agitate polymer matrices and a cell suspension. These methods show promise in building cell-seeded biodegradable scaffolds for clinical application.

An important limitation of our study is the lack of mechanical measurements of scaffold tensile strength and elasticity.[23] Without mechanical measurements the contribution of the cell seeding to the in vitro and in vivo mechanical properties cannot be directly measured. However, cell seeding of the GEL and PGA patches significantly prevented their stretching and thinning during 8 weeks of in vivo testing compared with their respective non-seeded scaffolds. Because the fibrous layer of the PCLA patch was unchanged during the 8 weeks of in vivo testing, the relative contributions of the fibrous layer, the cells, and extracellular matrix to tensile strength cannot be assessed.

We have previously reported that fetal rat ventricular cardiomyocytes can be grown in three dimensions in GEL and that the cardiomyocyte-seeded GEL contracted regularly and spontaneously in vitro and in vivo.[7,9] Although the GEL showed excellence in cell attachment and cell growth, its fragility and softness limit its usefulness as a scaffold unless reinforced with an additional polymer. Also of concern was that a graft-induced inflammatory reaction, which might be caused by the presence of a foreign protein in GEL, was observed in 2 of the GEL grafts as shown in our previous report.[7]

PGA is one of the most common and attractive synthetic biodegradable polymers used clinically because of its capacity to permit cell growth[5,21] and because of its biodegradation rate.[6] It has been used in combination with another biodegradable polymer in some tissue engineering studies,[23–26] because the PGA fibers lack structural stability and often cannot maintain their original structure during tissue development.[27,28] Our findings support the results of these researchers.

The PCLA spongy matrices made of ϵ-caprolactone-co-L-lactic acid were biodegraded, whereas the fibrous matrices made of poly-L-lactic acid (PLLA) persisted and remained intact in this study. The reason that the PCLA patch sustained its physical dimensions for at least 2 months regardless of cell seeding can be attributed to the mechanical property of the fibrous PLLA matrices resistant enough to circumstances in the RVOT and to the prolonged degradation term. In fact, Rajasubramanian and associates, who produced resorbable endoluminal stents from co-polymers, reported that polymer blends with higher PLLA proportions exhibit higher elastic moduli, ultimate tensile strength, and lower elongation and degradation rates.[29] Tamai et al implanted coronary PLLA biodegradable stents with no metallic components into 15 patients with coronary artery stenosis and reported that the PLLA stents were feasible, safe, and effective in humans.[30] The reports support our outcomes that the reliable polymer characteristics of PLLA matrices of the PCLA patch contributed to the physical performance as a RVOT patch. Our results do not distinguish between the contributions of the PLLA polymer, the smooth muscle tissue, and the extracellular matrix formation.

Kim and Mooney[27] have shown that the PLLA-bonded PGA matrices have a high cellularity and maintained their predefined structure more than the unbonded PGA matrices during the process of tissue development. Their report indicated that the combined or hybrid scaffold, such as the PCLA, should permit the development of a new tissue in a predefined 3-dimensional structure. We found that the PCLA spongy matrices made of ϵ-caprolactone-co-L-lactide facilitated in

vitro and in vivo cell growth. The honeycomb structure of the GEL also encouraged cellular proliferation. The PCLA graft had better in vivo cellularity than the GEL grafts possibly because its copolymer sponge matrices absorbed faster than the gelatin scaffolds or the difference in the biochemical properties of the polymers influenced cell growth in vivo.

An angiogenesis that was most evident at the border zone between the patch and host myocardium was present in both the control and SMC-seeded patches. During the first 8 weeks, the patch's nutrient supply and waste removal might depend on diffusion between the blood and the right ventricle. We anticipate that long-term studies will demonstrate that the patch will permit the formation of a well-organized muscular tissue supported by a vascular system and a more structured extracellular matrix.

In conclusion, SMC-seeded bioabsorbable patches were employed to repair the defect of RVOT. The seeded SMCs survived in scaffolds for the experimental period and led to new muscular and elastin formation. Because the PCLA patch possesses the unique hybrid structure, our results suggested that the fibrous PLLA outer layer contributed to the physical patch performance in cardiac milieu whereas the spongy copolymer matrices favored cell proliferation.

Acknowledgments

We thank Dr. Yoshito Ikada and Shin'ichiro Morita for providing PCLA material, and Dr. Tetsuro Sakai for his technical advice. Dr. R-K Li is a Career Investigator of the Heart and Stroke Foundation of Ontario. Funding was provided by the HSFO (NA4603) and CIHR (MOP14795) to RKL.

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