CARDIOTHORACIC TRANSPLANTATION

FETAL CELL TRANSPLANTATION: A COMPARISON OF THREE CELL TYPES

Tetsuro Sakai, MD
Ren-Ke Li, MD, PhD
Richard D. Weisel, MD
Donald A. G. Mickle, MD
Zhi-Qiang Jia, MD, MSc
Shinji Tomita, MD
Eung-Joong Kim, MD, PhD
Terrence M. Yau, MD, MSc

Objective: We have previously reported that fetal cardiomyocyte transplantation into myocardial scar improves heart function. The mechanism by which this occurs, however, has not been elucidated. To investigate possible mechanisms by which cell transplantation may improve heart function, we compared cardiac function after transplantation of 3 different fetal cell types: cardiomyocytes, smooth muscle cells (nonstriated muscle cells), and fibroblasts (noncontractile cells). **Methods:** A left ventricular scar was created by cryoinjury in adult rats. Four weeks after injury, cultured fetal ventricular cardiomyocytes ($n = 13$), enteric smooth muscle cells ($n = 10$), skin fibroblasts ($n = 10$), or culture medium (control, $n = 15$ total) were injected into the myocardial scar. All rats received cyclosporine A (INN: ciclosporin). Four weeks after transplantation, left ventricular function was evaluated in a Langendorff preparation. **Results:** The implanted cells were identified histologically. All transplanted cell types formed tissue within the myocardial scar. At an end-diastolic volume of 0.2 mL, developed pressures in cardiomyocytes group were significantly greater than smooth muscle cells and skin fibroblasts groups (cardiomyocytes, $134\% \pm 22\%$ of control; smooth muscle cells, $108\% \pm 14\%$ of control; skin fibroblasts, $106\% \pm 17\%$ of control; $P = .0001$), as were $+dP/dt_{\max}$ (cardiomyocytes, $119\% \pm 37\%$ of control; smooth muscle cells, $98\% \pm 18\%$ of control; skin fibroblasts, $92\% \pm 11\%$ of control; $P = .0001$) and $-dP/dt_{\max}$ (cardiomyocytes, $126\% \pm 29\%$ of control; smooth muscle cells, $108\% \pm 19\%$ of control; skin fibroblasts, $99\% \pm 16\%$ control; $P = .0001$). **Conclusions:** Fetal cardiomyocytes transplanted into myocardial scar provided greater contractility and relaxation than fetal smooth muscle cells or fetal fibroblasts. The contractile and elastic properties of transplanted cells determine the degree of improvement in ventricular function achievable with cell transplantation. (J Thorac Cardiovasc Surg 1999;118:715-25)

Molecular and cellular biologic evidence offer the promise of new therapeutic approaches to heart failure. Cell transplantation has recently been advocated as a means to repair failing hearts.^{1,2} In animal models, cultured fetal cardiomyocytes have been successfully transplanted into ischemic myocardium, which resulted in

improved cardiac function.^{3,4} On the basis of the initial success of cell transplantation with fetal cardiomyocytes, transplantation of other types of cultured cells has also been investigated, and some types have resulted in significant improvement of cardiac function.^{5,6}

There is, at present, only limited data comparing the

From the Division of Cardiovascular Surgery and The Center for Cardiovascular Research, Toronto General Hospital, University Health Network, Department of Surgery, University of Toronto, Toronto, Ontario, Canada.

R.K.L. is a Research Scholar of the Heart and Stroke Foundation Canada. R.D.W. is a Career Investigator of the Heart and Stroke Foundation of Ontario.

Read at the Seventy-ninth Annual Meeting of The American Association for Thoracic Surgery, New Orleans, La, April 18-21, 1999.

Received for publication March 19, 1999; revisions requested April 29, 1999; revisions received May 24, 1999; accepted for publication June 16, 1999.

Address for reprints: Ren-Ke Li, MD, PhD, Toronto Hospital—General Division, CCRW 1-815, 101 College St, Toronto, Ontario, Canada, M5G 2C4.

Copyright © 1999 by Mosby, Inc.

0022-5223/99 \$8.00 + 0 12/6/100750

degree of improvement in heart function that can be achieved by the transplantation of various cell types, because published reports have examined only individual cell types. No study has yet identified the cell type that may offer superior functional augmentation or whether cardiomyocytes are more effective than other cell types. In addition, the mechanisms by which transplanted cells may enhance ventricular function have not been fully investigated.

The purpose of our study was therefore to assess the relative merits of cardiomyocytes for cell transplantation and to address some possible mechanisms of the functional improvement noted after cell transplantation. We compared 3 different fetal rat cell types: ventricular cardiomyocytes, enteric smooth muscle cells (as nonstriated muscle cells), and skin fibroblasts (as noncontractile cells).

Material and methods

Experimental animals. The Animal Care Committee of The Toronto Hospital 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" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. Nineteen-day fetuses were harvested as cell donors from dated pregnant Sprague-Dawley rats (Lewis; Charles River Canada Inc, Quebec, PQ, Canada). Adult male Sprague-Dawley rats weighing 300 to 350 g served as cell transplantation recipients.

Cell culture and preparation for transplantation.

Ventricular cardiomyocytes, gastric smooth muscle cells, and skin fibroblasts were isolated and cultured by methods we have previously described.⁷ In brief, for cardiomyocytes, the fetal hearts were washed 3 times in phosphate-buffered saline solution (PBS; NaCl, 136.9 mmol/L; KCl, 2.7 mmol/L; Na₂HPO₄, 8.1 mmol/L; and KH₂PO₄, 1.5 mmol/L; pH 7.3). After the atria and great vessels were removed, the ventricles were minced with fine scissors and incubated in PBS solution containing trypsin (0.5%), collagenase (0.1%), and glucose (0.02%) at 37°C. Smooth muscle cells from the stomach and skin fibroblasts were also processed as described. Digestion with trypsin and collagenase was performed for 10 minutes for cardiomyocytes and for 30 minutes for smooth muscle cells and fibroblasts. The clumps of cells were separated by gentle agitation during the digestion. The cell suspension was transferred into a tube containing 20 mL of culture medium supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. Iscove's modified Dulbecco's medium (Gibco Laboratory, Life Technologies, Grand Island, NY) containing 0.1 mmol/L β-mercaptoethanol was used for cardiomyocytes. Dulbecco's modified Eagle's medium (Sigma Chemical Co, St Louis, Mo) was used for smooth muscle cells and fibroblasts. The cell suspension was

centrifuged at 600g for 5 minutes, and the cell pellet was resuspended in culture medium. At this stage, cardiomyocytes were purified by a preplating method.⁷ The freshly isolated myocardial cells were plated on dishes and cultured 2 hours to let noncardiomyocytes attach to the surface of the dishes. Then the supernatant containing floating cardiomyocytes was transferred into another dish for further culture. The isolated cells were cultured at 37°C in 5% carbon dioxide and 95% air. Twenty-four hours after seeding, the cells were detached from the culture dishes with 1 mL of 0.05% trypsin in PBS solution with glucose (0.02%). The reaction was stopped by the addition of culture medium, and the cell suspension was then centrifuged at 600g for 5 minutes. The cell pellet was resuspended in culture medium at a concentration of 1.6×10^7 cells/mL. A volume of 0.25 mL of the cell suspension was used for each transplantation.

Cell purity

Cardiomyocytes. The cultured cardiomyocytes beat spontaneously and regularly at a rate of 67 to 70 beats/min in the culture dishes. At the time of transplantation, the purity of the cardiomyocytes in culture was evaluated by staining with a monoclonal antibody against myosin heavy chain (Rougie Bio-Tech Ltd, Quebec, Canada), with a technique that we have previously described.⁷

Smooth muscle cells. The purity of the smooth muscle cells in culture was evaluated with a monoclonal antibody against alpha-smooth muscle actin (Sigma Diagnostics, St Louis, Mo). In brief, the cells were washed with PBS and fixed in 100% methanol at -20°C for 15 minutes. After being washed with PBS 3 times, the cells were incubated with the first antibody against smooth muscle cell actin at 37°C for 30 minutes followed by an overnight incubation at 4°C. The control sample was incubated with PBS. After samples were washed with PBS (3 times, for 5 minutes each), a biotin-labeled secondary antibody (1:250; Ector Lab Inc, Burlingame, Calif) was added to the specimens and incubated at room temperature for 1 hour. The samples were rinsed 3 times for 5 minutes each in PBS and incubated with an avidin-biotin complex conjugated with peroxidase at room temperature for 45 minutes. Visualization was accomplished with diaminobenzidine solution (0.25 mg/mL in 0.05 Tris-HCl buffer containing 0.02% H₂O₂) for 10 minutes. The cellular nuclei were counterstained with hematoxylin for 1 minute. The samples were covered with crystal mounts and photographed.

Fibroblasts. Fibroblasts were identified morphologically by light microscopy by their characteristic spindle-shaped appearance.

Anesthesia and postoperative care of the recipients rats.

Adult male rats were anesthetized with an intramuscular injection of ketamine hydrochloride (22 mg/kg), followed by an intraperitoneal injection of sodium pentobarbital (30 mg/kg). The rats were endotracheally intubated and ventilated with a Harvard ventilator (model 683; Harvard Apparatus Co, Inc, S Natick, Mass). Positive-pressure ventilation was maintained at a rate of 60 cycles/min with a tidal volume of 3 mL with room air supplemented with oxygen (2 L/min).

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 body weight) was given subcutaneously every 8 hours for the first 48 hours after the operation. After the operation, rats were monitored until they fully recovered from the anesthesia and were then returned to their cages.

Myocardial scar formation. After the rat received general anesthesia as described, the heart was exposed through a 2-cm left lateral thoracotomy. An 8 × 10-mm metal probe was cooled to -190°C by immersion in liquid nitrogen and was applied with gentle pressure on the left ventricular free wall to produce a cryoinjury. After 1 minute, the cryoprobe was removed from the surface of the heart and immersed in liquid nitrogen. When the frost on the cryoinjured area of the heart had disappeared, the cryoprobe was reapplied to the same area of the heart. This procedure was performed a total of 10 times. Our previous study has shown that cryoinjury generated a scar comprising 36% ± 4% of the left ventricular free wall at 4 weeks. At 8 weeks, the scar had expanded to 55% ± 3% of the left ventricular free wall.³

Cell transplantation. Four weeks after cryoinjury, rats were randomly assigned either to a cell-transplantation group or to a control group. Rat hearts were exposed with a median sternotomy after general anesthesia was administered. In the transplantation groups (cardiomyocytes, n = 13; smooth muscle cells, n = 10; fibroblasts, n = 10), 0.25 mL of cells suspended in culture medium (4 × 10⁶ cells) was injected into the center of the scar tissue of the left ventricular free wall with a tuberculin syringe. In control rats (total number = 15; 5 control rats for each cell type), the same volume of culture medium was injected into the scar in the same manner. All the rats received daily subcutaneous injections of cyclosporine A (INN: ciclosporin), at a dose of 5 mg/kg body weight per day.

Myocardial function study. Four weeks after transplantation, heart function of the transplanted and control rats was evaluated in a Langendorff preparation.⁸ After general anesthesia was administered, heparin sodium (200 units) was administered intravenously to the rats. With a repeated sternotomy, the heart was quickly excised and perfused in a Langendorff apparatus with filtered Krebs-Henseleit buffer (NaCl, 118 mmol/L; KCl, 4.7 mmol/L; KH₂PO₄, 1.2 mmol/L; CaCl₂, 2.5 mmol/L; MgSO₄, 1.2 mmol/L; NaHCO₃, 25 mmol/L; glucose, 11 mmol/L; pH 7.4) equilibrated with 5% carbon dioxide and 95% oxygen. A latex balloon was inserted into the left ventricle through the mitral valve and connected to a pressure transducer (model p10EZ; Viggo-Spectramed, Oxnard, Calif), a transducer amplifier, and a differentiation amplifier (model 11-G4113-01; Gould Instrument System, Inc, Valley View, Ohio). After 30 minutes of stabilization at 37°C, coronary flows were measured by repeated timed collections. Balloon volume was adjusted to produce an end-diastolic pressure of -25 mm Hg, which was set as the baseline. The balloon size was then increased in 0.02 mL increments from 0.04 mL to 0.48 mL by the stepwise addition of saline solution. Systolic and diastolic pressure, the maximal rate of myocardial contraction (+dP/dt_{max}),

and the maximal rate of myocardial relaxation (-dP/dt_{max}) were recorded at each balloon volume, and the developed pressure was calculated as the differences between the peak-systolic and the end-diastolic pressures. After the measurements were completed, the heart was arrested with 10 mL infusion of a 20% KCl solution into the aortic root.

Measurement of left ventricular remodeling. After the heart was arrested, we measured the ventricular volumes as described by Pfeffer and associates.⁹ The volume of the intraventricular balloon required to distend the arrested left ventricle to a pressure of 30 mm Hg was recorded. The heart was then fixed with 10% phosphate-buffered formalin solution in a distended state. Two days after fixation, the balloon was removed, and the atria and great arteries were excised. The heart was weighed. The epicardial and the endocardial surface areas of the left ventricular free wall and those of the cryoinjury-derived scar tissue were measured by planimetry as previously described.³ In brief, the heart was cut into 5 slices each 3-mm thick. The areas and lengths of interest were traced and quantified by computed planimetry (Jandal Scientific Sigma-Scan, Corte Madera, Calif). The total endocardial and epicardial surface area of the scar tissue was measured as the sum of the epicardial length and the endocardial length × the thickness (3 mm).

For comparisons between the 3 transplantation groups, “the left ventricular volume index,” “the scar area index,” and “the scar thickness index” were used. The *left ventricular volume index* was defined as the left ventricular volumes of each transplantation group divided by the mean left ventricular volumes of their control rats. The *scar area index* was defined as the percent scar area of the left ventricular free wall of each transplantation group by the mean percent scar area of the left ventricular free wall of each transplantation group by the mean percent scar area wall of its control group. The *scar thickness index* was calculated by dividing the scar thickness of the transplantation group by the mean scar thickness of its control group. In addition, the percentage of the area of the myocardial scar occupied by the transplanted cells was recorded.

Histologic studies. The heart sections were fixed in 5% glacial acetic acid in methanol, embedded in paraffin, and sectioned to produce 10-μm thick specimens. The sections were stained with hematoxylin and eosin as described in the manufacturer’s specifications (Sigma Diagnostics).

Data analysis. All data are expressed as the mean ± the standard deviation. The heart rate, coronary flow, heart weight, left ventricular volume index, scar area index, scar thickness index, and percentage of scar area occupied by transplanted cells were analyzed with the SPSS software package for Windows v8.0 (SPSS Inc, Chicago, Ill). Comparisons of continuous variables among the 3 groups were performed by a 1-way analysis of variance, and Scheffé’s test was used to specify differences among groups.

Functional data were evaluated with the SAS software package for Windows v6.12 (SAS Institute Inc, Cary, NC). To compare the results of different studies, we carefully indexed the results from each transplantation group to its own control group and expressed the results as the differ-

Fig 1. Indices of (A) developed pressure, (B) $+dP/dt_{\max}$, (C) $-dP/dt_{\max}$, and (D) end-diastolic pressure in hearts transplanted with cardiomyocytes (CM), smooth muscle cells (SM), and fibroblasts (FB) at intraventricular balloon volumes of 0.1 and 0.2 mL. By analysis of covariance, the indices of developed pressure, $+dP/dt_{\max}$, and $-dP/dt_{\max}$ in cardiomyocytes were significantly greater than in smooth muscle cell and fibroblast ($^{**}P = .0001$). Smooth muscle cell had significantly greater $+dP/dt_{\max}$ index and $-dP/dt_{\max}$ index than fibroblast ($^{*}P = .0024$, $.014$, respectively), but there was not a significant difference in developed pressure index ($P = .63$). There were no significant differences in end-diastolic pressure index ($P = .22$).

Table I. Heart rate, coronary flow, and heart weight at the time of functional study

	Cardiomyocytes	Smooth muscle cells	Fibroblasts	P value
Heart rate (beats/min)	400.0 $\pm$ 28.4	351.0 $\pm$ 47.0	361.0 $\pm$ 43.6	.12
Coronary flow (mL/min)	19.4 $\pm$ 1.9	21.3 $\pm$ 3.4	18.6 $\pm$ 2.3	.12
Heart weight (g)	1.60 $\pm$ 0.08	1.68 $\pm$ 0.34	1.55 $\pm$ 0.15	.46

ence from control (end-diastolic pressure) or percent difference from control (developed pressure, $+dP/dt_{\max}$, and $-dP/dt_{\max}$). The 3 transplantation groups were compared by analysis of covariance, with intraventricular balloon volume as the covariate and developed pressure index, end-diastolic pressure index, $+dP/dt_{\max}$ index, and $-dP/dt_{\max}$ index as dependent variables. Main effects were group, volume, and interaction of group $\times$ volume. If a significant difference was identified among groups by analysis of covariance, pair-wise comparisons were performed to specify differences.

Results

Cryo-injury model. The rat mortality rate in the process of scar generation was 2%.

Ventricular function. There were no significant differences in heart rate, coronary flow, or heart weight among the 3 groups (Table I).

Developed pressure (expressed as a percentage of control values) was significantly greater in the cardiomyocyte group than in the smooth muscle cell

Fig 2. Fetal cardiomyocyte transplantation. Transplanted cardiomyocytes formed cardiac tissue (*T*) in the myocardial scar tissue (*S*). *L*, Lymphocytes; *H*, host myocardium. (Hematoxylin and eosin stain; original magnifications: **A**, $\times 100$; **B**, $\times 400$.)

group or the fibroblast group ($P = .0001$), although there was no significant difference between the smooth muscle cell and fibroblast groups ($P = .63$; Fig 1, *A*).

The $+dP/dt_{\max}$ (expressed as a percentage of control values) was significantly higher in cardiomyocyte group than in the other groups ($P = .0001$), and smooth muscle cell group was higher than fibroblast group ($P = .0024$; Fig 1, *B*).

The $-dP/dt_{\max}$ (expressed as a percentage of control values) was significantly greater in the cardiomyocyte group than in the other groups ($P = .0001$), and smooth muscle cell group was greater than fibroblast group ($P = .014$; Fig 1, *C*).

The end-diastolic pressures (expressed as differences from their control values) were not significantly different among the 3 groups ($P = .22$; Fig 1, *D*).

Histologic studies. The purity of the cultured cells, evaluated by immunohistochemistry, was $94.0\% \pm 3.5\%$ for the fetal rat cardiomyocytes and $86.0\% \pm 5.3\%$ for the fetal rat smooth muscle cells. Fibroblasts tended to

grow easily and quickly, resulting in greater than 95% purity.

All the transplanted cells formed a block of tissue in the myocardial scar. Photomicrographs of the transplanted hearts showed a characteristic morphologic condition for each cell type, with infiltration of lymphocytes around the transplanted cells in spite of the administration of cyclosporine A. All the transplanted cells were completely surrounded by host scar tissue. A number of capillaries were seen in the area of transplantation, for all cell types. The transplanted fetal cardiomyocytes formed striated myocardium. The alignment of the tissue formed by the transplanted cardiomyocytes appeared to be somewhat disorganized, compared with the native myocardium (Fig 2, *A* and *B*). This tissue, when excised en bloc, appeared to beat spontaneously and regularly at the time of explantation. The transplanted fetal smooth muscle cells (Fig 3, *A* and *B*) stained positively with antibodies against smooth muscle actin (Fig 3, *C*). The transplanted fetal

Fig 3. Fetal smooth muscle cell transplantation. (Hematoxylin and eosin stain; original magnifications: **A**, $\times 40$; **B**, $\times 400$.) Transplanted smooth muscle cells formed tissue (T), which were stained positively for smooth muscle specific actin (**C**, $\times 400$; arrows). S, Myocardial scar; L, lymphocytes.

Fig 4. Fetal fibroblast transplantation. A dense fibrotic tissue (*T*) with hair follicles (*arrows*) was formed in the myocardial scar (*S*). (Hematoxylin and eosin stain; original magnifications: **A**, ×40; **B**, ×400.)

fibroblasts formed a dense fibrous structure with what appeared to be hair follicles (Fig 4). The transplanted smooth muscle cells and fibroblasts within the scar tissue did not align normally. The control group, which had been injected only with culture medium but no cells, had a thin layer of fibrous scar (Fig 5).

Left ventricular remodeling. There were not statistically significant differences in left ventricular volume indices between groups (Table II). The scar thickness index and the scar area index were significantly lower in the cardiomyocyte transplantation group than in the other 2 groups. In all of the transplanted groups, transplanted cells occupied more than 30% of the scar area. The fibroblast transplantation group demonstrated the greatest cell proliferation within the scar.

Discussion

Cell transplantation has been advocated as an alternative therapy to restore heart function after a myocardial infarction. We³ and other investigators⁴ have found

that transplanted fetal cardiomyocytes improved heart function in a rat model. We have reported improvement in left ventricular function after transplantation of cultured fetal enteric smooth muscle cells into the cryoinjury-induced scar tissue of rat hearts.⁵ Taylor and associates⁶ demonstrated improved regional systolic and diastolic function after transplantation of cultured autologous skeletal satellite cells in a cryoinjured rabbit heart model. These findings support the effectiveness of noncardiomyocyte cells for cell transplantation, which may permit wider application of cell transplantation techniques in the clinical setting.

The mechanisms of improvement of ventricular function after cell transplantation have not, however, been fully elucidated. In addition, the effect of different cell types on the functional outcome has not yet been addressed. We hypothesized that cells with contractile proteins, such as cardiomyocytes, may offer greater functional recovery because of their elastic and contractile properties.¹⁰ However, none of the published

Fig 5. Control group. A thin fibrous cardiac scar was produced by cryoinjury. (Hematoxylin and eosin stain; original magnifications: **A**, $\times 40$; **B**, $\times 400$.)

Table II. Left ventricular remodeling after cell transplantation

	Cardiomyocytes	Smooth muscle cells	Fibroblasts
Left ventricular volume index*	1.00 ± 0.04	0.91 ± 0.32	0.84 ± 0.20
Scar thickness index	1.13 ± 0.25	$2.10 \pm 0.68^{\dagger}$	$2.40 \pm 0.86^{\dagger}$
Scar area index	0.78 ± 0.08	$1.27 \pm 0.28^{\ddagger}$	$1.37 \pm 0.34^{\S}$
Transplantation area/scar area	37.0 ± 8.9	32.2 ± 4.8	$51.3 \pm 14.6^{\P}$

Each index was calculated as a ratio of control values.

*No significant difference ($P = .44$) by analysis of variance.

[†] $P = .005$, versus cardiomyocytes.

[‡] $P = .023$, versus cardiomyocytes.

[§] $P = .003$, versus cardiomyocytes.

^{||}Percentage of the area of scar tissue occupied by the transplanted cells.

[¶] $P = .04$, versus smooth muscle cells.

reports have demonstrated the significance of cells with contractile proteins or any superiority of cardiomyocytes over other cell types.

To address these issues, we transplanted 3 kinds of fetal cells (cardiomyocytes, smooth muscle cells, and fibroblasts) into the cryoinjured scar of rat hearts and

compared their functional outcomes and the left ventricular remodeling process. We chose fetal cells because of their superior growth potential compared with adult or pediatric cells.¹¹ Ventricular cardiomyocytes served as a standard cell type, because the transplantation of cardiomyocytes has been previously

reported to result in improvement in ventricular function. Smooth muscle cells derived from the stomach were chosen as a nonstriated muscle cell with contractile proteins that differed from those of cardiomyocytes. We did not use vascular smooth muscle cells because of the difficulties in obtaining sufficient cells from the great vessels of fetal rats. Skin fibroblasts were selected as a cell with little, if any, contractile protein. We used a Langendorff perfusion model to assess the heart function. Although this *ex vivo* method has limitations compared with *in vivo* measurements by echocardiography or conductance catheters, this technique was technically straightforward and allowed identification of differences in systolic and diastolic function between our 3 transplantations.

Histologic studies demonstrated that the transplanted cells formed a block of tissue composed of their specific cell types in the myocardial scar. In a Langendorff preparation, the cardiomyocyte transplantation group had the greatest developed pressure, $+dP/dt_{\max}$, and $-dP/dt_{\max}$. The smooth muscle cell transplantation group had significantly better improvement than fibroblast transplanted hearts in $+dP/dt_{\max}$ and $-dP/dt_{\max}$ but not in developed pressure. Fibroblast transplantation resulted in the greatest scar thickness because of the growth of the transplanted fibroblasts and a relatively smaller left ventricular volume but showed the least functional improvement in systolic function.

These observations may clarify some of the mechanisms by which ventricular function is improved after cell transplantation. A fundamental mechanism for this functional improvement seems to be related to the law of Laplace: $\tau = Pr/w$, where τ is wall stress; P is transmural pressure; r is radius, and w is wall thickness. All the cell transplantation groups, including the fibroblast transplanted rats, showed increased scar thickness and relatively smaller left ventricular volumes than control rats, which would decrease left ventricular wall stress and contribute to this functional improvement. By this mechanism, even noncontractile cells such as fibroblasts can offer some improvement.

A second mechanism seems to be related to the elastic properties of the tissue formed by the transplanted cells. Although transplanted fibroblasts formed the thickest scar, they resulted in the least improvement in function. The rigidity of the thick, fibrotic scar seen after fibroblast transplantation may impair diastolic ventricular filling. On the other hand, the tissue formed by transplantation of cells with contractile proteins (cardiomyocytes and smooth muscle cells) may have greater elasticity, and this elasticity may partly explain the greater improvement in function noted

after cardiomyocyte or smooth muscle cell transplantation.

In this study, transplanted cardiomyocytes offered significantly greater functional improvement than smooth muscle cells. These observations are consistent with those of Kumar and associates,¹² who demonstrated the importance of cardiac-specific actin, compared with the enteric smooth muscle-specific actin, in the function and development of the heart.

In our study, the greater functional benefit of cardiomyocytes over smooth muscle cells may be due to the fact that the cardiomyocyte transplantation group had significantly smaller scar areas than the smooth muscle cell transplantation group. However, because the scar thickness was greater in the smooth muscle cell group and the left ventricular volume indexes were identical between the 2 groups, the first potential mechanism, related to the law of Laplace, may not fully explain this difference. Smooth muscle cells have been reported to have passive characteristics, including stress-length relationships, hyperbolic velocity-load relationships, and power output curves and the ability to resist imposed loads, similar to those of striated muscle.¹³ The potential active contraction and relaxation of transplanted cardiomyocytes may explain the differences noted between this group and the smooth muscle cell-transplanted group. We have previously demonstrated that fetal rat cardiomyocytes, when injected into the subcutaneous tissue of rats, formed a block of tissue that could be visualized grossly and by echocardiography beating at the rate of 73 ± 12 beats/min, 21 days after injection.⁷ In this study, we found that the transplanted fetal cardiomyocytes formed a block of striated myocardium and that this tissue, when excised en bloc, appeared to beat spontaneously and regularly at the time of explantation. To address this issue fully, however, further studies that use imaging modalities that will permit evaluation of regional wall motion will be required.

In conclusion, we transplanted 3 different fetal cell types into the cryoinjury-induced myocardial scar of rats. Cardiomyocyte transplantation offered the best functional improvement, followed by smooth muscle cell transplantation. Fibroblast transplantation had the greatest effect on the left ventricular remodeling process but resulted in minimal functional improvement. The contractile and elastic properties of transplanted cells determine the degree of improvement in ventricular function achievable with cell transplantation.

We thank Dr Guang Ming Li, Ms Dev Olshansky, and Mr James C. Ho for the preparation of the histologic materials.

REFERENCES

1. Li R-K, Yau TM, Sakai T, Mickle DAG, Weisel RD. Cell therapy to repair broken hearts. *Can J Cardiol* 1998;14:735-44.
2. Leor J, Prentice H, Sartorelli V, et al. Gene transfer and cell transplant: an experimental approach to repair a "broken heart." *Cardiovasc Res* 1997;35:431-41.
3. Li R-K, Jia Z-Q, Weisel RD, et al. Cardiomyocyte transplantation improves heart function. *Ann Thorac Surg* 1996;62:654-61.
4. Scorsin M, Hagege AA, Marotte F, et al. Does transplantation of cardiomyocytes improve function of infarcted myocardium? *Circulation* 1997;96(Suppl):II188-93.
5. Li R-K, Jia Z-Q, Weisel RD, Merante F, Mickle DAG. Smooth muscle cell transplantation into myocardial scar tissue improves heart function. *J Moll Cell Cardiol* 1999;31:513-22.
6. Taylor DA, Atkins BZ, Hungspreugs P, et al. Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nature Med* 1998;4:929-33.
7. Li R-K, Mickle DAG, Weisel RD, Zhang J, Mohabeer MK. In vivo survival and function of transplanted rat cardiomyocytes. *Circ Res* 1996;78:283-8.
8. Fremes SE, Furukawa RD, Zhang J, et al. Cardiac storage with University of Wisconsin solution and a nucleoside transport blocker. *Ann Thorac Surg* 1995;59:1127-33.
9. Pfeffer JM, Pfeffer MA, Fletcher PJ, Braunwald E. Progressive ventricular remodeling in rat with ventricular infarction. *Am J Physiol* 1991;264:H1727-33.
10. Klung MG, Soonpaa MH, Koh GY, Field LJ. Genetically selected cardiomyocytes from differentiating embryonic stem cells form stable intracardiac grafts. *J Clin Invest* 1996;98:216-24.
11. Li R-K, Mickle DAG, Weisel RD, et al. Human pediatric and adult ventricular cardiomyocytes in culture: assessment of phenotypic changes with passaging. *Cardiovasc Res* 1996;32:362-73.
12. Kumar A, Crawford K, Close L, et al. Rescue of cardiac α -actin-deficient mice by enteric smooth muscle γ -actin. *Proc Natl Acad Sci U S A* 1997;94:4406-11.
13. Murphy RA. Smooth muscle. In: Berne RM, Levy MN, editors. *Physiology*. 3rd ed. St Louis: Mosby; 1993. p. 309-24.

Discussion

Dr Juan C. Chachques (*Paris, France*). We are working in a similar protocol using satellite cells (myoblasts), so I want to ask you a technical question. If you perform Langendorff hemodynamic studies, you cannot study the hearts histologically because the isolated beating heart at 37°C would have histologic damage, making a histologic study impossible. How did you perform in your protocol Langendorff studies followed by histologic studies?

Dr Sakai. Our studies of ventricular function in our Langendorff preparation lasted about 45 minutes, during which time the hearts were perfused with filtered Krebs-Henseleit buffer solution equilibrated with 5% carbon dioxide and 95% oxygen at 37°C. Because the hearts were well perfused, we did not anticipate significant histologic changes, especially in the aspects we studied: identification of the transplanted cells and their structure. We do not think that a 45-minute period of perfusion should affect these histologic findings.

Dr Si M. Pham (*Miami, Fla*). It has been shown recently that the nonhemopoietic stem cell from the bone marrow can

differentiate into different cells, myocytes, smooth muscle cells, a variety of cells. Because it is difficult to grow the adult cardiac myocytes. I wonder whether it would be more clinically relevant if you can use some of those nonhemopoietic stem cells from the bone marrow to inject it into the myocardial scar? Have you done it or have you considered doing it?

Dr Sakai. Immunorejection is a major problem in the potential application of cell transplantation in a clinical setting. The use of multipotential stem cells from autologous bone marrow, and their induction to differentiate into cardiomyocytes, is one of the several promising ways to avoid immunorejection. Our research group has transplanted autologous bone marrow cells into a cryoinjured rat heart model. In that study, we found that the transplanted bone marrow cells survived in the scar tissue, limited scar expansion, and improved heart function compared with a control group. In our current study, we demonstrated the relative merits of fetal cardiomyocytes, compared with other fetal cells, for cell transplantation. It will be interesting to compare the functional improvement after cardiomyocyte transplantation with that with bone marrow cell transplantation.

Dr John E. Mayer, Jr (*Boston, Mass*). Other than histologic appearance, what evidence do you have, if any, that the cardiomyocytes are in fact really cardiomyocytes? Once they get into the tissue, is there any evidence that they might change their phenotype to become a different type of cell or is there any evidence that they would change phenotype while you were culturing them? The same questions, I think, would apply for the smooth muscle cells and for the fibroblasts. It sounds like the fibroblasts still remember how to make skin, but I wondered if you had any other specific kinds of cell markers that would help you with that kind of question?

Dr Sakai. In our previous studies, we identified the cultured cardiomyocytes by positive staining with antibodies against troponin I and myosin heavy chain. We also found that these cells expressed the myosin heavy chain gene in culture. Electron microscopy revealed myofilaments in the cells. These cultured cardiomyocytes underwent marked time-dependent dedifferentiation and lost the normal structure of contractile proteins when they were maintained in cell culture for up to 6 months. In the present study, however, the duration of cell culture was 24 hours, and the cultured cardiomyocytes did not undergo significant phenotypic changes during this period. After transplantation of cultured fetal cardiomyocytes into the cryoinjured scar, electron microscopy showed organized sarcomeres and intercalated discs in the transplanted cardiomyocytes. The smooth muscle cells stained positively with an antibody against α -smooth muscle actin, both in culture and in the tissue.

Dr Fred A. Crawford, Jr (*Charleston, SC*). You injected these cells into a scar. Where did the blood supply come from? How did they survive?

Dr Sakai. We have studied angiogenesis in the scar area where we transplanted cultured cardiomyocytes, smooth muscle cells, fibroblasts, or endothelial cells. We found capillaries and arterioles in the scar after transplantation of all of these cell types. We speculate that this angiogenesis was

stimulated by cell transplantation or by growth factors secreted by the transplanted cells. It seemed that the vessels grew inward from the surrounding myocardium.

Dr Jakob Vinten-Johansen (*Atlanta, Ga*). Have you done studies to determine how long the myocytes retain their phenotype in the cryoinjured area? How long does the improvement in function last? Have you done a time-course study and determined whether functional recovery wanes after 8 weeks or whether it continues or is maintained?

Dr Sakai. In our previous long-term study of fetal cardiomyocyte transplantation, the tissue formed from allotransplanted cells gradually decreased in size over 24 weeks

because of immunorejection. The ventricular chamber size increased with time compared with that at 4 weeks after transplantation. The ventricular scar also became thinner. These data suggested that allogenic cell transplantation was unlikely to improve heart function in the long term. We have focused on autologous cell transplantation to try to bypass this problem. Transplantation of cultured autologous adult cardiomyocytes and autologous bone marrow cells resulted in survival of the transplanted cells and improvement of heart function at 5 weeks after cell transplantation. We are currently performing longer term studies of autologous cell transplantation to determine the time course of this effect.