Elastin Stabilizes an Infarct and Preserves Ventricular Function

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Background—After a myocardial infarction, the injured region becomes fibrotic and the myocardial scar may expand if the ventricular wall lacks elasticity. Cardiac dilatation may precipitate the vicious cycle of progressive heart failure. The present study evaluated the functional benefits of increasing elastin within a myocardial scar using cell based gene therapy.

Methods and Results—A myocardial infarction was generated by ligation of the left anterior descending artery in rats. Six days later, $2 \times 10^6$ syngeneic rat endothelial cells transfected with the rat elastin gene (elastin group, n=14) or an empty plasmid (control group, n=14) were transplanted into the infarct scar. Cardiac function, left ventricular (LV) volume, and infarct size were monitored over 3 months by echocardiography, Langendorff measurements, and planimetry. Elastin deposition was evaluated in the cells and in the infarct region by Western blot assay and by histological examination. Recombinant elastin was found in the scar in the elastin group but not the control group during the 3 months after cell transplantation. Histological assessment demonstrated organized elastic fibers within the infarct region. LV volume and infarct size were significantly smaller ($P<0.05$) in the elastin group than in the control group. Cardiac function evaluated by echocardiography and during Langendorff perfusion was significantly better ($P<0.05$) in the elastin group than in the control group.

Conclusions—Expressing recombinant elastin within the myocardial scar reduced scar expansion and prevented LV enlargement after a myocardial infarction. Altering matrix remodeling after an infarct preserved the LV function for at least 3 months. (Circulation. 2005;112[suppl I]:I-81–I-88.)

Key Words: genes ■ myocardial infarction ■ cells ■ transplantation ■ remodeling

Cardiomyocytes lost during a myocardial infarction are usually replaced by fibrotic tissue, including type III collagen. After the infarct, the affected region does not contract and has less elasticity than the normal myocardium or other elastic tissues, such as the walls of arteries. The lack of elastic recoil contributes to the thinning and expansion of the infarct region, which frequently progresses after a myocardial infarction and results in cardiac enlargement and cardiac failure with time.$^{1-5}$

Recent research has suggested that cardiac function may be improved after an infarction by modifying the myocardial scar. For example, increasing regional perfusion by angiogenic factors salvaged dying myocardium. Cell transplantation into myocardial scar tissue increased regional angigenesis and myogenesis, which prevented scar thinning and expansion and left ventricular dilatation and preserved cardiac function.$^{4-6}$ Although some reports suggested that the implanted cells form junctions with host myocardium and might contract in vivo, no studies have documented an improvement in cardiac function resulting from the contraction of the implanted muscle cells. Cell transplantation may alter ventricular remodeling by modifying the extracellular matrix, which permits the preservation of the scar size, left ventricular geometry, and cardiac function.

Elastin is one of the major insoluble extracellular matrix components. Elastic fibers laid down in blood vessels, lungs, and skin remain for the lifetime of the organism to maintain the tissue structure. Mature elastic fibers consist of a core of tropoelastin surrounded by fibrillin and microfibrils. The elastin fiber network provides tissue with a critical property of elasticity and resilient recoil and maintains the architecture against repeated expansion.$^{7-11}$ The elasticity of the myocardium depends on the ratio of muscle fibers to fibrotic tissue and the density of the cross-linked collagen.$^{12,13}$ Expression of elastin in the myocardial scar may be able to change the composition of the extracellular matrix of the infarct region and preserve the elasticity of the infarcted heart. We hypothesized that these alterations could prevent scar expansion and preserve cardiac function after a myocardial infarction.

In this study, we used syngeneic rat endothelial cells to deliver the elastin gene into the infarcted myocardium because we previously reported that endothelial cell implanta-
tion increased vascularity but did not improve ventricular function. Therefore, any improvement in cardiac function would result from the expression of the recombinant elastin and not cell transplantation. The results of this study were intended to determine whether elastin production could be a contributor to improved function after cell transplantation. If elastin production in the infarct region improved ventricular function, then elastin production could be induced in cells implanted into the heart and further improve the functional benefit of cell transplantation. This study is unique because it evaluates a novel proof of concept, but it does not evaluate a treatment, which would be used in patients.

**Methods**

**Preparation of Rat Elastin Genes**

Total ribonucleic acid (RNA) was isolated from aortas of male Lewis rats (Charles River, Quebec, Canada) and used in a reverse transcription-polymerase chain reaction as a template to generate the complementary DNA (cDNA) of a segment of rat tropoelastin, which was located from 1201 to 1800 base-pair (bp). The cDNA of the elastin segment was ligated with a plasmid vector with an N-terminal LP60 tag (pcDNA3.0, Invitrogen), which was used for identification of synthesized elastin molecules in vitro and in vivo. The modified plasmids were transformed into *Escherichia coli* and the successful transformed bacteria were selected with Luria Broth agar plates containing 100 μL/mL ampicillin. The selected recombinant constructs were sequenced to ensure that the correct fragment of the tropoelastin gene had been cloned into the constructs.

**Transfection of Rat Elastin Gene**

The endothelial cells were isolated from aortas of 8- to 9-week-old male Lewis rats and cultured. The purity of the endothelial cells was 92.4% by immunohistological evaluation with anti-factor VIII antibody. The cultured cells (4.5 × 10⁶) were transfected with the plasmid containing the elastin gene or the empty plasmid vector (100 μg) using Lipofectamine 2000 (Invitrogen Life Technologies) in serum-free M199. The transfected cells were then cultured in M199 including 20% fetal bovine serum for 48 hours before cell transplantation.

**Assessment of In Vitro Gene Expression**

Before cell transplantation, 1 × 10⁶ cells and 1 mL of the media cultured with transfected cells were separately collected. The cells were lysed in 0.5 mL of lysis buffer. After centrifugation, the supernatants of both the lysate and the media were used for Western blot analysis. The protein sample (30 μg) was loaded onto an agarose gel for electrophoresis. The gels were blotted onto nitrocellulose membranes. After blocking with 10% milk protein, the membranes were incubated with a primary antibody against the LP60 tag (a gift from Dr. Yang, BB, Sunnybrook and Women's College Health Sciences Centre, Toronto, Canada) at 4°C overnight. After washing, the membranes were incubated with goat anti-mouse immunoglobulin G antibody (Biorad) at room temperature for 1 hour, followed by washing for 1 hour. The membranes were then exposed to enhanced chemiluminescence reagents (Amersham) and exposed to x-ray films in a dark room, followed by the film development.

**Generation of Myocardial Infarction and Animal Selection**

All experimental procedures were approved by the Animal Care Committee of the University Health Network and conformed to the guidelines in the *Guide to the Care and Use of Experimental Animals* published by the National Institutes of Health (NIH publication 85-23, revised 1985). The syngeneic male Lewis rats (340 to 380 g) were used in this experiment.

Under general anesthesia (ketamine, 20 mg/kg body weight, pentobarbital, 22 mg/kg body weight, and inhaled isoflurane, 1% to 2%), the left anterior descending artery (LAD) of each rat was ligated via a left thoracotomy. The incision was closed with 3-0 silk sutures. Each rat was given antibiotics (150 000 IU/kg, Duplocillin LA, Intervet Canada Limited) and buprenorphine (0.01 to 0.05 mg/kg body weight) subcutaneously after the surgery.

Five days after LAD ligation, echocardiography was performed as described below, and the animals used for the study were selected to minimize the variation in infarct size and cardiac function according to the following criteria: Rats were included in the study if the infarct size as measured by 2-dimensional echocardiography was longer than 0.8 cm but shorter than 1.2 cm and if percent fractional area shortening (%FAS) was higher than 20% but lower than 40%. These selection criteria reduced the variability in infarct size in both groups and facilitated comparison of the 2 groups, but the results cannot be extrapolated to larger or smaller infarcts.

**Cell Transplantation**

Twenty-eight rats met these criteria and were randomly allocated to the elastin or control groups. Cell transplantation was performed 6 days after LAD ligation, because we have demonstrated that inflammation had decreased but the heart had not yet thinned or dilated. The heart was exposed via median sternotomy under general anesthesia as described above. Two million elastin gene transfected rat endothelial cells (elastin group) or empty plasmid vector transfected endothelial cells (control group) were injected into both the scar tissue and the border area using an insulin syringe with a 28G needle (Becton Dickinson). The sternum and skin were closed with 3-0 silk sutures. Postoperative care was provided as described above.

**Figure 1.** Left ventricular function, evaluated by echocardiography. We evaluated %FAS (A) by 2-dimensional echocardiography, and %FS (B) and EF (C) were estimated by M-mode echocardiography. Percent FAS, %FS, and EF in the elastin group was preserved to a greater degree compared with the control group.
Two rats in each group were euthanized at 2 weeks and 1, 2, and 3 months after cell transplantation for evaluation of histology and elastin gene expression by Western blot analysis.

Assessment of Cardiac Function

Eight rats in each group were used for functional assessment throughout the 3 months after cell transplantation. Echocardiography was performed 5 days after LAD ligation and 1, 2, and 3 months after cell transplantation by an experienced echocardiographer who was blinded to the group to which the animals had been allocated and using a Sequoia Echocardiography System (ACUSON Corporation). Anesthesia was induced and maintained with 1.5% to 2.0% inhaled isoflurane and the rats were placed in the left lateral decubitus position. The echo transducer was placed on the left hemithorax and short axis views were recorded at the site where the maximal infarct area could be seen. Infarct length was determined as the maximum length of the akinetic wall at end-diastole in the short axis view. We calculated %FAS as [(LV end-diastolic area−LV end-systolic area)/(LV end-diastolic area)]×100 (%). LV end-diastolic volume (LVEDV) and LV systolic volume (LVESV) were measured with the M-mode tracing. Percent fractional shortening (%FS) was determined as [(LVEd−LVs)/(LVEd)×100 (%)]. LV diastolic and systolic pressures, max dP/dt (maximal rise in pressure during systole) and min dP/dt (minimal decrease in pressure in diastole) were recorded at each balloon volume. Developed pressure was calculated as the difference between the systolic and diastolic pressures.

Planimetry and Histological Assessment

After the Langendorff evaluation, the hearts were fixed with 10% formalin at a pressure of 30 mm Hg, and then sliced into sections 3-mm thick. Each section was photographed and the endocardial and epicardial borders of the left ventricle and the infarcted scar were traced onto a transparency and quantified using computed planimetry by experienced individuals who were blinded to the group to which the animals were allocated. After that, all sections were embedded in paraffin.

Samples were collected at 2 weeks and 1, 2, and 3 months after cell transplantation for evaluation of histology and elastin gene expression by Western blot analysis. The scar and border zone were resected and separated into 2 pieces. One piece was embedded in paraffin, and another piece was stored at a temperature of −80°C until use. All the paraffin-embedded tissues were cut into 4-μm thick

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Left ventricular function, evaluated in a Langendorff apparatus. Developed pressure (A), maximum −dP/dt (B), and minimum −dP/dt (C) were all significantly greater in the elastin group than in the control group.

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** The scar dimensions and left ventricular dimensions evaluated by echocardiography. Infarct length (A), LV diastolic and systolic dimensions (B), and LV diastolic and systolic volumes (C). Infarct lengths increased in both groups after myocardial infarction, but the rate of expansion in the elastin group was less than that in the control group. The elastin group had smaller LV dimensions and LV volumes than the control group. The hearts in the elastin group dilated to a lesser degree over the 3-month duration of the study compared with the hearts in the control group.
slices, and the slices were stained with hematoxylin and eosin and elastic von-Giesson staining.

Assessment of In Vivo Gene Expression
The frozen scar tissues resected 2 weeks and 1, 2, and 3 months after cell transplantation were minced and homogenized with lysis buffer. After centrifugation, the supernatant was used for Western blot analysis as described above.

Data Analysis
All results are presented as mean±standard error of the mean. Comparisons between the 2 groups were performed by Student’s t test. Comparisons of LV function data obtained from echocardiography were performed by 2-way repeated measures analysis of variance using SPSS system software. Data on LV function obtained from the Langendorff apparatus were analyzed by analysis of covariance. A value of P<0.05 was considered statistically significant.

Results
Cardiac Function
Twenty-eight rats met the echocardiographic inclusion criteria for this study. There was no difference in body weight between the elastin and control groups before LAD ligation (345±5.9 g versus 349±4.8 g, respectively; P=0.57). There were also no differences in any of the echocardiographic parameters, such as infarct length (1.04±0.04 cm versus 1.04±0.04 cm, P=0.94) and %FAS (29.3±0.8% versus 28.2±1.2%, P=0.49) between the groups before cell transplantation.

Echocardiography showed that FAS of the control group declined from 28.2±1.2% to 14.6±0.8% over the 3 months of the study, and the decrease in FAS of the elastin group was significantly attenuated (declining only from 29.3±0.9% to 21.0±1.4%, P=0.002; Figure 1A). The %FS and EF of the elastin group were also better preserved than in the control group (P=0.046, Figure 1B and 1C).

At the conclusion of the study, LV function was also evaluated in a Langendorff apparatus (Figure 2). We found that the elastin group had greater developed pressures and maximum +dP/dt (P<0.001, P<0.001, respectively; Figure 2A and 2B) than the control group. The elastin group also had a lower minimum −dP/dt than the control group (P<0.001; Figure 2C).

Left Ventricular Remodeling
Echocardiography was used to evaluate scar lengths and left ventricular dimensions and volumes at different times after LAD ligation and cell transplantation (Figure 3). The infarct length in both groups expanded over the 3 months after myocardial infarction. This expansion, however, was significantly less in the elastin group (22.8%), than in the control group.

Figure 4. The scar size (A) and left ventricular volumes (B) evaluated by planimetry. The elastin group had smaller scar sizes and LV volumes 3 months after cell transplantation than the control group. *P<0.05.

Figure 5. Evaluation of transgene expression of the recombinant elastin in vitro (A) and in vivo (B) by Western blotting. The recombinant elastin was detected in the lysate and media of the elastin group but not in those of the control group. Recombinant elastin was detected by antibody staining against Tag Marker LP60 in the scar at every evaluated time point over the 3-month duration of this study in the elastin group but not in the control group.
group (52.1%, \( P=0.004 \); Figure 3A). Left ventricular diastolic and systolic dimensions in the elastin group were smaller than those of the control rats (\( P=0.043 \) versus \( P=0.028 \), respectively; Figure 3B). Left ventricular diastolic and systolic volumes in the elastin group were also smaller than those in the control group (\( P=0.035 \) versus \( P=0.035 \), respectively; Figure 3C).

In agreement with the echocardiographic results, the scar size estimated by planimetry was also significantly smaller in the elastin group (0.96±0.07 cm\(^2\)) than in the control group (1.26±0.11 cm\(^2\)) (\( P=0.049 \); Figure 4A), and the LV volume in the elastin group was significantly smaller (0.87±0.05 cm\(^3\)) than in the control group (1.03±0.03 cm\(^3\), \( P=0.021 \); Figure 4B).

**In Vitro and In Vivo Gene Expression**

The recombinant elastin was detected in both the lysates and the supernatant medium of the elastin gene transfected cells, but not in empty vector transfected cells of the control group before cell transplantation (Figure 5A). The recombinant elastin in the infarct scar was detected at 2 weeks and 1, 2, and 3 months after cell transplantation in the elastin group (Figure 5B). However, no recombinant elastin was found in the center of the scar of the control group at any time during the 3-month duration of this study. Elastin incorporation in the border zone was more prominent in the elastin than the control group.

**Histology**

The transplanted endothelial cells were difficult to identify by hematoxylin and eosin staining (Figure 6A). The transplanted endothelial cells in the scar were stained red with anti-factor VIII antibody (Figure 6B), pink with Masson-Trichrome staining (Figure 6C), and gray with elastic von-Giesson staining (Figure 6D). The recombinant elastin could not be stained with Masson-Trichrome staining (Figure 6C). With elastic von-Giesson staining, the recombinant elastin stained black and was found deposited between the transplanted cells (Figure 6D).

In the elastin group, the elastic fibers were detected at every evaluation time point during the 3-month duration of the study, by elastic von-Giesson staining (Figure 7A, 7C, 7E, and 7G). Recombinant elastin was found widely spread throughout the scar area and border zone, forming an extensive elastin network all around the infarcted area (Figure 8A). At high magnification, the elastin formed long, wavy fibers (Figure 8B). Elastin fibers were not detected at any time point in the control group (Figure 7B, 7D, 7F, and 7H).

**Discussion**

Although preclinical studies of cell transplantation have consistently demonstrated myogenesis and limitation of adverse postinfarction LV remodeling after transplantation of cardiomyocytes,\(^4\) skeletal myoblasts,\(^5\) and smooth muscle cells,\(^6\) there has as yet been no convincing evidence that the transplanted myocytes can achieve synchronous contraction with surrounding native cardiomyocytes.\(^4\)–\(^6,16\) There is, however, emerging evidence that cell transplantation may exert part of this effect on remodeling through alteration of the extracellular matrix, which may occur both locally and globally by a paracrine mechanism involving inhibition of matrix metalloproteinases by their tissue inhibitors.\(^17,18\) We hypothesized, therefore, that alteration of the extracellular matrix after cell transplantation by other means might also enhance LV function by further limiting remodeling.

Because the elastic properties of arteries and other structures are dependent on elastin fibers, which consist of a tropoelastin core surrounded by fibrillin and microfibrils, we chose to evaluate the effect of an elastin transgene on LV function. The entire length of rat tropoelastin (2595 bp) would be difficult to clone and express. Keeley and colleagues\(^19\) have reported, however, that the extensile and resilient properties of elastin can be localized to a fragment of the entire molecule. We therefore used only a 600 bp segment of the rat tropoelastin gene, comprising the fragment from 1201 bp to 1800 bp. This
fragment was cloned into a plasmid, which was transfected into rat endothelial cells with a lipid-based technique, with approximately 15% transfection efficiencies.

We chose endothelial cells as carrier cells because our previous studies demonstrated that transplantation of endothelial cells resulted in angiogenesis but no improvement in LV function after myocardial cryoinjury. Therefore, any improvement in LV function after transplantation of elastin-overexpressing endothelial cells could reasonably be attributed to the elastin transgene rather than cell transplantation itself. In addition, endothelial cells do not normally express elastin, and comparison between elastin-transfected endothelial cells and unmodified endothelial cells would therefore be simpler.

We were able to induce detectable overexpression of the recombinant elastin transgene in vitro and in vivo for up to 3 months after cell transplantation in the elastin group, though the expression at 3 months decreased significantly. The limited duration of elastin production may have had a prolonged influence on ventricular remodeling because of long life time of elastin molecules. Histological examination of hearts in the elastin group showed accumulation of elastin deposition both in the center of the scar and extending to the border zone. These elastin fibers were configured in a wavy pattern resembling that of normal elastin in an arterial wall. In contrast, the scar of control hearts had substantially less elastin fiber, which may be produced by the myofibroblasts in the border zone of the infarct. The accumulated elastin fibers in the elastin group could be contributed by the production of elastin by the transplanted cells. It could be also possible that secreted elastin molecules have stimulated viable cells or myofibroblasts in the infarct border zone to produce and/or assemble and incorporate elastin fibers into the remodeling scar matrix, altering the physical properties of the infarct region, which may have added elastic recoil to the infarct

**Figure 7.** Elastin deposition in the scar. The elastin group at 2 weeks (A), 1 month (C), 2 months (E), and 3 months (G), and the control group at 2 weeks (B), 1 month (D), 2 months (F), and 3 months (H). The recombinant elastin was seen in the elastin group over 3 months. Little elastin could be detected in the control group. Red arrow indicates recombinant elastin.
region. Future biochemical measurements or mechanical analysis may provide a more reliable quantitative assessment of elastin within the infarct region. In addition, long-term studies will be required to determine the long-term effect of limited elastin production.

As we hypothesized, elastin overexpression had a significant impact on adverse LV remodeling. Rats in the elastin group had significantly lower infarct lengths, LV dimensions, and LV volumes as early as 1 month after transplantation, and this benefit continued to increase at 2 and 3 months. The addition of elastin to the infarct region and the border zone may have added recoil to the infarct scar, which may have modified the matrix remodeling. In the present study, cell transplantation was carried out 6 days after LAD ligation, after postinfarction inflammation had subsided, but before the scar had fully matured, thinned, and dilated. It seems reasonable to assume that greater delays in cell transplantation and greater adverse remodeling in that interval would limit the potential benefits of this strategy. Further studies will be required to determine the optimal timing of this therapy.

Elastin overexpression also had a dramatic effect on LV systolic function, which was greater in the elastin group at all time points, reflected in greater fractional area shortening, fractional shortening, and ejection fraction. Three months after transplantation, improved systolic function was confirmed by greater developed pressures and greater maximum +dP/dt in the elastin-transfected group, and greater active relaxation during early diastole by a lower minimum −dP/dt. The mechanisms responsible for the improved ventricular function have not been elucidated, but the addition of elasticity into the infarct region may permit recoil in response to the stresses of contraction, which may reduce the stresses on the infarct scar and reduce the scar thinning and dilatation.

We have previously reported that endothelial cell transplantation induces angiogenesis but does not preserve LV function after cryoinjury.14 Although we did not directly evaluate angiogenesis in this study, we would not anticipate that expression of an elastin transgene would alter the degree of angiogenesis induced by the transplantation of endothelial cells. We have, however, demonstrated that transient expression of a vascular endothelial growth factor transgene in transplanted cells significantly enhanced angiogenesis,20 an effect that outlasted the vascular endothelial growth factor transgene expression and was limited spatially to the scar and
border zone and temporally to 4 weeks. Similarly, transient expression of elastin in this experiment may exert an effect that transcends the duration of elastin expression. Further studies, however, will be required to determine whether this effect can persist for years and not just months.

This study is the first to attempt to directly evaluate one of the potential mechanisms of the beneficial effects of cell transplantation on LV function after infarction. Previous studies have demonstrated that the implantation of myocytes4–6 or bone marrow cells22 into an infarct prevented scar thinning and dilatation and LV dysfunction. The mechanisms responsible for the beneficial effect have not been adequately elucidated but likely include angiogenesis,14,19,20 limitation of apoptosis,23,24 matrix remodeling,17,18 and myogenesis.4–6,16,22 This study evaluated the benefits of adding elastin to the infarct independent of the effects of cell engraftment alone. The improved ventricular function in the elastin group suggests that this strategy was successful and may be one method to improve the results of cell transplantation. The addition of the elastin gene to a cell type, which also improves ventricular function, may permit complete restoration of ventricular function after a myocardial infarction.

This preliminary study did not include a control group that received media injection without cells. The study was intended to evaluate the proof of concept of elastin expression in the infarct region. The ventricular function observed in the control group did not differ from that which we have previously reported in media-injected control groups from other studies.20,21,22 We have previously reported that endothelial cell transplantation does not preserve LV function after myocardial injury.14 This preliminary report suggests that increasing the elastin content in the infarct will prevent thinning and dilatation and improve function. Further studies are necessary, however, to determine whether enhancing elastin production will improve the functional benefit of myocyte or progenitor cell transplantation.

In summary, our study demonstrates that overexpression of elastin in transplanted endothelial cells significantly improves LV systolic and diastolic function for at least 3 months. Further studies will be required, however, to determine whether the beneficial effect of elastin overexpression is durable over the long term and to define the optimal interval after myocardial infarction for a therapy designed to limit myocardial repair.

In addition, the interaction of elastin overexpression with other cell or gene therapeutic strategies for myocardial repair remains to be determined.

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References
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