

Cell Transplantation Improves Ventricular Function After a Myocardial Infarction

A Preclinical Study of Human Unrestricted Somatic Stem Cells in a Porcine Model

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Background—Cell transplantation offers the promise in the restoration of ventricular function after an extensive myocardial infarction, but the optimal cell type remains controversial. Human unrestricted somatic stem cells (USSCs) isolated from umbilical cord blood have great potential to differentiate into myogenic cells and induce angiogenesis. The present study evaluated the effect of USSCs on myocardial regeneration and improvement of heart function after myocardial infarction in a porcine model.

Method and Results—The distal left anterior descending artery of Yorkshire pigs (30 to 35 kg) was occluded by endovascular implantation of a coil. Four weeks after infarction, single-photon emission computed tomography technetium 99m sestamibi scans (MIBI) and echocardiography were performed. USSCs (100×10^6) or culture media were then directly injected into the infarcted region ($n=8$ per group). Pigs were immunosuppressed by daily administration of cyclosporin A. At 4 weeks after transplantation, MIBI and echocardiography were repeated and heart function was also assessed with a pressure-volume catheter. The infarcted myocardium and implanted cells were studied histologically. MIBI showed improved regional perfusion ($P<0.05$) and wall motion ($P<0.05$) of the infarct region in the transplant group compared with the control. Ejection fraction evaluated by both MIBI and echocardiography decreased in the control group but increased in the transplant group ($P<0.01$). Scar thickness of the transplant group was higher than the control. The grafted cells were detected 4 weeks after transplantation by both immunohistochemistry and in situ hybridization.

Conclusion—Engrafted USSCs were detected in the infarct region 4 weeks after cell transplantation, and the implanted cells improved regional and global function of the porcine heart after a myocardial infarction. This study suggests that the USSC implantation will be efficacious for cellular cardiomyoplasty. (*Circulation*. 2005;112[suppl I]:I-96–I-104.)

Key Words: myocardial infarction ■ heart failure ■ cells ■ transplantation

After a myocardial infarction, some heart cells in the defect area are lost and others hibernate because they are underperfused. Implantation of healthy cells into the damaged myocardium offers the promise to replace the lost cells (myogenesis) and perfuse the hibernating cells (angiogenesis). Transplantation of cells, including cardiomyocytes,^{1–5} skeletal myoblasts,⁶ bone marrow cells,^{7–9} and smooth muscle cells,¹⁰ has been demonstrated to be of potential therapeutic value for the repair of damaged myocardium in animal models. The beneficial effect of cell transplantation on cardiac functions in preclinical studies has led to several phase I and II clinical trials.^{11–13}

Although several cell types implanted into the infarct region have improved ventricular function after a myocardial infarction,^{4,6,7} the cell source might become an issue for a broad

clinical application of cardiac cell therapy because expansion of autologous cells could be problematic. Recent evidence suggests that age and disease states affect the collection of sufficient healthy autologous bone marrow-derived cells for transplantation, which will decrease the ability of autologous cells to improve ventricular function after a myocardial infarction.¹⁴ Heesch and colleagues¹⁵ also reported that bone marrow mononuclear cells from patients with ischemic heart disease have a significantly lower regeneration potential than cells from healthy controls. The availability of pluripotent cells, which can be implanted without manipulation or expansion in an allogenic setting, would permit rapid cardiac restoration after a myocardial infarction.

Mesenchymal stem cells (MSCs) isolated from adult bone marrow have shown a great potential for cell therapy because

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these cells possess pluripotent capabilities,¹⁶ proliferate rapidly, induce angiogenesis, and differentiate into myogenic cells.¹⁷⁻¹⁹ Recently, a pluripotent stem cell population with high proliferative potential, unrestricted somatic stem cell (USSCs), was isolated from human umbilical cord blood as described by Kogler and colleagues.²⁰ USSCs have been suggested to be an earlier cell type than multipotent MSCs, possibly representing the precursor cells for MSCs as shown by comparing the differentiation potentials of USSCs²⁰ and MSCs.²¹ In vitro and in vivo studies showed that the USSCs have the potential to differentiate into osteoblasts, chondrocytes, adipocytes, neurons, and cardiomyocytes.²⁰ In a sheep model, the application of USSCs did not induce macroscopic or microscopic tumors in a long-term study after transplantation.²⁰ Therefore, USSCs could be highly promising precursor cells for cardiac implantation after a myocardial infarction.

To explore the clinical application of human stem cells, we transplanted USSCs into porcine hearts after a myocardial infarction to investigate the effects of the implanted USSCs on regional perfusion, wall motion, and global heart function. We found that the transplanted USSCs survived in the infarct region. Regional myocardial perfusion and wall motion were improved and global heart function was preserved after USSC transplantation.

Methods

Experimental Animals

Adult female domestic Yorkshire swine (30 to 35 kg, Charles River, Quebec, Canada) were used in the study. All procedures were approved by the Animal Care Committee and Radiation Safety Committee of the Toronto General Hospital and 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* published by the National Institutes of Health.

Creation of the Myocardial Infarction

All surgical procedures were performed when pigs were under general anesthesia and electrocardiographic monitoring as we described previously.^{4,22} Animals were premedicated with ketamine (20 to 25 mg/kg, intramuscularly) and 4% to 5% isoflurane and oxygen. After intubation, anesthesia was maintained with 1.5% to 2.5% isoflurane and oxygen at 2 to 3 L/min.

Under the guide of fluoroscopy C-arm, a coronary guiding catheter was introduced into distal left anterior descending coronary artery (LAD) through the right carotid artery. A superselective infusion catheter was inserted into the LAD or its diagonal branch. A myocardial infarction was created by occlusion of the LAD or the branches using an intraluminal coil (Boston Scientific Corporation) and a piece of gelatin sponge. An ST-T change of the ECG and a follow-up coronary arteriography were recorded to confirm the occlusion of the vessel. The carotid artery was then repaired and the animals were recovered. The animals were treated with antibiotics (cefazolin, 1g), analgesics (nurmorphin 1 to 2 mg, buprenorphine, 0.3 mg). A nitroglycerin ointment 2% (Nitrol, Paladin Labs Inc) was applied on the postauricular skin postoperatively. Brevibloc 0.1 mg · kg⁻¹ · min⁻¹ was used intravenously during the recovery period.

Human Unrestricted Somatic Stem Cell Preparation

USSCs were isolated, cultured, and prepared in Kourion Therapeutics AG as previously described.²⁰ The USSCs were characterized by cell surface markers using flow cytometry with antibodies against CD13, CD14, CD29, CD31, CD33, CD 34, CD44, CD45, CD49e, CD56, human leukocyte antigen class II (Beckmann Coulter) and

CD133 (Miltenyi Biotecy) according to the manufacturer's protocol. The cells were transported to Toronto at 4°C and arrived on the day of cell transplantation.

The cells were washed with serum free Iscove's Modified Dulbecco's Medium (IMDM) twice by centrifugation, the supernatant was discarded, and the cells were resuspended in the serum free media. The number of cells was counted using a cell counter and 100×10⁶ cells were prepared in 1 mL of serum free IMDM for transplantation. For evaluation of survival rate after transportation from Germany to Toronto, 100 μL of cell suspension were transferred into 2 cell culture dishes and cultured in IMDM containing 2% fetal bovine serum. Twenty-four hours later, the number of cells in each dish was counted. The data were presented as number of attached-growth cells/number of seeded cells × 100.

Cell Transplantation

Four weeks after myocardial infarction, the animals were randomly assigned to transplantation and control groups (n=8 each). Under anesthesia, the heart was exposed through a left intercostal incision, as previously described. The infarcted area was visually identified and either 100×10⁶ cells/mL or media (1 mL) was injected at 5 locations with a tuberculin syringe within 3 minutes. The animals recovered from the procedure and were treated with antibiotics and analgesics as described above. Three days before cell transplantation, the pigs in both groups were daily immunosuppressed with cyclosporine A (5 mg · kg⁻¹ · d⁻¹, Novartis Pharmaceuticals) until the end of the study.

Cardiac Function Measurement

Echocardiography

Echocardiography was performed before coil occlusion, before cell transplantation, and 4 weeks after transplantation using a SEQUOIA Echocardiography System (ACUSON Corporation,) with a 5-MHz transducer. The animals were sedated with the inhalation of isoflurane (2%) and placed at the left lateral decubitus position. Parasternal long- and short-axis views were obtained with both M-mode and 2-dimensional echo images. Left ventricle dimensions (end-diastolic diameter [LVEDD] and end-systolic diameter [LVESD]) were measured perpendicular to the long axis of the ventricle at the midchordal level. Fractional shortening (%) was calculated as (LVEDD-LVESD)/LVEDD × 100. Left ventricular ejection fraction (LVEF) (%) was calculated automatically by the echocardiography system by the equation:

$$(1) \quad LVEF = (LVEDV - LVESV) / LVEDV \times 100,$$

where $LVEDV = 7.0 \times LVEDD^3 / (2.4 + LVEDD)$ and $LVESV = 7.0 \times LVESD^3 / (2.4 + LVESD)$.

Technetium 99m-Sestamibi Single Photon Emission Tomography

Technetium 99m-sestamibi single photon emission tomography (^{99m}Tc-SPECT MIBI) measurement was performed 3 days before cell transplantation and at 4 week after cell transplantation. After anesthesia with ketamine, 18 to 20 mCi of ^{99m}Tc-MIBI solution (E.I. du Pont de Nemours & Co) was administered intravenously 30 minutes before ECG-gated ^{99m}Tc-SPECT MIBI scan acquisition was performed with Autospect software and quantified by Cedars-Sinai Quantitative Gated SPECT software.

The data analysts independently reviewed each ^{99m}Tc-SPECT MIBI scan in a blinded fashion, performing a semiquantitative assessment of perfusion and wall motion in each segment in cross-sectional views at the apical (segments 1 to 6) and midventricular (segments 7 to 12) levels and in a vertical long-axis view (segments 19 and 20) as we described previously.^{4,22} Segments at the basal region (segments 13 to 18) were excluded because they were normal regions. The perfusion was graded from 0% to 100%, indicating no perfusion to normal perfusion. The wall motion was graded from 0 to 10, indicating dyskinesia, varying levels of hypokinesia, up to normal wall motion. The LVED and LVES volumes were measured with a count-based technique, and LVEF was calculated by the computer program.

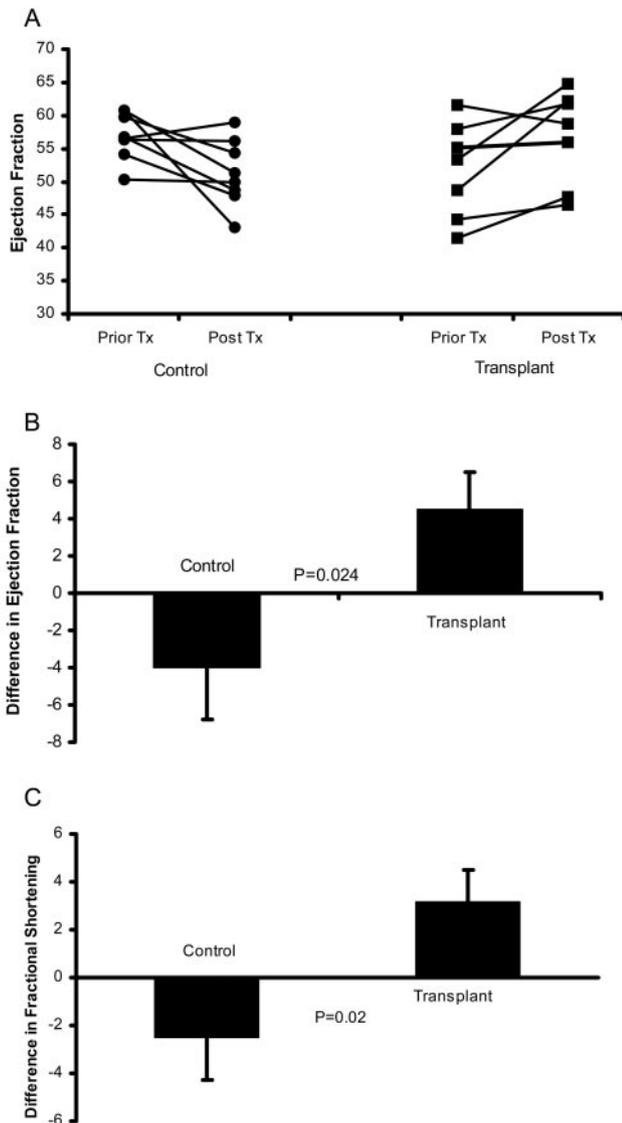


Figure 1. A, Left ventricular function (ejection fraction (%)) evaluated by echocardiography before and 4 weeks after cell transplantation. The difference was calculated between pre- and post-transplantation values. B, Ejection fraction (%) increased in the cell transplantation group but decreased in the media control group. The difference between pre- and post-transplantation was of statistical significance. The difference of percentage fractional shortening (C) in transplant group was also better than the controls.

Evaluation of Ventricular Function by Pressure-Volume Catheter

At end of the study (4 weeks after cell transplantation), ventricular function in the control (n=4) and transplantation (n=4) animals was evaluated by inserting conductance and Millar catheters (Millar Instruments) into left ventricle through the apex.^{4,22} Pressure and volume data were collected with the Conduct-PC software (CardioDynamics). Three baseline values were obtained and 3 more values were then recorded after clamping of the superior and inferior vena cavae until the left ventricle had emptied, as indicated on the volume tracing. Parallel conductance was evaluated after the injection of hypertonic saline solution into the pulmonary artery, and the volume measurements were corrected for the parallel conductance. Preload recruitable stroke work and end-systolic pressure-volume relation were calculated as we described previously.^{4,22}

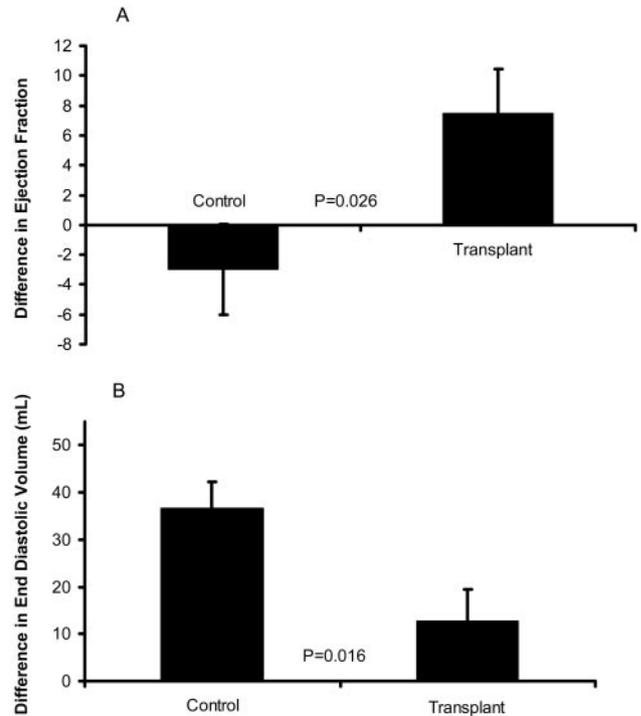


Figure 2. Left ventricular function and volume evaluated by ^{99m}Tc-SPECT MIBI scan before and 4 weeks after cell transplantation. The difference was calculated between pre- and post-transplantation values. Ejection fraction (%) (A) increased in transplantation group, but decreased in control group at 4 weeks after cell transplantation. The difference was of statistical significance. Left ventricular volume (B) in the control group increased significantly over 4 weeks compared with that of the transplantation group.

Morphological and Histological Studies

After completion of all measurements, the hearts were arrested with potassium chloride and rapidly excised. The coronary arteries were then perfused with 100 mL 10% formaldehyde, and the heart was fixed in diastole position with an intraventricular pressure of 30 mm Hg in formaldehyde solution. The fixed heart was sliced into 5-mm thick slices and photographed. Cross-sectional areas of the ventricular muscle, scar area, and scar thickness were measured with computed planimetry as we described previously.⁴ A cube of tissue (5 mm) from the center of the infarct zone was embedded in paraffin and cut into 10- μ m sections. Serial cutting sections were used for immunohistochemical studies to localize the transplanted cells, infiltrated cells, and contractile protein positive cells.

For immunofluorescent staining, formalin-fixed paraffin embedded tissue sections were serially rehydrated in 100%, 95%, and 70% ethanol after deparaffinization with xylene. The antigen retrieval procedures were then used. Slides were microwaved in 10 mmol/L citrate buffer (pH 6) 3 times for 2 minutes each and washed in phosphate buffered saline (PBS) 3 times for 5 minutes each. Tissue sections were incubated in a blocking solution containing 2% goat serum and 5% bovine serum albumin in PBS to reduce nonspecific binding. Double-labeled tissue sections were incubated with goat anti-myosin heavy chain (1:200, Santa Cruz) and mouse anti-human mitochondria (1:100, Chemicon International) overnight at 4°C. Sections were then incubated for 1 hour at room temperature with a Cy3 conjugated donkey anti-mouse immunoglobulin G and an alexa fluor 647 conjugated chicken anti-goat immunoglobulin G. Nuclei were counterstained with Hoechst 33342 (2.5 μ g/mL in PBS; Molecular Probes). Slides were mounted using an anti-fading aqueous mounting medium (Dakocytomation). Slides were then visualized using an Olympus Fluoview 1000 confocal microscope. Background levels of autofluorescence on unstained sections and those

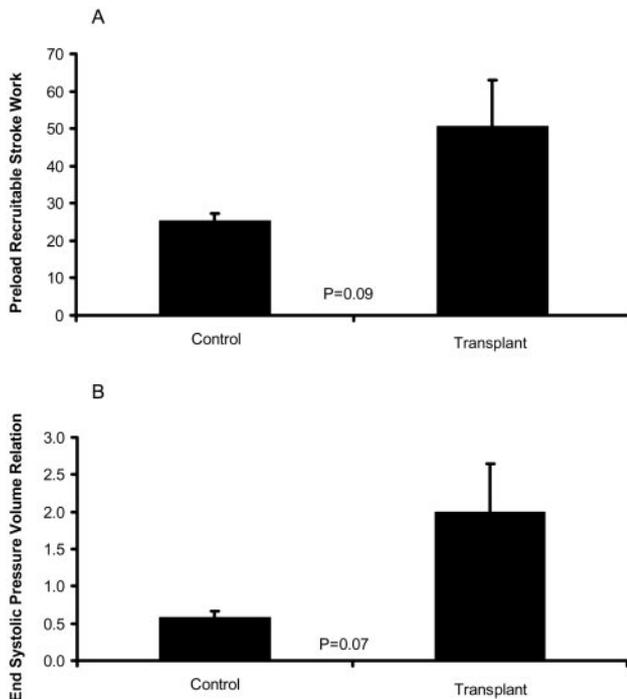


Figure 3. Left ventricular function evaluated by pressure-volume catheter 4 weeks after transplantation. A, Preload-recrutable stroke work (the slope of the stroke work-end diastolic volume relationship) was greater in cell transplantation group than in media control group. B, The end-systolic pressure-volume relationship shows that cell transplantation preserved heart function compared with the media control group. However, there was no statistical difference.

incubated with secondary antibodies only were used as controls to set the laser power and gain.

For immunohistochemical studies, endogenous peroxidase and biotin activities in the rehydrated sections were blocked with 3% aqueous hydrogen peroxide and Avidin/Biotin blocking kit for 15 minutes. The sample was treated for 10 minutes with 10% normal serum from the species where the secondary antibodies were generated. To retrieve antigens, the slides were pre-incubated with either pepsin (for CD3) or trypsin (for macrophage) or heated in a microwave (for mitochondria, troponin I, and myosin heavy chain) for 3 minutes in 10 mmol/L citrate buffer (pH 6.0). After washing, the sample was incubated overnight with the antibodies against human mitochondria (1:100; Chemicon), myosin heavy chain (1:400, Santa Cruz), troponin-I (1:800, Covance), CD3 (1:50; Dako), or macrophages (1:50; Neomarker) at room temperature in a moist chamber. After rinsing well with PBS, the sample was incubated for 30 minutes at room temperature with a species-specific biotinylated secondary linking antibody and then washed well with PBS. The sample was incubated with labeling reagent (Ultra Streptavidin-Horseradish Peroxidase Complex) at room temperature for 30 minutes, washed in PBS, developed with a freshly prepared working solution of Novared (Vector Laboratories) for 5 to 10 minutes, and rinsed in running tap water. The sample was counterstained with Hematoxyline. After rinsing, the sample was dehydrated through alcohols, cleared in xylene, and mounted in Permount (Fisher).

For in situ hybridization, deparaffinized samples were digested with a pepsin solution (Zytomed) for 40 minutes at 37°C. To stop the proteolysis reaction, the samples were covered with ethanol (100%), washed with distilled water, and dried again. For DNA hybridization, the samples were preincubated to denature DNA at 83°C for 10 minutes and then incubated with biotinylated DNA probe targeting specific human Alu-repeat DNA sequence (Texogene) for 1 hour at 37°C. After washing, the samples were rehydrated with Tris-buffered saline 0.05 mol/L and incubated with 3% aqueous hydrogen

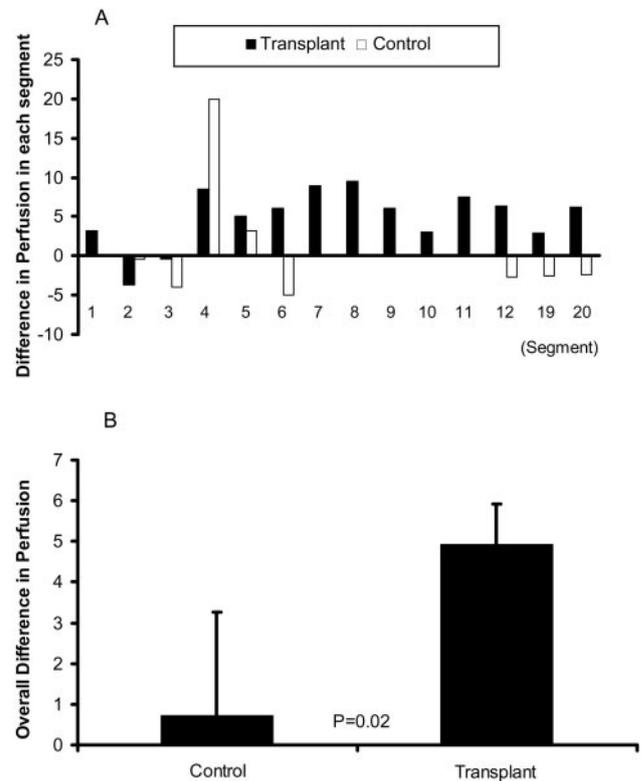


Figure 4. Regional perfusion (segments 1 to 12 and 19 and 20) evaluated by ^{99m}Tc -SPECT MIBI scan before and 4 weeks after transplantation. The difference was calculated between pre- and post-transplantation values. Cell transplantation increased myocardial perfusion at transplanted areas (A), but regional perfusion did not change after media injection. The difference of overall regional perfusion in the cell transplant area (B) was statistically better than that of the control.

peroxide and an Avidin/Biotin blocking kit to inactivate endogenous peroxidase and to block biotin activities for 15 minutes. After washing with Tris-buffered saline, samples were incubated for 1 hour with rabbit antibody (1:50 dilution; Dako). To visualize bound rabbit antibodies, samples were incubated with a Dako EnVision kit according to manufacturer's protocol and developed with freshly prepared DAB solution. Samples were counterstained with Methyl-ene Green and mounted as described above.

For angiogenesis analysis, the myocardial biopsies were sectioned and immunohistochemically stained for factor VIII and α -smooth muscle actin. The number of large blood vessels (both factor VIII and smooth muscle actin positive) and capillaries (factor VIII positive only) were counted in a blinded fashion and compared between the groups.

Statistical Analysis

Data are presented as mean \pm standard error. Statistical analysis was carried out with the SPSS software package (SPSS Inc) and the data were subjected to Student's *t* test and ANCOVA.

Results

Unrestricted Somatic Stem Cells

The USSCs used in the study were negative for CD14, CD31, CD33, CD34, CD45, CD56, CD133 and human leukocyte antigen class II and positive for CD13, CD29, CD44, and CD49e. After transportation from Germany to Toronto, the survival rate of USSCs before implantation was $93.3 \pm 15.4\%$.

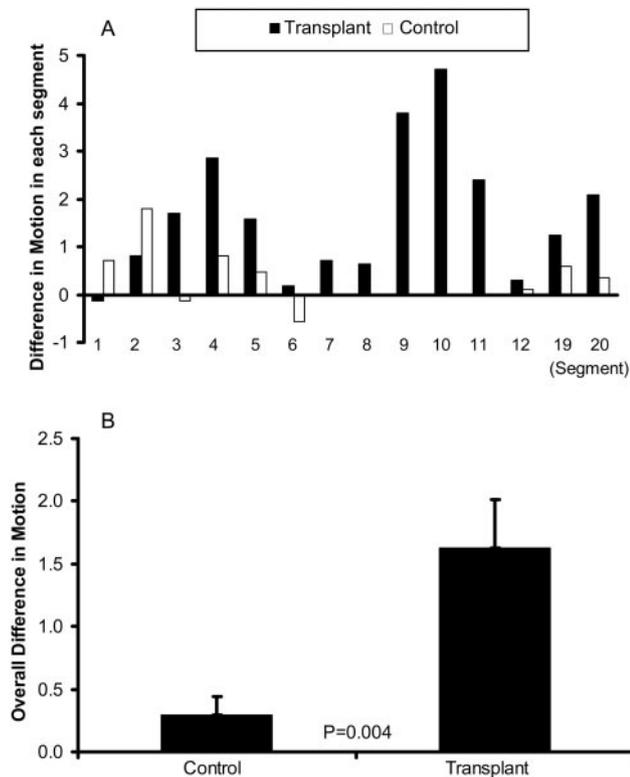


Figure 5. Difference of regional wall motion (segments 1 to 12 and 19 and 20) evaluated by ^{99m}Tc -SPECT MIBI scan before and 4 weeks after transplantation. The difference was calculated between pre- and post-transplantation values. Cell transplantation increased regional wall motion at the transplanted areas, but media injection did not change wall motion (A). The difference of overall wall motion in the transplantation group (B) was statistically better than that of the control.

Cardiac Function

The effect of USSCs on cardiac function at 4 weeks after cell transplantation was evaluated by echocardiography, ^{99m}Tc -SPECT MIBI scan, and pressure-volume measurements.

Echocardiography

Four weeks after myocardial infarction, cardiac ejection fraction decreased significantly from $61 \pm 0.9\%$ (baseline) to $54 \pm 1.4\%$ (before transplantation) ($P=0.004$). The LVEF improved in cell transplant group at 4 weeks after transplantation, whereas it decreased in the media control group ($P=0.02$) (Figure 1A and 1B). The fractional shortening in the transplantation group was also significantly better ($P=0.02$) than in the controls (Figure 1C). LVEDD in control group increased 0.37 ± 0.13 cm, but it only increased 0.12 ± 0.1 cm in the transplantation group over 4 weeks after transplantation. The increase of LVED volume in the control group was 3 times greater than that of the transplantation group (9.8 ± 8.5 versus 27.2 ± 9.3 cm^3 , respectively). However, there was no statistical difference.

^{99m}Tc -SPECT MIBI

The LVEF in the transplantation group increased significantly after cell transplantation, whereas a decrease was observed in the control group ($P=0.03$, Figure 2A). The end-diastolic volume increased significantly in control group ($P=0.02$) compared with the transplantation group (Figure 2B).

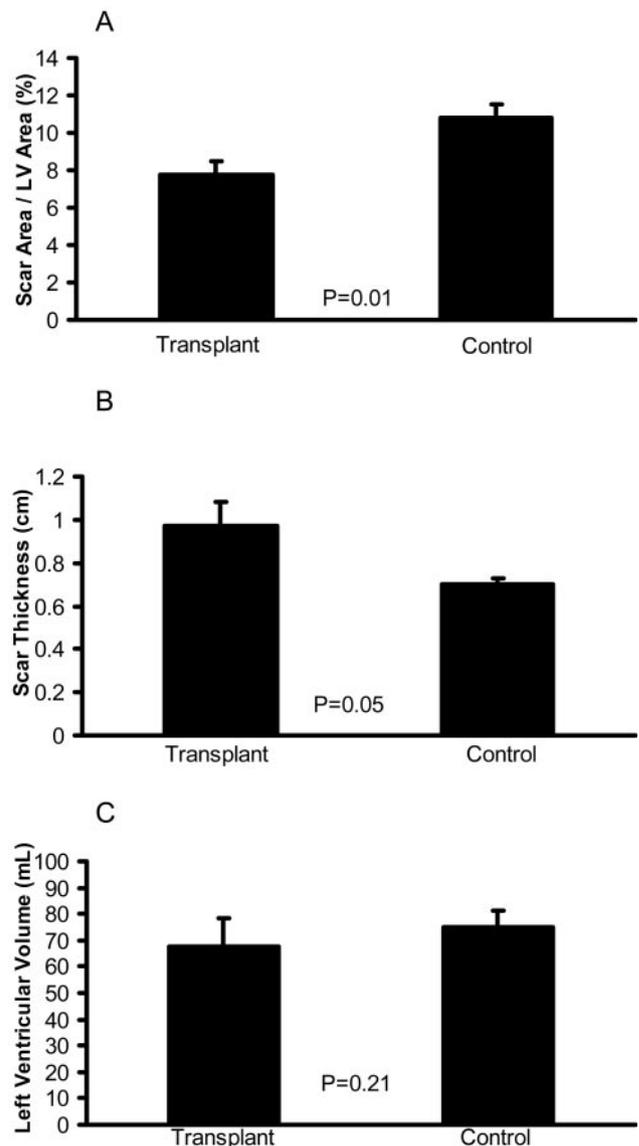


Figure 6. Scar area (A), scar thickness (B), and left ventricular volume (C) were evaluated by planimetry 4 weeks after transplantation. The ratio of scar area/left ventricular wall area in cell transplantation group was smaller than the ratio of the control. Cell transplantation increased scar thickness. However, there was no difference in ventricular volume between 2 groups.

Pressure-Volume Catheter Measurement

Figure 3 shows that preload recruitable stroke work was greater in the pigs receiving cell transplantation than in the control group ($P=0.09$). End-systolic elastance was also greater ($P=0.07$) in the transplantation group than in the controls.

Myocardial Perfusion and Wall Motion

Myocardial perfusion and wall motion were evaluated before and 4 weeks after cell transplantation by ^{99m}Tc -SPECT MIBI scan. Figure 4A shows the perfusion of each segment at the apical and midventricular levels before and after cell transplantation. The overall changes in myocardial perfusion were significantly improved ($P=0.02$) in the transplantation group compared with the changes in the control group (Figure 4B).

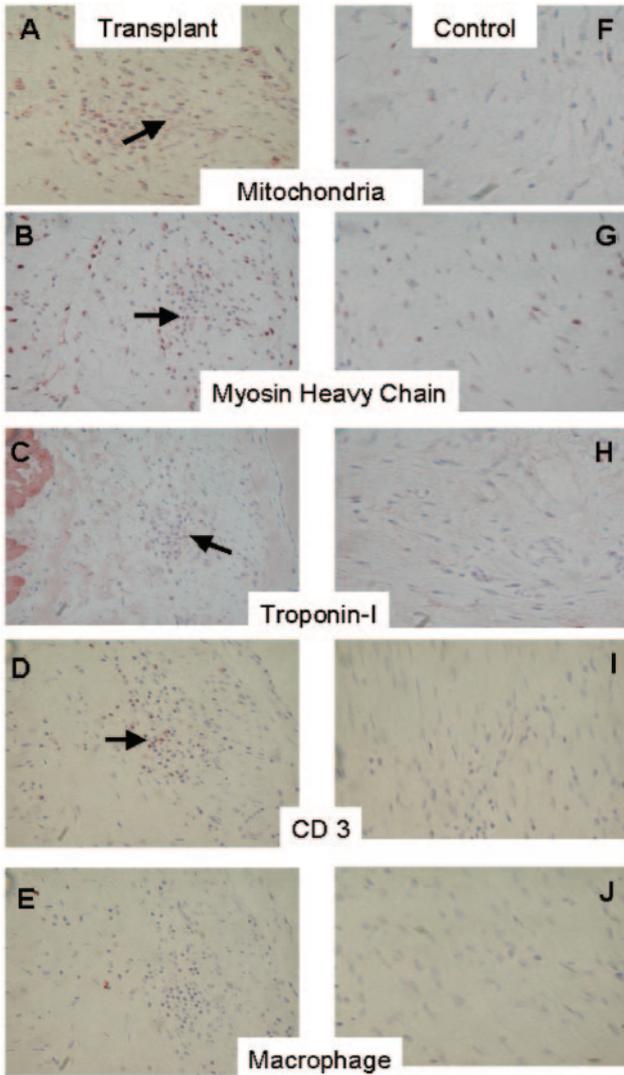


Figure 7. Photomicrographs of representative sections of a pig heart transplanted with USSCs (A through E) or media (F through J). The sections were stained with antibodies against human mitochondria antigen (A and F), myosin heavy chain (B and G), troponin-I (C and H), CD3 (D and I), or macrophage (E and J). USSCs were engrafted within the infarct zone. Myosin heavy chain and troponin-I positive cells were observed in the USSC transplanted group, suggesting that the transplanted cells differentiated into myogenic cells. Although the animals were immunosuppressed, CD3 (T-cell antigen) positive cells and macrophages were observed at implanted area. No human antigen, myosin heavy chain, troponin-I, CD3, or macrophage positive cells were observed in the control animals ($\times 100$).

In most animals, the infarcted regions were segments 19 and 20 (apex region) and the USSCs were transplanted into these regions. The myocardial perfusion rates in these regions were significantly different ($P=0.007$) in the transplantation and control groups (control, -2.4 ± 1.8 ; transplantation, 4.6 ± 1.6). Figure 5 shows the regional wall motion of the transplantation and control groups. The wall motion in the transplantation group was significantly greater ($P=0.004$) than in the control group (Figure 5B). The myocardial wall motions in segments 19 and 20 were different ($P=0.057$) in the transplantation and control groups (control, 0.47 ± 0.60 ; transplantation, 1.66 ± 0.47).

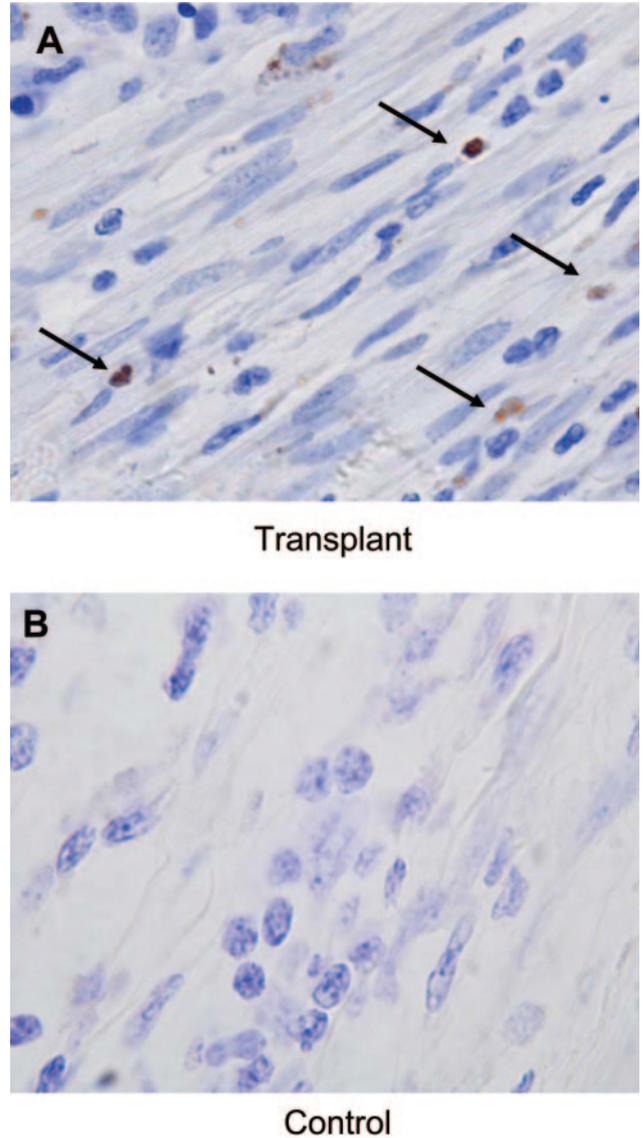


Figure 8. Photomicrographs ($\times 400$) of representative sections of a pig heart transplanted with unrestricted somatic stem cells (A) or media (B). The samples were in situ hybridized with a biotinylated DNA probe targeting a specific human *Alu*-repeat DNA sequence. Human stem cells were observed at implanted area of transplantation group (arrows) but not the control group.

Morphological and Histological Studies

Figure 6 shows scar size, scar thickness, and ventricular volumes of the hearts that received USSCs or culture media. The scar size was smaller ($P=0.01$) and scar thickness was greater ($P=0.05$) in the transplantation group than the control group. However, there was no difference in left ventricular volume between the groups ($P=0.21$).

Myocardial tissues from the infarct regions of both the transplantation and control groups were evaluated histologically (Figure 7). To identify the transplanted USSCs, we used independent methods: Antibodies against human mitochondria protein (Figure 7A and 7F) and DNA probes targeting human *Alu*-repeat DNA sequence (Figure 8), which are species-specific and do not cross-react with porcine mitochondria or chromosome DNA, respectively (data not

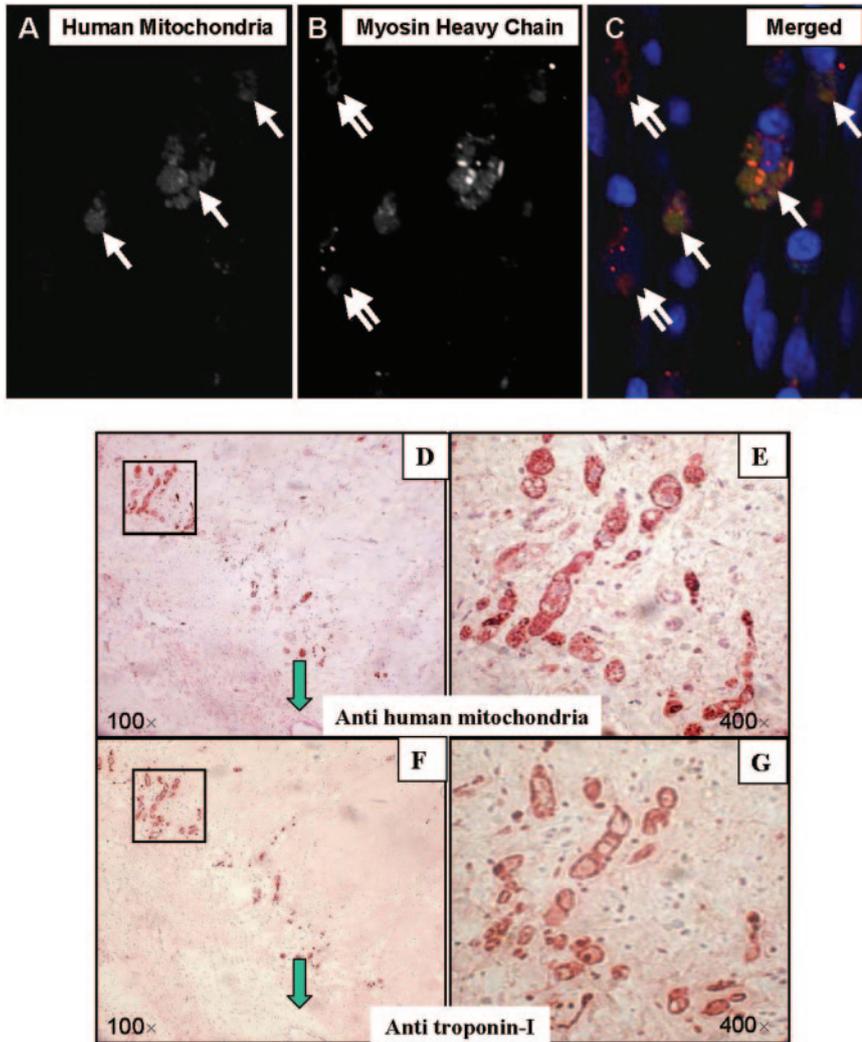


Figure 9. Photomicrographs of transplanted section of a pig heart transplanted with USSCs. Confocal images showed that implanted human cells (A, small arrows) expressed contractile protein (myosin heavy chain, B and C). Double arrows indicate cells in the scar area that do not coexpress both these proteins. The colocalization was also illustrated by immunohistochemical staining of serial cutting sections. The sections were stained with antibodies against human mitochondria antigen (D, $\times 100$; E, $\times 400$) and troponin-I (F, $\times 100$; G, $\times 400$). USSCs engrafted within the infarct zone expressed troponin-I protein. Large arrows show a marker of the serial tissue section.

shown). Both methods showed positive cells in the myocardial scar tissue of the USSC transplantation group (Figures 7A and 8A) but not in the control group (Figures 7F and 8B). Antibodies against myosin heavy chain (Figure 7B and 7G) and troponin-I (Figure 7C and 7H) demonstrated some positive cells in the scar tissue from the transplantation group (Figure 7B and 7C) but not in the scar area of the control group (Figure 7G and 7H). In some engrafted cells, colocalization of human cell markers and cardiomyocyte-specific markers (myosin heavy chain [Figure 9A through 9C] and troponin I [Figure 9D through 9G]) were observed. Antibodies against CD3 (Figure 7D and 7I) and macrophages (Figure 7E and 7J) showed CD3⁺ positive cells and macrophages in the infarcted region of the transplantation group (Figure 7D and 7E) but not the control group (Figure 7I and 7J).

Blood vessels were found in the myocardial scar tissue in both the transplantation and control groups (Figure 10A and 10B), and total number of blood vessels in the cell transplantation group was significantly greater ($P=0.02$) than that of the control group (Figure 10C). Density of large blood vessels in transplantation group was 17.3 ± 2.6 vessels/ 0.2 mm^2 , which was greater ($P=0.05$) than the 12.0 ± 0.7 vessels/ 0.2 mm^2 of the control group. Capillary density of the

transplantation group was also higher ($P=0.02$) than that of the control group (26.8 ± 2.5 versus 17.8 ± 1.6 vessels/ 0.2 mm^2 , respectively).

Discussion

Although cardiomyocyte regeneration has recently been reported in the myocardium after infarction, it cannot prevent the injured myocardium from being replaced by nonfunctional fibrous tissue, which may contribute to the onset and progression of ventricular dysfunction. The high morbidity of congestive heart failure and the shortage of donor hearts for transplantation demand a search for new approaches to prevent heart failure after a myocardial infarction. Recently, a number of studies have demonstrated that implantation of cells into damaged myocardia could regenerate the infarct, and the accumulated evidence suggests that cell transplantation may prevent heart failure.¹⁻⁹

When the cell transplantation was proposed, the implanted muscle cells were believed to improve ventricular function by synchronous contraction with the host myocardium. None of the recent studies, however, has demonstrated that the implanted cells beat synchronously with the recipient heart. In addition, cardiac function has been shown to improve after the transplan-

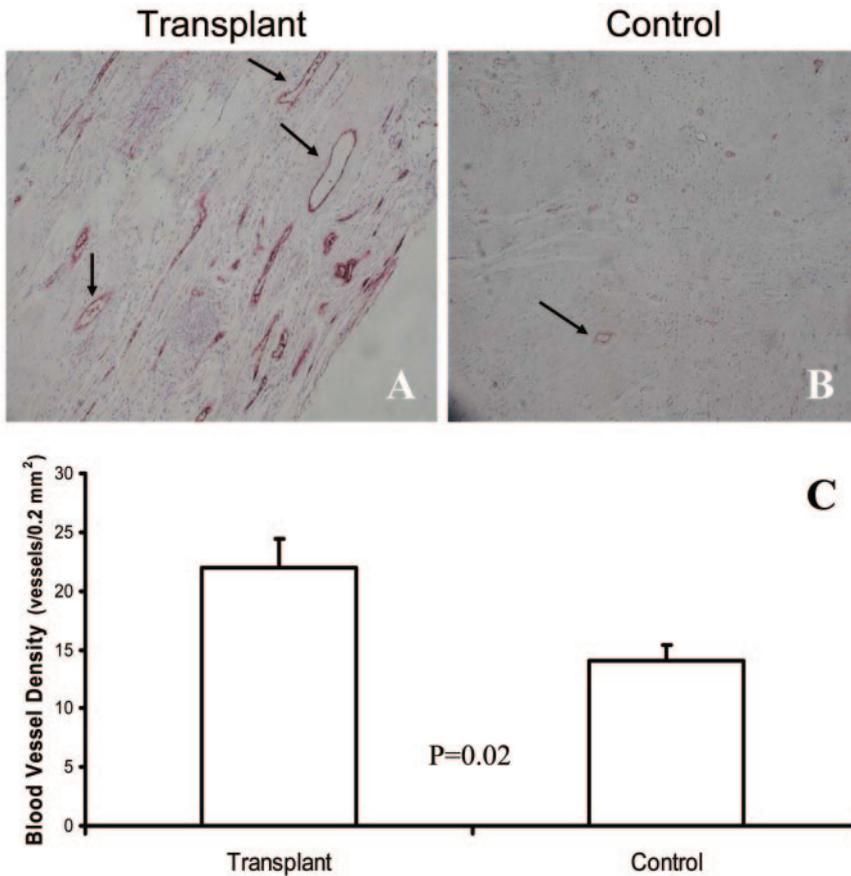


Figure 10. Photomicrographs ($\times 100$) of representative sections of pig hearts transplanted with USSCs (A) or media (B). The sections were stained with antibodies against factor VIII to identify the blood vessels. The vascular endothelial cells in the blood vessels were positively stained (brown in color, arrows). More blood vessels were identified in cell transplantation group than in the control (C).

tation of cardiomyocytes as well as noncontractile cells, such as smooth muscle cells.¹⁰ Recently, a number of studies demonstrated that stem cells, such as bone marrow mononuclear cells or bone marrow-derived mesenchymal stem cells, could be used to regenerate damaged myocardia.^{8,22,23} In the present study, we evaluated unrestricted somatic stem cells as a cell source for myocardial regeneration.

Human umbilical cord blood contains USSCs, a population of pluripotent stem cells. USSCs are different from mesenchymal stem cells, however, and may be less mature and thus have a higher proliferative potential, an extended life span, and longer telomeres. In culture, these cells grow adherently and can be expanded up to 10^{15} cells without losing pluripotency, as they can differentiate into osteoblasts, chondroblasts, adipocytes, hematopoietic cells, and neural cells.²⁰ The differentiation of USSCs along mesodermal and endodermal pathways was demonstrated in animal models. In addition, USSCs are abundantly available, can be routinely harvested without risk to the donor, and are seldom infected with agents, which gives them a definite advantage for the development of cell therapeutics in regenerative medicine. Umbilical cord stem cells are routinely frozen and therefore can be readily available for transplantation into an infarcted region without having to wait for expansion in a cell culture facility. Hence, the USSCs offer the promise of an “off the shelf” therapy for cardiac regeneration.

In the present study, we demonstrated that human USSCs transplanted into the infarcted myocardium in a porcine model survived in the myocardial scar tissue and prevented scar thinning and ventricular dilatation. The implanted cells

also increased regional perfusion and wall motion and improved cardiac function. A number of cell clusters were observed in the myocardial scar tissue of transplanted area in the cell transplantation group. Many of the cells in the cell islands stained positive for human mitochondrial proteins and for human *Alu*-repeat chromosomal DNA, which suggested that they were the implanted cells derived from human USSCs. Serial section technique of the histological samples allowed us to identify expression of the cardiomyocyte-specific markers (myosin heavy chain and troponin I) of these implanted USSCs. In addition, costaining of human mitochondria and troponin I revealed the expression of cardiomyocyte markers in some engrafted human USSCs. Similar results were also observed in our previous study, which demonstrated that some of the cultured stem cells converted to myogenic progenitor cells within the infarct region and could contribute to preservation of ventricular function.²² These additional muscle-like cells increased scar thickness and prevented scar expansion, which might be a possible mechanism to improve cardiac function by USSCs. Supporting this hypothesis, echocardiography and ^{99m}Tc-SPECT MIBI scans showed that the LVED volume in the transplantation group was significantly smaller than that in the control group. According to Laplace Law, the transplanted heart should have better cardiac function.

The beneficial effect of implanted USSCs on global cardiac function might also be explained by an improvement in myocardial perfusion. The cell transplantation group had better regional perfusion, especially within the infarcted

region where the cells were directly injected. The engrafted cells may provide progenitor cells, which participate in the formation of new blood vessels,²⁴ or they may release vascular growth factors to increase regional perfusion by either induction of new vessel formation or vessel growth. The regional wall motion in the transplantation group was also better preserved than in the control group, especially within the transplanted area. Both improved regional perfusion and increased regional wall motion may have contributed to the improvement of global cardiac function.

A major limitation of current study and future clinical trials using USSCs to regenerate damaged myocardium is immunorejection. Although we have identified implanted cells within the transplanted area, the animals were immunosuppressed during the study. Immunohistochemical staining showed T-cells (CD3-positive cells) and macrophages in the vicinity of the transplanted cells. Future studies will be required to evaluate the long-term beneficial effect of USSCs in animal models with and without immunosuppression. The survival rate of implanted cells in the transplanted area and distribution of implanted cells in other organs should be evaluated. Finally, a dose-response relationship will need to be established between the number of implanted USSCs and the recovery of cardiac function after a myocardial infarction.

In conclusion, unrestricted somatic stem cells from human umbilical cord blood were successfully transplanted into myocardial scar tissue. The transplanted USSCs engrafted in a cluster-like pattern, expressed muscle-specific antigens, increased myocardial perfusion, prevented ventricular remodeling, and improved ventricular function. These data suggest that USSCs could be a promising candidate for the cellular cardiomyoplasty in the future.

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