Novel regenerative therapy using cell-sheet covered with omentum flap delivers a huge number of cells in a porcine myocardial infarction model

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Objective: A key challenge to applying cell transplantation to treat severely damaged myocardium is in delivering large numbers of cells with minimum cell loss. We developed a new implantation method using skeletal myoblast (SMB) sheets, wrapped with an omentum flap as a blood supply to deliver huge numbers of SMBs to the damaged heart. We examined whether this method could be used to deliver a large amount of cells to deteriorated porcine myocardium.

Methods: Cell sheets were obtained by culturing mini-pig autologous SMB cells on temperature-responsive culture dishes. Myocardial infarction was induced by placing an ameroid constrictor around the left anterior descending artery. The mini-pigs were divided into 4 treatment groups ($n = 6$ in each): cell sheets with omentum, cell sheets only, omentum only, and sham operation. Each animal implant consisted of 30 cell sheets (1.5×10^7 cells per sheet). Six 5-layer constructs were each placed on a different area, immediately adjacent to but not overlapping one another, to cover the infarct and border regions.

Results: The new regenerative cell delivery system using SMB sheets covered and wrapped with omentum resulted in (1) a significantly reduced infarct size causing, at least in part, a thin scar with thick well-vascularized cardiac tissue; (2) increased angiogenesis, as determined by a significantly higher vascular density; and (3) improved cardiac function, as determined by echocardiography, compared with the conventional method (SMB sheet implantation).

Conclusions: This cell delivery system shows potential for repairing the severely failed heart. (J Thorac Cardiovasc Surg 2011;142:1188-96)

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Heart failure is a frequent and life-threatening disorder, despite recent medical and surgical advances. Myocardial regenerative therapy is gaining interest as a means for improving left ventricular (LV) function in patients with end-stage heart disease.[1-3] However, a recent clinical trial of cell transplantation by needle injection reported slightly disappointing results.[2-4] The main drawbacks of cell transplantation by needle injection appear to be poor retention and survival of the injected cells, local mechanical myocardial damage owing to injury by the needle itself, and the potential for lethal arrhythmias. We have been investigating cell-sheet techniques for delivering cells to severely damaged myocardium more efficiently, without damaging the myocardium, and, consequently, more effectively. This technique provides better improvement of cardiac function than obtained with the needle cell-injection method.[5-7]

The greatest advantage of the cell-sheet technique is that the sheet consists only of cells, which produce an extracellular matrix without requiring an artificial scaffold. The cell sheet has a high ability to integrate with native tissues, because the adhesion molecules on its surface are preserved.[5-7] The layered grafts must be carefully prepared to avoid tearing, but they themselves are strong, flexible, and easy to work with.

It has been suggested that an increased number of implanted skeletal myoblast (SMB) sheets is related to better results, such as improved cardiac function and angiogenesis, less fibrosis, and less hypertrophy, with the amounts of secreted cytokines dependent on the number of cell sheets used.[7] However, cell sheets with more than 5 layers show areas with disorganized vasculature, presumably because of insufficient supplies of blood, oxygen, and nutritients.[7,8] Thus, in applying cell transplantation to the severely damaged myocardium, a key challenge is in improving the blood perfusion of the implanted cells so

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Abbreviations and Acronyms

LAD = left anterior descending coronary artery
LV = left ventricular
LVEDV = left ventricular end-diastolic volume
LVEF = left ventricular ejection fraction
LVESV = left ventricular end-systolic volume
MI = myocardial infarction
O group = omentum only
RT-PCR = real-time polymerase chain reaction
S group = cell sheets only
SMB = skeletal myoblast
SO group = cell sheets wrapped with omentum
STAT3 = signal transducer and activator of transcription 3
VEGF = vascular endothelial growth factor

that large numbers of regenerative cells can be delivered with minimal cell loss.

The omentum is reported to potentially provide revascularization for the ischemic myocardium,[9] release a number of angiogenic cytokines,[10,11] supply stem cells, and attenuate inflammation.[12] The omentum was once commonly used in surgical revascularization to treat ischemic heart disease; however, omentopexy alone is not very effective for supporting the angiogenesis needed in the infarcted area for rapid recovery.[9] On the basis of those findings, we speculated combining SMB sheets with omentum might enhance survival of the implanted cells by improving angiogenesis. Thus, as a novel method for implanting large amounts of cells, we developed a cell-delivery system using SMB sheets wrapped and covered with omentum flap as an external source for blood flow. We hypothesized that this method could replace the myocardial infarction (MI) scar with cell-sheet–based cardiac tissue in the pig heart.

MATERIALS AND METHODS

All studies were performed with the approval of the institutional ethics committee of Osaka University. Humane animal care was used in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Animal Resources and published by the National Institutes of Health (Publication No 85-23, revised 1996). The authors had full access to the data and take full responsibility for its integrity. All the authors have read and agreed to the manuscript as written. All procedures and evaluations, including the assessment of cardiac parameters, were carried out in a blinded manner.

Animal Models and Study Protocol (Figure 1)

Thirty-seven female mini-pigs (8-10 months old; Japan Farm Co Ltd, Kagoshima, Japan) weighing 20 to 25 kg were used in these experiments.

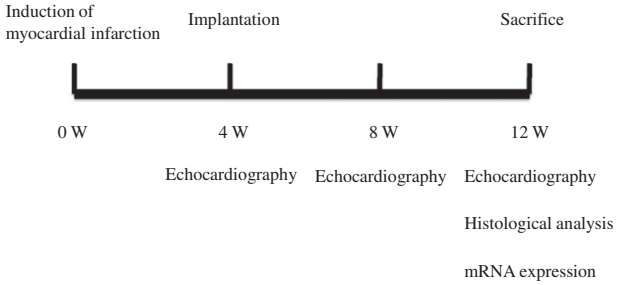

FIGURE 1. Study protocol for the assessment of cardiac function and histologic analysis.

The mini-pigs were anesthetized with an intravenous administration of ketamine (6 mg/kg) and sodium pentobarbital (10 mg/kg) for endotracheal intubation and then maintained with inhaled sevoflurane (15%-2%). The pericardial space was exposed by left thoracotomy through the fourth intercostal space. The distal portion of the left anterior descending coronary artery (LAD) was directly ligated as ischemic preconditioning to reduce the occurrence of lethal ventricular arrhythmia, followed by placement of an ameroid constrictor around the LAD just distal to the left circumflex coronary artery branching.[5,13] The muscle and skin were closed in layers, and the mini-pigs were then taken off the anesthetics. Eleven (30%) of the study animals died in the early postoperative period. This technique produced an ischemic cardiomyopathy model that reflected clinical relevance and can be used for appropriate preclinical studies with minimal procedure-related mortality.

Computer-generated random allocation generated 4 randomized study groups 1 week after MI. Autologous cells were isolated and grown in culture for 3 weeks to prepare samples for implantation. Four weeks after MI induction, the mini-pigs were again placed under general anesthesia for echocardiography followed by either cell-sheet implantation or a sham operation. Two mini-pigs whose LV ejection fractions (LVEFs) were above 40% before treatment, as measured by transthoracic echocardiography using the Simpson method, were excluded from the study. At 4 and 8 weeks after either cell-sheet implantation or sham operation, the mini-pigs again underwent general anesthesia for echocardiographic examination. The mini-pigs were humanely killed after the 8-week echocardiography measurements for histologic and biochemical analyses of the heart tissue.

Preparation and Grafting of SMB Cell Sheets

Autologous skeletal muscle weighing approximately 10 to 15 g was removed from the quadriceps femoris muscle, and purified autologous SMB cells were cultured for 3 weeks to prepare them for implantation, as described previsously.[5] Autologous SMBs are precursor cells of adult myofibers and feature several advantages, including autologous origin, high in vitro scalability, lack of tumorigenicity (owing to their myogenic lineage restriction), and strong resistance to hypoxia after ischemia. The cells were incubated in 60-mm temperature-responsive culture dishes (UpCell; Cellseed, Tokyo, Japan) at 37°C for 24 hours (1.5×10^7 cells per dish). The dishes were then transferred to another incubator, set at 20°C, for 1 hour to release the cultured cells as intact cell sheets. Under this protocol, the SMBs spontaneously detached from the plate as a free-floating monolayer cell sheet.

Grafting the SMB Cell Sheet Wrapped With Omentum

The mini-pigs with MI were divided into 4 treatment groups (n = 6 in each): cell sheets wrapped with omentum (SO group), cell sheets only (S group), omentum only (O group), and sham operation (sham group). Each animal in the SO and S groups received approximately 30 cell sheets (1.5×10^7 cells per sheet) with the total cell number being 4.5×10^8.

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