A novel vascularized patch enhances cell survival and modifies ventricular remodeling in a rat myocardial infarction model

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Objective: Although stem cells hold a great therapeutic potential for injured tissues, limited survival of transplanted stem cells has hindered the clinical application of this technology. We hypothesized that an omentum-based stem cell–supporting patch could provide adequate nutrients and microenvironment to prolong cell survival. We examined this hypothesis in rats with experimental myocardial infarction.

Methods: The omentum-based supporting patch was constructed by stitching polylactic acid-co-glycolic acid polymer seeded with mesenchymal stem cells from male Sprague–Dawley rats. Eight weeks after the experimental myocardial infarction, which was created by ligating the left coronary artery of female Sprague–Dawley rats, mesenchymal stem cells were transplanted with ($n = 16$) or without ($n = 14$) the supporting patch. After 4 weeks, transplanted mesenchymal stem cell survival, ventricular remodeling, and cardiac performance were examined.

Results: Significantly more cells survived after 4 weeks in rats transplanted with mesenchymal stem cells on the supporting patch assessed by means of polymerase chain reaction detection of the *Sry* gene than seen in those without the supporting patch (2.61 ± 0.40 vs 1.19 ± 0.12, $P < .05$). Rats with myocardial infarction that received mesenchymal stem cells with the patch also had significantly improved ventricular remodeling and cardiac function than those without the patch. Wrapping infarcted myocardium with omentum alone did not change the myocardial function.

Conclusions: The omentum-based cell-supporting patch provided a favorable microenvironment for transplanted mesenchymal stem cell survival, which resulted in favorable ventricular remodeling and restoration of cardiac function in rats with experimental myocardial infarction. Further validation of the technique in human subjects could make mesenchymal stem cell transplantation a viable therapeutic option for patients with cardiac disease. (J Thorac Cardiovasc Surg 2010;140:1388-96)

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Despite a continuous decrease in recent decades, myocardial infarction (MI) remains the leading cause of mortality and morbidity in most countries. After MI, cardiomyocytes die, and fibrous scars form in the infarction zone. Although antithrombotic medical treatment, percutaneous coronary intervention, and coronary artery bypass grafting (CABG) have been successful in revascularizing the ischemic tissues, there is thus far no effective treatment to regenerate cardiomyocytes that have dies as a result of infarction.

Pluripotent mesenchymal stem cells (MSCs) have been shown to potentially serve as a cell source for cardiac tissue regeneration.[1] Recently, stem cell–based treatment for MI or heart failure has been tested in human patients worldwide. The clinical outcome, however, was inconsistent and short lived.[2] Although many reasons could contribute to the lack of consistent therapeutic efficacy, early ischemia is the leading cause of transplanted cell apoptosis in host tissue. Early ischemia can readily induce graft cell death, which markedly reduces the number of surviving donor cells.[3] It therefore becomes a technical challenge to find ways to prolong the survival of the transplanted stem cells.

Among several techniques reported, engineered heart tissue to replace damaged myocardium has been constructed. Scientists have found that cell-loaded scaffold was more effective at reducing the infarction area than results seen in those with regular cell transplantation because the 3-dimensional scaffolds could provide biomechanical support for the transplanted cells.[4] However, the polymeric fibers cannot provide a vascularized environment to nourish the cells carried, and cell survival was still poor.[5] Historically, omentopexy was used to provide revascularization for the patient with ischemic heart disease.[6] However, further studies have shown that

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Abbreviations and Acronyms

CABG = coronary artery bypass grafting
Cx43 = connexin 43
DAPI = 4′-6-diamidino-2-phenylindole dihydrochloride
LVEDD = left ventricular end-diastolic diameter
LVEF = left ventricular ejection fraction
LVESD = left ventricular end-systolic diameter
LVFS = left ventricular fractional shortening
MI = myocardial infarction
MSC = mesenchymal stem cell
PCR = polymerase chain reaction
PLGA = polylactic acid-co-glycolic acid
SCF = stem cell factor
TUNEL = terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling
VEGF = vascular endothelial growth factor

omentopexy was insufficient to revascularize the myocardium.[7] In recent years, omentopexy combined with basic fibroblast growth factor or bone marrow mononucleic cells has been used to promote angiogenesis in the infarcted myocardium.[8,9] However, none of these studies focused on improving the survival of the engrafted stem cells and were not shown to ameliorate the undesirable ventricular remodeling process.

In the current study we developed a new hybrid approach by including omentum, MSCs, and biodegradable scaffold. We hypothesized that omentum can improve the microenvironment of the cell-seeded patch by providing blood supply and that the improved microenvironment would ensure greater cell survival. To test the hypothesis, an MI model was established in female rats, and an omentum-wrapped polylactic acid-co-glycolic acid (PLGA) patch seeded with male rat MSCs was grafted onto the epicardium of the infarction area. The survival and apoptosis of the transplanted cells, the density of the microvessels, ventricular remodeling, and cardiac function were evaluated. We found that this process provided a favorable growth environment for survival of the transplanted MSCs and demonstrated beneficial effects on ventricular remodeling and cardiac function.

MATERIALS AND METHODS

Animals

The animal investigation conforms to the ''Guide for the care and use of laboratory animals'' published by the US National Institutes of Health (publication no. 85-23, revised 1996). Sprague–Dawley rats were obtained from Beijing WTLH Experimental Animal Corporation (Beijing, China; certificate no. SCXK2004-2005 [Beijing]). All animal experimental procedures were approved by the Institutional Animal Care and Use Committee, Fu Wai hospital, and the Beijing Council on Animal Care, Beijing, China.

PLGA Scaffold

PLGA copolymer scaffold was obtained from Professor Ping Hu, Department of Chemical Engineering, Tsinghua University, Beijing, China. It was manufactured based on an electrospinning technique, as described previously.[10] (The details of the PLGA copolymer are presented in the Materials and Methods section of this article's Online Repository.) The copolymer scaffolds were cut into 10-mm-diameter pieces for cell seeding and implantation as a cardiac patch.

Creation of the Rat MI Model and Grouping

Experiments were performed in 8-week-old female Sprague–Dawley rats with an initial body weight of 280 to 300 g. The detailed procedure for creating the rat MI model has been described previously[11] (see also this article's Materials and Methods section in the Online Repository). Eight weeks after MI, the cardiac function of the surviving rats was evaluated by means of echocardiographic analysis (GE Vivid 7; GE, Fairfield, Conn) to confirm MI status. Those with infarction areas of greater than 25% of the left ventricular free wall and a left ventricular fractional shortening (LVFS) of less than 0.25 were selected for the following experiments.

MSC Isolation, Culture, and Seeding on PLGA Scaffold

MSCs were isolated and harvested on the basis of their preferential adherence to the plastic surface of cell-culture flasks, and the phenotypes were analyzed on the basis of the positive expression of CD29 and CD44 but negative for CD34 and CD45, as previously described in our laboratory.[12] MSCs at the second or third passages were used for the experiments. The morphologic appearance showed that the MSCs had normal proliferative capability and no evidence for chromosomal abnormalities at the time of transplantation. After labeling with 4′-6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma–Aldrich, St Louis, Mo),[13] the MSCs were injected into the PLGA scaffold in 0.5-mL cell suspension (4.0×10^6 cells/ml), and the MSC–PLGA patches were then incubated in a humidified 5% CO_2 atmosphere at 37°C for 3 days until use.

Implantation of MSC–PLGA Patches and Omentum Wrapping

All rats were anesthetized with ketamine before the hearts were exposed through the fifth left intercostal space. The infarction area was identified visually on the basis of surface scarring and abnormal wall motion. For group P, MSC–PLGA grafts were implanted onto the epicardium of infarcted myocardium with interrupted sutures. For group O, the omentum was exposed by means of an upper middle laparotomy before an omental flap (approximately 1×1 cm^2) was made carefully to protect its artery and vein as blood supply. The pedicled omental flap was passed through the diaphragm into the pericardial space and then covered the infarction area and sutured. For group P+O, the MSC–PLGA patch was implanted first, and the same surgical omentopexy process was conducted to cover the patch. After the operation, the thoracic and abdominal incisions were closed, and animals were allowed to recover in their cages.

Rats of group P were killed at 1 (n = 4), 2 (n = 3), and 4 (n = 7) weeks, respectively, after MSC–patch implantation. Rats of group O were killed at 1 (n = 5), 2 (n = 4), and 4 (n = 9) weeks, respectively, after omentopexy. Rats of group O+P were killed at 1 (n = 3), 2 (n = 3), and 4 (n = 10) weeks, respectively, after MSC–PLGA patch transplantation with omentopexy. Rats of group MI were killed at 1 (n = 7), 2 (n = 6), and 4 (n = 9) weeks, respectively.

Echocardiographic Assessment of Cardiac Function

Transthoracic echocardiographic analysis was performed on all animals before and 4 weeks after implantation. A standard protocol was used by 2 different echocardiography physicians during the study.[14] Each echocardiography physician was blinded to the treatment. Details are presented in the Materials and Methods section of this article's Online Repository.

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