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Transplantation of mesenchymal stem cells within a poly(lactide-co-ε-caprolactone) scaffold improves cardiac function in a rat myocardial infarction model

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Aims Cardiac tissue engineering has been proposed as an appropriate method to repair myocardial infarction (MI). Evidence suggests that a cell with scaffold combination was more effective than a cell-only implant. Nevertheless, to date, there has been no research into elastic biodegradable poly(lactide-*co*-ε-caprolactone) (PLCL) scaffolds. The aim of this study was to investigate the effect of mesenchymal stem cells (MSCs) with elastic biodegradable PLCL scaffold transplants in a rat MI model.

Methods and results Ten days after inducing MI through the cryoinjury method, a saline control, MSC, PLCL scaffold, or MSC-seeded PLCL scaffold was transplanted onto the hearts. Four weeks after transplantation, cardiac function and histology were evaluated. Transplanted MSCs survived and differentiated into cardiomyocytes in the injured region. Left ventricular ejection fraction in the MSC + PLCL group increased by 23% compared with that in the saline group; it was also higher in the MSC group. The infarct area in the MSC + PLCL group was decreased by 29% compared with that in the saline group; it was also reduced in the MSC group.

Conclusion Mesenchymal stem cells plus PLCL should be an excellent combination for cardiac tissue engineering.

Keywords Myocardial infarction • Heart failure • Mesenchymal stem cell • Poly(lactide-*co*-ε-caprolactone) • Cardiac tissue engineering • Scaffold

Introduction

Despite advances in medical science, coronary artery disease remains a major cause of mortality. Myocardial infarction (MI) is defined as death or necrosis of the myocardium due to decreased nutrients and oxygen supply to the heart muscle. Following injury, the myocardium cannot replicate and is replaced with fibrous tissue, eventually inducing ventricular remodelling and dilation, and ultimately leading to heart failure. Therefore, the question of how to restore the injured myocardium and ameliorate cardiac dysfunction is a major issue. Mesenchymal stem cells (MSCs) are pluripotent progenitor cells able to differentiate into vascular endothelial cells and cardiomyocytes. Many researchers have demonstrated that MSC implantation induces myocardial regeneration and improves cardiac function through myogenesis and angiogenesis.[1–3] Even though implanted cells can survive and differentiate into cardiomyocytes in the injured myocardium, because of the lack of oxygen and adequate nutrients, the infarcted myocardium is not an environment conducive to cell survival.

To resolve these problems, cardiac tissue engineering has become a promising strategy. Cardiac tissue engineering is a relatively new discipline that combines isolated, functional cells and

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three-dimensional (3-D) polymeric scaffolds. These scaffolds temporarily provide the biomechanical support for cells until they are able to produce their own extracellular matrix.[4–6] Many scaffolds have been used for cardiac tissue engineering, and they have shown beneficial effects on myocardial regeneration.[7–10] However, there are some possible problems associated with the use of these scaffolds. First, due to the repeated contraction and relaxation of the heart muscle, appropriate biomaterials for cardiac tissue engineering need to be elastic and strong enough to resist ventricular mechanical dilation. In addition to having enough elasticity for mechanical support, a good biomaterial must provide an excellent environment for cell survival. Furthermore, the ideal biomaterial should be capable of being safely replaced by newly formed tissue and it should degrade at an appropriate time point without producing any toxic products.[11] Despite the promise of scaffold technology and the importance of these considerations, there have been few studies to evaluate the use of elastic and biodegradable scaffolds for cardiac tissue engineering.

Poly(lactide-*co*-ε-caprolactone) (PLCL) is a synthetic biocompatible polymer, which has elastic and biodegradable characteristics. Because of these features, it has been used for artificial vessels and other tissues that require such characteristics in a scaffold.[11–13] PLCL not only has elastic and biodegradable properties, but also promotes good cellular interaction and degrades in a set time period without toxicity.[11,14,15]

Therefore, PLCL could be an appropriate scaffold candidate for cardiac tissue engineering. However, to our knowledge, there has been no study using a PLCL scaffold for cardiac tissue engineering.

In this study, we investigated whether a bone marrow-derived MSC (BMMSC)-seeded PLCL scaffold could repair injured myocardium and improve left ventricular function in a rat MI model.

Methods

Model of acute myocardial infarction

Male Lewis rats weighing 250–300 g (SLC, Hamamatsu, Japan) were used in this study. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No.85-23, revised 1996), and all protocols were approved by the Hanyang University Animal Care and Use Committee.

To standardize infarct size, MI induction was performed by the cryoinjury method. This standardized procedure (using the same size probe) minimizes the variation in infarct size commonly seen with the ligation of coronary artery induced infarct.[16] The rats were anaesthetized with a mixture of ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg). The anaesthetized rats were ventilated with a rodent ventilator (Model 683, Harvard Apparatus, USA). A left thoracotomy was performed in the fourth intercostal space, and the pericardium was removed. Cryoinjury was conducted with a metal probe (8 mm in diameter) cooled by immersion in liquid nitrogen. The cooled metal probe was applied to the left ventricle free wall for 10 s each time and applied three times.

Synthesis of poly(lactide-*co*-ε-caprolactone) scaffold

The polymerization of PLCL was carried out as described by S.I.J.[11,12] The ampoule containing L-lactide (100 mmol), ε-caprolactone (100 mmol), 1,6-hexanediol (0.5 mmol), and stannous octoate (1 mmol) was sealed under vacuum after purging three times with nitrogen at 90°C and subsequently heated to 170°C in an oil bath for 24 h, with stirring. After the reaction, the product was dissolved in chloroform and micro-filtered through a 0.45 μm pore membrane filter. The polymer was precipitated with an excess of methanol, filtered, and dried under vacuum. The overall reaction yield was ~90%. The molar ratio of L-lactide to ε-caprolactone was determined by nuclear magnetic resonance spectroscopy (5.1:4.9—L-lactide to ε-caprolactone).

Mesenchymal stem cell culture and seeding on poly(lactide-*co*-ε-caprolactone) scaffold

Bone marrow cells were obtained by flushing femurs and tibias with phosphate-buffered saline (PBS) and were fractionated over 1.073 g/mL Percoll solution (Sigma, USA). The obtained bone marrow cells were cultured in Dulbecco's Modified Eagle's Medium (Gibco BRL, USA) supplemented with 10% foetal bovine serum (FBS) and 50 IU/mL penicillin–streptomycin (Gibco BRL) and was kept at 37°C in humidified air with 5% CO_2. The non-adherent cells were discarded with the media changes, which were performed every 3 days. When the cultures became nearly confluent, adherent cells were detached with trypsin–EDTA and subsequently passaged. After four to five passages, cells were harvested and then used for cell implantation. Cultured MSCs were labelled with the carbocyanine dye CellTracker CM-DiI (Molecular Probes, Eugene, OR, USA) according to the manufacturer's protocol. In brief, 2 mg/mL of CM-DiI was diluted into Hanks' balanced salt solution to a final concentration of 5 μg/mL. The cells were incubated in the diluted CM-DiI solution for 5 min at 37°C, followed by 15 min at 4°C. After labelling, the cells were washed with PBS. After overnight culturing, the cells were examined by fluorescence microscopy with a rhodamine filter to determine the labelling efficiency. MSCs (1×10^6 cells) were seeded onto PLCL scaffolds (10 mm × 10 mm) and cell-seeded scaffolds were maintained in FBS in preparation for implantation. Cell-free scaffolds were produced by the same method but without cells.

Mesenchymal stem cell and poly(lactide-*co*-ε-caprolactone) implantation

Ten days after MI generation, rats were randomly assigned into four groups: the PLCL + MSC group received implantation of MSC-seeded scaffold ($n = 8$), the PLCL group received a cell-free scaffold ($n = 8$), the MSC group had only MSCs implanted ($n = 8$), and the control group was injected with saline ($n = 8$). MSCs (1×10^6 cells) in 100 μL medium or saline were injected into the border of the infarction using a 30 gauge needle. An MSC (1×10^6 cells)-seeded PLCL scaffold and a cell-free PLCL scaffold were sutured with 7-0 silk onto the epicardial surface over regions of infarcted myocardium and adjacent infarction border zones.

Echocardiographic examination

Transthoracic echocardiography was performed by a blinded investigator 4 weeks after treatment. Rats were anaesthetized with ketamine hydrochloride and xylazine hydrochloride for echocardiography examination. Two-dimensional targeted M-mode traces were obtained at the papillary muscle level using an echocardiography system (128 XP, Acuson, Mountain View, CA, USA) equipped with a 7.0 MHz transducer. Left ventricular end-systolic diameter

(LVESD) and left ventricular end-diastolic diameter (LVEDD) were measured by the American Society for Echocardiography leading-edge method from at least three consecutive cardiac cycles. LV volume and ejection fraction were calculated on the basis of the Teichholtz formula.

Analysis of protein expression

After echocardiographic examination, heart tissue was obtained from the infarct area in individual rats and was used for comparison between the four groups ($n = 3$ each). These samples were homogenized on ice in 0.1% Tween 20 homogenization buffer with a protease inhibitor. Then, 40 μg of protein was transferred into sample buffer, loaded onto a 7.5% sodium dodecyl sulphate–polyacrylamide gel, and blotted onto a polyvinyledene fluoride membrane. After being blocked for 120 min, the membrane was incubated with primary antibody at a dilution of 1:200. The membrane was then incubated with peroxidase-labelled secondary antibody at a dilution of 1:1000. Positive protein bands were visualized with an ECL kit. Western blotting was performed with rabbit polyclonal antibody against cardiac α-actin, myosin heavy chain (MHC), and troponin I (TnI). A mouse polyclonal antibody raised against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a protein-loading control.

Analysis of gene expression

Total RNA was extracted from infarcted heart tissue with Trizol reagent (Invitrogen, Carlsbad, CA, USA) for reverse transcription–polymerase chain reaction (RT–PCR). The reverse transcription reaction was performed with 5 μg of pure total RNA using SuperScript II reverse transcriptase (Invitrogen). Synthesized cDNA was amplified by PCR using the following primer: GATA-4, sense 5′-CTGTCATCTC ACTATGGGCA-3′, antisense 5′-AAATTCCTGCTCGGACTTGG-3′; and (GAPDH), sense 5′-CCTTCATTGACCTCAACTACA-3′, antisense 5′-CTGGCGTCTTCACCACCATG-3′. PCR was carried out for 30 cycles of denaturing (94°C, 30s), annealing (60°C, 30s), and extension (72°C, 60s), with a final extension at 72°C for 7 min. The PCR products were visualized by electrophoresis on 2% (w/v) agarose gels. The sizes of the RT–PCR products for GATA-4 and GAPDH are 257 and 168 bp, respectively.

Histological and immunohistochemical analysis

Heart tissue was fixed in 10% buffered formaldehyde, embedded in paraffin, and sections were cut from the injured site at 1 mm intervals vertical to the long axis of the heart. Five sections were selected at the middle of the injury site and stained with Masson's trichrome. Each stained section was scanned and computerized with a digital image analyzer (ImagePro plus, USA). The infarct area was measured as the ratio (%) of the injured area divided by the whole LV area and averaged over the five sections in each rat. For immunofluorscence staining, heart tissues were embedded in an optimal cutting temperature compound and snap frozen in liquid nitrogen. Four micrometer-thick tissue sections were stained with antibodies against TnI (Serotec, NA, USA) using fluorescein isothiocyanate-conjugated anti-goat immunoglobulin G antibody as a secondary antibody.

Statistical analysis

Numerical values are expressed as mean ± SEM unless otherwise indicated. Comparisons of parameters among the four groups were performed with one-way ANOVA. A value of $P < 0.05$ was considered statistically significant.

Results

Improvement in cardiac function

Four weeks after treatment, an echocardiography study showed that the MSC-seeded PLCL scaffold and implanted MSCs attenuated LV dilation and improved cardiac function compared with saline and PLCL only implantation. LVEDD in the MSC + PLCL group was significantly decreased compared with the saline and PLCL groups (6.7 ± 0.3 vs. 8.6 ± 0.4 mm, $P = 0.02$; 6.7 ± 0.3 vs. 8.4 ± 0.5 mm, $P = 0.03$). LVEDD in the MSC group was also significantly decreased compared with the saline and PLCL groups (7.2 ± 0.2 vs. 8.6 ± 0.4 mm, $P = 0.03$; 7.2 ± 0.2 vs. 8.4 ± 0.5 mm, $P = 0.03$). Even LVEDD in the MSC + PLCL group was smaller than that in the MSC group, but there was no difference between the two groups (6.7 ± 0.3 vs. 7.2 ± 0.2 mm, $P = 0.06$). LVEDD in the saline group was similar to the PLCL group (8.6 ± 0.4 vs. 8.4 ± 0.5 mm, $P = 0.80$) (*Figure 1A*). LVESD in the MSC + PLCL group was significantly reduced compared with the saline and PLCL groups (5.2 ± 0.3 vs. 6.7 ± 0.2 mm, $P = 0.017$; 5.2 ± 0.3 vs. 6.4 ± 0.2 mm, $P = 0.02$). Also, LVESD in the MSC group was smaller than that in the saline and PLCL groups (5.7 ± 0.2 vs. 6.7 ± 0.2 mm, $P = 0.035$; 5.7 ± 0.2 vs. 6.4 ± 0.2 mm, $P = 0.03$). However, LVESD in the MSC and MSC + PLCL groups (5.7 ± 0.2 vs. 5.2 ± 0.3 mm, $P = 0.18$) showed no difference. LVESD in the PLCL group showed no difference compared with the saline group (6.4 ± 0.2 vs. 6.7 ± 0.2 mm, $P = 0.35$) (*Figure 1B*). Left ventricular ejection fraction (LVEF) in the MSC + PLCL group was significantly increased compared with the saline and PLCL groups (52.5 ± 2.4 vs. 40.1 ± 1.6%, $P = 0.021$; 52.5 ± 2.4 vs. 42.4 ± 2.1%, $P = 0.03$). The LVEF in the MSC group was also higher than in the MSC and PLCL groups (48.7 ± 2.3 vs. 40.1 ± 1.6%, $P = 0.03$; 48.7 ± 2.3 vs. 42.4 ± 2.1%, $P = 0.04$). The LVEF in the MSC + PLCL group was slightly higher than in the MSC group; nevertheless, there was no difference between the two groups (52.5 ± 2.4 vs. 48.7 ± 2.3%, $P = 0.07$). The LVEF in the saline group was similar to that in the PLCL group (40.1 ± 1.6 vs. 42.4 ± 2.1%, $P = 0.64$; *Figure 1C*).

Induction of myogenesis

Fluorescence microscopy showed that MSCs labelled with the fluorescent dye CM-DiI prior to implantation were detected within the injured region and were positive for cardiac TnI 4 weeks after implantation (*Figure 2*). These implanted MSCs were engrafted into the myocardium and expressed cardiac markers. The MSC-seeded PLCL scaffold implantation expressed more α-actin and TnI than the MSC-only implantation.

Western blot analysis demonstrated that MHC, α-actin, and troponin-I cardiac markers were detected in the injured heart tissue. These cardiac markers were expressed more in the MSC + PLCL group than in the other group (*Figure 3*).

The RT–PCR analysis showed that implanted MSCs expressed cardiac transcription factor GATA-4 in the injured myocardium. Furthermore, the MSC-seeded PLCL scaffold implant expressed more GATA-4 than any other group (*Figure 4*).

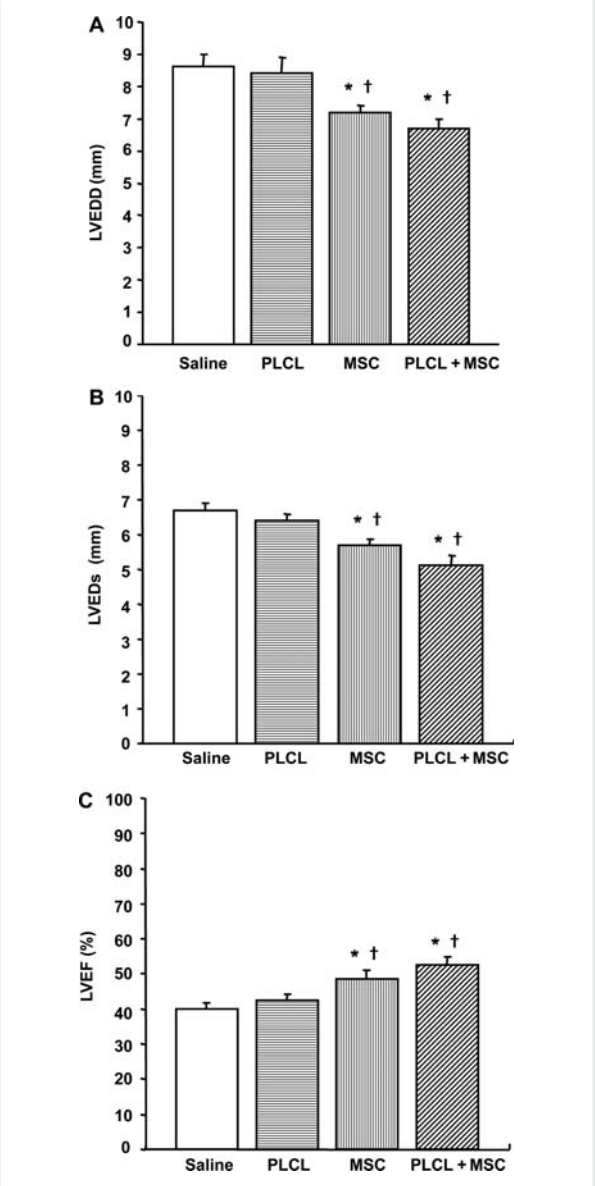

Figure 1 Echocardiography 4 weeks after treatment. (*A*) Left ventricular end-diastolic diameter (LVEDD) in the MSC + PLCL group and the MSC group was significantly decreased compared with that in the saline and PLCL groups, but no significant difference was seen between the MSC and PLCL + MSC groups. There was no difference in the LVEDD between the saline and PLCL groups. (*B*) The left ventricular end-systolic diameter (LVESD) in the MSC + PLCL and MSC groups was significantly decreased compared with that in the saline and PLCL groups, but no significant difference was seen between the MSC and PLCL + MSC groups. There was no difference in the LVESD between the saline and PLCL groups. (*C*) Left ventricular ejection fraction (LVEF) in the MSC + PLCL and MSC groups was significantly increased compared with that in the saline and PLCL groups, but no significant difference was seen between the MSC and PLCL + MSC groups. There was no difference in the LVEF between the saline and PLCL groups. $^{*}P < 0.05$ vs. saline group. $^{\dagger}P < 0.05$ vs. PLCL group. Data shown are mean $\pm$ SEM.

Reduction of myocardial infarct area

Masson's trichrome staining showed the amount of viable tissue and fibrous tissue present in the heart tissue samples. Injured myocardium, which is replaced with fibroblasts and collagen, appears blue, and viable myocardium appears red (*Figure 5A*). The infarct area in the MSC + PLCL group was significantly reduced compared with that of the saline, PLCL, and MSC groups (30.2 ± 2.6 vs. $38.8 \pm 4.3\%$, $P = 0.01$; 30.2 ± 2.6 vs. $37.8 \pm 3.2\%$, $P = 0.018$; 30.2 ± 2.6 vs. $34.2 \pm 3.6\%$, $P = 0.03$). The MSC implantation also significantly decreased the infarct area compared with saline and PLCL implantation (30.2 ± 2.6 vs. $38.8 \pm 4.3\%$; 30.2 ± 2.6 vs. $37.8 \pm 3.2\%$, $P < 0.05$, respectively). The infarct area in the saline group was similar to that in the PLCL group (38.8 ± 4.3 vs. $37.8 \pm 3.2\%$, $P = 1.00$; *Figure 5B*).

Discussion

In this study, we showed that a BMMSC-seeded, elastic, biodegradable PLCL scaffold repaired injured myocardium and effectively improved LV function in an MI rat model. The PLCL served as a mechanical extracellular matrix, allowing the seeded BMMSCs to survive and differentiate into cardiomyocytes, thereby regenerating the myocardium and ultimately ameliorating cardiac dysfunction.

After injury, the myocardium was replaced with fibrous tissue. Fibrous tissue lacks elasticity, so it becomes thin and dilates due to ventricular pressure and this ultimately leads to heart failure. Therefore, finding a way to restore the injured myocardium and reverse ventricular dilation induced cardiac dysfunction is a key goal for cardiac research. According to our findings, stem cell therapy may be a possible strategy for treatment of MI.

Mesenchymal stem cells represent a stem cell population present in adult tissues that can be isolated, cultured, and characterized *in vitro* and *in vivo*.[3,18,19] Some studies have demonstrated that MSCs can be detected at the site of implantation, can differentiate into cardiomyocytes, and effectively regenerate the myocardium.[1,17,20] Additionally, despite expressing major histocompatibility complex I (MHC I), MSCs have low levels of MHC II, meaning they do not initiate an allogeneic immune response,[21,22] making MSCs a promising stem cell candidate for cardiac cell therapy. In our study, LVEF in the MSC group was increased by 18% compared with LVEF in the saline group. The LVEDD and LVESD in the MSC group were attenuated by 11% and 9%, respectively, compared with the same measurement in the saline group. These findings indicate that MSC therapy effectively regenerates myocardium and improves cardiac function.

Even though implanted stem cells survived and regenerated myocardium at the site of injury, the site of MI is a poor environment for cell growth. To increase cell viability, some factors to improve such an infertile environment are required. There have been several studies of different scaffolds for cardiac tissue engineering,[23–25] but there has been little research focused on elastic, biodegradable scaffolds. An elastic scaffold can resist pressure-induced ventricular dilation while providing a structure that enhances the potential for excellent cell growth.

Unexpectedly, the differences in the LVEDD, LVESD, and LVEF between the MSC + PLCL group and the MSC group were not

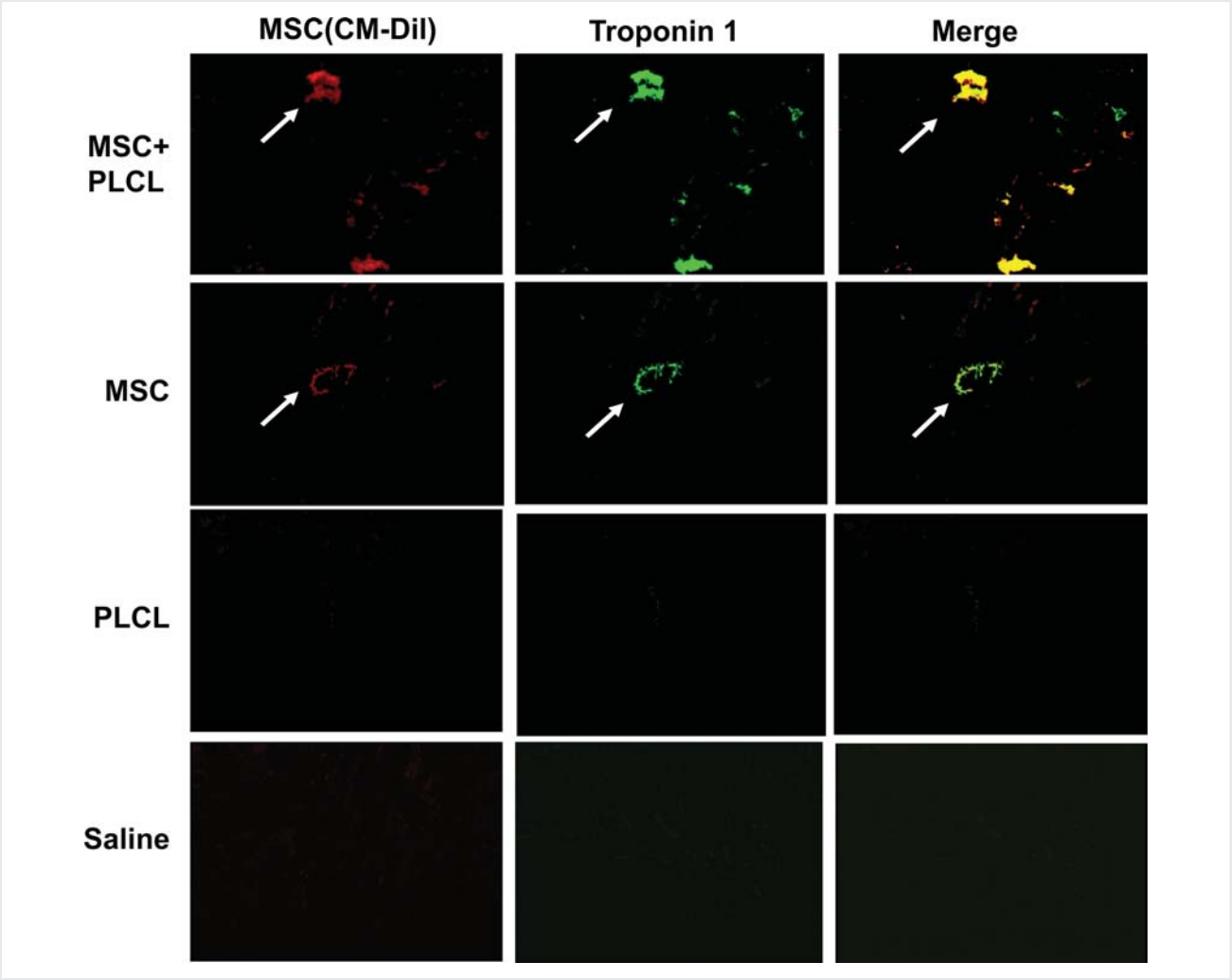

Figure 2 Fluorescent immunohistochemical staining of CM-DiI-labelled MSCs in infarcted myocardium for cardiac troponin I. CM-DiI-labelled MSCs appear red (white arrows); cardiac troponin I appears green (white arrows). Merged image shows transplanted MSCs differentiated into cardiomyocytes.

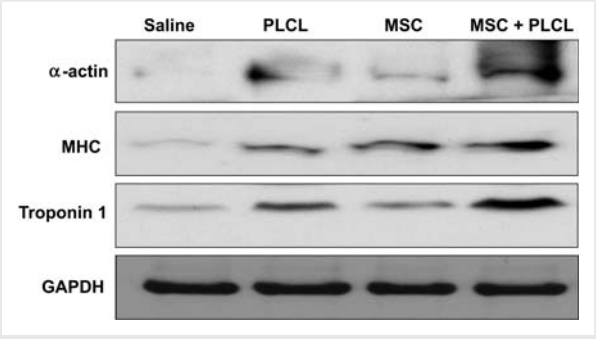

Figure 3 Western blot analysis of myosin heavy chain (MHC), α-actin, and troponin-I expression in injured myocardium. MHC, α-actin, and troponin-I were more abundantly expressed in the MSC + PLCL group than in the other three groups. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control.

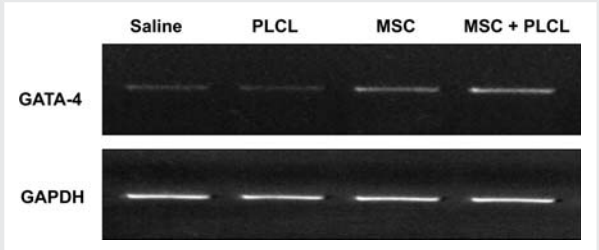

Figure 4 Reverse transcriptase–polymerase chain reaction analysis of GATA-4 expression in injured myocardium. GATA-4 was more abundantly expressed in the MSC + PLCL group than in the other three groups. GAPDH was used as a loading control.

statistically significant (6.7 ± 0.3 vs. 7.2 ± 0.2 mm, $P = 0.061$; 5.2 ± 0.3 vs. 5.7 ± 0.2 mm, $P = 0.17$; 52.5 ± 2.4 vs. $48.7 \pm 2.3\%$, $P = 0.073$). The lack of significance may be due to the fact that

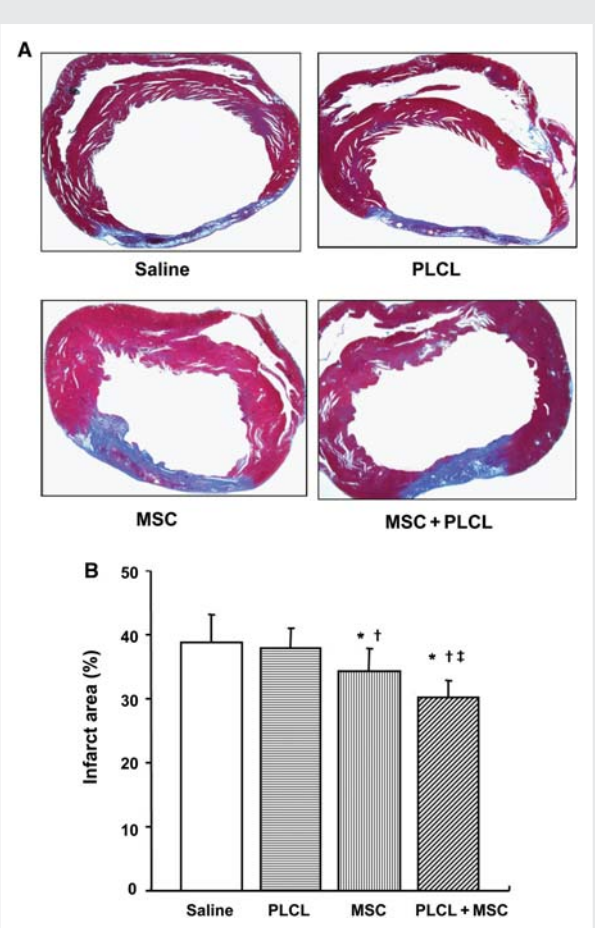

Figure 5 Effects of MSC + PLCL, MSC, and PLCL transplantation on myocardial infarct area. (*A*) Masson's trichrome staining showed the injured myocardium blue, and viable myocardium red. Infarct area in the MSC + PLCL group was smaller than that in other three groups. (*B*) Quantitative analysis of infarct area. Infarct area in the MSC + PLCL group was significantly reduced compared with the saline, PLCL, and MSC groups. The infarct area in the MSC group was also less than the saline and PLCL groups. $^{*}P < 0.05$ vs. saline group. $^{\dagger}P < 0.05$ vs. PLCL group. $^{\ddagger}P < 0.05$ vs. MSC group. Data shown are mean ± SEM.

our study involved only a small number of subjects (10 in each group) and used a relatively short-term time scale (4 weeks) for observation. With a larger sample size or different observation time points, it is likely that the increased effectiveness of the MSC + PLCL treatment observed in our study would become statistically significant.

In addition to regeneration of the myocardium, decreasing the size of the infarct is also an important step towards improving cardiac function. In this study, the infarct areas of the MSC + PLCL group and the MSC group were decreased by 29% and 18%, respectively, compared with those in the saline group. In addition, the infarct area in the MSC +PLCL group was significantly reduced compared with that in the MSC group (30.2 ± 2.6 vs. $34.2 \pm 3.6\%$, $P = 0.03$). Also, the MSC-seeded PLCL scaffold implantation expressed more cardiac markers (MHC, α-actin, troponin-I) and cardiac transcription factor (GATA-4) than the MSCs implanted without a scaffold. PLCL scaffold has some desirable physical characteristics including large pore size, interconnected pore structure, and a lack of skin layer on the external surfaces, all of which assist in induction of tissue formation via nutritional diffusion and cell migration.[11] These scaffold characteristics should increase the survival and differentiation of MSCs.

Unexpectedly, the cell-free PLCL group showed similar results to those of the saline group, which is not in agreement with the findings of another study. Piao *et al.*[26] demonstrated that cell-free scaffold implantation could affect the resident cardiac stem cells and recruit influent stem cells to the injured myocardium, showing that cell-free scaffolds also have a beneficial effect on MI. According to our knowledge, even if scaffolds have such positive effects, the implanted stem cells would be expected to play a key role in myocardial regeneration. In fact, many researchers doubt that the implanted stem cells are enough to directly affect myocardial regeneration and believe that the paracrine effects of the implanted cells are more likely to influence the growth of myocardium than any direct effect of the implanted stem cells. Our findings indicate that stem cells and scaffold in combination have synergistic effects and are more effective than stem cells or scaffolds alone.

Some reports have claimed that the appropriate biomaterials for cardiac tissue engineering should not have elastic characteristics because non-elastic scaffolds are not compliant with the ventricular pressure thus could resist ventricular dilation.[27] However, because of the heart's physiological characteristics of repeated contraction and relaxation, a non-elastic patch could resist the heart's natural motion. Therefore, a non-elastic scaffold would not be appropriate for cardiac tissue engineering.

In this study, we investigated only one elastic biodegradable scaffold in subjects with MI. Further study should investigate the difference between elastic and non-elastic scaffolds in myocardial regeneration. To our knowledge, this is the first report to evaluate the effects of BMMSC-seeded, elastic, biodegradable PLCL scaffolds in a rat MI model.

In conclusion, a BMMSC-seeded, elastic, biodegradable PLCL scaffold implant effectively attenuated LV dilation and improved LV function in a rat MI model. The PLCL served as a mechanical extracellular matrix where seeded BMMSCs survived and differentiated into cardiomyocytes, ultimately regenerating the myocardium and improving cardiac function. Therefore, the stem cell-seeded elastic biodegradable scaffold can be considered as an alternative therapeutic strategy for MI-induced heart failure.

Conflict of interest: none declared.

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References

1. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation* 2002;**105**:93–98.

2. Le Blanc K, Pittenger M. Mesenchymal stem cells: progress toward promise. *Cytotherapy* 2005;**7**:36–45.
3. Pittenger MF, Martin BJ. Mesenchymal stem cells and their potential as cardiac therapeutics. *Circ Res* 2004;**95**:9–20.
4. Leor J, Aboulafia-Etzion S, Dar A, Shapiro L, Barbash IM, Battler A, Granot Y, Cohen S. Bioengineered cardiac grafts: a new approach to repair the infarcted myocardium? *Circulation* 2000;**102**(Suppl. 3):III56–61.
5. Zimmermann WH, Fink C, Kralisch D, Remmers U, Weil J, Eschenhagen T. Three-dimensional engineered heart tissue from neonatal rat cardiac myocytes. *Biotechnol Bioeng* 2000;**68**:106–114.
6. Zimmermann WH, Melnychenko I, Eschenhagen T. Engineered heart tissue for regeneration of diseased hearts. *Biomaterials* 2004;**25**:1639–1647.
7. Dar A, Shachar M, Leor J, Cohen S. Optimization of cardiac cell seeding and distribution in 3D porous alginate scaffolds. *Biotechnol Bioeng* 2002;**80**: 305–312.
8. Christman KL, Vardanian AJ, Fang Q, Sievers RE, Fok HH, Lee RJ. Injectable fibrin scaffold improves cell transplant survival, reduces infarct expansion, and induces neovasculature formation in ischemic myocardium. *J Am Coll Cardiol* 2004;**44**: 654–660.
9. Li RK, Jia ZQ, Weisel RD, Mickle DA, Choi A, Yau TM. Survival and function of bioengineered cardiac grafts. *Circulation* **100**(Suppl.):II63–II69.
10. Shin M, Ishii O, Sueda T, Vacanti JP. Contractile cardiac grafts using a novel nanofibrous mesh. *Biomaterials* 2004;**25**:3717–3723.
11. Jeong SI, Kim SH, Kim YH, Jung Y, Kwon JH, Kim B-S, Lee YM. Manufacture of elastic biodegradable PLCL scaffolds for mechano-active vascular tissue engineering. *J Biomater Sci Polym Ed* 2004;**15**:645–660.
12. Xie J, Ihara M, Jung Y, Kwon IK, Kim SH, Kim YH, Matsuda T. Mechano-active scaffold design based on microporous poly(L-lactide-*co*-ϵ-caprolactone) for articular cartilage tissue engineering: dependence of porosity on compression force-applied mechanical behaviors. *Tissue Eng* 2006;**12**:449–458.
13. Burks CA, Bundy K, Fotuhi P, Alt E. Characterization of 75:25 poly(*l*-lactide-*co*-ϵ-caprolactone) thin films for the endoluminal delivery of adipose-derived stem cells to abdominal aortic aneurysms. *Tissue Eng* 2006;**12**: 2591–2600.
14. Jeong SI, Kim BS, Kang SW, Kwon JH, Lee YM, Kim SH, Kim YH. *In vivo* biocompatibilty and degradation behavior of elastic poly (L-lactide-*co*-ϵ-caprolactone) scaffolds. *Biomaterials* 2004;**25**:5939–5946.
15. Jeong SI, Lee AY, Lee YM, Shin H. Electrospun gelatin/poly(L-lactide-*co*-ϵcaprolactone) nanofibers for mechanically functional tissue-engineering scaffolds. *J Biomater Sci Polym Ed* 2008;**19**:339–357.
16. van den Bos EJ, Mees BM, de Waard MC, de Crom R, Duncker DJ. A novel model of cryoinjury-induced myocardial infarction in the mouse: a comparison with coronary artery ligation. *Am J Physiol Heart Circ Physiol* 2005;**289**:H1291–H1300.
17. Shake JG, Gruber PJ, Baumgartner WA, Senechal G, Meyers J, Redmond JM, Pittenger MF, Martin BJ. Mesenchymal stem cell Implantation in a swine myocardial Infarct model: engraftment and functional effects. *Ann Thorac Surg* 2002;**73**: 1919–1926.
18. Alhadlaq A, Mao JJ. Mesenchymal stem cells: isolation and therapeutics. *Stem Cells Develop* 2004;**13**:436–448.
19. Meirelles Lda S, Nardi NB. Murine marrow derived mesenchymal stem cell: isolation, *in vitro* expansion, and characterization. *Br J Haematol* 2003;**123**:702–711.
20. Xu W, Zhang X, Qiang H, Zhu W, Sun X, Hu J, Zhou H, Chen Y. Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype *in vitro*. *Exp Biol Med* 2004;**229**:623–631.
21. Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogeneic rejection. *J Inflamm* 2005;**2**:8.
22. Schuleri KH, Boyle AJ, Hare JM. Mesenchymal stem cells for cardiac regenerative therapy. *Handb Exp Pharmacol* 2007;**180**:195–218.
23. Kellar RS, Landeen LK, Shepherd BR, Naughton GK, Ratcliffe A, Williams SK. Scaffold-based three-dimensional human fibroblast culture provides a structural matrix that supports angiogenesis in infarcted heart tissue. *Circulation* 2001;**104**: 2063–2068.
24. Fukuhara S, Tomita S, Nakatani T, Fujisato T, Ohtsu Y, Ishida M, Yutani C, Kitamura S. Bone marrow cell-seeded biodegradable polymeric scaffold enhances angiogenesis and improves function of the infarcted heart. *Circ J* 2005;**69**: 850–857.
25. Zammaretti P, Jaconi M. Cardiac tissue engineering: regeneration of the wounded heart. *Curr Opin Biotechnol* 2004;**15**:430–434.
26. Piao H, Kwon JS, Piao S, Sohn JH, Lee YS, Bae JW, Hwang KK, Kim DW, Jeon O, Kim BS, Park YB, Cho MC. Effects of cardiac patches engineered with bone marrow derived mononuclear cells and PGCL scaffolds in a rat myocardial infarction model. *Biomaterials* 2007;**28**:641–649.
27. Siepe M, Giraud MN, Pavlovic M, Receputo C, Beyersdorf F, Menasche P, Careel T, Tevaearai HT. Myoblast-seeded biodegradable scaffolds to prevent post-myocardial infarction evolution toward heart failure. *J Thorac Cardiovasc Surg* 2006;**132**:124–131.