A new transmyocardial degradable stent combined with growth factor, heparin, and stem cells in acute myocardial infarction

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Aims We developed a new method—transmyocardial drilling revascularization (TMDR) with absorbable stent incorporated with basic fibroblast growth factor (bFGF) and heparin. The present study tested the effect of this method with transplantation of bone marrow-derived stem cells (BMSCs) in acute myocardial infarction.

Methods and results Infarction was produced in mini-swine by ligating the left anterior descending (LAD) coronary artery. TMDR of 3.0 mm in diameter was made by mechanical drilling in the infarcted area. The animals that had LAD ligation were divided into six groups according to the procedures followed (n = 6 in each): control; T (TMDR); C (cell implantation); TS (TMDR + stent implantation); TC (TMDR + cell implantation); TSC (TMDR + stent implantation + cell implantation). Left ventricular (LV) function, myocardial perfusion, vascular density, and histological and morphological analyses were evaluated pre-operatively and at 30 min and 6 weeks post-operatively. Six weeks after operation, the above indices were significantly better in the TSC group than in other groups (P < 0.001 compared with the control group, and P < 0.05 or 0.01 compared with the TS and TC groups), although TS and TC also showed better results than the control group (P < 0.05).

Conclusion We have demonstrated in a pig model that an intramyocardial stent implanted with slow release of bFGF, heparin, and BMSC transplantation may significantly increase LV function, cardiac blood flow, and vascular density. Therefore, the present study may provide a new method for the surgical treatment of myocardial infarction.

KEYWORDS
Myocardial infarction; Transmyocardial revascularization; Heparin; Basic fibroblast growth factor; Stem cell transplantation

1. Introduction
Bone marrow stem cells have been widely studied for the treatment of ischaemic heart disease for their possible effects in improving myocardial function1,2 and reversing ventricular remodelling.3 Foetal Flk1 + CD34−CD31− bone marrow-derived stem cells (BMSCs) have been proved to have haemangioblastic characteristics.4 However, the effect of cell transplantation is largely influenced by inadequate perfusion in the infarcted region.5 Basic fibroblast growth factor (bFGF) belongs to a family of heparin-binding growth factors which has been shown to greatly contribute to neovascularization6 and to maintain cell propagation.7 However, the effects of bFGF are largely reduced by the disadvantage of its short half-life in vivo. Protein-controlled release systems8 are now used to overcome such a disadvantage by releasing the protein persistently and providing a relatively steady concentration for a certain period.

Transmyocardial revascularization is a promising therapy for patients with end-stage coronary artery disease who are not eligible for surgical intervention and other conventional treatments. It has been reported that transmyocardial...
revascularization is effective in refractory angina because of its effect in angiogenesis. The main problem for transmyocardial revascularization, however, is occlusion of the transmural channels.

We therefore developed a new method—transmyocardial drilling revascularization (TMDR)—that combines a mechanical drilling procedure in the myocardium with the implantation of a stent, newly developed in our institution, into the hole made by TMDR. The new stent is composed of poly-γ, l-lactic/glycolic acid (PLGA) as a controlled bFGF release system. We expect that this new method may avoid laser-caused heat injury in the usual laser transmyocardial revascularization and that it will be effective in neovascularization. The present study was designed to test the hypothesis that the effect of this new method may be enhanced by Flk1+CD34−CD31− BMSCs transplantation.

2. Methods

Mini-swine weighing 25–30 kg were used in this study. All animal experiments were approved by Tianjin Administration Committee for Laboratory Animals. All animals received humane care, and experimental procedures were carried out strictly in compliance with the 1996 Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, USA, and the related ethical regulations of our university.

2.1 Flk1+CD34−CD31− BMSCs preparation and immunophenotyping analysis

Mini-swine were anaesthetized with intramuscular administration of ketamine (10 mg/kg) followed by an intravenous drip of sodium pentobarbital (30 mg/kg). Under general anaesthesia, bone marrow was aspirated from the anterior iliac crest with a syringe containing 6000 U heparin with a myeloid puncture needle. The marrow sample was diluted with phosphate-buffered saline (PBS). To separate BMSCs and other cells, the gradient centrifugation method was used: cells suspension was loaded onto 1.077 g/mL Ficoll solution in 15 mL centrifuge tubes; the tubes were centrifuged at 1500 r.p.m. for 10 min at 20°C. The white coat composed of mononuclear cells from the upper layer and interface was carefully collected into a tube and then diluted with two volumes of D-Hanks to remove the Ficoll. The cell pellet was then resuspended in culture medium. The cells were incubated in a 95% humidified incubator at 37°C and 5% CO₂.

The culture medium contained 58% DMEM/F12, 40% MCDB-201 (Sigma, St Louis, MO, USA), 2% foetal bovine serum (Gibco Life Technologies, Paisley, UK), 100 μg/mL penicillin, and 100 U/mL streptomycin sulfate (Gibco). After being incubated with these chemicals for 24–48 h, the suspension cell population was removed, and the adherent layer was washed with fresh medium every 3 days. The cells were then continuously cultured until ~90% confluence (approximately 1 week). Cells were harvested by trypsinization (0.25% tryspin) before transplantation.

For immunophenotyping analysis, cells were washed twice with PBS containing 0.5% bovine serum albumin (BSA, Sigma) and then resuspended in PBS and incubated with primary antibodies (10–20 ng/mL) against human CD34, Flk1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), CD44, CD31, CD29, CD105, CD106, and HLA-ABC (BD-PharMingen Biotechnology, CA, USA) for 30 min at 4°C. Cells were then washed with PBS containing 0.5% BSA and incubated with fluorescein isothiocyanate (FITC)-conjugated secondary antibodies for 30 min at 4°C. For intracellular antigen detection, cells were fixed in 2% paraformaldehyde for 15 min at 4°C and then permeabilized with 0.1% saponin (Sigma) for 1 h at room temperature. The same isotype irrelevant antibody of the same species was used as negative control. After being washed, cells were resuspended in PBS for fluorescence-activated cell sorting (FACS) analysis.

2.2 Cell label in vitro

The cells were washed with PBS twice and stained with red-orange fluorescent dye CM-Dil (3 μM, Invitrogen Corporation, CA, USA) according to the protocol of the supplier. Briefly, 2 × 10⁷ cells were incubated at 37°C for 5 min, and then for an additional 15 min at 4°C. After labelling, cells were washed with PBS and resuspended in 100 μL saline and kept on ice. The labelling efficiency reached 95%, as observed in vitro under a fluorescence microscope.

2.3 Preparation of bFGF and heparin anticoagulated degradable stent

In this study, recombinant human bFGF (molecular mass 17.4 kDa, purity >97%) was produced by R&D (USA). With a giant molecular material 50:50 PLGA (Chinese Academy of Sciences, Chengdu, China), a novel heparinized degradable polymer stent was developed in our institution. To sustain the shape of the stent, polyacaprolactone (PCL, Chinese Academy of Sciences) was used as inner layer of the stent, composed of PLGA, bFGF, and heparin sodium powders. This stent may continuously release bFGF and heparin for 4–6 weeks with gradual degradation of PLGA polymer as exothecium of the stent. The procedure was briefly performed as follows: PLGA 0.8 g, bFGF 100 μg, and heparin sodium 200 mg (150 μg/mg, Dingguo Biotechnology, Beijing, China) were dissolved in 10 mL dichloromethane solution. The liquor mixture was then spread into a thin and flexible film by casting technique and rolled into a hollow tube with an outside/inside diameter of 3.0 mm/2.8 mm. The tubular PLGA/PCL was then dried (25°C for 24 h) in vacuum and cut into 10 segments of 8 mm in length for each. Therefore, each stent approximately had 10 μg bFGF and 20 mg heparin sodium. The actual releasing curve for bFGF could not be obtained because the amount coated in the stent is too small to be detected in the solution by usual methods. However, in our institution (Institute of Bioengineering, Peking Union Medical College, Beijing, China), we have developed a standard releasing curve of proteins coated in the stent presented in this study by using BSA, as shown in Figure 1. The release of coated proteins may last for at least 25 days.

To facilitate blood permeation through stent wall, regular micropores were produced through the stent wall by mini-power drill equipped with 0.5 mm bit in diameter (Sandvik, Sweden). The stents were sterilized by Co⁶⁰ and kept at 4°C. Figure 2 (left panel, A) shows the actual stent and the device (right panel, B) to drill a hole in the myocardium.
2.4 Acute myocardial infarction model
Under general anaesthesia, mini-swine were intubated, and positive pressure ventilation was maintained. The heart was fully exposed via a median sternotomy. The mid-third of the left anterior descending (LAD) artery was ligated after three intermittent brief preconditioning occlusions, each for 5 min. A bolus dose of lignocaine was given intravenously (1 mg kg$^{-1}$) and was then maintained at 1 mg min$^{-1}$ kg$^{-1}$ by intravenous dripping. Following the successful establishment of the model, mini-swine ($n = 36$) were randomly assigned to six groups ($n = 6$ in each group) as follows:

Control group: had only LAD ligation;
Group T: LAD ligation immediately followed by TMDR;
Group C: LAD ligation immediately followed by cell implantation;
Group TS: LAD ligation immediately followed by TMDR + stent implantation;
Group TC: LAD ligation immediately followed by TMDR + cell implantation;
Group TSC: LAD ligation immediately followed by TMDR + stent implantation + cell implantation.

2.5 Stent implantation and cell transplantation
After coronary ligation, two transmyocardial tunnels were created through the left ventricle (LV) ischaemic area (in T, TS, TC, and TSC groups) by using a hollow drill bit with 3.0 mm diameter at 13 000 r.p.m. Immediately after the creation of the channel, a stent was manually implanted and fixed on the epicardium by a purse-string stitch prepared before this procedure (in TS and TSC groups). Cells ($2 \times 10^7$) in 100 μL saline were subsequently injected with a sterile microinjection at five sites in the ischaemic area (in the C group) or around the channels (in TC and TSC groups). The needle was advanced 5 mm into the myocardium and the cells were injected 1.5 mm away from the centre of the channel. Haemostasis was performed, and the chest was closed in layers. Post-operatively, penicillin G benzathine (30 000 U/day) was given intravenously for 3 days.

2.6 LV function
LV fractional shortening (FS%) was determined by an echocardiography system (Philips SONOS 7500, Agilent Technologies, Andover, MA, USA) equipped with a 1.6–3.2 MHz transducer, for the assessment of the LV function at 30 min and 6 weeks post-operatively. The echocardiographer was blinded to the experimental groups, and the echocardiographic images were obtained by directly placing the transducer on the epicardial surface to obtain the best images. This was performed before the closure of the chest and at 6 weeks by reopening the chest.

2.7 Assessment of myocardial perfusion
Myocardial perfusion was evaluated by intravenous injection of 14.8 MBq kg$^{-1}$ 99mTc- sestamibi (Institute of Atomic Energy, Beijing, China) at 30 min and 6 weeks post-operatively. Myocardial perfusion images were acquired at 6’ per frame, totally for 180’ by rotating a 64 x 64 matrix detector in a 20% energy window using ECG-gated single photon emission computed tomography (SPECT, GE Millenium vg-5, CT, USA). Quantitative analysis of changes of mass defect percentage (MDP) was performed by Emory Cardiac Toolbox software. MDP was used as an index of ischaemic myocardium mass [MDP = (Md/Mt) x 100%], where the mass of infarct-related defect myocardium (Md) and that of total myocardium (Mt) were calculated by tomographic reconstruction. Changes in MDP were calculated by values at 30 min and 6 weeks post-operatively.

Figure 1 In vitro release curve of BSA coated in the stent. A stent was placed in a test tube containing 3 mL PBS (0.15 M, pH 7.4). Each tube was attached at the paddle end of a rotating paddle apparatus (Sotax AT7, OSI, Paris, France) and agitated at a speed of 100 rad/min at 37°C for 5 weeks. At specified times, the solution was removed completely for the measurement of BSA by using the Coomassie brilliant blue assay; the tube was immediately filled with the same volume of fresh PBS again for the next measurement. Each measurement was repeated in three stents.

Figure 2 (A) A hollow tubular bilayer PCL/PLGA stent with 3.0 mm/2.8 mm (outside/inside diameter). (B) The drill (Beijing Kai Step Medical Equipment Technical Center) used for making transmyocardial channels in the present study. The drill is equipped with a hollow bit of 3.0 mm in diameter.
2.8 Immunohistochemistry and analyses of vascular density

Six weeks after operation, animals were anaesthetized as described previously and the hearts were removed. Tissue samples around the stent or channels were collected and fixed with 10% formaldehyde. The samples were then embedded and cut to 4 μm-thick sections. The cross-sections were stained with haematoxylin–eosin and Masson’s stain (Baso Biotechnology, Shenzhen, China). To evaluate the effect of combined therapy on angiogenesis, sections of each group were stained with rabbit anti-human von Willebrand factor (vWF) antibody (Dako Cytomation, CPH, Denmark) and anti-human smooth muscle actin (SMA, Dako) antibody counterstained with diaminobenzidine.

Five non-overlapping fields at ×100 magnification in transverse sections per sample were randomly captured with a video camera and digitized into tagged image file format. New vessels were quantified with Image Pro Plus 4.5 software package (Media Cybernetics, MD, USA). The positively staining areas were padded with a single colour and converted to pixels through optical density (OD) calibration.

2.9 Identification of the transplanted cells

Cells labelled with CM-DiI in vitro were examined under fluorescent microscopy. Immunofluorescence was then carried out with rabbit anti-human factor vWF antibody, anti-human SMA antibody, and anti-human heavy chain cardiac myosin antibody (Abcam Ltd, Cambridge, UK) to identify the grafted cells and their types. The red fluorescence-positive sections were followed by incubation with FITC-conjugated secondary antisera in myocardial paraffin sections. In this study, every antibody used has cross-reactivity with mini-swine. After rinsing with PBS, sections were observed and photographed under fluorescent as well as light microscopy.

2.10 Statistical analysis

Statistical analyses were performed with one-way analysis of variance followed by Bonferroni test or t-test when appropriate by using Statistical Product and Service Solutions 13.0 software package software analysis. Data (mean ± SD) were considered statistically significant at a value of $P < 0.05$.

3. Results

3.1 Morphous and immunophenotypes of mini-swine cells

After being cultured for 7 days, the cells appeared as colonies of large flat cells or spindle-like cells (Figure 3). FACS showed that the cells did not express haematopoietic markers CD34, CD45, and endothelial markers CD31. The proportion of Flk1+ cells was ~60%.

3.2 LV function

The LV function was described by FS%. FS% was significantly decreased after the ligation of the LAD (44.5 ± 2.8 vs. 26.6 ± 1.6%, $P < 0.001$; Figure 4A). There were no significant differences in baseline data among the six groups after acute myocardial infarction (AMI) (Figure 4B).

Six weeks after treatment, there were no differences among T (26.57 ± 1.92%), C (26.74 ± 1.68%), and control groups (24.61 ± 1.71%, $P > 0.05$). However, TC (30.78 ± 0.93%) and TS (31.13 ± 0.99%) groups had significantly higher FS, compared with the control group ($P < 0.001$; Figure 4B), although there were no differences between
TC and TS groups \( (P > 0.05) \). Importantly, the TSC group \( (33.46 \pm 1.13\%) \) showed further improvement compared with TC and TS groups \( (P < 0.05; \text{Figure 4B}) \).

3.3 Regional myocardial blood flow

The improvement of regional myocardial perfusion was reflected by MDP, as summarized in Figure 5. Six weeks after treatment, changes in MDP showed no differences between control \( (1.93 \pm 0.26\%) \) and T groups \( (2.06 \pm 0.20\%; P > 0.05) \). However, there was a significant decrease in C \( (-0.32 \pm 0.39\%) \), TS \( (-1.79 \pm 0.45\%) \), and TC \( (-1.74 \pm 0.40\%) \) groups, compared with control and T groups \( (P < 0.001) \). There was also a significant difference between C and TC groups \( (P < 0.001) \). Interestingly, there was a further decrease in the TSC group \( (-2.45 \pm 0.20\%) \), compared with C \( (P < 0.001) \), TS \( (P = 0.028) \), and TC \( (P = 0.014) \) groups, indicating that treatment with cell transplantation combined with TMDR and stent implantation (TSC) had significantly increased regional myocardial blood flow.

3.4 Vascular density

Findings in histological staining are shown in Figure 6. It was revealed that new vessel density showed no difference between T \( (OD = 2581 \pm 428 \text{ pixels/hpf}) \) and control groups \( (OD = 2434 \pm 442 \text{ pixels/hpf}, P = 1) \), and a significant increase in C \( (OD = 4416 \pm 408 \text{ pixels/hpf}) \), TS \( (OD = 6201 \pm 443 \text{ pixels/hpf}) \), TC \( (OD = 6157 \pm 291 \text{ pixels/hpf}) \), and TSC groups \( (OD = 6447 \pm 403 \text{ pixels/hpf}) \), compared with control and T groups \( (P < 0.001) \). Further, there were significant increases in TS, TC, and TSC groups, compared with the C group \( (P < 0.001) \). These results demonstrated that angiogenesis was induced by TMDR, bFGF stent, and cell transplantation after acute myocardial ischaemia and that the combined therapy may have better effect in promoting angiogenesis.

3.5 Histological analyses

Microscopically, there was fibrosis with scattered chronic inflammatory cells composed predominantly of lymphocytes around TMDR channels in the control group. In the groups with stent implantation, stent degradation was observed. Vessels were also seen in remnants of stents (Figure 7A). There was evidence of surviving myocardium around the stent by Masson’s stain in the TSC group (Figure 7B). The surrounding area of the channel showed an increase in the number of vessels within the stent and the area immediately surrounding the stent using factor vWF stain. These vessels were of varying sizes including capillaries, small arterioles, and larger arteries with several layers of smooth muscle stained by SMA (Figure 7C and D). Compared with the other three groups, the number of larger arteries and small arterioles significantly increased in the TSC group. Red blood cells were also seen in the vessels, indicating the existence of blood flow within the vessels.

3.6 Identification of the transplanted cells

We tracked the engraftment of transplanted undifferentiated cells 6 weeks after AMI. Paraffin sections within the core of the stent showed red fluorescence-positive cells distributed in all regions especially in and around the stents in the TSC group, whereas most transplanted cells were found in the peri-infarct regions in the TC group (Figure 8A and B). The distribution of red fluorescence-positive cells demonstrated survival of transplanted cells. Immunofluorescence of cross-sections of the cell-transplanted groups showed

Figure 5  MDP in the six groups. (A) Examples of myocardial SPECT perfusion images (LV short axis) in each group immediately before the chest closure (pre) and 6 weeks (post) post-operatively. (B) Comparison of changes of MDP in each group 6 weeks after treatment. There were no significant differences between control and T groups and between TS and TC groups. The MDP was significantly decreased in the C group compared with control and T groups, and significantly decreased in TS and TC groups compared with the C group. The MDP was further decreased in the TSC group compared with TS and TC groups. \( ^\star P < 0.05 \) vs. control and T groups; \( ^\# P < 0.05 \) vs. C group; \( ^* P < 0.05 \) vs. TS and TC groups. See Figure 4 for the abbreviations of the groups.
that red fluorescence- and vWF-antibody-positive cells were negative for heavy chain cardiac myosin and SMA. In many regions, the red fluorescence-positive spots were observed coincident with the green fluorescence spots of vWF antibody (Figure 8C–G), indicating that labelled BMSCs were actively differentiated into endothelial cells in vivo and formed network structure although they did not actively survive as myoblasts or smooth muscle cells. In contrast, sections from control, T, and TS groups had no evidence of red and green fluorescence.

4. Discussion

The present study for the first time demonstrated that bone marrow-derived Flk1+CD34−CD31− stem cell transplantation may enhance the myocardial angiogenesis in addition to the effect of the newly developed bFGF and heparin degradable stent in the animal model of AMI.

Cell transplantation provides a promising therapeutic option for patients with ischaemia and LV dysfunction, although there are still many unknown aspects. It has been
Figure 7  Histological findings 6 weeks after treatment. (A) Remnant of the stents (asterisk) and new vessels (arrow) are shown (haematoxylin–eosin staining, magnification ×200). (B) Survival myocardium surrounded the stent in the TSC group is shown (Masson’s staining, magnification ×200). (C) Vessels increased around the stent (factor vWF staining, magnification ×100) is shown. (D) Vessels of varying sizes are shown. Note arteries with several layers of smooth muscle in the TSC group (SMA staining, magnification ×100). The length of scale bars in (A) and (B) represents 30 μm; in (C) and (D) 60 μm (the scale proportion is enlarged to three-fold). See Figure 4 for the abbreviations of the groups.

Figure 8  Distribution and differentiation of transplanted cells. (A) Red fluorescence-positive cells distributed in and around the stent in the TSC group. (B) Most transplanted cells were in the peri-infarction regions in the TC group (magnification ×100). (C) Survived transplanted cells labelling with red fluorescence are factor vWF-positive. (D) Merged picture of (B) and (C) (magnification ×100). (E and F) Cells in the TSC group. (G) Merged picture of (E) and (F) (magnification ×200). The length of scale bars in (A)–(D) represents 60 μm; in (E)–(G) 30 μm (the scale proportion is enlarged to three-fold). See Figure 4 for the abbreviations of the groups.
suggested that the infarcted region may not be adequate for the survival of transplanted cells. In the present study, we transplanted Flk1+CD34−CD31− BMSCs with the combination of TMDR and a new controlled release system for bFGF and heparin. Our study has shown that this new method is more effective than any single method to improve the LV function and the survival of the transplanted cells. Our results also suggest the importance of angiogenesis in cell transplantation therapy. Previous studies have shown that transmyocardial laser revascularization may be effective for patients with refractory angina and end-stage coronary disease. However, disadvantages such as the heat injury to surrounding myocardium and fibrosis formation in the channel limited its application. We therefore developed this new method of TMDR, using mechanical drilling instead of laser and creating larger diameter (3.5 mm) channels in order to overcome the shortages in conventional transmyocardial laser revascularization as mentioned earlier. Our preliminary studies (data not shown) already demonstrated that TMDR was effective in angiogenesis with little damage to the myocardium. In addition, by implanting the bFGF and heparin-incorporated stent, the transmyocardial channels are kept patent at least for 6 weeks, and neovascular network formation in the vicinity of the channel is further promoted. In the present study, transplanted cells were found in and around the stent, with some of them even in the scar tissue. We speculate that the following mechanisms may account for these results. First, TMDR in 3.5 mm diameter with stent implantation supplies more blood flow to the ischemic region. Second, bFGF has strong effect on angiogenesis by stimulating the proliferation of endothelial and smooth muscle cells. Therefore, our new method by promoting angiogenesis may increase perfusion in the ischemic zone and provide adequate oxygen and nutrition to help transplanted cell survival. In fact, BMSCs secretes a wide array of arteriogenic cytokines contributing to collateral remodeling. It has been demonstrated that the intra-myocardial injection of stem cells prolonged the upregulation of vascular endothelial growth factor (VEGF) expression, which might contribute to further enhancement of angiogenesis. Third, we used the bFGF and heparin stent as a controlled release system that may slowly release bFGF and heparin for 6–8 weeks. As bFGF is a family of heparin-binding growth factors and the activation of fibroblast growth factor (FGF) receptors is sensitive to heparin, the use of heparin may prolong biological effect of bFGF by stabilizing its molecular conformation. The present study has clearly indicated that myocardial perfusion and heart function in the experimental group with the combination of the aforementioned factors were better than those in any other groups.

Flk1+CD31−CD34− cells as a type of pluripotent stem cells derived from foetal bone marrow could differentiate into endothelial cells and haematopoietic cells, suggesting that these cells may have potential application for vascular diseases. Flk1 as the receptor of VEGF plays an important role in the differentiation of Flk1+CD31−CD34− cells into the endothelial cell. The morphological data of the present study confirmed, by the identification of red fluorescence-positive cells, that the transplanted cell survived in the stenting and the surrounding area. In our experiments, these cells were observed to be aligned and tightly juxtaposed with host endothelial cells, as demonstrated by immunofluorescence, although immunohistological co-staining as a means of defining cell type has some limitations, as there could be potential false positives.

4.1 Clinical implications
In severe coronary artery disease, patients may have myocardial infarction as the result of the occlusion of some coronary artery branches. In the infarcted area, the number of living myocardial cells is reduced and the contractility of this area is therefore compromised, causing myocardial dysfunction. By using the combined methods presented in the present study, restoration of myocardial blood flow will be greatly benefited. It is also possible that the contraction of the myocardium may be further enhanced by the stem cell transplantation. However, this needs to be further investigated. Therefore, this combined method may open a new area of treatment for severe coronary artery disease.

In conclusion, we have demonstrated in a pig myocardial infarction model that combined use of bFGF and heparin-incorporated stent through TMDR and BMSCs transplantation may significantly increase LV function, cardiac blood flow, and vascular density. The effect of BMSC transplantation is synergistic with that of bFGF and heparin-incorporated stent through TMDR. Therefore, the present study may provide a new method for the surgical treatment of myocardial infarction.

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References


