Degradable scaffolds combined with basic fibroblast growth factor for myocardial angiogenesis☆

Zhao Jian[1], Cheng Zhao-yun[1], Lü Feng[2], Liu Tian-jun[2], Liu Xiao-cheng[3]

Abstract

BACKGROUND: Studies have shown that basic fibroblast growth factor has effects on stimulating vessel regeneration and collateral reconstruction. However, administration was performed mostly by peripheral vein, left atrium or percutaneous coronary intervention, and it is difficult to achieve an effective therapeutic concentration in the local myocardium.

OBJECTIVE: Based on the property of poly(D, l-lactic-coglycolic acid) (PLGA), to investigate outcomes of inducing neovascularization in the myocardium in combination of basic fibroblast growth factor (bFGF) by ensuring target release of protein growth factor in local tissue.

METHODS: PLGA and bFGF were dissolved in dichloromethane. This liquid mixture was rolled into the form of a hollow tube (3.0 mm outer diameter, 2.8 mm inner diameter, 0.1 mm thick, 10 mm length) for further use. The middle third of the left anterior descending coronary artery of mini-swines was ligated, and the local myocardium became dark purple. After the successful establishment by abnormal regional wall motion in the cardiac apex at anterior wall using ultrasound, the mini-swines were assigned to channels and bare scaffolds (BS) group and channels and bFGF-incorporating scaffolds (FS) group. The scaffold was implanted in the myocardium using self-made hollow bit. At 6 weeks, the number of proliferative cells was quantified by immunohistochemical staining. New vessels were quantified utilizing Image-Pro Plus software package in both groups. Quantitative analysis of changes in mass defect percentage was performed by Emory Cardiac Toolbox software combined with single-photon-emission computed tomography.

RESULTS AND CONCLUSION: At 6 weeks, number of proliferative cells and the density of new vessels were significantly increased in the FS group compared with BS group ($P < 0.001$). Single-photon-emission computed tomography illustrates that MDP was significantly lower in the FS group compared with the BS group ($P < 0.001$). Results have suggested that PLGA scaffolds that incorporate bFGF were able to induce angiogenesis and enhance blood-flow perfusion.

[1]Department of Cardiovascular Surgery, Henan Provincial People's Hospital, Zhengzhou 450003, Henan Province, China; [2]Tianjin Key Laboratory of Biomaterial Research, Institute of Biomedical Engineering, Chinese Academy of Medical Sciences, Tianjin 300193, China; [3]Department of Cardiovascular Surgery, TEDA International Cardiovascular Hospital, Tainjin 300457, China

Zhao Jian☆, Doctor, Attending physician, Department of Cardiovascular Surgery, Henan Provincial People's Hospital, Zhengzhou 450003, Henan Province, China
doctorzhao66@126.com

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INTRODUCTION

Patients who are poor candidates for coronary artery bypass grafting surgery or percutaneous coronary intervention have few options to restore cardiac perfusion[1-2]. Preliminary clinical experience with therapeutic angiogenesis has suggested that this new therapeutic strategy, "angiogenic biologic bypass[3-4]", may provide additional blood flow for underperfused regions, and thus be valuable in the management of these patients.

Basic fibroblast growth factor (bFGF), as a pluripotent mitogenic polypeptide for fibroblasts, smooth muscle cells, and vascular endothelial cells, is involved in neovascular formation[5], scar contraction[6], and collateral development[7]. Nonetheless, the short biological half-life of free bFGF *in vivo*[8] limits its ability to continuously induce therapeutic angiogenesis. In this circumstances, slow-release carrier systems for bFGF have been explored[9-10]. However, these delivery strategies to achieve clinically significant angiogenic responses are controversial.

Recently, we have developed a biodegradable scaffold composed of poly(D, l-lactic-coglycolic acid) (PLGA) to enable bFGF to be released at the site of action for an extended time period. The present study was designed to evaluate whether this bFGF-incorporating scaffold promotes therapeutic angiogenesis around the scaffold and to further improve blood-flow perfusion of ischemic myocardium.

MATERIALS AND METHODS

Design

A randomized, controlled, animal study.

Time and setting

This study was performed at the Department of Cardiovascular Surgery, Henan Provincial People's Hospital, Tianjin Key Laboratory of Biomaterial Research, Institute of Biomedical Engineering, Chinese Academy of Medical Sciences, and Department of Cardiovascular Surgery, TEDA International Cardiovascular Hospital, China from May 2008 to July 2009. Protocols were performed in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of the People's Republic of China in 2006[11].

Materials

Animals

A total of 14 mini-swines were secured for the experiment. All experimental animals were cared for in accordance with institutional guidelines and with the 1996 "Guide for the Care and Use of Laboratory Animals," published by the National Institutes of Health (NIH publication 85–23, revised 1996). Two of the 14 mini-swines were subsequently excluded because of intractable ventricular fibrillation after ligation.

Methods

Preparation of the models

A total of 12 mini-swines survived for the subsequent

procedures. These remaining animals, weighing 25 to 35 kg, were anesthetized intramuscularly with ketamine (10 mg/kg) and midazolam (0.2 mg/kg). After oral endotrachial intubation, anesthesia was maintained with 1% to 2% inhaled isoflurane. The 12 subjects were monitored *via* electrocardiography. Three million units of penicillin were given intravenously before skin incision. Under sterile conditions, the heart was fully exposed *via* a median sternotomy. The middle third of the left anterior descending coronary artery was ligated after three intermittent periods, and brief preconditioning occlusions were performed to prevent malignant ventricular dysrhythmias. Lidocaine was given by direct intravenous injection (1 mg/kg) and then by intravenous drip 1 mg/min per kg. After the successful establishment of the model by left anterior descending coronary artery occlusion, the mini-swines were assigned to 2 groups (*n* = 6) at random: channels and bare scaffolds (BS) group, and channels and bFGF-incorporating scaffolds (FS) group.

Preparation of scaffolds

In this study, a porous hollow biodegradable polymer scaffold (Figure 1) was developed. It was composed of a 50: 50 moL ratio of polylactic to glycolic acid (Chinese Academy of Science, Chengdu, China). The procedure was briefly performed as follows: 0.8 g PLGA and 150 μg bFGF (molecular mass, 17.4 kD; purity >97; R & D Systems, Minneapolis, MN, USA) were dissolved in a 10-mL solution of dichloromethane. Subsequently, this liquid mixture was spread into a thin film and then rolled into the form of a hollow tube (outer diameter, 3.0 mm; inner diameter, 2.8 mm) by a mandrel casting technique. It was then dried (25 ℃ for 24 hours) in a vacuum and cut into 10 segments of 10 mm each in length. Therefore, each segment (or scaffold) had 15 μg bFGF. In BS group, the bare scaffolds had no bFGF. A total of 16 regularly aligned micropores were produced within each scaffold wall by a mini power drill equipped with a 1.0-mm bit (Sandvik, Sandviken, Sweden). The scaffolds were then sterilized by cobalt-60 radiation and maintained at 4 ℃.

Channel-producing procedure

Within 6 hours of the onset of infarction (that is, ligation of the left anterior descending coronary artery), we drilled transmural channels in the myocardium using self-made hollow bit of 3.0 mm in diameter. The depth of the channel was monitored by echocardiography. Two channels in each heart were created and then the scaffolds were immediately implanted into the channels. Bleeding was controlled by shallow epicardial pursestring stitches, which also served as markers of the channel sites 6 weeks later. In the BS group, the entire procedure, excluding the scaffold placement, was performed. The chest was closed in layers in routine fashion, and anesthesia was reversed. Antibiotics were administered intramuscularly for 3 days postoperatively.

Assessment of myocardial perfusion

Myocardial perfusion was evaluated by intravenously injecting 99mTc-sestamibi (14.8 MBq/kg) before implantation and again 6 weeks postoperatively. Myocardial perfusion images were acquired at 6° per frame, totally for 180° by rotating a 64×64 matrix detector in a 20% energy window using echocardiographicgated single-photon-emission computed tomography (SPECT, Millennium VG-5, GE Healthcare; Chalfont St. Giles, UK), with reconstruction parameters as follows: pre-filter, Butterworth; critical frequency, 0.52; and power, 5.0. Quantitative analysis of changes in mass defect percentage (MDP) was performed by Emory Cardiac Toolbox software (Syntermed, Inc., Atlanta, GA). Mass defection of percentage was used as an index of perfusion defect: MDP = (myocardial defect/total myocardium) ×100. The mass of ischemic-related myocardial defect and that of total myocardium were calculated by tomographic reconstruction. Changes in MDP were calculated as MDP at 6 weeks postoperatively minus baseline MDP before implantation.

Histological staining

At 6 weeks postoperatively, the animals were sacrificed with an overdose of potassium chloride, and their hearts were harvested for histological analysis. The locations of the channels were readily identified by the sutures on the epicardial surface. Heart samples were immediately immersed into 4% formaldehyde in phosphate-buffered saline of pH 7.4 at 4 ℃ for 24 hours. After fixation, the samples were embedded in paraffin and sectioned in 5-μm-thick slices. Routine staining was performed utilizing hematoxylin-eosin. Vascular endothelial cells were identified by von Willebrand factor immunohistochemical staining, which was performed as follows: the sections were incubated with 1: 100 von Willebrand factor antibody (Dako Denmark A/S, Glostrup, Denmark) in 0.1% phosphate-buffered saline for 1 hour, and then counter-stained with diaminobenzidene. A total of 50 non-overlapping fields per group were randomly captured with a video camera at 100 × magnification in transverse sections and then digitized into tagged-image file format. New vessels were quantified utilizing Image-Pro Plus 4.5 software package (Media Cybernetics, Inc., Bethesda, MD). The positively stained areas were padded with a single color and converted to pixels through optical density calibration. In addition, tissue sections were stained with Ki-67 antibody (Dako Denmark A/S, Glostrup, Denmark) to reveal the number of proliferating cells. A total of 50 non-overlapping fields per group were randomly captured at × 400 magnification in transverse sections.

Main outcome measures

Endpoints included proliferating cells counts for Ki-67 antigen and local vessel counts for von Willebrand factor. Myocardial perfusion was documented by nuclear scanning.

Statistical analysis

The data were presented as Mean ± SD. *t*-test for comparison between both groups was used to evaluate differences in vascular density, proliferating cells, changes in MDP. Statistical tests were two-tailed and $P < 0.05$ was regarded as statistically significant.

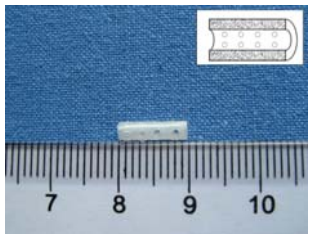
RESULTS

Death and injury assessment

Aside from the 2 mini-swines that were excluded from the experiment because of intractable ventricular fibrillation after ligation, there were no subsequent problems with injury, and no deaths. Scaffold implantation did not induce severe events involving malignant arrhythmias, embolization, bleeding, and so on.

Histological analysis

In the FS group, the hematoxylin-eosin images displayed a large number of new vessels. There also occurred some small vesiculous polymer remnants, which appeared to act as pro-angiogenic cores and to provide a suitable platform for the attachment of endothelial cells and for vascular remodeling. Perivascular spindle-like endothelial cells formed round or oval lumina with the passage of red-stained blood components, although the original lumina of the scaffolds were almost completely obliterated. In Ki-67 immunohistological staining, the number of proliferative cells in the FS group (21.3 ± 3.6 cells/ high-power field, $P < 0.001$) significantly increased compared with the BS group (13.7 ± 3.6 cells/ high-power field). Image-Pro Plus software analysis revealed that vascular density in the FS group (5 934 ± 313 pixels/ high-power field, $P < 0.001$) increased significantly in comparison with the BS group (2 655 ± 373 pixels/ high-power field) (Figures 1–4).



Inset: the scaffold in profile

Figure 1 Poly(D, L-lactic-coglycolic acid) scaffold is 10 mm in length, with 16 regularly aligned micropores

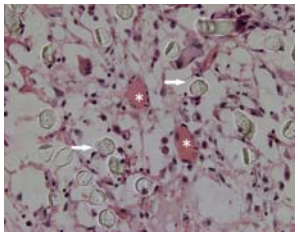
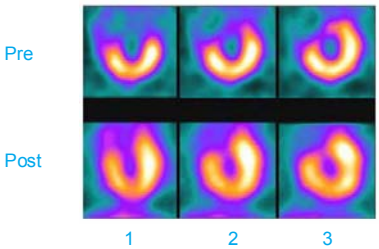


Figure 2 New vessels within the basic fibroblast growth factor-incorporating scaffold group (Hematoxylin-eosin staining,×200)



1: vertical short axis; 2: horizontal long axis; 3: vertical long axis

Figure 3 Representative single photon emission computed tomography images in basic fibroblast growth factor-incorporating scaffold group

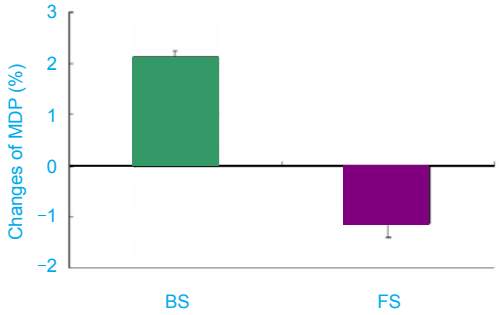


Figure 4 Changes in mass defect percentage (MDP) of channels and bare scaffolds (BS) and channels and bFGF-incorporating scaffolds (FS) groups, as indicated by Emory Cardiac Toolbox™ software analysis

SPECT evaluation

Figure 3 illustrates SPECT measurements for all groups. The SPECT images showed perfusion improvements in the FS group at 6 postoperative weeks, compared with perfusion before therapy. Figure 4 illustrates that MDP was significantly lower in the FS group (- 1.12% ± 0.28%, $P < 0.001$), compared with the BS group (2.12% ± 0.13%).

DISCUSSION

Many experimental and clinical trials concerning angiogenic therapy using protein formulations have been reported to induce coronary collateral blood supply to the ischemic myocardium[12]. However, these therapies have many new and presently unsolved problems. The most important limitation is thought to be the limited tissue half-life of growth factors. In view of this problem, gene therapy may be preferred. However, gene approach may be hampered by unexpected expression *in vivo*. Based on this consideration, our delivery system using PLGA scaffolds can fulfill an extended and predictable tissue exposure coinciding with the period of vascular formation[13], by adjusting the content ratio of polylactic acid to glycolic acid[14]. Degradable polymeric materials, including hydrogel[15], chitosan hydrogel[16], alginate[17], collagen[18], and PLGA, were used as carriers for local delivery of the bioactive agent. Of these, PLGA, due to its superior biodegradability and biocompatibility *in vivo*, has been approved by the U.S. Food and Drug Administration and is widely used as a controlled-release carrier of various exogenous agents. In this study, PLGA scaffolds are almost completely degraded, which did not cause pharmacological side-effect responses.

With the aid of the PLGA carrier, bFGF continuously acts in both mitogenic and chemoattractant roles to induce S-stage cellular proliferations and extracellular matrix ingrowth. Previous studies have shown that both recruited and proliferative cells—whether monocytes and macrophages[19] or endothelial cells and fibroblasts[20]—are involved in vascular reconstruction[21]. We observed the same result in sites where scaffolds had been implanted. Consequential to the proliferative "seed cells[22]", therapeutic angiogenesis was significant, largely attributable to the revascularization of underperfused myocardium. This observation was also supported by the

results of high-resolution SPECT assessments.

This study initially designed a hollow tubular scaffold to mimic myocardial sinusoids, directly conveying oxygenated blood to ischemic tissue. However, the channels were eventually obliterated due to the recurrent cardiac squeezing. Considering this limitation, by further fabricating bilayer-degradable scaffolds, composed of inner layer of poly (ε-caprolactone) material with good elasticity and strength, and outer layer of PLGA loaded with growth factors, we investigate neovascular development under the pressure gradient from patent channels.

In conclusion, PLGA scaffolds that incorporate bFGF were able to induce neovascular formation, enhance blood-flow perfusion, although the original scaffold channels were eventually occluded.

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缓释降解支架复合碱性成纤维细胞生长因子诱导心肌血管再生的作用☆

赵 健[1]，程兆云[1]，吕 峰[2]，刘天军[2]，刘晓程[3]([1]河南省人民医院心血管外科，河南省郑州市 450003；[2]中国医学科学院天津生物医学工程研究所生物医学材料重点实验室，天津市 300193；[3]天津泰达国际心血管病医院心血管外科，天津市 300457)

赵 健☆，男，1978年生，河南省济源市人，汉族，2008年北京协和医学院毕业，博士，主治医师，主要从事冠心病心肌血运重建的研究。

摘要

背景：研究证实碱性成纤维细胞生长因子具有刺激血管再生及侧支重建的作用，但是，以往多通过外周静脉、左心房或冠脉介入途径给药，心肌局部难以达到有效治疗浓度。

目的：基于高分子材料聚乳酸/乙醇酸的降解特性，复合碱性成纤维细胞生长因子，保证蛋白生长因子在局部组织中靶向释放，观察其诱导心肌血管再生效果。

方法：以聚乳酸、乙醇酸为原料，二氯甲烷溶解后加入重组人碱性成纤维细胞生长因子，通过模具塑形，制成外径/内径分别为3.0/2.8 mm、壁厚0.1 mm、长度10 mm的圆柱形中空管状支架备用。于小型猪冠脉前降支中、远端1/3交界处阻断，局部心肌颜色变为暗紫色，超声观察前壁心尖局部室壁运动异常证实模型制作成功，随机分至空白支架对照组和碱性成纤维细胞生长因子支架组，支架通过自主设计的机械打孔装置植入。6周后，免疫组织化学染色量化分析血管重建的增殖细胞数量，并结合Image Pro Plus软件量化各组新生血管密度，SPECT结合软件Emory Cardiac Toolbox分析灌注缺损区域质量百分率的变化。

结果与结论：6周后碱性成纤维细胞生长因子支架组增殖细胞数量、新生血管密度较空白支架组显著增加($P < 0.001$)，心肌核素显像显示灌注质量缺损百分率较空白支架组显著减少($P < 0.001$)。结果证实，复合碱性成纤维细胞生长因子的聚乳酸/乙醇酸支架能够显著增加新生血管密度，进而改善缺血部位心肌血流灌注。

关键词：心肌缺血；药物缓释；碱性成纤维细胞生长因子；支架；血管再生；生物医用可降解高分子材料

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