Prevention of arterial graft spasm in rats using a vasodilator-eluting biodegradable nano-scaled fibre[†]

Kei Yagami^a, Aika Yamawaki-Ogata^a, Makoto Satake^b, Hiroaki Kaneko^b, Hideki Oshima^a,
Akihiko Usui^a, Yuichi Ueda^a and Yuji Narita^{a,*}

^a Department of Cardiac Surgery, Nagoya University Graduate School of Medicine, Nagoya, Japan

^b Technology Innovation Center, Teijin Limited, Hino, Japan

* Corresponding author. Department of Cardiac Surgery, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan. Tel: +81-52-7442376; fax: +81-52-7442383; e-mail: ynarita@med.nagoya-u.ac.jp (Y. Narita).

Received 16 October 2012; received in revised form 28 January 2013; accepted 8 February 2013

Abstract

OBJECTIVES: Arterial graft spasm occasionally causes circulatory collapse immediately following coronary artery bypass graft. The aim of this study is to evaluate the efficacy of our developed materials, which were composed of milrinone (phosphodiesterase III inhibitor) or diltiazem (calcium-channel blocker), with nano-scaled fibre made of biodegradable polymer to prevent arterial spasm.

METHODS: Milrinone- or diltiazem-releasing biodegradable nano-scaled fibres were fabricated by an electrospinning procedure. *In vivo* milrinone- or diltiazem-releasing tests were performed to confirm the sustained release of the drugs. An *in vivo* arterial spasm model was established by subcutaneous injection of noradrenalin around the rat femoral artery. Rats were randomly divided into four groups as follows: those that received 5 mg of milrinone-releasing biodegradable nano-scaled fibre (group M, $n = 14$); 5 mg of diltiazem-releasing biodegradable nano-scaled fibre (group D, $n = 12$); or those that received fibre without drugs (as a control; group C, $n = 14$) implanted into the rat femoral artery. In the fourth group, sham operation was performed (group S, $n = 10$). One day after the implantation, noradrenalin was injected in all groups. The femoral arterial blood flow was measured continuously before and after noradrenalin injection. The maximum blood flow before noradrenalin injection and minimum blood flow after noradrenalin injection were measured.

RESULTS: *In vivo* drug-releasing test revealed that milrinone-releasing biodegradable nano-scaled fibre released 78% of milrinone and diltiazem-releasing biodegradable nano-scaled fibre released 50% diltiazem on the first day. The ratios of rat femoral artery blood flow after/before noradrenalin injection in groups M (0.74 ± 0.16) and D (0.72 ± 0.05) were significantly higher than those of groups C (0.54 ± 0.09) and S (0.55 ± 0.16) ($P < 0.05$).

CONCLUSION: Noradrenalin-induced rat femoral artery spasm was inhibited by the implantation of milrinone-releasing biodegradable nano-scaled fibre or diltiazem-releasing biodegradable nano-scaled fibre. These results suggested that our materials might be effective for the prevention of arterial graft spasm after coronary artery bypass graft.

Keywords: Spasm • Milrinone • Diltiazem • Drug delivery system • Electrospinning

INTRODUCTION

Coronary artery bypass graft (CABG) is among the most frequently performed cardiac surgical procedures in Western countries [1]. Arterial grafts of, for instance, the internal thoracic artery (ITA) is used for the bypass graft in most cases, because it has excellent documented clinical results and satisfactory short- and long-term patency rates [2–4]. Meanwhile, arterial graft-related complications are relatively infrequent; however, arterial graft spasm causes severe adverse complications (e.g. circulatory collapse, graft failure) after CABG. To prevent arterial graft spasm, vasodilators, such as calcium (Ca) blockers (e.g. diltiazem, verapamil) and phosphodiesterase (PDE) inhibitors (e.g. milrinone,

papaverine), have been administered locally to arterial grafts during harvesting and anastomosis, and systemically administered during or after surgery [3]. However, local administration of vasodilators has a short-term effect and may not prevent post-operative spasm. Moreover, systemic administration of Ca blockers sometimes causes adverse effects such as depression of cardiac function [5]. Therefore, locally sustained administration of a vasodilator would be an ideal strategy for preventing perioperative arterial spasm.

We developed novel sustained drug-releasing materials composed of biodegradable polymers with a vasodilator for the prevention of arterial spasm. The sustained release of the vasodilator was accomplished by hydrolysis of the biodegradable polymer. We chose to use milrinone and diltiazem as vasodilators because local administration of these drugs has been evaluated in previous studies [3, 6–8]. The aim of this study was to

[†]Presented at the 26th Annual Meeting of the European Association for Cardio-Thoracic Surgery, Barcelona, Spain, 27–31 October 2012.

evaluate the efficacy and safety of milrinone-releasing biodegradable nano-scaled fibres (MRBNFs) and diltiazem-releasing biodegradable nano-scaled fibres (DRBNFs) for the prevention of arterial spasm in a rat femoral arterial spasm model.

MATERIALS AND METHODS

Animals

The animals were cared for in accordance with the 'Guide for the Care and Use of Laboratory Animals,' published by the US National Institutes of Health (Publication 85-23, National Academy Press, Washington, DC, revised in 1996). All procedures involving animals were approved by the Animal Experiment Advisory Committee of the Nagoya University School of Medicine. In this study, we used male C57BL6 mice 4–8 weeks old, and male Sprague–Dawley (SD) rats 4–8 weeks old, which were purchased from the Chubu Kagaku Shizai Corporation (Nagoya, Japan).

Fabrication of drug-releasing biodegradable nano-scaled fibre

We have been developing a drug-containing biodegradable nano-scaled fibre, using an electrospinning technique to achieve a local and sustained release of drugs [9–11]. In this study, we applied this technology for the controlled release of vasodilators to prevent arterial spasm. We chose to use milrinone (Wako Jyunyaku) and diltiazem (Wako Jyunyaku) as vasodilators, because the local administration of milrinone is clinically performed during bypass surgery for the prevention and treatment of arterial spasms, in the ITA, gastroepiploic artery (GEA) and radial artery (RA) [7]. Systemic administration of diltiazem is also commonly used in bypass surgery to prevent spasm [12].

The method of fabricating drug-releasing biodegradable nano-scaled fibre was described in previous studies [9, 10, 13, 14]. Briefly, milrinone or diltiazem was mixed in a biodegradable polymer. Poly-L-lactide-co-glycolide, which consists of ~50% poly lactide and 50% poly glycolide, was prepared as the biodegradable polymer. The polymer solution was filled in a 1-ml syringe with a right angle-shaped metal capillary attached to it. The circular orifice of the capillary had an inner diameter of 1.2 mm. A flat counter electrode was located 35 cm from the capillary tip. Pressure was applied to the solution in the syringe, gradually forcing the piston to maintain a steady flow of solution from the capillary outlet. The flow rate of the polymer solution was 0.3 ml/min. The applied voltage was in the range of 10–15 kV. The fibres released in the atmosphere were electrostatically removed and trapped with a rod-shaped collector. The nano fibres ranged from 100 to 800 nm in diameter. The shape of the MRBNF and DRBNF showed a 'cotton-wool'-like formation (Fig. 1). This configuration was flexible and easy to handle for any type of arterial graft. The milrinone and diltiazem content in the fibre was 1.0 wt%. Figure 2 shows the scanning electron microscopic findings of our materials.

Drug-releasing test

We performed a 'milrinone- or diltiazem-releasing test' to examine the degree of drug release from MRBNF and DRBNF *in*

Figure 1: Overall appearance of a biodegradable nano-scaled fibre. This material had a 'cotton wool'-like appearance.

vivo. Five milligrams of MRBNF or DRBNF were implanted into the subcutaneous space of a C57BL6 mouse (Chyubu Kagaku Shizai Corporation, Nagoya) under general anaesthesia. Then, we took out only MRBNF or DRBNF at postoperative days 1 ($n = 3$), 2 ($n = 3$), 3 ($n = 3$) and 7 ($n = 3$), and measured the quantity of milrinone or diltiazem which remained in MRBNF or DRBNF by the use of high-performance liquid chromatography (HPLC) [15, 16].

Surgical protocol: establishment of rat femoral arterial spasm models

Rat femoral arterial spasm models were established by injection of 0.1 mg/0.1 ml of noradrenalin [(NA) Daiichi Sankyo Seiyaku, Japan] to the subcutaneous tissue around the femoral artery of the rats. We measured the blood flow of the femoral artery by using a Pulse-Doppler rheometer (SCPD-20, Prime Tech Co., Tokyo, Japan) before and after NA injection. The detailed procedure for the measurement of femoral arterial blood flow including the timing of NA injection is described as follows: the femoral arterial blood flow was measured continuously before and after NA injection. Because it was quite difficult to obtain stable rat femoral blood flow, the probe was fixed using a micro manipulator (UMM-3c, NARISHIGE, Tokyo, Japan) at the point at which maximum blood flow could be obtained before NA injection. NA was injected into the femoral subcutaneous tissue with the probe kept fixed. Then, we determined the minimum blood flow during measurement.

The right or left inguinal region of the rat was cut and dissected to expose the femoral artery. Experimental groups were divided into four groups: Group M was implanted with 5 mg of MRBNF into the femoral artery ($n = 14$), Group D was implanted with 5 mg of DRBNF ($n = 12$), Group C was implanted only with biodegradable nano-scaled fibre without drugs as a control ($n = 14$) and a sham operation was performed for Group S ($n = 10$), after which the wounds were closed. Some cases in each group (1 for group M, 3 for group D and 1 for group C) were omitted because it was impossible to obtain arterial blood flow due to instability of the blood flow. A summary of the surgical protocol and the timing of blood flow measurement is given in Fig. 3.

As for determining the dosage of milrinone, reportedly 90% of the vascular relaxation for a vasoconstricted rabbit artery was

Figure 2: Scanning electron microscope views of an electrospun biodegradable nano-scaled fibre, (A) without drug, $\times 200$, scale bar = $50\text{ }\mu\text{m}$, (B) $\times 1000$, scale bar = $10\text{ }\mu\text{m}$, (C) MRBNF, $\times 200$, scale bar = $50\text{ }\mu\text{m}$, (D) $\times 1000$, scale bar = $10\text{ }\mu\text{m}$, (E) DRBNF, $\times 200$, scale bar = $50\text{ }\mu\text{m}$, (F) $\times 1000$, scale bar = $10\text{ }\mu\text{m}$. MRBNF: milrinone-releasing biodegradable nano-scaled fibre; DRBNF: diltiazem-releasing biodegradable nano-scaled fibre.

Figure 3: Surgical protocol and timing of blood flow measurement.

induced by potassium chloride (KCl), and relaxation was provided by a milrinone concentration of $100\text{ }\mu\text{g/ml}$ [17]. Therefore, at least $20\text{ }\mu\text{g}$ of milrinone is required for a fixed period,

assuming that the volume of the circumference tissue of the rat femoral artery (RFA) is $200\text{ }\mu\text{l}$. The dose of milrinone in this study was considered, and because MRBNF released $\sim 95\%$ milrinone in 2 days, we decided to implant 5 mg of $1\text{ wt}\%$ MRBNF containing $50\text{ }\mu\text{g}$ of milrinone. Regarding the determination of dosage, diltiazem promoted vascular relaxation for the vasoconstriction status of porcine gastropiploic artery induced by NA *in vitro*. That relaxation provided a concentration of $\sim 200\text{ }\mu\text{g/ml}$ [18]. Knowing that DRBNF released $\sim 60\%$ diltiazem in 2 days, we decided to implant 5 mg of $1\text{ wt}\%$ DRBNF containing $50\text{ }\mu\text{g}$ of diltiazem. Twenty-four hours after operation (implantation), femoral arterial blood flow (ml/min) was measured before and after NA injection. As for the comparison among the groups, we calculated the ratio of the femoral arterial blood flow after NA injection, which indicated post-NA injection blood flow/pre-NA injection blood flow (defined as the 'ratio of maintained blood flow').

Evaluation of adverse effects

To verify any adverse effects that would be induced by the systemically circulated drugs, and to confirm the safety of our materials, the materials were implanted in the backs of the rats. The serum concentration of drugs in rats was measured by HPLC. Blood examination including complete blood count, and liver function [serum concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT)], renal function [serum concentration of blood urea nitrogen (BUN) and creatinine (Cre)] and pancreatic enzyme (amylase; AMY) tests were also performed in these rats at 1 ($n=3$), 2 ($n=3$), 3 ($n=3$) and 7 ($n=3$) days after material implantation. The results were compared with those of sham-operated rats.

Statistical analysis

The statistical analyses were performed using Graph Pad Prism software programs. Comparisons of pre- and post-NA injection blood flow were made using a paired Student's *t*-test. The difference in the ratio of blood flow among the groups was determined by a one-way factorial analysis of variance with Bonferroni correction. Experimental results are expressed as mean $\pm$ standard error, with a *P* value of <0.05 was considered to be statistically significant.

RESULTS

All animals survived to the end of our protocol, and no signs of infection were detected.

Drug release from materials

An *in vivo* (a mouse subcutaneous) release of the drugs from the materials (MRBNF and DRBNF) is shown in Fig. 4. MRBNF released 78% of its milrinone in a day. Furthermore, 95% of its milrinone was released by the second day. On the other hand, DRBNF released 50% of its diltiazem in a day, and 60% by the

second day. Because almost all cases of arterial graft spasm occur within 24 h after operation [19], we considered that the drug-releasing kinetics would be suitable for the prevention of arterial graft spasm.

Rat femoral artery spasm model

All groups (groups M, D, C and S) had a significant decrease in blood flow by NA injection when compared with pre-injection (Fig. 5, Table 1). Within each group, there was no significant difference between pre-NA blood flow. The 'ratio of maintained blood flow' of group M (0.74 ± 0.16) and D (0.72 ± 0.05) was significantly higher than that of groups C (0.54 ± 0.09) and S (0.55 ± 0.16) ($P < 0.05$) (Fig. 6). On the other hand, there was no significant difference in the ratio of the maintained blood flow in groups M and D, and in groups C and S. These results revealed that MRBNF and DRBNF showed similar preventive effects for experimental arterial spasm.

Verifying adverse effects of materials

There was no difference between the material-implanted group and the sham operation group in terms of complete blood count, blood concentration of liver enzymes (AST and ALT), blood renal function test (BUN, Cre) and leakage of blood pancreatic enzymes (AMY). There was no blood concentration of drugs in either group (Table 2).

DISCUSSION

Insufficient arterial graft flow caused by arterial graft spasm after CABG could increase perioperative morbidity and mortality. Arterial graft spasm from bypass surgery is mainly caused by mechanical stimuli associated with graft manipulation at harvesting, and anastomosis. In addition, perioperative changes in graft temperature and the dosage of adrenoceptor agonists cause endothelium disorder and the release of the endogenous vasoconstriction factor [20]. Therefore, surgeons must take special

Figure 4: (A) Milrinone releasing test. The longitudinal axis of this graph indicates the remaining percentage of milrinone in MRBNF (%). The abscissa axis indicates days. MRBNF eluted 95% milrinone in 2 days. MRBNF: milrinone-releasing biodegradable nano-scaled fibre. (B) Diltiazem-releasing test. The longitudinal axis of this graph indicates the remaining percentage of diltiazem in DRBNF (%). The abscissa axis indicates days. DRBNF eluted 60% diltiazem in 2 days. DRBNF: diltiazem-releasing biodegradable nano-scaled fibre.

Figure 5: Blood flow of the femoral artery pre- and post-injection of NA. The longitudinal axis of the graph indicates blood flow of rats’ femoral artery (ml/min). The abscissa axis indicates pre- and post-NA injection. By NA injection, blood flow significantly declined in every group (NA: noradrenalin).

Table 1: Blood flow of rat femoral artery (RFA) (ml/min)

	Blood flow of RFA		P value
	Pre-injection NA	Post-injection NA	
group M	3.29 ± 0.92	2.40 ± 0.72	0.05
Group D	3.37 ± 0.17	2.41 ± 0.11	0.05
Group C	3.20 ± 0.80	1.72 ± 0.59	0.05
Group S	3.73 ± 0.85	2.02 ± 0.57	0.05

NA: noradrenalin.

Figure 6: Post/pre-injection NA blood flow ratio. The longitudinal axis of the graph indicates the ratio of blood flow after/before NA injection (%). Groups M and D were significantly higher than groups C and S.

care when handling arterial grafts and adjusting temperature, and must avoid drying and contact with the blood ingredient to prevent spasm [21]. On the other hand, we have a pharmacological approach for preventing graft spasm, for instance by filling up vasodilators such as papaverine or milrinone in an

arterial graft lumen, and by placing a vasodilator-soaked gauze around the outside of the arterial graft. Furthermore, systemic administration of diltiazem or nicorandil has been used for the prevention of spasm, because both are nitric oxide donors and adenosine triphosphate-sensitive potassium-channel openers [3]. However, topical use of a vasodilator cannot prevent post-operative arterial graft spasm because of the short duration of a drug’s effect. Moreover, systemic administration of a vasodilator decreases systemic blood pressure and/or causes bradycardia. Therefore, we consider placement of ‘vasodilator-sustained-releasing materials’ the ideal strategy for preventing perioperative arterial spasm without systemic adverse side effects.

Milrinone is a specific inhibitor of phosphodiesterase III, and exerts its pharmacological effect by increasing cyclic adenosine monophosphate levels in the myocardium and vascular smooth muscle cells, which causes an increase in the contractility of heart muscle and reduction in peripheral vascular resistance. Although milrinone was developed for the treatment of heart failure, it has also been used as an arterial graft vasodilator in CABG [6–8, 20, 21]. There is a report asserting milrinone is more effective than other PDE III inhibitors (amrinone and olprinone) for the relaxation of the internal thoracic arterial graft, which is most frequently used as an arterial graft in CABG [8]. On the other hand, systemic infusion of diltiazem, which was started prior to harvesting, provided increased internal thoracic arterial blood flow by prevention spasm [12]. Diltiazem relaxes the contraction of the ITA induced by KCL, or human urotensin II and diltiazem are more effective than glyceryl trinitrate for preventing the contraction *in vitro* [22]. Therefore, we consider that locally sustained release of these agents might be effective in preventing arterial graft spasm.

Although various agents with biodegradable polymers have been used for establishment of a drug delivery system (DDS) by using an electrospinning procedure [9, 10, 23], few reports have focused on the area of cardiovascular diseases. Therefore, it may be important and meaningful to describe here the electrospinning DDS used for cardiovascular treatment. The advantages of

Table 2: Blood examination after implantation of material

	Control (n = 3)	Day 1 (n = 3)	Day 2 (n = 3)	Day 3 (n = 3)	Day 7 (n = 3)	P value
WBC	3767 ± 203	5600 ± 603	4000 ± 300	4100 ± 306	3567 ± 233	0.11
RBC	765 ± 3.2	809 ± 27	772 ± 28	820 ± 5.6	785 ± 4.9	0.27
Hb	13.3 ± 0.1	13.3 ± 0.1	13.0 ± 0.2	13.5 ± 0.5	13.4 ± 0.4	0.42
PLT	73.3 ± 0.89	66.7 ± 8.2	76.8 ± 8.8	81.3 ± 2.7	80.2 ± 10.9	0.66
AMY	714.3 ± 6.3	711.7 ± 20.7	729 ± 11.3	770.7 ± 26.3	754 ± 42.1	0.4
BUN	20.9 ± 0.15	24.2 ± 0.41	19.2 ± 0.48	20.1 ± 1.15	20.7 ± 1.7	0.1
Cr	0.29 ± 0.01	0.27 ± 0.003	0.28 ± 0.01	0.3 ± 0.02	0.29 ± 0.02	0.41
AST	105.6 ± 11.3	85.3 ± 4.3	102.3 ± 24.1	93.7 ± 5.2	74.3 ± 4.1	0.25
ALT	57.6 ± 7.3	43.7 ± 5.8	49.7 ± 9.6	55.7 ± 6.7	36.3 ± 1.9	0.33
Drugs	Not detected	Not detected	Not detected	Not detected	Not detected	

WBC: white blood cell; RBC: red blood cell; Hb: haemoglobin; plt: platelet; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; Cr: creatinine; AMY: amylase; NS: non-significant.

this strategy are as follows: (i) it does not require a complex apparatus to create the materials; (ii) it has a topical/local effect that minimizes toxic side effects; and (iii) our materials have a peculiar configuration (cotton wool-like formation), and so they can flexibly fit any type of artery (ITA, GEA and RA) or anastomosis site (such as end-to-side anastomosis). On the other hand, the disadvantage of this strategy is that surgical procedures are necessary to implant the materials. Therefore, conversely, these materials may be useful tools in addition to surgical intervention.

It is important to understand how many doses are required, and over how long a period the dose of the drug is released from the materials. It is considered that the drug release ratio will depend on the degradation period of the polymer and the content of the drug in the material. Therefore, it can be controlled by the type of biodegradable polymer and the ratio of co-polymerization (if a co-polymer was used). However, although both our materials (MRBNF and DRBNF) are composed of the same co-polymer (PLA: PGA = 50:50) and the drug content is the same (1 wt%), the drug release patterns are quite different. These differences may depend on the characteristics of the drugs such as solubility of the drug, water solubility or liposolubility. Further study is necessary to optimize the release ratio of each drug.

There are some limitations in our study.

- (i) We first used a rat femoral arterial spasm model induced by NA injection. The femoral artery is not one of the peripheral arteries used as a bypass graft (e.g. ITA, GEA and RA).
- (ii) NA-induced enforced arterial spasm is not a physiological arterial graft spasm.

Our murine model did not reflect human arterial graft spasm after CABG. Therefore, for clinical practice, we considered that a large animal experiment would be necessary to confirm the reproducibility of our results and also closely resemble a clinical setting including the use of the ITA.

CONCLUSION

Our materials (MRBNF and DRBNF) could inhibit the reduction of arterial blood flow in the NA injection-induced rat femoral arterial spasm model. These results suggested that our materials

might be useful for the prevention of perioperative arterial graft spasm in bypass surgery.

Funding

The authors gratefully acknowledge the grant support from a Grant-in-Aid for Science Research (no. 21659328) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and the Japan Foundation for Clinical Pharmacology.

Conflict of interest: none declared.

REFERENCES

- [1] Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB *et al.* Writing Group Members. Heart disease and stroke statistics—2012 update: a report from the American Heart Association. *Circulation* 2012; 125:e2–20.
- [2] Montgomery D, Spence PA, Hansen SB, Randsbek F, Santamore WP. Arterial bypass graft spasm: an examination of the role of high flow demands and endothelial function in the porcine GEA. *Eur J Cardiothorac Surg* 1996;10:12–19.
- [3] Attaran S, John L, El-Gamel A. Clinical and potential use of pharmacological agents to reduce radial artery spasm in coronary artery surgery. *Ann Thorac Surg* 2008;85:1483–9.
- [4] Grondin CM, Campeau L, Lespérance J, Enjalbert M, Bourassa MG. Comparison of late changes in internal mammary artery and saphenous vein grafts in two consecutive series of patients 10 years after operation. *Circulation* 1984;70:1208–12.
- [5] Shapira OM, Alkon JD, Macron DS, Keaney JF Jr, Vita JA, Aldea GS *et al.* Nitroglycerin is preferable to diltiazem for prevention of coronary bypass conduit spasm. *Ann Thorac Surg* 2000;70:883–9.
- [6] Garcia-Rinaldi R, Soltero ER, Carballido J, Mojica J. Intraluminal milrinone for dilatation of the radial artery graft. *Tex Heart Inst J* 1999;26:189–91.
- [7] Liu JJ, Doolan LA, Xie B, Chen JR, Buxton BF. Direct vasodilator effect of milrinone, an inotropic drug, on arterial coronary bypass grafts. *J Thoracic Cardiovasc Surg* 1997;114:302–4.
- [8] Onomoto M, Tsuneyoshi I, Yonetani A, Suehiro S, Matsumoto K, Sakata R *et al.* Differential pharmacologic sensitive of phosphodiesterase-3 inhibitors among human isolated gastroepiploic, internal mammary, and radial arteries. *Anesth Analg* 2005;101:950–6.
- [9] Mutsuga M, Narita Y, Yamawaki A, Satake M, Kaneko H, Suematsu Y *et al.* A new strategy for prevention of anastomotic stricture using tacrolimus-eluting biodegradable nanofiber. *J Thorac Cardiovasc Surg* 2009;137:703–9.

- [10] Yamawaki-Ogata A, Hashizume R, Satake M, Kaneko H, Mizutani S, Moritan T *et al.* A doxycycline loaded, controlled-release, biodegradable fiber for the treatment of aortic aneurysm. *Biomaterials* 2010;31:9554–64.
- [11] Wadagaki R, Mizuno D, Yamawaki-Ogata A, Satake M, Kaneko H, Hagiwara H *et al.* Osteogenic induction of bone marrow-derived stromal cells on simvastatin-releasing, biodegradable, nano-microscale fiber scaffolds. *Ann Biomed Eng* 2011;39:1872–81.
- [12] Tabel Y, Hepguslar H, Erdal C, Catalyurek H, Acikel U, Elar Z *et al.* Diltiazem provides higher internal mammary artery flow than nitroglycerin during coronary artery bypass grafting surgery. *Eur J Cardiothorac Surg* 2004;25:553–9.
- [13] Chakraborty S, Liao IC, Adler A, Leong KW. Electrohydrodynamics: a facile technique to fabricate drug delivery systems. *Adv Drug Deliv Rev* 2009;61:1043–54.
- [14] Vasita R, Katti DS. Nanofibers and their applications in tissue engineering. *Int J Nanomed* 2006;1:15–30.
- [15] Brocks DR, Spencer TJ, Shayeganpour A. A sensitive and specific high performance liquid chromatographic assay for milrinone in rat and human plasma using a commercially available internal standard and low sample volume. *J Pharm Pharmacol Sci* 2005;8:124–31.
- [16] Bhamra RK, Ward AE, Holt DW. HPLC measurement of diltiazem and desacetyldiltiazem in serum or plasma. *Biomed Chromatogr* 1987;2:180–2.
- [17] Saitoh M, Sato S, Taguchi K, Asano M. Vasodilatory effect of milrinone in isolated rabbit aorta and canine saphenous vein. *Kiso to Rinsho* 1996;30:999–1006.
- [18] Kim DC, Chen D, Perrotta VJ, Shuman CA, Wendling WW, Harakal C *et al.* Porcine gastroepiploic artery as an in vitro experimental model to study vasodilators in microsurgery. *Ann Plast Surg* 1996;36:502–7.
- [19] Lorusso R, Crudeli E, Lucà F, De Cicco G, Vizzardi E, D'Aloia A *et al.* Refractory spasm of coronary arteries and grafted conduits after isolated coronary artery bypass surgery. *Ann Thorac Surg* 2012;93:545–51.
- [20] Hayashida N, Kawara T, Tomoeda H, Oda T, Akasu K, Kosuga T *et al.* Influence of milrinone on internal mammary artery grafts. *Kyobu Geka* 1999;52:993–7.
- [21] Yaginuma G, Abe K, Ottomo M, Okada Y, Ota K, Fujimori S *et al.* Use of the radial artery graft in coronary artery bypass grafting: harvesting technique and spasm prevention. *Kyobu Geka* 1998;51:823–8.
- [22] Bai XY, Liu XC, Yang Q, Tang XD, He GW. The interaction between human urotensin II and vasodilator agents in human internal mammary artery with possible clinical implications. *Ann Thorac Surg* 2011;92:610–6.
- [23] Chen P, Wu QS, Ding YP, Chu M, Huang ZM, Hu W. A controlled release system of titanocene dichloride by electrospun fiber and its antitumor activity in vitro. *Eur J Pharm Biopharm* 2010;76:413–20.