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Protocols

Coaxial electrospinning multicomponent functional controlled-release vascular graft: Optimization of graft properties

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ABSTRACT

Small diameter vascular grafts possessing desirable biocompatibility and suitable mechanical properties have become an urgent clinic demand. Herein, heparin loaded fibrous grafts of collagen/chitosan/poly(L-lactic acid-*co*-ε-caprolactone) (PLCL) were successfully fabricated via coaxial electrospinning. By controlling the concentration of heparin and the ratio of collagen/chitosan/PLCL, most grafts had the heparin encapsulation efficiency higher than 70%, and the heparin presented sustained release for more than 45 days. Particularly, such multicomponent grafts had relative low initial burst release, and after heparin releasing for 3 weeks, the grafts still showed good anti-platelet adhesion ability. In addition, along with the excellent cell biocompatibility, the fabricated grafts possessed suitable mechanical properties including good tensile strength, suture retention strength, burst pressure and compliance which could well match the native blood vessels. Thus, the optimized graft properties could be properly addressed for vascular tissue application via coaxial electrospinning.

1. Introduction

To fabricate a functional small diameter vascular graft that possesses desirable biocompatibility and suitable mechanical properties has become an urgent clinic demand. Synthesized polymeric materials of Dacron and expanded poly tetrafluoroethylene (ePTFE) have been successfully applied in clinics as large-diameter vascular grafts [1,2], yet they are failed to be utilized as small diameter vascular grafts due to the acute thrombogenicity and poor mechanical properties [3].

Grafts loading with heparin have been studied over the past decades in order to promote the antithrombotic performance [4–9]. After graft implantation, sustained release of heparin is desired during the entire endothelialization process of the lumen. Unfortunately, fast release of heparin and incomplete endothelialization are the main obstacle in current vascular grafts design, which could ultimately result in the graft occlusion.

Coaxial electrospinning is a facile technique which could make core-shell fibrous graft with drugs encapsulated into the inside of the fibers, inducing prolonged release time in vivo [7,10–13]. In these studies, poly(L-lactic acid-*co*-ε-caprolactone) (PLCL) grafts could load various drugs by coaxial electrospinning, and drugs could be sustained with relatively lower initial burst release compared to PLCL grafts blended with drugs. However, drug releasing study showed that most of the drugs were fully released within two weeks, indicating further research is still needed for better controlled release. It is likely that multicomponent grafts loaded with drugs constructed by coaxial electrospinning might be able to improve the release behavior through adjusting the degradation rate of polymers and diffusion rate of drugs. So it is speculated that heparin would be encapsulated into multicomponent vascular grafts fabricated by coaxial electrospinning with controlled released to achieve long-term antithrombotic performance in vivo. Apart from anti-thrombosis property, it is also very important to have desired mechanical properties for a vascular graft, which is crucial for the performance of graft after implantation. A functional vascular graft should possess enough strength to withstand arterial pressure as well as be elastic to match the compliance of native blood vessels [14–16].

In our previous study, electrospun collagen/chitosan/PLCL nanofibrous graft had been fabricated which had great physical properties and biocompatibility [17]. However, the antithrombotic property of the graft had not been considered comprehensively. In this paper, we fabricated a heparin incorporated collagen/chitosan/PLCL vascular graft with both good antithrombotic performance and good mechanical properties. Heparin with different concentration encapsulated into the vascular graft was

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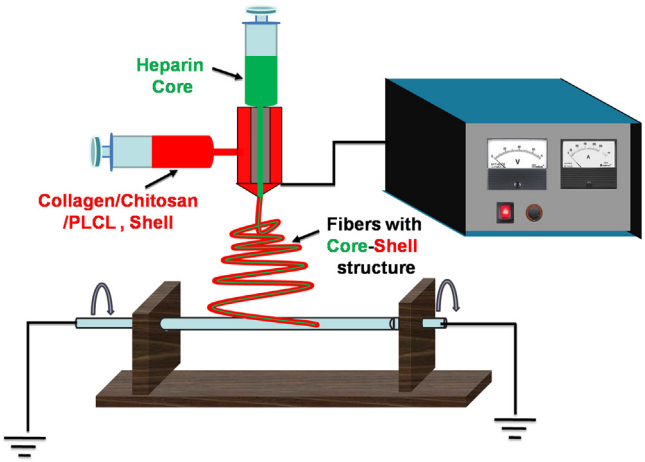

Fig. 1. Schematic diagram of the coaxial electrospinning process.

fabricated by coaxial electrospinning, and the effect of different concentration of collagen/chitosan/PLCL and heparin on the structure of fibrous grafts was further investigated in detail. Meanwhile, heparin release behavior and endothelial cells growth in vitro were examined. In addition, the mechanical properties of grafts in terms of tensile strength, suture retention strength, burst pressure, and compliance were also measured.

2. Materials and methods

2.1. Materials

Poly(L-lactic acid-*co*-ε-caprolactone) (PLCL) polymer (MW: 300,000 Da, LA to CL mole ratio at 50:50, Gunze Limited, Japan) and collagen type I (MW ~10^5 Da, Sichuan Minrang Biotechnology, China) were dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, TCI America) at 80 mg/mL, respectively. Chitosan (molecular weight ~10^6 Da, 85% deacetylated, Jinan Haidebei Marine Bioengineering, China) was dissolved in HFP and 2,2,2-trifluoroacetic acid (TFA) (Sinopharm Chemical Reagent, China) (v/v, 9:1) at 80 mg/mL. Heparin (13 kDa, Runjie Medicine Chemical, China) was dissolved in distilled water at different concentration. All reagents used for cell culture were purchased from Gibco Life Technologies, USA, unless stated otherwise.

2.2. Fabrication of grafts by coaxial electrospinning

Before electrospinning, the source solutions (collagen, chitosan and PLCL) were blended at different volume ratios of collagen/chitosan/PLCL. Heparin-loaded grafts were fabricated using a special setup depicted in Fig. 1. For coaxial electrospinning, the core solution (heparin) was injected at a flow rate of 0.2 mL/h which constituted the inner layer of the fibers, while the shell solution (collagen/chitosan/PLCL) was injected at a rate of 1.0 mL/h which formed the outer layer of the fibers. The needle tip was subjected to +12 kV with an air gap distance of 12 cm between the needle and the grounded aluminum foil collector. To fabricate the tubular grafts, the collector was a solid cylindrical stainless mandrel with 4 mm in diameter. The deposited fibrous sheet or conduit was dried in a vacuum oven at room temperature for up to 7 days to remove any residual solvents.

2.3. Morphology characterization of the graft

The morphology characterization was performed using scanning electron microscopy (SEM, JEOL JSM-5610LV, Japan). SEM micrographs were analyzed with a software Image-J (National Institutes of Health). The average fiber diameter was determined by measuring 50 randomly selected fibers in the SEM image. Calibration of the Image Tool software was achieved by using the scale bar on each image. Verification of the core-shell structure of the heparin-loaded fiber was conducted by TEM (H-800, Hitachi) at 100 kV. The sample was prepared by collecting the fibers onto carbon-coated copper grids.

2.4. Mechanical properties of grafts

2.4.1. Uniaxial tensile testing

For tensile testing, specimens in a "dog bone" shape were punched from electrospun mats (sample size: 2.75 mm wide at their narrowest point with a length of 7.5 mm) and were hydrated in PBS for 6 h before testing. Uniaxial tensile testing was performed on a MTS Bionix 200 testing system with a 100 N load cell (MTS Systems Corp.) at an extension rate of 10.0 mm/min. Modulus, peak stress, and strain at break were calculated using Test Works version 4 (software).

2.4.2. Suture retention strength

Suture retention strength was measured with a rectangular test sample (10 mm in width/20 mm in length). Before testing, one end of the graft was clamped to one arm of the micro material testing machine (MMT-250N, Shimadzu Co., Japan). A loop of a 5-0 polyester suture (Shanghai Pudong Jinhuan Medical Products Co., Ltd., China) was placed 2 mm from the edge of the free end of the sample and clamped to the other arm which moved at a constant speed of 120 mm/min until failure. The suture retention strength was defined as the peak force obtained during the procedure. All samples were kept hydrated throughout the testing protocols.

2.4.3. Dynamic compliance

Dynamic compliance was determined for tubular grafts with a length of 4 cm under simulated physiological conditions in accordance with Section 8.10 of ANSI/AAMI VP20:1994.32,33 [3]. Grafts were soaked in PBS for 6 h before testing. The specimens were tested in a bioreactor developed by Tissue Growth Technologies (Minnetonka, MN) filled with PBS. The bioreactor provided a cyclic (1 Hz, representing 60 beats per minute) pressure change to the inside of the graft at a pressure level of 120/80 mmHg systolic/diastolic. Prior to compliance measurements, all grafts were allowed to stress relax for 600 cycles. Internal pressure was measured with a pressure transducer capable of measuring dynamic pressure up to 200 ± 2 mmHg, while the external diameter of the graft was recorded with a laser micrometer system with an accuracy of ±0.001 mm. Compliance was calculated through recording of pressure and inner diameter as:

$$\%\,\text{Compliance} = \frac{R_{P_2} - R_{P_1}}{R_{P_1}} \frac{1}{P_2 - P_1} \times 10^4$$

while R is the internal radius, P_1 is the lower internal pressure, and P_2 is the higher internal pressure.

2.4.4. Burst pressure

Burst strength testing of electrospun grafts was completed using a device designed in accordance with Section 8.3.3.3 of ANSI/AAMI VP20:1994.31,32 [3]. Tubes with 4 cm in length were hydrated in PBS for 6 h, fitted over 2.5 mm diameter nipples attached to the device, then a thin latex balloon (Party Like Crazy, Target) was inserted, and the balloon/graft was secured with 2-0 silk suture to the nipples. At last, pressurized air was introduced into the system with increased pressure at a rate of 5 mmHg/s until the tubes

ruptured. Burst pressure (mmHg) was recorded when the structure ruptured.

2.5. In vitro release of heparin & blood compatibility testing

Heparin-loaded collagen/chitosan/PLCL membranes (2 cm × 2 cm) were suspended in 4 mL PBS (pH 7.4) solution in glass vials. All samples were put in shaking water bath at 37 °C. At pre-determined time points, 2.0 mL supernatant was taken from the vial and an equal volume of fresh medium was replaced. The concentration of each derived heparin solution was then determined by toluidine blue method [7]. Briefly, toluidine blue (3.0 mL) was added into the supernatant which was drawn from the vial and reacted adequately with heparin for 2 h at 37 °C. After that, hexane (3.0 mL) was added, following with vigorous stirring to separate the heparin-toluidine blue complex. The aqueous solution of the samples was tested at 630 nm by a microtiter plate reader (MultiskanMK3, Thermo, USA).

The loading capacity of heparin was determined by dissolving the membranes (2 cm × 2 cm) in 4 mL HFP in a screw cap tube. The solution was analyzed by toluidine blue method to determine the amount of heparin encapsulated in the fibrous membranes.

For blood compatibility testing, the whole rabbit blood was collected with sodium citrate, 10 mm diameter samples were punched from the grafts, and then were placed into centrifuge tube (15 mL) filled with 4 mL of fresh blood, and incubated for 3 h at 37 °C. The samples were rinsed with PBS and then immersed in a 2.5% glutaraldehyde solution, following dehydrated with gradient ethanol solutions, and finally the samples were sputter-coated with gold for SEM analysis.

2.6. Cell analysis

In order to study the proliferation of cells in grafts, porcine iliac artery endothelial cells (PIECs) (Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, China) were seeded on the grafts. DMEM medium with 10% fetal bovine serum and 1% antibiotic-antimycotic was used. Coaxial electrospun fibrous membranes were prepared on circular glass cover slips (14 mm in diameter) and then were fixed into 24-well plates with stainless rings. Before seeding the cells, membranes and controls (electrospun fibrous membranes without heparin) were disinfected by immersing in 75% ethanol for 2 h, washed three times with PBS, and then seeded with 100 μL of the cell suspension (cell concentration of 100,000 cells/mL) for each well, and placed in an incubator at 37 °C with 5% CO_2.

Cell proliferation on grafts was determined by the standard MTT assay [17]. After 1, 4, and 7 days post-seeding, the cells and matrices were incubated with 5 mg/mL 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) for 4 h. Thereafter, the culture media were extracted and 400 μL of dimethylsulfoxide (DMSO) was added and slightly vibrated for 20 min. After that, 100 μL supernatant was transferred to a 96-well plate, read at an absorbance of 492 nm by a microtiter plate reader (MultiskanMK3, Thermo, USA).

For cell adhesion and morphology analysis, PIECs were seeded onto grafts at a density of 10^4 cells/well for 3 days. After 3 days, the cell-graft cultures were rinsed twice with PBS and fixed in 4% glutaraldehyde solution for 2 h. After that, the fixed samples were rinsed twice with PBS and then dehydrated in graded concentrations of ethanol (30, 50, 70, 80, 90, 95, and 100%). Finally, they were dried under vacuum overnight. The samples were then gold sputter coated and observed under the SEM at a voltage of 10 kV.

2.7. Statistics analysis

All the data were obtained at least in triplicate and expressed as means ± standard deviation (SD). The one-way ANOVA at a significance of $p < 0.05$ was performed using Origin 8.0 (Origin Lab, USA).

3. Results and discussion

3.1. Morphology of fibrous graft

To fabricate coaxial electrospun heparin-loaded collagen/chitosan/PLCL grafts, various volume ratios of collagen/chitosan/PLCL (shell) solutions and heparin (core) concentration were controlled (Table 1). Fig. 2 presents the SEM images of five different kinds of fibers with controlled properties. These SEM images were analyzed to determine average fiber diameters of the grafts (Fig. 2f). Overall, fibrous grafts appeared macroscopically smooth without any gross defects. As shown in Fig. 2a–c, with the same core concentration of 15%, the average diameters gradually deceased from 938 ± 281 nm to 584 ± 189 nm with the increased collagen and chitosan content. Meanwhile, in Fig. 2b, d and e, at the same shell ratios, the average diameters were 769 ± 234 nm, 744 ± 198 nm, 517 ± 112 nm with the core concentration of 5%, 15% and 30%, respectively. This phenomenon could be explained by the conductivity increase of the electrospinning solution with the increase of collagen, chitosan and heparin contents, leading to more ions formation. Similar results were reported as the formed ions could increase the conductivity of the electrospun solution, as well as cause the increased elongational forces and thus produced smaller fibers [18,19]. At the same time, grafts with different fibrous structure probably affect the loading capacity of heparin (see the results in 3.3).

Fig. 3a shows the TEM micrographs of the core-shell structure of heparin loaded collagen/chitosan/PLCL fiber (taking 40:10:50–15% for example, including TEM and SEM). The shell and the core in the image showing a clear interface indicated that heparin was encapsulated well into the fiber. As shown in Fig. 3b and c, the heparin-loaded collagen/chitosan/PLCL fibrous graft with a length of 7 cm, an inner diameter of 2.5 mm and a wall thickness of 400 μm could be well fabricated, and these characters could match the human blood vessels very well. To further demonstrate the encapsulation of heparin into the tubular graft, a cross section of graft was made. After that, the graft was soaked into distilled water for 10 min and then dried in the fume hood for SEM test. Fig. 3d–g depicted the fiber cross section, which obviously showed some fiber sections with holes, causing by the heparin diffused into water and formed the hollow fiber (showed in red arrow sections). In the meanwhile, the diameter of the fibers are different, the heparin diffusing time from the fiber inside to outside are different under normal condition, therefore, with larger fiber diameter, heparin diffused from the inside of fiber to outer side need more time, and would get slower heparin releasing. As a result, we speculated that the drug release rate could be controlled via the different ration of collagen, chitosan and PLCL, which mainly lead to different fiber diameters.

3.2. Mechanical properties of grafts

3.2.1. Tensile testing

The quantitative data for tensile strength was shown in Table 2. As for graft with heparin core concentration of 15%, the higher content of PLCL lead to a significantly stronger graft, especially when the shell ratio was 20:5:75 where the peak tensile stress reached a value of 12.0 ± 1.8 MPa. As a comparison, the peak tensile stress of 60:15:25–15% graft was only 2.5 ± 1.1 MPa, which was too weak to

Table 1
Fabrication parameters of coaxial electrospun heparin-loaded collagen/chitosan/PLCL grafts.

Fabrication condition	volume ratios of collagen/chitosan/PLCL (Shell)	Concentration of heparin (w/v) (Core)
20:5:75–15%	20:5:75	15%
40:10:50–15%	40:10:50	15%
60:15:25–15%	60:15:25	15%
40:10:50–5%	40:10:50	5%
40:10:50–30%	40:10:50	30%

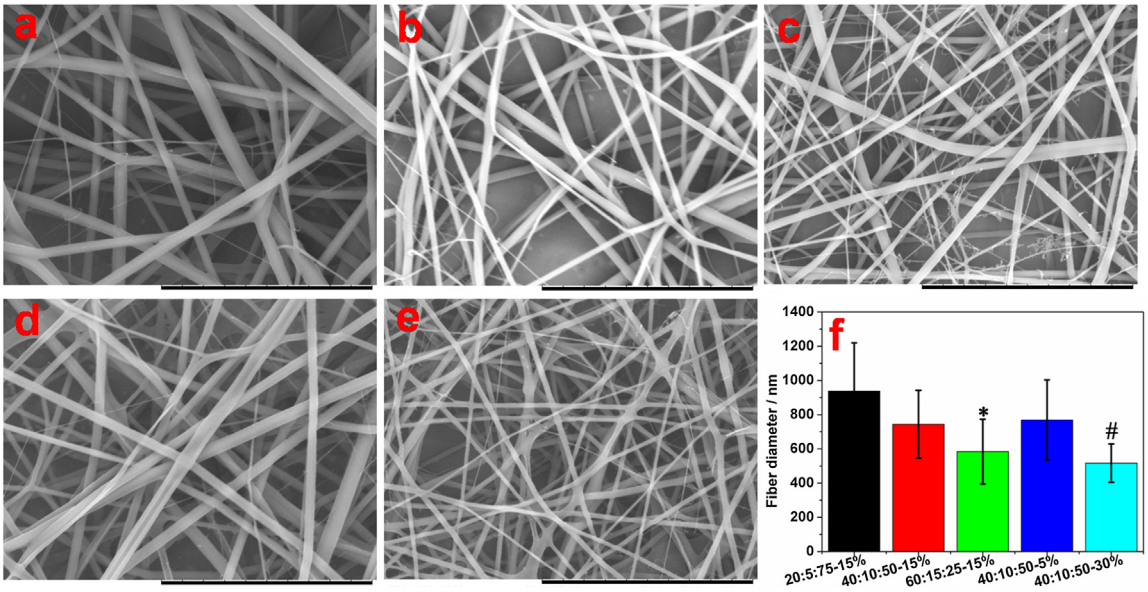

Fig. 2. SEM micrographs of fibers with different core-shell concentration and ratios. (a) 20:5:75–15% (b) 40:10:50–15% (c) 60:15:25–15% (d) 40:10:50–5% (e) 40:10:50–30% (f) fiber diameter distribution of different grafts. * indicated a significant difference from 20:5:75–15%, while # indicated a significant difference from 20:5:75–15%, 40:10:50–15% and 40:10:50–5%, $p < 0.05$. (Scale bar = 20 μm).

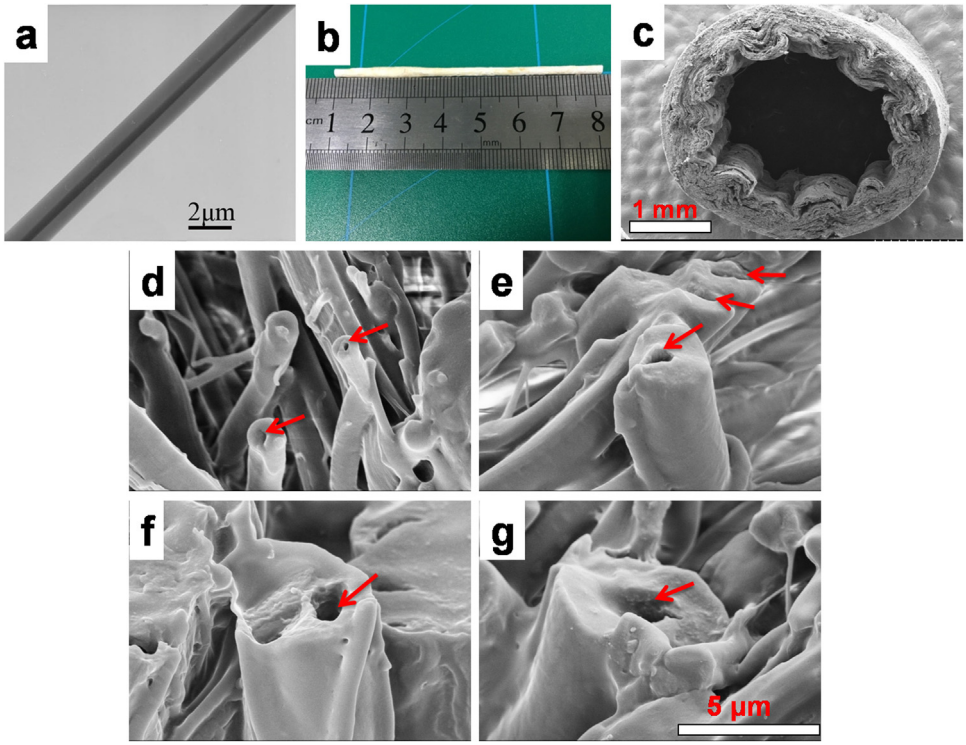

Fig. 3. TEM micrograph of fiber with core-shell structure (a), and the digital camera image of small diameter electrospun tubular graft (b). SEM micrograph of the cross section of tubular graft (c) and the high resolution of fiber cross section (d–g) of tubular graft section. The scale bar for d–g = 5 μm. (For interpretation of the references to colour in the text, the reader is referred to the web version of this article.)

Table 2
Mechanical properties of different grafts.

Sample	Peak Stress/MPa	Modulus/MPa	Strain At Break/%	Suture strength/N	Compliance/(%/100 mmHg)	Burst pressure/mmHg
20:5:75–15%	12.0 ± 1.8	5.4 ± 1.6	73 ± 14	1.8 ± 0.1	1.1 ± 0.3	3350 ± 106
40:10:50–15%	8.8 ± 2.2	5.7 ± 1.1	74 ± 16	2.1 ± 0.2	1.1 ± 0.4	3000 ± 72
60:15:25–15%	2.5 ± 1.1*	2.2 ± 1.0#	59 ± 11	0.9 ± 0.1*	–	–
40:10:50–5%	7.8 ± 0.9	2.5 ± 0.3#	184 ± 14*	3.8 ± 0.1*	1.8 ± 0.2$	2200 ± 105&
40:10:50–30%	8.8 ± 2.5	7.8 ± 1.6	87 ± 7	2.2 ± 0.1	0.9 ± 0.4	3280 ± 176

* indicates a significant difference from other grafts, # indicates a significant difference from 20:5:75–15%, 40:10:50–15%, and 40:10:50–30% grafts. $ indicates a significant difference from 40:10:50–30% grafts, while & indicates a significant difference from 40:10:50–30% and 20:5:75–15% grafts.

match that of the native artery (4.4 MPa) [20]. For grafts with the shell ratio of 40:10:50, in spite of different heparin concentration, all three grafts had no significant difference for tensile strength. These results indicated that the ultimate stress of the graft was mainly influenced by shell ration rather than the core concentration.

For grafts with different PLCL content at 15% heparin concentration, the ultimate strain of grafts had the similar tendency as the peak tensile, which reduced gradually with the decreased content of PLCL. While for the shell ratio of 40:10:50, grafts with different core concentration obtained different strain (40:10:50–5% could especially reach to 184 ± 14%). It was demonstrated that the core heparin concentration affected the ultimate strain of grafts obviously, and this might be ascribed to the small amount of heparin encapsulation in promoting the ductility of the fibers, where larger number of heparin loading would hinder the mobility of molecular chain of fibers. Overall, the ultimate strain of these grafts (except 60:15:25–15%) was comparable to the human coronary artery(45–99%) [21].

In terms of elastic modulus, grafts held higher ultimate strain could be more flexible with lower modulus (eliminate 60:15:25–15%).

3.2.2. Suture retention strength

The ability for suture retention was investigated with the results presented in Table 2. The results showed that at a low core heparin concentration (5%), grafts had the highest suture retention strength with an average value of 3.8 ± 0.1 N. Overall, all the grafts except 60:15:25–15% (0.9 ± 0.1 N) had a bit higher suture retention strength than native blood vessels (1.7 N) [20].

3.2.3. Dynamic compliance

Results from Table 2 showed the compliance values with a range from 0.9 to 1.8%/100 mmHg. Although the one of 60:15:25–15% had also been measured, unfortunately the grafts were ruptured before reaching 600 cycles. The 40:10:50–5% grafts possessed the highest compliance value which was significantly higher than that of 40:10:50–30% grafts. The flexibility of grafts changed probably due to the heparin addition. After adding a small amount of the hydrophilic heparin, the flexibility of grafts was enhanced to a certain extent. However, the presence of a large number of heparin in grafts will hinder the fibrous molecular movement, therefore, the compliance value of graft decreased. Whatever, according to a prior study, grafts produced similar compliance values as saphenous vein (0.7–1.5%/100 mmHg) [3,17]. Besides, the grafts possess higher compliance values than standard ePTFE grafts (0.1%/100 mmHg) [3].

3.2.4. Burst pressure

The burst pressure of a vascular graft is one of the most important parameters which determine the suitability of its use as a vascular graft for implantation [22]. According to the burst pressure results shown in Table 2, the average values for all grafts were more than 2200 mmHg except the 60:15:25–15% (the grafts were ruptured after few seconds in the very beginning procedure). Along with the increased heparin content, the burst pressure resistance for grafts was gradually increased. The 20:5:75–15% possessed the highest measurement values due to holding more PLCL component which had good mechanical properties. It has been reported that saphenous vein and mammary artery have burst pressure values of 1680–2273 and 2031–4225 mmHg, respectively [22,23]. Therefore, all the grafts excepting the 60:15:25–15% one revealed potential applications as an artery bypass graft.

3.3. In vitro drug delivery & blood compatibility testing

For various heparin-loaded fibrous membranes, the real loaded heparin amount were 7.7, 7.9, 7.2, 6.8, and 10.5 mg, respectively, the theoretical weight of heparin were calculated according to the concentration of solution and electrospinning time (data were not show here), and the encapsulation efficiency for these five fibrous grafts was about 75%, 77%, 56%, 75%, and 70%, respectively. Obviously, 60:15:25–15% had low loading capacity, and the encapsulation efficiency for others was more than 70%.

In order to check whether heparin was properly loaded, the in vitro release measurement was conducted for 45 days, and the results were shown in Fig. 4. In the aqueous solution, the release of heparin was found to experience two stages: the initial burst release at day 1 and the continuous release from day 2 to day 45. When exposed to PBS buffer, the heparin was immediately released from these five fibrous membranes (18%, 23%, 40%, 25% and 31%, respectively) by the end of the first day (Fig. 4b), and the diffusion of heparin near the fiber surface was the main factor leading to the initial burst release.

After the initial burst release, sustained release could be observed as the curve showed stable ascending trend. The total amount of the released heparin was approximately 60%, 80%, 95%, 96% and 61% after 45 days, respectively. In this stage, the mechanism of heparin release would involve [12,24]: (1) heparin diffusion through the polymer fibers and release into the media; (2) the erosion and swelling of the chitosan and collagen; (3) the degradation of the polymer. For this study, the mechanism of heparin release mainly got through heparin diffusion from the pores of the polymer and the erosion/swelling of chitosan and collagen. The degradation for PLCL was quite slow, and was not likely to occur within 45 days, while collagen and chitosan would degrade in the later phase. Overall, the relative rate of heparin release was faster with the lower amount of heparin-loading (60:15:25–15%, load amount of 7.2 mg), and slower with the higher drug-loading (40:10:50–30%, load amount of 10.5 mg). While for 20:5:75–15% graft (load amount of 7.7 mg), it obtained the slowest heparin release rate as well as the lowest release amount due to the high content of PLCL which had no obvious mass loss during early in vitro release experiment period in this study.

In our early research, heparin was loaded into PLCL fibrous graft via coaxial electrospinning, the heparin released from PLCL fibers was shown in the bottom right corner of Fig. 4b [7]. It had a high initial burst release at about 40%, and almost 80% of heparin was released within 14 days. That's probably because the shell was only PLCL, and the conductivity was different from col-

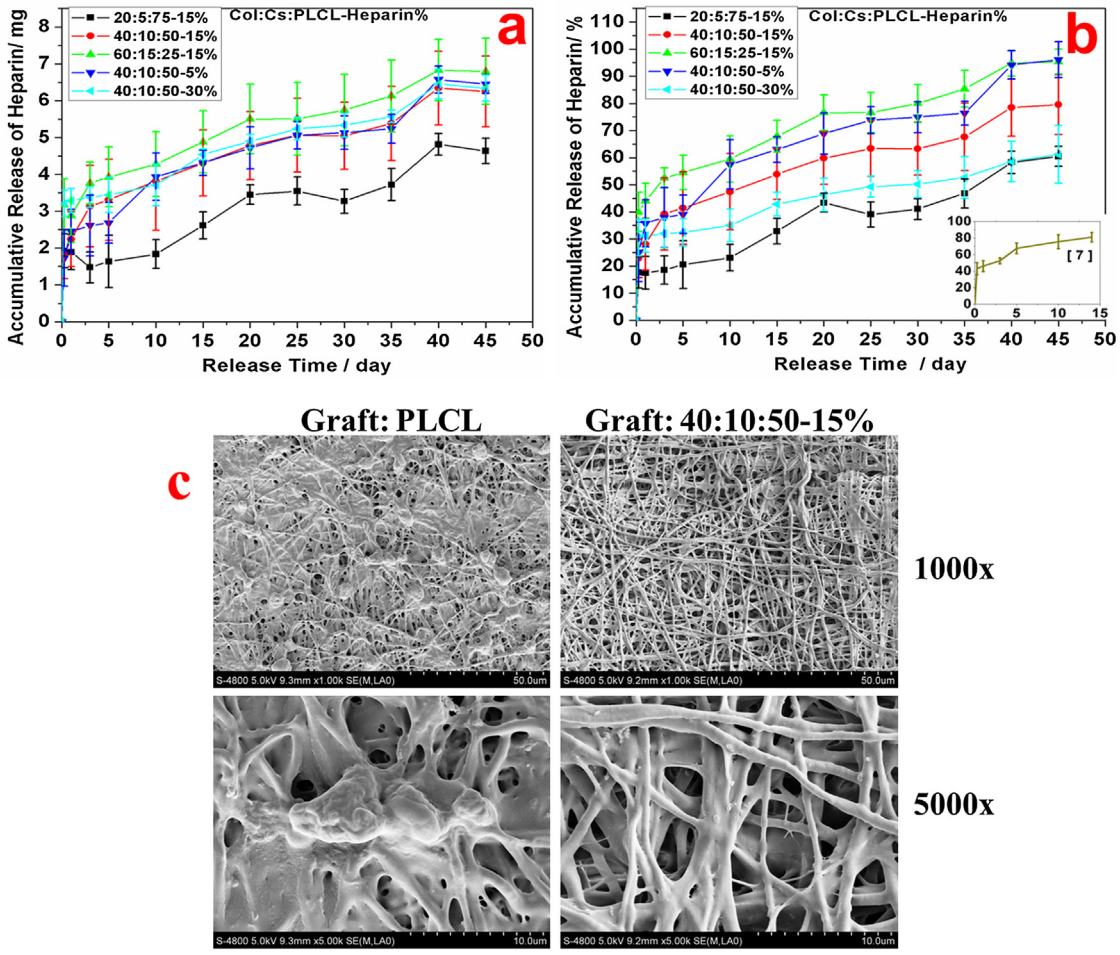

Fig. 4. Accumulative release of heparin from different grafts. Accumulative release amount (a) and accumulative release rate (b). Platelet adhesion on the grafts after 3 weeks of drug delivery in vitro (c).

lagen/chitosan/PLCL which led to the different fiber structure and affected the loading capacity of heparin. Therefore more heparin distributed on the surface or under very near the surface of PLCL fibers, which contributed to the high burst release and fast release rate. By adding collagen and chitosan into PLCL as shell via coaxial electrospinning, heparin release could be controlled not only in low initial burst release but also in long-term sustained release which reached up to 45 days.

Above results displayed that the 40:10:50–15% graft got high drug-load amount (7.9 mg), high encapsulation efficiency (77%), very stable sustained release rate, and more importantly, relatively low initial burst release (23%). This kind of graft might be more suitable for vascular tissue application.

Platelet adhesion testing have been done on 40:10:50–15% graft comparing to previous graft which was also fabricated by coaxial electrospinning (PLCL as shell, and heparin as core). Both of grafts have heparin released for 3 weeks in vitro before testing the platelet adhesion. As showed in Fig. 4c, a lot of platelets adhered on heparin-loaded PLCL graft after heparin releasing for 3 weeks, while less platelet adhered on 40:10:50–15% graft. These data showed this multicomponent graft (40:15:50–15%) with heparin loading by coaxial electrospinning did have good blood compatibility.

3.4. Cell behavior

Grafting for tissue engineering was typically designed to promote cell growth, gain physiological functions, and maintain normal states of cell differentiation [25–27]. The proliferation of PIECs on days 1, 4, and 7 after being seeded on the various grafts was shown in Fig. 5. Overall, all the grafts with heparin-loading were conducive to cell proliferation. In comparison with controls (graft without heparin), the cell proliferation in all grafts have no significant difference in 1 or 4 days. On day 7, cell proliferation rate on 60:15:25–15% was significantly lower than 40:10:50–15% and 60:15:25 (Fig. 5a). Linking to the results in Fig. 4, it was assumed that the experimental phenomenon was probably caused by the introduction of heparin, the fastest relative rate of heparin release resulted high concentration of heparin in media which would slow down the cell growth rate. On the other hand, cell proliferation rate for 40:10:50 with different core heparin concentration had no significant difference (Fig. 5b), that probable because these three grafts had almost the same amount of heparin release.

SEM micrographs of cell morphology and the interaction between cells and grafts were shown in Fig. 6. After 3 days, PEICs were more easily spread to develop an endothelial cell layer on the surface of 40:10:50–5%, 40:10:50–15%, and 40:10:50–30% grafts compared to others. Combined the results above (Figs. 4 and 5), on day 3, heparin released from 40:10:50–5%, 40:10:50–15%, and 40:10:50–30% grafts had almost the same tendency, while a lot of heparin released from 60:15:25–15%. Such results further demonstrated that heparin in a proper concentration could enhance PIECs to grow, while very high heparin concentration could partially hinder cell proliferation.

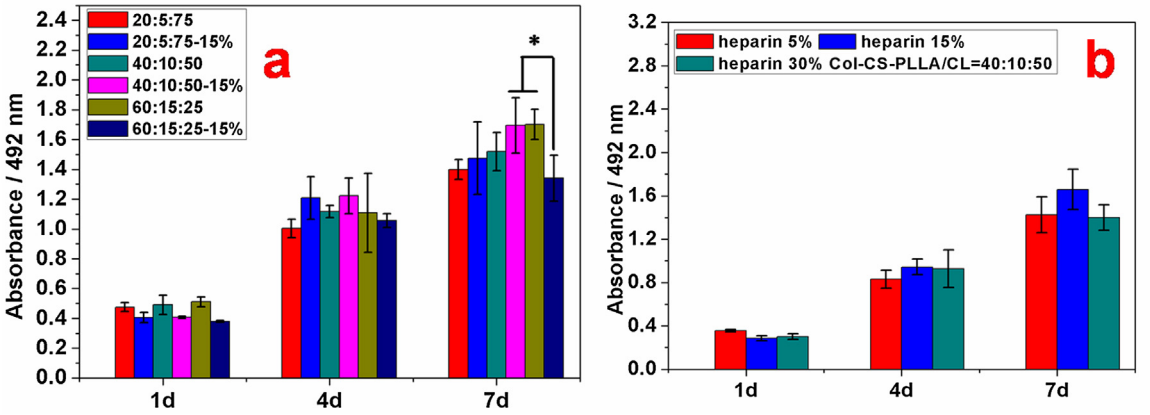

Fig. 5. Proliferation of PIECs cultured on different grafts and cover slips for 1, 4, 7 days. Statistical difference between groups is indicated.

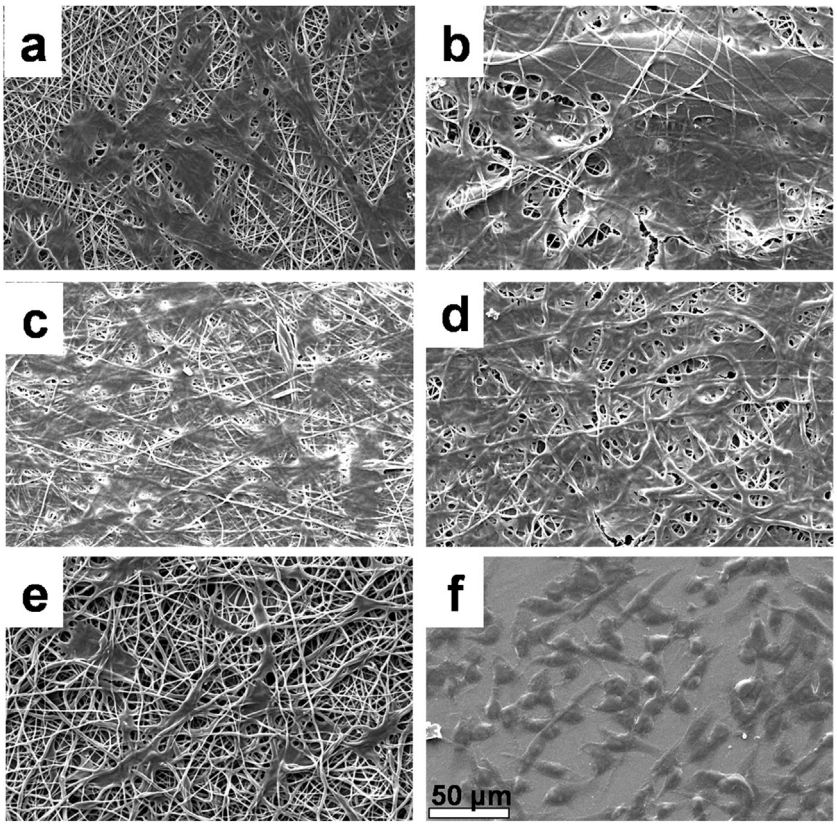

Fig. 6. SEM micrographs of endothelial cells grown on different grafts. (a) 20:5:75–15%, (b) 40:10:50–5%, (c) 40:10:50–15%, (d) 40:10:50–30%, (e) 60:15:25–15%, (f) cover slip. The scale bar = 50 μm.

4. Conclusion

Fibrous grafts with heparin-loading were successfully fabricated via coaxial electrospinning, and the heparin encapsulation efficiency was high over than 70% except 60:15:25–15%. Moreover, the heparin could sustain release for more than 45 days. Especially for the 40:10:50–15% graft, it got high drug-load amount (7.9 mg), high encapsulation efficiency (77%), very stable sustained release rate as well as release amount, and it had low initial burst release (23%), and more importantly, it still had good performance of antiplatelet adhesion after heparin releasing for 3 weeks. In addition, it possessed excellent cell biocompatibility and suitable mechanical properties including tensile strength, suture retention strength, burst pressure and compliance which could match the native blood vessels. Thus, through controlling the parameter of fabrication process, this kind of graft might be a promising candidate for vascular tissue application.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2017.01.045.

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