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A novel heparin loaded poly(L-lactide-co-caprolactone) covered stent for aneurysm therapy

Chunchen Wu [a,1], Qingzhu An [b,1], Dawei Li [a], Jing Wang [a], Liping He [a], Chen Huang [a], Yu Li [c], Wei Zhu [b,*], Xiumei Mo [a,*]

[a] Biomaterials and Tissue Engineering Lab. Faculty of Chemistry, Chemical Engineering & Biotechnology, Donghua University, Shanghai 201620, China
[b] Clinical Center of Neurosurgery, Shanghai Neurosurgical Emergency Center, Department of Neurosurgery, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai 200040, China
[c] MicroPort Medical (Shanghai) Co. Ltd, Shanghai 201230, China

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ABSTRACT

The metallic stents covered with heparin loaded poly(L-lactide-co-caprolactone) nanofibers via emulsion electrospinning have been fabricated as a novel covered stent. The morphology and inner-structure of core–shell nanofibers were respectively ion observed by scanning electron microscopy and transmission electron microscopy. The distribution of heparin aqueous solution and chemical component in nanofibers was separately determined by fluorescence microscopy and Fourier transform infrared spectrum. The results showed that the nanofibrous matrix successfully encapsulated with heparin would not rupture with the expansion of metallic stent, which could effectively separate the aneurysm dome with bloodstream in the rabbit model. The aneurysm was immediately obliterated after the stenting and angiogram at 14 days follow-up showed that the aneurysm was still obliterated. No obvious stenosis and intima hyperplasia in parent artery were found. Therefore, this work provides a promising approach to fabricate covered stent for aneurysm treatment.

1. Introduction

Endovascular interventions, including detachable balloons, coils, liquid embolic and covered stent, have become an alternative therapy for wide-necked aneurysm. The covered stents could reduce intimal hyperplasia by inhibiting cellular migration into stent lumen [1] and decrease the recanalization rate by separating the aneurysm dome from the blood circulation [2]. Thus, the covered stent has been widely employed in obliterating a wide-necked or giant aneurysm with a simple procedure [3–5].

However, conventional vascular prostheses working well in large-diameter vascular such as expanded polytetrafluoroethylene (ePTFE) and polyethyleneterephtalate (Dacron) were not suitable for small vascular treatment [6], which were prone to acute thrombosis and intimal hyperplasia [7] and delayed the development of small-diameter covered stent [8,9]. Thus, a novel covered stent with anticoagulant matrix is needed to avoid these disappointing results.

In this study, the endovascular metallic stent covered with heparin loaded poly(L-lactide-co-caprolactone) P(LLA-CL) core–shell nanofibers was fabricated as a new type of covered stent for aneurysm treatment. The localized delivery of heparin to the site of aneurysm dome could effectively avoid the formation of acute thrombus [10]. Heparin loaded P(LLA-CL) core–shell nanofibers have been prepared in our previous work by co-axial electrospinning [11]. To avoid the co-axial spinning nozzle used in electrospinning, the emulsion electrospinning technique has been developed to fabricate the heparin loaded P(LLA-CL) core–shell nanofibers and formed anticoagulant matrix. Moreover, the novel nanofibrous matrix could not only avoid recanalization but also promote endothelialization in the treatment of aneurysms with giant size, wide-neck or fusiform morphologies, which could avoid the stenosis in a long time for covered stent [12]. Therefore, the novel heparin loaded P(LLA-CL) covered stent has a great potential for aneurysm treatment.

2. Materials and methods

Materials and reagents: P(LLA-CL) (M_w=300 kDa, LA:CL=50:50) was obtained from Nara Medical University, Japan. Heparin (≥150 U/mg), sorbitan trioleate (Span-80) and dichloromethane were respectively purchased from Shanghai CaiYou Industrial Co, Ltd. (Shanghai, China), Sigma-Aldrich Co, Ltd. (St. Louis, USA) and

* Corresponding authors. Tel.: +86 21 67792653; fax: +86 21 62492884.
E-mail addresses: weszhu398@yahoo.com (W. Zhu), xmm@dhu.edu.cn (X. Mo).
[1] These authors contributed equally to this work.

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Shanghai FineChemicals Co., Ltd. (Shanghai, China). Bare metal stents, stent delivery balloon and guiding catheter were kindly provided by Shanghai MicroPort Co., Ltd. (Shanghai, China). All reagents for aneurysm creation were offered from HuaShan Hospital of FuDan University (Shanghai, China).

The fabrication of the heparin loaded P(LLA-CL) covered stent: 25% (w/v) heparin aqueous solution (HAS)[2] (0.5 ml) and Span-80 (0.2 ml) were added dropwise into dichloromethane (10 ml) in the meantime. With magnetic stirring at 300 rpm, HAS gradually dispersed in dichloromethane and formed uniform water-in-oil emulsion eventually. P(LLA-CL) (0.8 g) was then added into the emulsion and dissolved in the dichloromethane phase. The resulting solution was employed to fabricate P(LLA-CL)/heparin core–shell nanofibers (PLCL-Hep-NF)[3] with the voltage of 20 kV and feed rate of 1.5 ml/h, which were collected by a rotating rare metal stent (600 rpm, Φ=2.7 mm, and l=13 mm) and formed a covered stent with nanofibrous matrix. The rare metal stent was made up of stainless steel, whose deployment pressure, resistance of the stent to external compressive forces, foreshortening and coverage area were respectively 9 atm, 100 kPa, 9% and 19%. Deionized water was used to replace the HAS to fabricate P(LLA-CL) nanofibers (PLCL-NF) with the method mentioned above. To determine the distribution of water phase in nanofibers, HAS containing oil-insoluble fluorescein isothiocyanate (FITC, 0.5 mg) was used to fabricated core–shell nanofibers with the same method. The heparin loaded covered stent was then crimped on crimping machine.

Characterization of heparin loaded nanofibrous matrix: The morphology of heparin loaded matrix composed of PLCL-NFs (PLCL-Hep-M) (see footnote 2) was observed by scanning electron microscopy (SEM, Hitachi TM-100, Japan) and the inner-structure of core–shell of PLCL-Hep-NF was taken by transmission electron microscopy (TEM, Hitachi H-800, Japan). The distribution of water phase in PLCL-Hep-NF was determined by fluorescence microscopy (FM, Olympus IX71-A12FL, Japan). The chemical components and tensile property of PLCL-Hep-M were respectively characterized by Fourier transform infrared spectrum (FTIR, Thermo Electro AVATAR 380, USA) and universal materials tester (H5K-S, Hounsfield, UK).

Aneurysm creation: Aneurysms were created in three New Zealand white rabbits (2–3 kg). The procedure was performed as described [13]. Briefly, the right common carotid artery (CCA) of each animal was surgically exposed and injected of an approximately 75 U solution of porcine elastase to establish aneurysm models. Every surgical procedure was conducted under anesthesia and sterile conditions.

Stent implantation and angiographic follow-up: After allowing sufficient time for the aneurysm to mature, the animals were brought back to the angiography suite for stent implantation. Treated animals were orally given aspirin (20 mg per day) from 7 days before to 14 days after stenting to mitigate acute thrombotic events. A 5F vascular sheath was inserted retrograde into the right femoral artery. A 5F guiding catheter was advanced into the descending aorta. After successful aneurysm creation was confirmed with angiography, the covered stent was implanted in the innominate-to-subclavian artery with the intent of maintaining equal lengths on either side of the aneurysm ostium. The stent delivery balloon was then inflated, covering aneurysm neck and occluding flow into the aneurysm. Angiographic analysis was again used to evaluate aneurysm blood flow immediately after stenting. All the animals were followed up with angiography at 14th day after stenting. The procedure was performed via left femoral artery approach.

3. Results and discussion

Characterization of heparin loaded P(LLA-CL) nanofibers: The heparin loaded covered stents were prepared using a conventional electrospinning setup (Fig. 1a). An earthed rotating bare metal stent (Fig. 1b) was used to collect PLCL-Hep-NF directly. Digital images (Fig. 1c) showed that bare metal stent would be fully covered with PLCL-Hep-M composed of PLCL-Hep-NF depositing on it. According to the results of tensile test, the stress and strain of the PLCL-Hep-M were respectively 11.78 ± 1.45 MPa and $182.34 \pm 11.90\%$. Thus, PLCL-Hep-M could keep its morphology and structure and would not rupture with the expansion of covered stent (from 2.7 mm to 4.0 mm) (Fig. 1d) and in the aneurysm site, which could effectively separate aneurysm dome with bloodstream.

Fig. 2a illustrated the heparin loaded matrix possessed nanofibrous structure. However, it could be seen that nanofibers had been fused together at the junction position, that was because the P(LLA-CL) employed in this paper was rubbery material. The nanofibrous structure of matrix was profit for adhesion and proliferation of cells compared with film of conventional covered stent. Thus, the covered stent was believed to be conducive to the adhesion and proliferation of endothelial cells [14,15] and the formation of endothelialization [16,17]. Moreover, porous structure of the matrix would impede the occurrence of intimal hyperplasia compared with a bare stent [18]. FM image (Fig. 2b) demonstrated that HAS containing oil-insoluble FITC had been incorporated and well distributed in each part of nanofibers. TEM image (Fig. 2c) revealed that PLCL-Hep-NF had a novel multi-cores inner-structure, which was different from conventional core–shell structure of coaxial nanofibers. Emulsion droplets with tiny size (100–300 nm) would keep independence with each other due to the high negative charge density of heparin. Thus, emulsion droplets would not assemble together to form a continuous core, but formed multi-cores inter-structure. Moreover, the charged emulsion droplets were elongated in the process of electrospinning and presented stick-shaped. According to FTIR spectrum (Fig. 2d), the typical absorption peak at 1640 cm^{-1} (amide I) caused by –C=O stretching of $-CONH_2$ group in heparin appeared in PLCL-Hep-NF FTIR spectrum demonstrated heparin had been loaded into the nanofibers. Encapsulated heparin in the fibers could be released stably and sustainedly [11], which was benefit for the sustained anticoagulation.

Aneurysm creation and DSA follow-up: Saccular aneurysms were successfully created in all cases without morbidity or mortality (Fig. 3a). The rabbit saccular aneurysm models simulated the morphology and hemodynamics of human cerebral aneurysms. All rabbits were healthy without any observable neurological deficit over the course of experiments. No difference was found in diet intake and behavior before and after surgery. The initial angiograms before stenting (Fig. 3b) revealed direct flow into the aneurysm of right CCA stump. Immediately after stenting (Fig. 3c), the aneurysm was obliterated (Fig. 3d). Angiogram at 14 days follow-up showed that the aneurysm was still invisible and no stenosis or intima hyperplasia in parent artery (Fig. 3e).

In future study, we would detect the effectiveness of these new covered stents with long-term follow-up. Besides the angiogram, histological analysis, including tissue reaction to the graft,

[2] Heparin aqueous solution (HAS) was prepared by dissolving 0.25 g heparin powder in 1 ml deionized water with magnetic stirring.

[3] The process of the fabrication of heparin loaded covered stents include two steps. In the first step, the heparin loaded poly(L-lactide-co-caprolactone) nanofibers were prepared by the conventional electrospinning setup via emulsion electrospinning which was named as PLCL-Hep-NF. In the second step, PLCL-Hep-NF were collected by the rotating bare stent directly. With enough PLCL-Hep-NF depositing on the rotating bare stents, a nanofibrous matrix formed and constructed the coating layer of the covered stent which was named as PLCL-Hep-M.

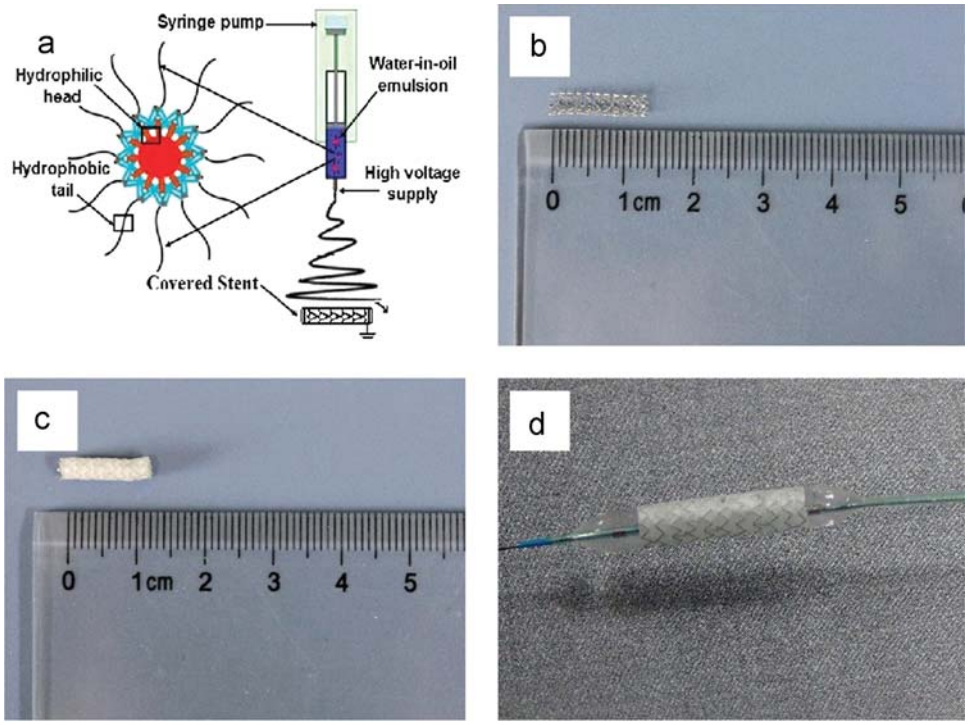

Fig. 1. The scheme of the fabrication of heparin loaded covered stent (a) and digital images of bare metal stent (b), covered stent (c) and expanded covered stent (d).

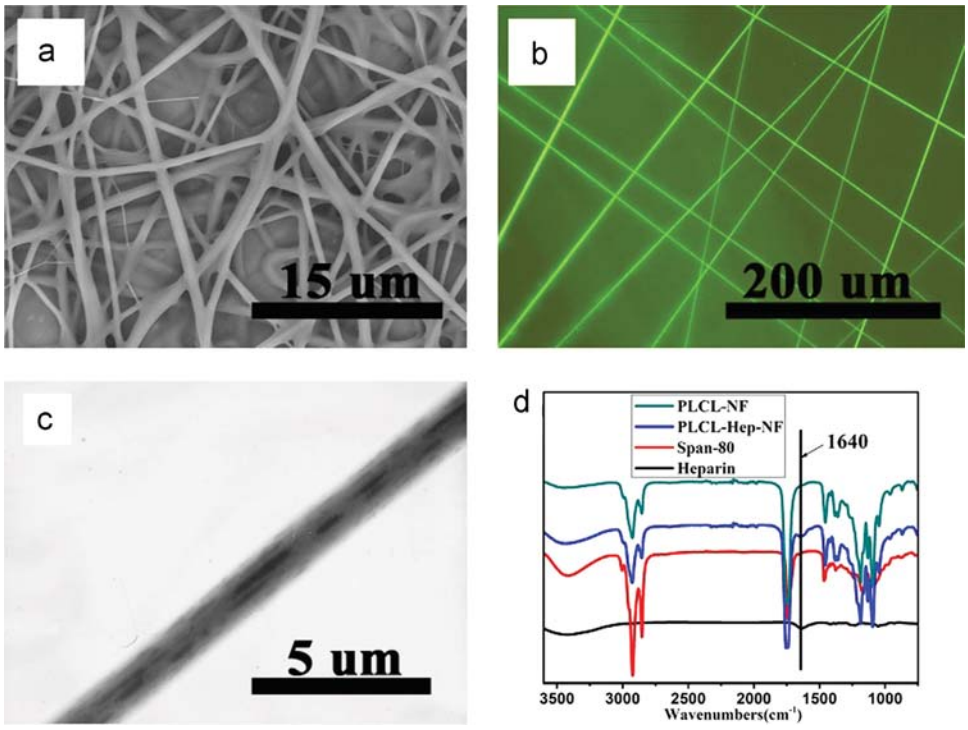

Fig. 2. SEM image (a), FM image (b), TEM image (c) of PLCL-Hep-NF and FTIR spectra of PLCL-NF, PLCL-Hep-NF, Span-80 and Heparin (d).

neointimal hyperplasia, endothelialization, smooth muscle regeneration need to be investigated.

4. Conclusion

We have developed a novel heparin loaded covered stent via emulsion electrospinning. The HAS could be incorporated into all parts of PLCL-Hep-NF with a novel multi-cores inner-structure, which avoids the formation of thrombus together with the structure of nanofibrous matrix. According to the result of DSA follow-up, the aneurysm was obliterated either immediately after stenting or 14 days follow-up by separated the aneurysm dome from the blood circulation. Such covered stent might become a potential candidate in the treatment of aneurism.

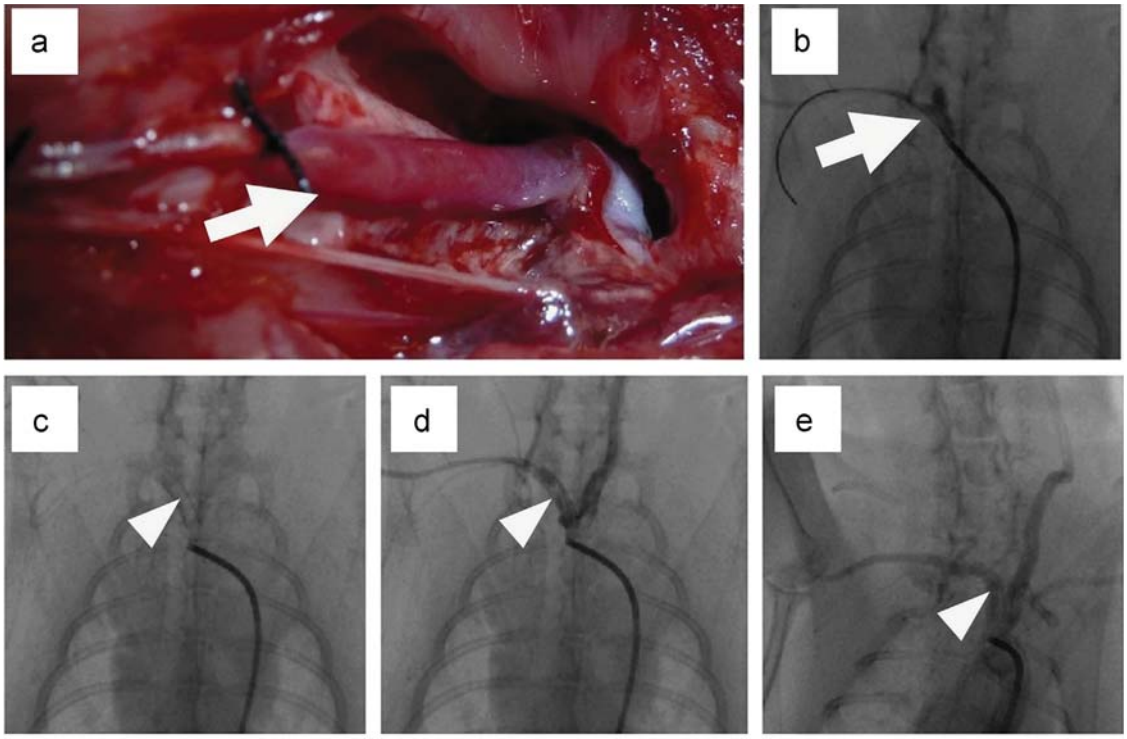

Fig. 3. (a) Aneurysm (arrow) was created in rabbit immediately after surgery. (b) DSA revealed direct flow into the aneurysm (arrow) of right CCA stump. (c) Stent (arrow head) located in parent artery. (d) The aneurysm was obliterated immediately after stenting (arrow head). (e) The aneurysm was still invisible and the parent artery was patent without obvious stenosis 14 days after stenting (arrow head).

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