Untangling the co-effects of oriented nanotopography and sustained anticoagulation in a biomimetic intima on neovessel remodeling

Zihao Wang, Chungeng Liu, Di Zhu, Xiang Gu, Yin Xu, Qinghua Qin, Nianguo Dong, Shengmin Zhang, Jianglin Wang

Article Info

Keywords:
- Vascular graft
- Oriented structure
- Anticoagulation
- Numerical simulation
- Electrospinning

Abstract

Constructing a small-diameter artificial blood vessel with biological functions and mechanical compliance comparable to native tissues is still a major challenge in vascular tissue engineering. To address the issues of severe thrombosis and unsatisfactory long-term patency in small-diameter vascular grafts, herein we designed a biomimetic intima with an oriented nanotopographical structure and covalently immobilized anticoagulant molecules. The mixture of heparinized silk fibroin (SF-Hep) and polycaprolactone (PCL) was used to produce oriented inner layer and pure PCL was used to fabricate vertically porous outer layer by a two-step cross-electrospinning. Our findings showed that the immobilized heparin significantly influenced adherence and activation of platelets while the oriented nanotopography mainly manipulated the elongation and aligned flow. More importantly, two factors of the oriented structure and anticoagulation presented the obviously synergistic effects on rapid endothelialization, long-term patency and remodeling of neovessel. Consequently, the current study successfully combined biochemical induction of heparin molecule and biophysical stimulation of oriented nanotopography to create an off-the-shelf small-diameter vascular graft with excellent antithrombosis in the early stage and long-term patency in the late stage.

1. Introduction

Cardiovascular disease (CVD) is still the leading cause of mortality all over the world, with around 17 million lives death each year based on the annual report of World Health Organization [1,2]. Most of CVD are directly associated with the vascular dementia [3,4]. The blocked or injured vessels replaced with autologous blood vessels remain the gold standard in the currently clinical treatment [5-7]. However, the autologous vessels are normally unavailable due to the limitations of vascular diseases, actual size and repeated requirement [8,9]. Thus, it is a pressing requirement to develop artificial blood vessels. To date, the replacement of large-diameter (Inner diameter > 6 mm) blood vessels have been successfully solved with non-degradable polymeric materials such as Dacron, expanded poly-tetrafluoroethylene (ePTFE), and polyurethane (PU) [10-12]. Nevertheless, for small-diameter blood vessels (Inner diameter < 3 mm), the above bioinert polymeric materials are not the ideal alternative owing to the drawbacks of thrombosis, hyperplasia, and poor patency [13,14]. Vascular grafts based on biodegradable materials can potentially serve as a better alternative of small-diameter blood vessels for clinical application.

Structural design of biodegradable vascular grafts are of great significance to manipulate the function of endogenous cells on inducing in-situ repair and regeneration of injured vessels [15,16]. Native vessels present the ordered multiple structures on the tubular geometry at a macroscopic level and the fibrous architecture at the nanometer scale [17,18]. Particularly, the oriented, compact and nanoscale intimal structural of native vessels can not only regulate hemocompatibility and inhibit blood leakage, but also promote endothelialization [19-21].
Despite there are many ongoing efforts to develop small-diameter vascular grafts with a certain of structural simulation, most of them remain to hardly create the ideal products because of the lacking on the innovation of fundamental materials and improvement of material processing techniques.

Both synthetic and natural biodegradable materials have been attempted to apply on vascular tissue engineering [22]. The former exhibits adequate mechanical strength to the benefit of surgery suturing and prevention of the burst of blood vessel but poor hemocompatibility in small-diameter artificial blood vessel [23,24]. In contrast, the latter provides excellent hemocompatibility but weak mechanical strength and easy disintegration [25,26]. Consequently, in this work we designed a novel small-diameter vascular graft by combining natural biomaterial of heparinized silk fibrin with synthetic polymer of polycaprolactone for the balance on material strength, bioactivity and degradation. Silk fibrin has been verified to promote rapid endothelialization of vascular graft in compare with ePTFE [27,28]. On this basis, the heparinized silk fibrin can significantly enhance the anticoagulation at the initial stage of implantation [29]. Polycaprolactone as a FDA-approved biodegradable polymer has been extensively applied in tissue engineering scaffolds, particularly its nanofiber form via electrospinning [30,31].

Material processing technique normally influences the function of 3D scaffolds since the same material composition with distinct micro/nano-scale structure eventually employs various functions [32,33]. Electrospinning as a routine processing technique to construct the artificial blood vessel has been extensively applied for porous structure and nano-structure [34–36]. In this study, we applied two-step cross-electrospinning to successfully produce a vascular graft with oriented intimal topography and vertically porous outer layer. The oriented topography is in favor of the ordered and rapid growth of endothelial cells whereas the vertically porous outer layer can maintain tubular stability and mechanical support, and stimulate the ingrowth of smooth muscle tissues [23]. Consequently, two-layer structure of vascular graft derived from two-step cross-electrospinning presents structural similarity to tunica intima and tunica media of native vessels. In this work, we aimed to develop an off-the-shelf small-diameter vascular graft with structural similarity and functional bionics of native blood vessels. Two-step cross-electrospinning was applied to fabricate the oriented intimal structure and vertically porous outer layer for offering the alignment regulation of endothelial cells and mechanical stability (Fig. 1). Integrating utilization of synthetic and natural biodegradable polymers attempts to achieve the balance between mechanical strength and hemocompatibility. As a result, we eventually created a well-designed small-diameter vascular graft with hierarchical structures and instructive biomaterials that enabled to promote rapid endothelialization, maintain long-term patency and remodel neovessel.

2. Materials and methods

2.1. Fabrication of heparinized silk fibrin

Heparin (sodium salt, Sigma–Aldrich) was immobilized onto silk fibrin using an N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC, Sigma–Aldrich) and N-hydroxysuccinimide (NHS, Sigma–Aldrich) covalent crosslinking method. The preparation process for heparinized silk fibrin was shown in (Fig. 1a). In brief, the heparin solution of 5% (w/v) was prepared with 2-morpholinoethane sulfonic acid (MES buffer, 0.05 M, pH 6.0). The carboxylic acid groups of heparin was added to a 50 ml volume of EDC/NHS-activated heparin solution (Hep-COOH) were activated by adding EDC and NHS to the heparin solution. The molar ratio of EDC:NHS:Hep-COOH was 2:1.3:1 for 1 h at 37 °C. Then, the platelet-poor plasma (PPP) was incubated with 2 ml diluted rabbit blood for 1 h at 37 °C. The activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT) were conducted to evaluate anticoagulant activity of the vascular grafts by thromboplastin, prothrombin and thrombin kit ( Jiancheng, Nanjing). The square pieces of 10 mm × 10 mm of each sample were tightly attached to the bottom of a 24-well culture plate and incubated with 2 ml diluted rabbit blood for 1 h at 37 °C. Then, the platelet-poor plasma (PPP) was collected by centrifuging at 3000 rpm for 20 min. Three sets of 200 µl PPP were treated separately activated partial thromboplastin, prothrombin and thrombin at 37 °C for 3 min.

2.2. Construction of small-diameter vascular grafts

We dissolved SF–Hep and poly[(ε-caprolactone) (PCL) (Mn = 80000 g/mol, Sigma-Aldrich, US) pellets in 1,1,1,3,3,3-Hexafluoro-2-propanol with a ratio of 1:1 (w/v) at 24 wt%. The mixture of SF-Hep fibrin solution was loaded into a 10-mL syringe with a 21-gauge needle and injected using a syringe pump (ET-1334H, Beijing Ucalery). The injection speed was 4 mm/min and the scope of translation was 35 mm. We employed a high-voltage generator to generate a voltage of 9 kV. The oriented SF-Hep nanofibers were collected by a rotator spinning at 2800 r/min. We subsequently rolled the films of oriented SF-Hep nanofibers with 3 mm-diameter stainless steel rod, and the rolled films were further fixed with vertically electrospun pure PCL fibers (12%, dissolve in 1,1,1,3,3,3-Hexafluoro-2-propanol). The oriented SF-Hep graft was successfully fabricated after the mandrel was taken off. The non-oriented SF-Hep graft was made with non-oriented SF-Hep and PCL nanofibers that were fabricated with the rotator spun at 100 r/min. The temperature and relative humidity were 37 °C and 60%, respectively. The oriented SF grafts without heparin were prepared by the same protocol for oriented SF-Hep.
lactate dehydrogenase (LDH, Dojindo Chemical Company, Shanghai, China). The LDH activity in the lysed platelet suspensions was measured using an LDH cytotoxic Assay Kit. Platelets adhered to the vascular grafts was observed by scanning electron microscopy. Simultaneously, the CD62 as an activation marker of platelets was measured to evaluate the platelet activation by ELISA (Qiyi Biological Technology, Shanghai China). Endothelial cells (ECs) were commercially purchased and used to evaluate the adhesion and proliferation of ECs on different luminal surfaces of vascular grafts. Cell adhered to the scaffolds was observed by laser scanning confocal microscopy (LSCM, Leica, TCS SP5, Germany) and scanning electron microscopy.

2.6. In vivo evaluation based on rabbit carotid artery model

In vivo performance of vascular grafts was further evaluated by rabbit carotid artery model. Animal studies were carried out in compliance with the protocol approved by the Institutional Animal Care and Use Committee of HUST. Twenty-one male mongrel rabbits (1.2–1.8 kg) were randomly divided into 3 groups of vascular grafts with the oriented SF-Hep vascular grafts and the controls. The rabbits were normally anesthetized by injecting 3–5 ml of 3% pentobarbital sodium solution from the auricular vein. After the rabbits become completely unconscious, we shaved heavy hair around the neck with depilatory cream and disinfected the region of neck and incised the epidermal layer to expose and separate the left internal carotid artery. A segment of artery (about 6 cm) was transected between the clamps, and the vascular grafts (about 2 cm in length) were implanted and sewn into the defect position of rabbit carotid artery. After removing the clamps, the blood flow was restored, and the wounds were closed with 3–0 monofilament nylon sutures. Aspirin was administrated daily as anticoagulant for 1 week (2 mg/kg).

2.7. Doppler ultrasound examination

Doppler sonographic assessments (EPIQ 5, Philips Ultrasound, Inc.) of the implanted vascular grafts were performed in different time intervals after implantation of 1 week, 1 month, 2 months and 3 months, respectively. At each time point, the rabbits were anesthetized with previously protocols, and 3D color ultrasonography and the real-time flow velocity were obtained by collected. The red and blue colors in Doppler flow spectrum represent the forward and reverse flow respectively.

2.8. Histological staining and immunofluorescence staining

At the endpoint of animal evaluation, all rabbits were sacrificed by
pentobarbital injection. The conduits were explanted and fixed for the histological analysis and immunofluorescence staining based on previous protocols.

2.9. Quantitative real-time PCR

Total RNA was harvested from native vessels and oriented SF-Hep grafts after explantation, respectively. Quantitative real-time PCR (qRT-PCR) was performed on a BIO-RAD CFX Connect RealTime PCR System (Bio-Rad, Hercules, CA) based on SYBR® Green Master Mix (Yeasen, China). The relative expression level of the mRNA of interest was expressed as $2^{\Delta\Delta CT}$ and normalized to GAPDH. The primer sequences used in this study are listed in Table S2.

2.10. Western blot analysis

The grafts and native carotids were homogenized in cold RIPA buffer (Servicebio, China) containing protease inhibitor cocktail. The homogenized tissues were centrifuged at 12000 rpm for 10 min at 4 °C and protein contents of the supernatants were measured by BCA Assay (Servicebio, China). After being boiled in water for 15min, the protein samples were separated by electrophoresis using 8% SDS–polyacrylamide gels. Then the separated proteins were transferred onto transfer membranes(Immobilon, Germany) and incubated with following primary antibody. The membranes were then incubated with an second antibody and washed with TPBS. The bands were detected by adding Super ECL Detection Reagent (Yeasen, China) and quantified using Image-J software. Antibodies used are listed in Supplemental Table S3.

2.11. Statistical analysis

Data for APTT, TT, PT, contact angle, platelet adhesion, platelet activation, mechanical tests, cell proliferation, luminal area were analyzed by SPSS 22.0 software. Comparison of means used samples t tests and one-wayanalysis of variance to determine the statistical difference. When values of $p < 0.05$, $p < 0.01$, and $p < 0.001$ were considered to indicate statistically significant (*), very significant (**), and extremely significant (***). The results were expressed as mean ± standard deviation (SD).
3. Results

3.1. Fabrication and characterization of heparinized silk fibroin (SF-Hep)

Silk fibroin (SF) and heparin (Hep) were covalently grafted to create a novel and fundamental anticoagulant biomaterial of heparinized Silk fibroin (SF-Hep) (Fig. 1a and Fig. S1a). FTIR results of SF, Hep and SF-Hep further showed that the classic absorption peaks of silk fibroin and heparin were detected in the spectrum of SF-Hep. The sulfate group of heparin was probed at the position of 1039 cm\(^{-1}\), which was displayed in SF-Hep. The characteristic amide groups of silk fibroin (amide I, 1629 cm\(^{-1}\) and amide II, 1532 cm\(^{-1}\)) were also present in SF-Hep (Fig. S1b). Results of elemental analysis (EA) demonstrated that heparin molecules were successfully immobilized onto the SF due to the

Fig. 3. Hemocompatibility evaluation of vascular grafts in vitro. (a) Schematic illustration showed that the platelets were isolated from the rabbit whole blood and seeded on different substrates to evaluate the platelet adhesion and activation. (b) SEM images of platelet adhesion on the three kinds of substrates demonstrated that biological cue (heparin) played a positive role in inhibiting platelet adhesion. (c) Quantitative analysis of platelet adhesion further confirmed that fewer platelets were attached on the surface of the SF-Hep graft, and (d) ELISA results demonstrated that the heparinized graft with the oriented intima was favorable to reducing platelet activation. Data were expressed as mean ± SD, n = 6 ***p < 0.001.
The presence of typical sulfur element in SF-Hep compared with SF (Fig. S1c). The grafting amount of heparin onto the heparinized silk fibroin was estimated by the content of elemental sulfur between the heparinized silk fibroin and pure heparin, and the Hep loaded onto the SF was 17.46 ± 0.40% (Table S1). Analysis of the XPS spectra revealed the presence of characteristic peak: S 2p of the Hep and SF-Hep (Fig. S1d). EA, FTIR and XPS results solidly verified the SF-Hep was successfully synthesized via the chemically covalent strategy.

3.2. Physicochemical characterization of vascular grafts

The artificial blood vessels with around 3 mm in diameter and 0.25 mm in wall thickness were constructed by a two-step cross-electrospinning (Figs. 2a and S2a). For the inner layer of vascular grafts, both SEM and Laser interferometry micrographs confirmed that the parallelly aligned structure was clearly observed in oriented SF and SF-Hep compared with non-oriented SF-Hep. We also found interface wettability of inner surface was obviously influenced by surface topology. The measurement of water contact angle (WCA) showed that the oriented surface exhibited higher hydrophobicity than that in the non-oriented surface (Fig. 2b). The WCA on the oriented SF graft and oriented SF-Hep graft were 69.67° ± 5.03° and 73.83° ± 7.15°, respectively. However, the WCA on the non-oriented SF-Hep graft declined to 21.67° ± 2.89°, indicating the orientation of nanofibers.
enhancing hydrophobicity (Fig. 2c). For the outer layer of vascular grafts, the porous structure was detected (Figs. S2b and c). Based on the results of mass loss in different time points of 1, 2, and 3 months, there was no significant difference in the mass loss among different vascular grafts (Fig. S3). Results of mechanical strength indicated that the oriented structure of nanofibers offered the higher elastic modulus than that in the non-oriented structure (Fig. 2d). Similarly, the other properties including tensile strength, elongation at break, and burst pressure were also significantly enhanced by the oriented alignment of nanofibers (Figs. S4a–d). Consequently, the oriented structure of vascular grafts could extremely improve their mechanical properties.

3.3. Anticoagulant evaluation of vascular grafts

APTT, TT and PT were separately measured to evaluate the anticoagulation of various vascular grafts (Fig. 2e). For the oriented SF, the anticoagulant time of APTT, TT and PT were 48 ± 7s, 38 ± 7s and 44 ± 4s, respectively. For the oriented SF-Hep, the anticoagulant time of APTT, TT and PT were 134 ± 5s, 124 ± 3s and 106 ± 11s, respectively. There was no significant difference on anticoagulant time between oriented SF-Hep and non-oriented SF-Hep. All those results showed that both oriented SF-Hep graft and non-oriented SF-Hep could extremely prolong anticoagulant time in comparison with the oriented SF graft without heparin immobilization. Thus, the immobilized heparin molecules made a primary contribution on anticoagulation while the oriented structure slightly affected on anticoagulation.

3.4. Regulation of platelet adherence and activation by vascular grafts

Rabbit platelet-rich plasma was prepared and used to investigate the adherence and activation of platelets (Fig. 3a). The results of SEM and LDH activity indicated that the number of adherent platelets on the oriented SF-Hep and non-oriented SF-Hep substrates was extremely less than that on oriented SF substrates (Fig. 3b and c). Compared with oriented SF substrates, the expression of CD62L extremely decreased in oriented SF-Hep and non-oriented SF-Hep grafts based on ELISA analysis (Fig. 3d). The results clearly indicated that biochemical cue of heparin molecule exerted the major influences on platelets adherence and activation.

3.5. Interaction between endothelial cells and vascular grafts

Endothelial cells (ECs) were cultured on three vascular grafts within 5 days, forming a cell monolayer on the substrates. We found that the ECs on non-oriented substrates exhibited the random distribution whereas the cells on oriented substrates presented highly alignment along the extended direction of nanofiber (Fig. 4a). The CCK-8 assay showed that three grafts were able to support cell adhesion and proliferation, and there was no significant ECs proliferation among various vascular grafts (Fig. 4b). SEM and LSCM images displayed that the resident cells on oriented grafts presented a significant spindle shape along the paralleled nanofibers while the polygonal shape of cells were displayed on non-oriented grafts, indicating the oriented structure extremely influenced the morphology of resident cells (Fig. 4c).

3.6. Numerical simulation of hemodynamics

Blood flow velocity in vascular grafts with oriented and non-oriented inner surfaces was investigated by numerical simulation. We firstly built a numerical model of hemodynamics according to SEM images of inner surface. Our findings showed that the surface topology significantly influenced blood flow velocity and hemodynamics. More intersections of fibers in non-oriented grafts extremely plummeted blood flow velocity compared with that in oriented grafts (Fig. 5 and Video.S1). Both surface streamline and 3D streamline clearly confirmed the severe turbulence caused by intersections in non-oriented grafts (Fig. 5 and Video.S1). In contrast, there was no obvious turbulence in oriented grafts that were paralleled to the direction of blood flow (Fig. 5 and Video.S2). Therefore, the oriented structure can not only induce the aligned growth of vascular cells, but also show a vital impact on blood flow velocity and hemodynamics.

Supplementary data related to this article can be found at https://doi.org/10.1016/j.biomaterials.2019.119654.

3.7. Long-term patency of graft implantation

In vivo implantation and replacement of artificial vascular grafts was conducted on the left carotid artery of the rabbits (Fig. 6a and b). The patency of implanted grafts was evaluated by Doppler ultrasound at four time points of 1, 2 months, and 3 months (Fig. 6c). At the first time point, all oriented SF-Hep grafts were patent while 3 of 7 non-oriented SF-Hep grafts and all oriented SF grafts got obstructed. The patency rate of the oriented SF-Hep was as high as 85.7% at timepoints of 1 and 2-month, but the patency rate of non-oriented SF-Hep grafts declined to zero after 2 months. At the endpoint of 3 months, the patency rate of oriented SF-Hep grafts was maintained with 71.4% (Fig. S5). Thus, to maintain a satisfactory long-term patency, two keys factors of heparinized graft and oriented topography should be considered simultaneously.

3.8. Histological analysis

The implants were withdrew to perform histological analysis within three months after implantation. Based on the observation of cross-section images, the patency of oriented SF-Hep was excellent comparable to native vessels whereas two controls of oriented SF and non-oriented SF-Hep were severely obstructed by embolisms (Fig. 7a). The results of Hematoxylin/Eosin (H&E) staining further exhibited that both native vessels and the oriented SF-Hep possessed a round-shaped lumen and a smooth inner side (Fig. 7b). New intact and dense lumen was completely formed in oriented SF-Hep vascular grafts, but the luminal area was smaller than that in native vessels (Fig. 7c, d). Also, the tube wall of oriented SF-Hep grafts was thicker than that in native vessels (Fig. 7d). Compared with the controls, the porous outer layer of oriented SF-Hep exhibited ingrowth of newly-formed tissues. Therefore, histological results confirmed that both oriented structure and immobilized heparin played a key role on manipulating luminal formation and sustained patency of small-diameter vascular grafts.

3.9. Remodeling of vascular grafts into neo vessel

Immunofluorescence of vascular inner and media layer was further investigated to evaluate the remodeling of oriented SF-Hep vascular grafts. Our findings showed that two typical markers of vascular ECs, CD31 and vWF, were positively stained and concentrated at the continuous basement membrane proving that complete endothelialization was generated (Fig. 8a). Smooth muscle cells, major components for vascular media layer, were confirmed to exist via the positive staining of α-SMA and meanwhile both collagen I and collagen III in the ECM were verified in the regenerated neovessel compared with native vessel (Fig. 8a). Cell number in the remodeled grafts was similar to that in the native vessels (Fig. 8b). Both gene and protein expressions of α-SMA, CD31, and collagen I were separately measured to assess the re-generation of the vascular inner and media layer using RT-qPCR and Western Blot (Fig. 8c and d). The results indicated that the oriented SF-Hep vascular grafts were capable of recruiting and remodeling endogenous endothelial cells and smooth muscle cells to promote endothelialization and ECM deposition in situ.

4. Discussion

Long-term patency of small-diameter vascular grafts remains a
major challenge due to the formation of thrombosis and the lack of endothelialization in current vascular tissue engineering [7,37]. Development of new materials with the ability of anti-thrombosis and promoting endothelialization has been considered as a desired solution. Some previous works have referred to the construction of specific surface nanotopography for manipulating the endothelialization, but most of them ignore the issue of thrombosis in the early stage [38,39]. On the contrary, some other works have successfully designed antithrombotic materials to overcome the early thrombosis, but they finally failed due to the lack or deficiency of endothelialization in the late stage [40,41]. In this study, we for the first time created a small-diameter vascular graft with the capacity of anti-thrombosis by means of heparinized silk fibroin and the promotion of endothelialization via the oriented intimal structure. Unlike routine strategies, our current design combines bio-physical and biochemical cues in one system to simultaneously inhibit platelets adhesion and activation and stimulate rapid endothelialization of vascular grafts.

Oriented structures of artificial vessels can drive the aligned adherence of endothelial cells to promote rapid endothelialization. Firstly, the aligned ECs guided by oriented nanofibers can form a dense monolayer cell-sheet to reduce the contact area between platelets and nanofibers, and eventually inhibiting the thrombus formation [34,42,43]. Secondly, the oriented growth of ECs along the direction of blood flow is based on the biomimetic intimal structure of native vessels.

Fig. 5. Numerical simulation of blood flow velocity on different inner surfaces. Simulation models for vascular grafts with oriented fibers and non-oriented fibers were built based on their SEM images. Non-oriented intimal surface presented more intersections compared with oriented one. The models of both surface streamline and 3D streamline showed that blood flow velocity extremely plummeted as flowing through these intersections in non-oriented surface (See Video S1 in SI). In contrast, there were less intersections blocking the blood flow due to oriented fibers paralleling to the direction of blood flow (See Video S2 in SI).
that consisting of the aligned ECs [18,44]. Moreover, ECs cultured on aligned nanofibrillar films remained well-aligned exposed to disturbed flow had significantly reduced inflammation and proliferation, while maintaining intact intercellular junctions [45]. And nanoscale signaling cues from aligned nanofibrillar scaffolds can induce improved neovascularization in vivo by mediating the process of integrin α1 activation which is benefit to ECs growth [46].

In this study, we similarly confirmed the aligned formation of resident ECs induced by the oriented grafts (Fig. 4). More importantly, we firstly applied the numerical simulation to elucidating the influence of non-oriented and oriented intimal structure on blood flow velocity and hemodynamics (Fig. 5). We found that the severe turbulence of blood flow caused by non-oriented grafts that might affect the anticoagulation and endothelialization of vascular grafts [21,47]. Consequently, our current work not only successfully constructs a biomimetic small-diameter vascular graft with oriented nanofiber intima, but also systematically extends the effects of oriented interface on tuning the behaviors of vascular cells as well as manipulating the hemodynamics (Fig. 5 and Videos S1 and S2).

To obtain a bioinspired vascular graft employing optimal
hemocompatibility and flexible mechanical strength, in this work we combined natural and synthetic materials via the electrospinning technique [22]. Heparin coated materials have been widely applied on vascular tissue engineering due to the excellent anticoagulation of heparin molecule [29]. Polycaprolactone as a FDA-approved degradable polymeric material is readily to produce nanofiber by electrospinning technique [30]. In this work, soft heparinized silk fibroin and stiff polycaprolactone were mixed to construct vascular grafts that can achieve flexible mechanical properties. Moreover, the composite material of silk fibroin and polycaprolactone can maintain a degradable balance prior to the remodel of neovessel.

5. Conclusions

In summary, we successfully fabricated a two-layer small-diameter vascular grafts through a two-step cross-electrospinning. The inner layer consisted of heparinized silk fibroin (SF-Hep) and polycaprolactone (PCL) employed the oriented structure and sustained anticoagulation. The outer layer fabricated by pure PCL presented the vertically porous structure. The results demonstrated that this biomimetic intima with oriented nanotopography and anticoagulant property played a pivotal role in the inhibition of platelet adherence and activation, induction of oriented and compact growth of ECs, reduction of the turbulence of blood flow, promotion of rapid endothelialization,
and maintenance of long-term patency. On the other hand, the vertically porous outer layer provided a stable mechanical support, exchange of bioactive molecules, and ingrowth of regenerated tissues. Therefore, our current work not only creates a cell-free small-diameter vascular graft with biochemical induction and biophysical stimulation in one system, but also firstly verifies the synergetic effects of oriented nanotopography and anticoagulant bioactivity on manipulating rapid endothelialization and long-term patency of small-diameter vascular grafts.

Fig. 8. Remodeling of vascular graft into neovessel. (a) Typical markers of endothelial layer, CD 31 (red) and vWF (red) were positively stained in both oriented SF-Hep grafts and native vessels, indicating the complete intima formation in oriented SF-Hep grafts. The smooth muscle cells layer was verified by the positive staining of α-SMA (green). The components of ECM were stained with collagen I (green) and collagen III (red). Cell nuclei was stained with DAPI (blue). (b) Quantitative analysis of cell counting based on five parts of whole section by DAPI staining. There was no significant difference between them. (c, d) Both gene and protein expression of α-SMA, CD31 and collagen I were investigated to confirm the formation of intimal and media layer of vascular grafts. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This work was financially supported by the National Key Research and Development Program of China (2017YFC1103900, 2018YFC1105700, 2016YFA0101100), the National Natural Science Foundation of China (31670968, 81601610, and 31800805), Sanming Project of Medicine in Shenzhen (SZSM201812055), and the