Fabrication of Multi-layered Composite Scaffolds by Bi-directional Electrospinning Method

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Abstract: A multi-layered composite scaffolds consisting of poly (L-lactide-co-ε-caprolactone) (P(LLA-CL)), collagen (COL) and chitosan (CS) were fabricated by a bi-directional electrospinning method. Synthetic P(LLA-CL) was used as the middle layer to enhance the strength, while natural COL/CS blending (9∶1, v/v) was used as the bioactive surfaces (inner and outer layers) to improve the biocompatibility. Each three transitional layers were set between inner/outer layer and middle layer for delamination resistance. Scanning electron microscopy (SEM) was used to observe the fiber morphology. The Fourier transform infrared attenuated total reflectance spectroscopy (FTIR-ATR) spectra, X-ray diffraction (XRD) and thermogravimetry (TG) tests were used to analyze the physical properties of the scaffolds. The results showed that the modified electrospinning method had no negative effect on the components, crystal structure and thermostability of the scaffolds, but could effectively combine the mechanical property of synthetic material and biocompatibility of natural materials. Such method could be applied to the fabrication of composite scaffolds for vascular, skin, and nerve tissue engineering.

Key words: *electrospinning; poly (L-lactide-co-ε-caprolactone) (P(LLA-CL)); collagen; chitosan; composite nanofibers*

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Introduction

Since pure synthetic materials do not offer clinically satisfactory bioactivity, the bionics design and fabrication of biocompatible scaffolds occur as core tasks from material and biology science perspectives [1]. Usually, synthetic and natural materials were directly utilized in combination, however, it still cannot be avoided that synthetic materials would directly contact with tissues. In this case, novel composite methods of mixing synthetic and natural materials should be eagerly developed.

Collagen (COL) and chitosan (CS) have been the common used natural polymers in clinical application, finding widespread utilization in tissue engineering to enhance bioactivity and biofunctionability as well [2-3]. Also, electrospun fibrous scaffolds have been widely applied in numerous tissue engineering approaches, because such fibrous scaffolds bear size scale and structural similarity to native extracellular matrix [4], and micro and nano scale fibers could be easily and effectively fabricated via electrospinning method [5-6].

As reported, two separate components and gradient fiber electrospinning could be developed to fabricatethe multilayered scaffolds with diverse fiber scale and combined properties [7-8]. In this study, a developed bi-directional electrospinning method was applied to fabricating multi-layered composite scaffolds. Through regulating the flow rates of both sides in electrospinning process, the fiber diameter and the components proportion of each layer could be controlled. The addition of poly (L-lactide-co-ε-caprolactone) (P(LLA-CL)) aimed at enhancing the strength support, and the natural interfaces of COL and CS were applied to improving the biocompatibility. Between inner/outer and middle layers, three transitional layers were set respectively to avoid delamination. The specific structure of composite scaffolds would combine the sufficient strength support of P(LLA-CL) and the optimal biocompatibility of COL and CS, which would be a promising candidate for tissue-engineered application.

1 Materials and Methods

1.1 Materials

P(LLA-CL) (50∶50) was purchased from Gunze Limited (Japan, molecular weight 3×10^5 Da). COL type I was purchased from Sichuan Ming-rang Bio-Tech Co., Ltd. (China, molecular weight 1×10^5 Da) CS was purchased from Sigma-Aldrich (UK, Brookfield viscosity 200 000 cps, medium molecular weight). 1, 1, 1, 3, 3, 3-hexafluoro-2-propanol (HFIP) was obtained from Fluorochem Ltd. (UK) 2, 2, 2-trifluoroacetic acid (TFA) and a crosslinking agent of aqueous glutaraldehyde (GA) solution (25% by volume) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China)

1.2 Fabrication of multi-layered composite scaffolds

P(LLA-CL) and COL were dissolved in HFIP at a concentration of 8%, respectively. CS was dissolved in HFIP/TFA blended solvent (9∶1, v/v) at a concentration of 6%. After stirred uniformly, P(LLA-CL) was set as component I, and COL/CS blending (9∶1, v/v) was set as component II. Each component was severally filled into a 5 mL plastic syringe with a blunt-ended needle. Then the syringes were respectively located in syringe pumps (789100C, Cole-Pamer, America) and dispensed at scheduled flow rates. High voltages of 14 and 20 kV from two high voltage power supplies (BGG 6-358, BMEICO, Ltd., China) were severally applied on the needles of components I and II, and collecting distances were both 12 cm.

After the flow rate groups of components I and II for each layer were confirmed, P(LLA-CL)/COL/CS multi-layered composite scaffolds were fabricated via continuous bi-directional electrospinning method. Two electrospinning equipments were set in opposite directions. High voltages and collecting distances were set according to the above parameters, and each flow rate group lasted for 30 min. After fabrication, multi-layered

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composite and COL/CS scaffolds were cross-linked in a sealed desiccator with GA vapor (evaporating from 10 mL of 25% GA aqueous solution) at room temperature for 24 h. Then the samples were placed in the vacuum oven for 7 d.

1.3 Characterization methods

To observe the morphologies of fibers and different structures of inner/outer and middle layer of composite scaffolds, scanning electron microscopy (SEM) (JSM-5600, Japan) was used at an accelerated voltage of 10 kV. Then Image J was used to analyze the average fiber diameter (n = 100).

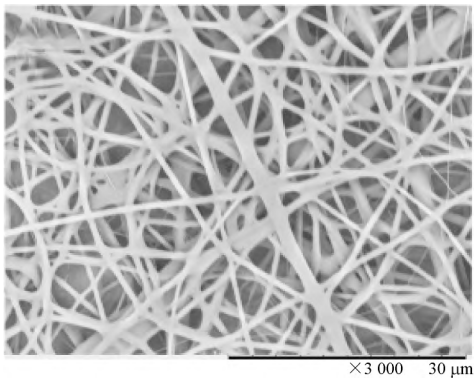
To analyze the components of composite scaffolds, the Fourier transform infrared attenuated total reflectance spectroscopy (FTIR-ATR) spectra were obtained at room temperature on FTIR instrument (NEXUS-670, America) in the range 4 000-600 cm^{-1} at a resolution of 4 cm^{-1}.

To analyze the crystal structure of composite scaffolds, X-ray diffraction (XRD) curves were obtained on an X-ray diffractometer (D/max-2500 PC, Japan) within the scanning region of 2θ (5° - 50°), with CuK_α radiation (1.541 8 Å) at 40 kV and 300 mA.

The thermogravimetry (TG) curves were acquired using a thermogravimetric analyzer (TG 209 F1, Germany) from room temperature to 600 ℃ at a rate of 10 ℃/min under a nitrogen atmosphere. The nitrogen gas flow rate was 40 mL/min.

2 Results and Discussion

SEM micrographs of P(LLA-CL) and COL/CS with different flow rates are shown in Figs. 1 and 2. Figure 1 shows that the diameter of P(LLA-CL) nanofibers increases with the increase of flow rates. With the flow rate changing from 0.1 to 3.2 mL/h, the average fiber diameter changes as (834 ± 135), (1 066 ± 226), (1 256 ± 289), (1 509 ± 201), (1 615 ± 164), (2 264 ± 382), and (2 581 ± 411) nm.



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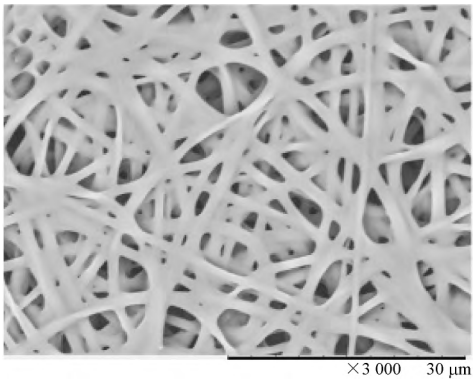

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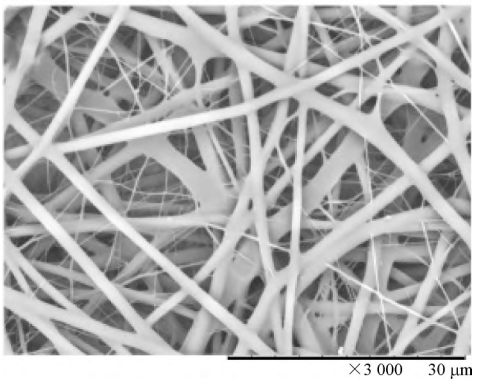

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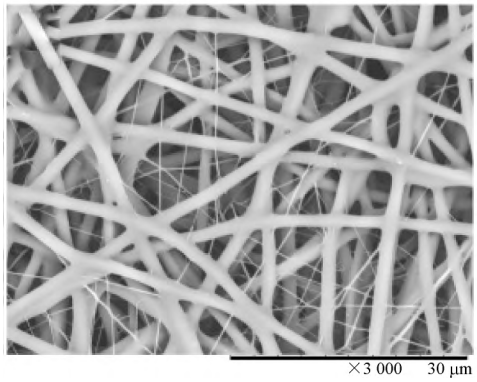

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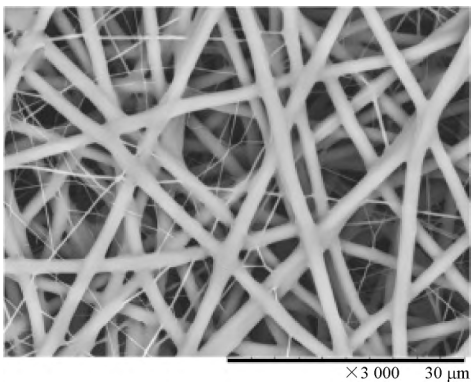

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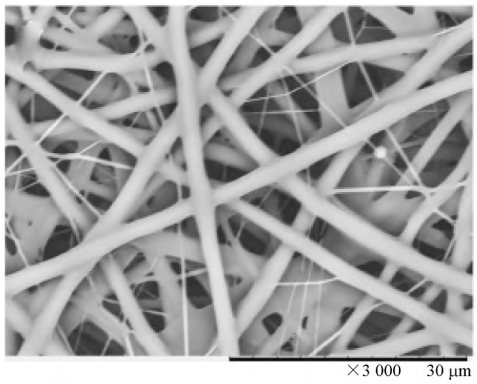

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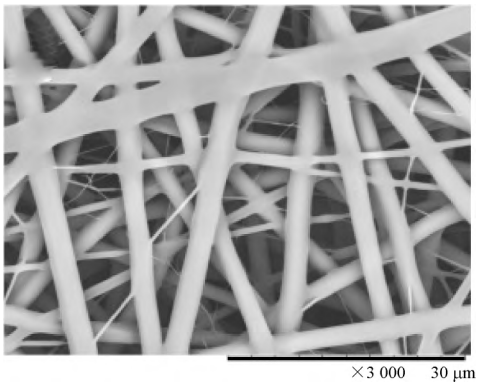

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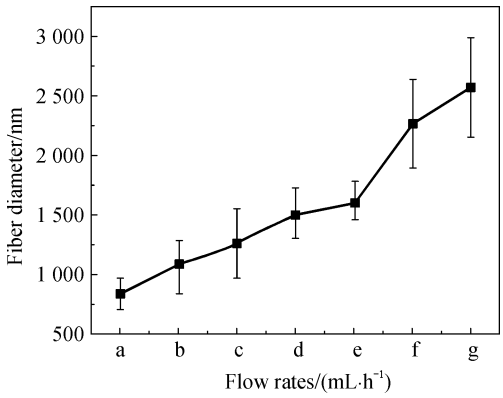
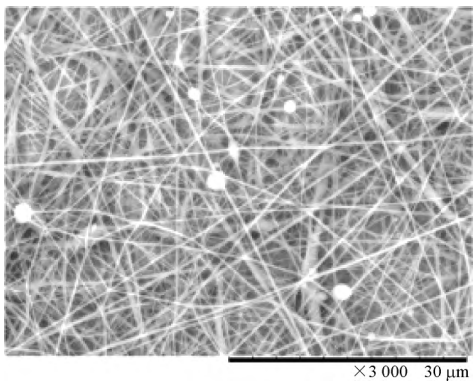


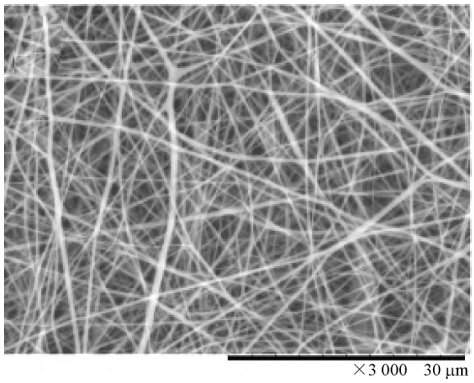
(h)

Fig. 1 SEM images of P(LLA-CL) with different flow rates ((a) 0.1; (b) 0.3; (c) 0.5; (d) 0.7; (e) 1.0; (f) 1.6; (g) 3.2) and average fiber diameter (h)

Figure 2 shows that the diameter of COL/CS nanofibers changes little with the increase of flow rates. With the flow rate changing from 0.1 to 2.0 mL/h, the average fiber diameter changes as (366 ± 117) , (419 ± 107) , (443 ± 113) , (445 ± 116) , (479 ± 106) , (483 ± 115) , and (521 ± 130) nm. As a result, to fabricate a symmetrically gradient structure, flow rate groups (mL/h) of components I and II from inner layer to outer layer are set as follows: 0.8/0, 0.4/0.2, 0.2/0.4, 0.1/0.8, 0/1.6, 0.1/0.8, 0.2/0.4, 0.4/0.2, and 0.8/0.



(a)



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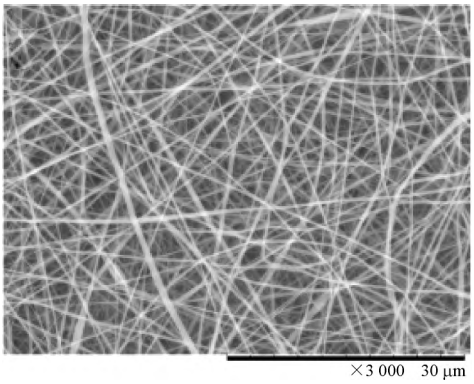

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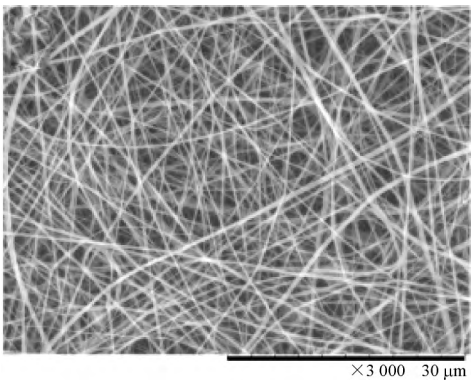

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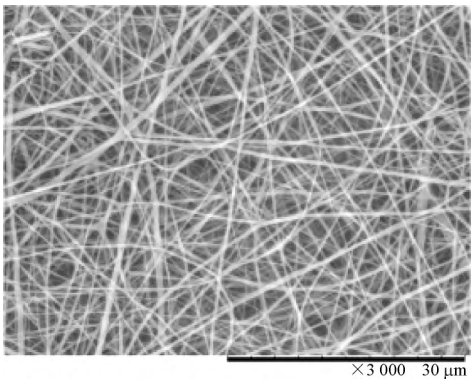

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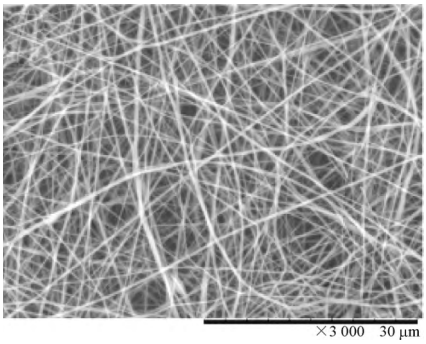

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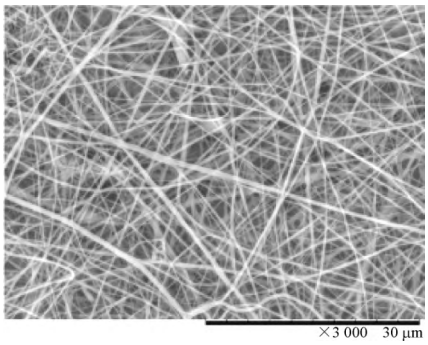

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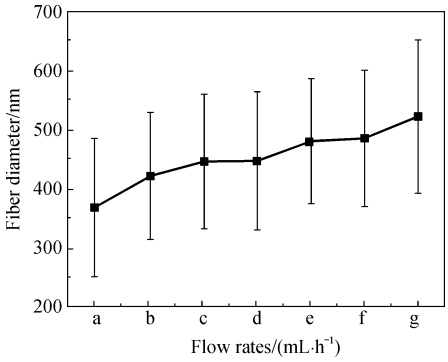
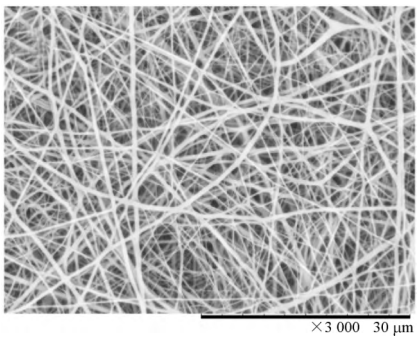


(h)

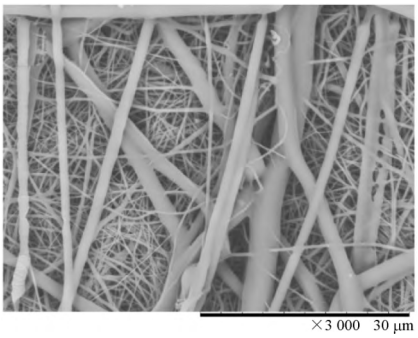
Fig. 2 SEM images of COL/CS with different flow rates ((a) 0.1; (b) 0.3; (c) 0.5; (d) 0.7; (e) 1.0; (f) 1.5; (g) 2.0) and average fiber diameter (h)

Continuous bi-directional gradient electrospinning was applied to fabricating multi-layered composite scaffolds, and each flow rate group lasted for 30 min. As shown in Fig. 3, the inner/outer and middle layers of composite scaffolds indicate significant difference in fiber diameter. Several thicker microfibers of middle layer covered on thinner nanofibers, which demonstrated the diverse fiber structure of composite scaffolds.

For tissue-engineered application, scaffolds with COL and CS should be cross-linked to avoid being dissolved in physiological environment, and GA vapor was an acceptable method [9]. Figure 4 (A) shows FTIR-ATR spectra of P(LLA-CL), composite and COL/CS scaffolds before and after cross-linking. The composite scaffolds remained the functional groups of both P(LLA-CL) and COL/CS, and the functional groups kept consistent before and after cross-linking. Figure 4 (B) shows XRD curves of different scaffolds. The composite scaffolds also remained the crystal feature of P(LLA-CL) electrospun scaffolds and GA crosslinking process had no negative effect on the scaffolds. In short, the developed electrospinning method and GA vapor crosslinking process would not change the components and crystal characteristics of composite scaffolds, indicating the stability of scaffolds preparation.

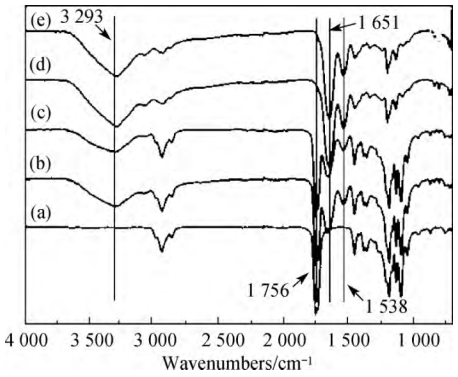


(a)

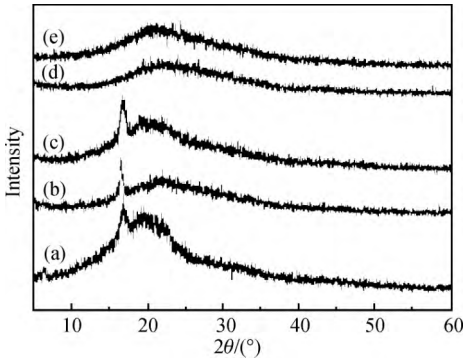


(b)

Fig. 3 SEM images of inner/outer layer (a) and middle layer (b) of multi-layered composite scaffolds



(A)



(B)

Fig. 4 FTIR-ATR spectra (A) and XRD curves (B) of P(LLA-CL) (a), composite (b, c) and COL/CS (d, e) scaffolds before GA crosslinking (a, b, d) and after GA crosslinking (c, e)

As shown in Fig. 5, P(LLA-CL) scaffolds had better thermostability than COL/CS scaffolds, because COL and CS were normally hydrophilic and would dehydrate at the beginning of thermal decomposition [10]. As for multi-layered composite scaffolds, the curve shape seemed to be similar to that of COL/CS scaffolds, however, with the mixture of P(LLA-CL), the composite scaffolds could stand higher temperature. Hence, with the combination of P(LLA-CL) and COL/CS, the thermostability of composite scaffolds was enhanced.

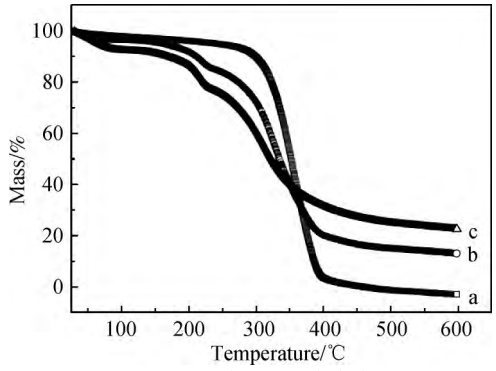


Fig. 5 TG curves of P(LLA-CL) (a), composite (b) and COL/CS (c) scaffolds

3 Conclusions

Multi-layered composite scaffolds were fabricated by the modified bi-directional electrospinning method. The inner and outer natural fiber-based surfaces avoided the direct contact of synthetic material with cells, which was found conducive to cells growth. The middle layer of the scaffold was formed by P(LLA-CL) fibers, which could provide sufficient mechanical support. Consequently, this kind of scaffolds combined the optimal properties of both natural and synthetic materials, which would be a promising candidate for vascular, skin, and nerve tissue engineering.

References

[1] Soliman S, Pagliari S, Rinaldi A, *et al*. Multiscale Three-Dimensional Scaffolds for Soft Tissue Engineering via Multimodal Electrospinning [J]. *Acta Biomaterialia*, 2010, **6**(4): 1227-1237.

[2] Elsabee M Z, Naguib H F, Morsi R E. Chitosan Based Nanofibers, Review [J]. *Materials Science and Engineering: C*, 2012, **32**(7): 1711-1726.

[3] Burck J, Heissler S, Geckle U, *et al*. Resemblance of Electrospun Collagen Nanofibers to Their Native Structure [J]. *Langmuir: the ACS Journal of Surfaces and Colloids*, 2013, **29**(5): 1562-1572.

[4] Metter R B, Ifkovits J L, Hou K, *et al*. Biodegradable Fibrous Scaffolds with Diverse Properties by Electrospinning Candidates from a Combinatorial Macromer Library [J]. *Acta Biomaterialia*, 2010, **6**(4): 1219-1226.

[5] de Valence S, Tille J C, Giliberto J P, *et al*. Advantages of Bilayered Vascular Grafts for Surgical Applicability and Tissue Regeneration [J]. *Acta Biomaterialia*, 2012, **8**(11): 3914-3920.

[6] Fridrikh S, Yu J, Brenner M, *et al*. Controlling the Fiber Diameter during Electrospinning [J]. *Physical Review Letters*, 2003, **90**(4): 144502-1-144502-4.

[7] Du F Y, Wang H, Zhao W, *et al*. Gradient Nanofibrous Chitosan/Poly ε-caprolactone Scaffolds as Extracellular Microenvironments for Vascular Tissue Engineering [J]. *Biomaterials*, 2012, **33**(3): 762-770.

[8] Grey C P, Newton S T, Bowlin G L, *et al*. Gradient Fiber Electrospinning of Layered Scaffolds Using Controlled Transitions in Fiber Diameter [J]. *Biomaterials*, 2013, **34**(21): 4993-5006.

[9] Chen Z G, Wang P W, Wei B, *et al*. Electrospun Collagen-Chitosan Nnanofiber: a Biomimetic Extracellular Matrix for Endothelial Cell and Smooth Muscle Cell [J]. *Acta Biomaterialia*, 2010, **6**(2): 372-382.

[10] Salomé Machado A A, Martins V C A, Plepis A M G. Thermal and Rheological Behavior of Collagen. Chitosan Blends [J]. *Journal of Thermal Analysis and Calorimetry*, 2002, **67**(2): 491-498.