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Fabrication of Patterned PDLLA/PCL Composite Scaffold by Electrospinning

He Xu,[1] Wenguo Cui,[1] Jiang Chang[1,2]
[1]School of Biomedical Engineering and Med-X Research Institute, Shanghai Jiao Tong University, Shanghai 200030, People's Republic of China
[2]State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, People's Republic of China
Correspondence to: J. Chang (E-mail: jchang@mail.sic.ac.cn)

ABSTRACT: Fabricating fibrous electrospun scaffolds with controllable fiber-arrangement have gained an increasing attention in the field of tissue engineering. In this study, the composite patterned D,L-poly(lactic acid)/poly(ε-caprolactone) (PDLLA/PCL) scaffolds were fabricated via electrospinning for the first time, and the order degree and contractibility of patterned composite scaffolds with different PDLLA/PCL ratios were further investigated. The results showed that the order degree of the pattern and *in vitro* shrinkage behaviors of PDLLA/PCL electrospun mats could be finely tuned by controlling blending ratios. The PDLLA/PCL electrospun mats with the ratio 50/50 showed the most balanced properties with controllable pattern structure and appropriate dimensional stability, and they might be a suitable candidate for tissue engineering application. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

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INTRODUCTION

The nanofibrous scaffolds obtained through electrospinning have similarity in morphology and fiber size scale to those of the native extracellular matrix (ECM), and have gained increasing popularity in the field of tissue engineering.[1–3] Current research have paid more and more attention on the control of electrospun fibers alignment and assembly accurately, since mimicking some aligned fibrous tissues like connective tissues[4,5] might positively affect cell behavior such as adhesion, growth, and proliferation, which is critical for tissue engineering applications of biomaterial scaffolds.[6,7]

Up to now, synthetic polymers such as poly(glycolide), poly(lactide), and poly(ε-caprolactone) (PCL) have been used for preparing electrospun scaffolds.[8,9] As an biocompatible polymer, the PCL material possesses good mechanical strength during the process of biological application, so that the PCL-engineered scaffolds have shown a great potential for tissue engineering applications.[10] However, it is a challenge in the control of PCL fiber alignment and assembly accurately. So far, PCL nanofibers have been collected as random or single directional aligned assembly by electrospinning, while little effort has been devoted to the preparation of PCL electrospun scaffold with complex ordered architecture,[11–13] and the fabrication of accurately controllable PCL fiber alignment and assembly remains an important issue.

Besides PCL material, D,L-poly(lactic acid) (PDLLA) is also a kind of synthetic polymer with good biocompatibility and has been widely used in tissue engineering applications.[14] Zhang and Chang had successfully fabricated PDLLA patterned electrospun mats with complex ordered patterns such as meshes and square matrix, and proved that the assembly of PDLLA nanofibers could be controlled accurately by changing the surface structures of collectors.[15,16] However, PDLLA electrospun mats have a very high shrinkage when they are immersed in culture medium at 37°C, which limits the application as tissue engineering scaffold.[17,18]

Considering the good mechanical strength and stability in culture medium of PCL and the controllable fabrication of patterned structure of PDLLA electrospun mats, it is assumed that the combination of PDLLA and PCL with certain mixture ratio would result in composite electrospun scaffolds with accurately controllable fiber assembly and good dimensional stability in culture medium condition. In order to test this hypothesis, patterned composite PDLLA/PCL scaffolds with different PDLLA/PCL ratios were fabricated via electrospinning, and the fiber order degree and contractibility of patterned composite scaffolds were evaluated.

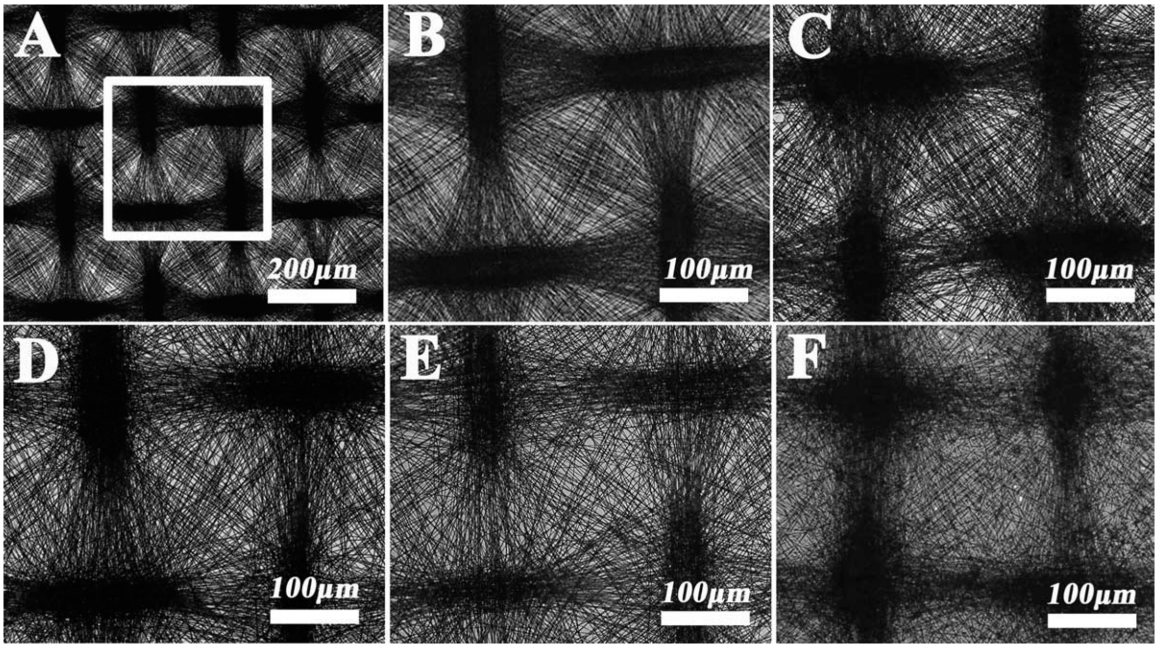

Figure 1. Optical images of the patterned electrospun membranes. A: The optical image of a pure PDLLA patterned electrospun membrane. B–F: A typical unit in the electrospun membranes (as shown in the white square region shown in image (A)) with different ratio of PDLLA/PCL: (B) 100/0; (C) 70/30; (D) 50/50; (E) 30/70; (F) 0/100.

EXPERIMENTAL

Materials

Polycaprolactone (PCL, MW = 50,000) was purchased from Shanghai Hosen Trade (Shanghai, China). PDLLA with an average molecular weight (MW) of 45 kDa was obtained from the Chengdu Institute of Organic Chemistry (Sichuan, China). Dimethylformamide (DMF) and tetrahydrofuran (THF) of analytical grade were purchased from A.R. Shanghai Chemical Reagent, (Shanghai, China). All the reagents in this experiment were used as received without any further treatment.

Electrospinning Process

The blend of PDLLA and PCL polymers with five different mass ratios (100/0, 70/30, 50/50, 30/70, 0/100) were dissolved in a mixture of DMF and THF (4 : 1,v/v), and then stirred for several hours to obtain a homogeneous and stable solution with polymer concentration of 4.8% (w/v). The feeding rate of the solution in the syringe (2 mL) was controlled at 0.02 mL/m by using a syringe pump (LSP01-1A, Baoding Longer Precision Pump, China). The voltages applied to the needle of the syringe were 6 kV. Distance between the tip of the needle and the collector was 12 cm. In this process, a stainless steel mesh with chess-like pattern was chosen as collector, the diameter of the wires in the collector is 100 μm, and the wire spacing is 220 μm. In addition, a patterned electroconductive collector with square-shaped protrusions was also used in the study. The width and height of the protrusions of the collector are 100 μm and 400 μm, respectively, and the spacing between protrusions is 200 μm.

The conductivity of the electrospinning solutions were measured by Five Easy^{TM} conductivity (FE30, Mettler Toledo, Switzerland), and the average value of conductivity was obtained through three repeated preparations. All the experiments were conducted at room temperature and the relative humidity was about 40–60%, and all the electrospun mats were vacuum dried for 24 h to completely remove any residual solvent prior to further characterization.

Morphology Observation of Electrospun Mats

The morphology of the obtained electrospun mats was observed by using an optical microscope (Leica DM2500 M). In addition, the order degrees of pattern electrospun mats with five different PDLLA/PCL ratios were evaluated from the optical images.

In Vitro Shrinkage Behaviors

The shrinkage behavior was evaluated from the morphological change after soaking in culture medium. The electrospun mats were first cut into squares with an average area of 10 × 10 mm^2 and then incubated in culture medium (2 mL) at 37°C for 7 days. After that, samples were washed out with large amount of water, and then dried under vacuum at room temperature for 24 h before surface area measurement. The contractibility rates of the samples were obtained by calculating the dimension shrinkage ratios of electrospun mats before and after incubation. The tests were done in triplicate and results averaged.

RESULTS

Electrospinning of PDLLA/PCL with Different Mixture Ratios

The electrospun mats with different PDLLA/PCL ratios were fabricated using an electroconductive collector with patterned structure, and Figure 1(A) shows an optical image of a pure PDLLA electrospun mat with low magnification. It was clear to see that a patterned architecture similar to the patterned

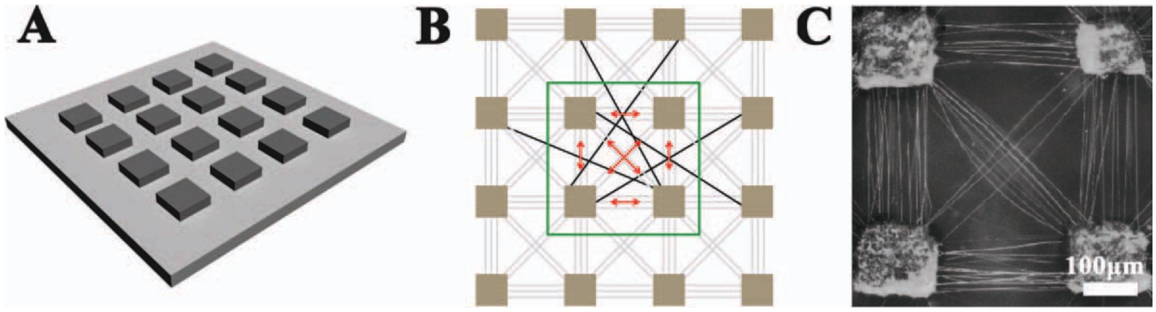

Figure 2. (A) Schematic illustration of the patterned collector with square-shaped protrusions. (B) Schematic illustration of a patterned electrospun membrane fabricated by the collector in (A). The green loop showed a typical unit. The fibers deposited in the 6 directions (red arrows showed the corresponding direction) are defined as regular, and the fibers out of the 6 direction displayed in black colour were considered as disordered. (C) The optical image of one patterned unit of an electrospun membrane. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

template collector was generated after electrospinning, and magnified images of the electrospun patterned mats with different PDLLA/PCL ratios were displayed in Figure 1(B–F), respectively. It was found that the electrospun mats were formed with a uniform crisscross pattern, the majority of the fibers were deposited onto the electroconductive areas of the collector, and the highest density of fibers were deposited on the cross points tending to arrange in a parallel manner, while there were also nanofibers deposited as sidelines and diagonals between the cross points.

Effect of PDLLA/PCL Mixture Ratios on the Order Degree of Patterned Electrospun Mats

As shown in Figure 1, the corresponding patterned mats became less ordered when the ratios of PDLLA/PCL decreased, the quantitative analysis of the fiber order degree was conducted on the mats collected using a more regular template [shown in Figure 2(A)] with a collected time of 15 s. The schematic illustration of the pattern membrane obtained through this process is shown in Figure 2(B), which demonstrate that numbers of fibers aligned in parallel between the most adjacent protrusions, while the others tend to be deposited as a cross between diagonally located protrusions (showed in light gray color). Taking four protrusions as a typical unit, the fibers deposited in the six different directions, which are displayed in the Figure 2(B) with red arrows, are defined as regular, and other fibers displayed in black color are disordered, the proportion that ordered fibers account for all the fibers in the image are defined as the value of order degree, the order degree is calculated using the following equation:

$$\varepsilon = 1 - N_1/N \tag{1}$$

where ε is the value of order degree, N is the number of all fibers within a four protrusions unit shown in Figure 2(C), and N_1 is the number of disordered fibers among them. To determine the order degree of a composite mat with a certain PDLLA/PCL ratio, 20 units from each mat were randomly selected in the optical images, and the average values of order degree were obtained.

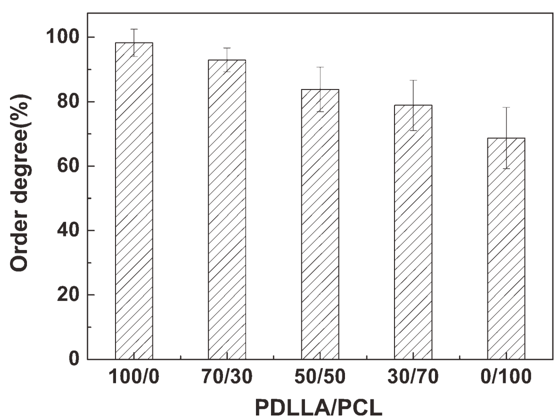

Figure 3. The relationship between PDLLA/PCL ratios and the order degree of corresponding patterned membranes. The values of order degree are calculated using the formula $\varepsilon = 1 - N_1/N$, in which N_1 is the number of disorder fibers, and the N is the number of total fibers in a typical unit showed in Figure 2(C).

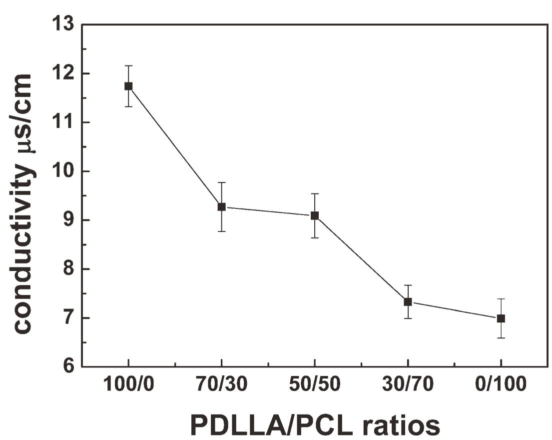

Figure 4. The conductivity of PDLLA/PCL composite solutions with five different ratios.

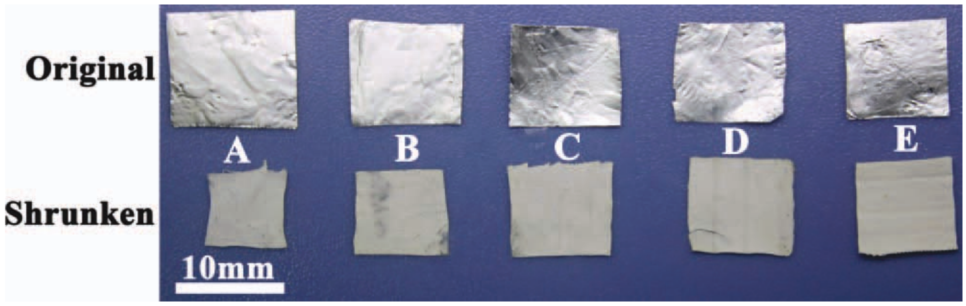

Figure 5. The optical images of electrospun membranes with different PDLLA/PCL ratios before and after soaking in culture medium: (A) 100/0; (B) 70/30; (C) 50/50; (D) 30/70; (E) 0/100. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The relationship between the ratios of PDLLA/PCL and the order degree of corresponding pattern mats were analyzed using the histogram (Figure 3). The results confirmed the fact that the order degree of patterned electrospun mats decreased with the reduction of the PDLLA/PCL ratios.

The conductivity of the PDLLA/PCL electrospun solution with different ratio is shown in Figure 4. A significant drop in the conductivity of the electrospun solutions could be observed with the decrease of the PDLLA/PCL ratios, and the pure PDLLA solution showed the highest conductivity.

Effect of PDLLA/PCL Mixture Ratios on the *In Vitro* Shrinkage Behaviors of Patterned Electrospun Mats

Figure 5 shows the shrinkage behaviors of patterned electrospun mats with different PDLLA/PCL mixture ratios. Significant surface area shrinkage was observed with electrospun PDLLA mat when it was immersed in culture medium, while the pure PCL mat showed almost no shrinkage, and with the decrease of the PDLLA/PCL ratios, the electrospun patterned mats were more stable in culture medium. The contractibility rates of the scaffolds were obtained by calculating the dimension ratios of electrospun mats before and after incubation. The results showed that the contractibility rates of the electrospun patterned mats decreased with the reduction of the PDLLA/PCL ratios (Figure 6).

DISCUSSION

Generally, electrospun fibers deposit randomly if a flat electroconductive plate is used as collector.[19] However, when collectors with different configurations such as conductive protrusions are used, the fibers prefer to deposit on the protrusions of collectors. It is reported that the arrangement of charged electrospun fibers is mainly affected by Coulomb interactions with collectors.[15,16] Since the Coulomb interactions is proportional to the positive charges on the fibers, surface charge density of fibers plays the most important role in the formation of electrospun mats with ordered architecture, when the other process parameters of electrospinning remain unchanged.[20]

In our study, it was clear to see that the order degree of fiber arrangement decreased with the reduction of PDLLA/PCL ratios, while the pure PDLLA mat revealed the most ordered pattern (Figure 1). The quantitative analysis of the fiber order degree confirmed these facts (Figure 3). Correspondingly, a significant drop in the conductivity of the electrospun solutions was observed when the PDLLA concentration in the composite mats decreased, and the pure PDLLA solution showed the highest conductivity (Figure 4). This phenomenon can be explained as the following. The charges carried by fibers mainly depend on the conductivity of the electrospun solution when the external electric field remains the same.[21] As the conductivity of the electrospun solutions decreases with the reduction of PDLLA/PCL ratios, the Coulomb interactions between the fibers and collector become weak because of the lower charge density of fibers, which results in the formation of electrospun mats with less ordered architecture. Whereas, the pure PDLLA solution shows the highest conductivity, leading to a higher charge density on the surface of fibers, and as a result, the Coulomb interactions are enhanced resulting in the pattern of PDLLA in a higher order degree.

It was also noticed that the shrinkage behaviors of the composite electrospun patterned mats were affected by the PDLLA/PCL ratios, and the contractibility rates decreased with the increase of PCL concentration. It is reported that the molecular chains of polymer will be elongated and orientated during the process of electrospinning. As an amorphous polymer, the PDLLA solution possesses a random molecular chain structure, and the chains will be elongated after electrospinning. When immersed

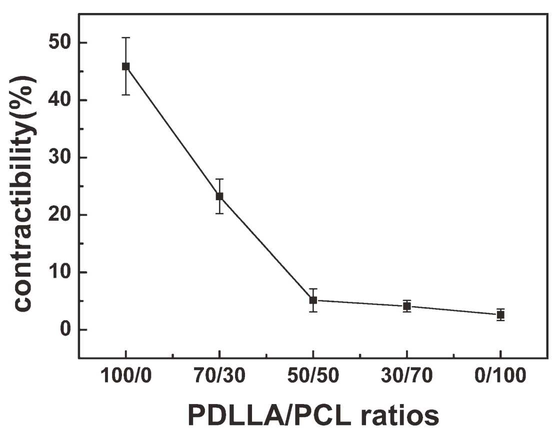

Figure 6. The contractibility of composite electrospun membranes with different PDLLA/PCL ratios, and the corresponding contractibility is 45.9%, 23.24%, 5.11%, 4.07%, 2.61%, respectively.

in the culture medium at a higher temperature (37°C), these stretched molecular chains may rapidly relax to random coil state, resulting in a dimensional shrinkage of the PDLLA fibrous mat.[17,18] The PCL semicrystalline polymer remains a partly ordered crystal structure after being fabricated into electrospun nanofibers, which possesses high dimensional stability in various kinds of solutions at 37°C.[22] When the composite PDLLA/PCL polymer mats were prepared via electrospinning, the chains of PDLLA in random coil state were constrained by the crystalline part of PCL, which hampered the relaxation and shrinkage process when soaked in culture medium.[23,24] Therefore, the addition of PCL reduced the surface shrinkage of PDLLA/PCL electrospun mats, and the contractibility rates decreased with the increase of PCL concentration.

CONCLUSIONS

In this study, the patterned composite PDLLA/PCL scaffolds were successfully fabricated via electrospinning. The order degree and contractibility of patterned composite scaffolds with different PDLLA/PCL ratios were evaluated. The results showed that the order degree of the pattern structure and *in vitro* shrinkage behaviors of PDLLA/PCL electrospun mats could be finely tuned with different blending ratios. In general, the order degree of pattern structure decreased while the dimensional stability increased with the reduction of PDLLA/PCL ratios, and it was found that the PDLLA/PCL electrospun mat with the ratio 50/50 possessed the most balanced properties with a controllable designed pattern and appropriate stability in culture medium, and it might be the best choice for tissue engineering application.

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