Electrospun nanofiber meshes with tailored architectures and patterns as potential tissue-engineering scaffolds

This article has been downloaded from IOPscience. Please scroll down to see the full text article.

2009 Biofabrication 1 015001

(http://iopscience.iop.org/1758-5090/1/1/015001)

The Table of Contents and more related content is available

Download details:
IP Address: 128.206.9.138
The article was downloaded on 20/04/2009 at 22:43

Please note that terms and conditions apply.
Electrospun nanofiber meshes with tailored architectures and patterns as potential tissue-engineering scaffolds

Yazhou Wang1,2, Guixue Wang1,4, Liang Chen2, Hao Li2, Tieying Yin1, Bochu Wang1, James C-M Lee3 and Qingsong Yu2,4

1 Bioengineering College of Chongqing University and ‘111 Project’ Laboratory of Biomechanics & Tissue Repair of Ministry of Education, Chongqing, 400044, China
2 Center for Surface Science and Plasma Technology, Department of Mechanical and Aerospace Engineering, University of Missouri, Columbia, MO 65211, USA
3 Department of Biological Engineering, University of Missouri, Columbia, MO 65211, USA
E-mail: wanggx@cqu.edu.cn and yuq@missouri.edu

Received 29 November 2008
Accepted for publication 10 February 2009
Published 20 March 2009
Online at stacks.iop.org/BF/1/015001

Abstract
Using a stainless steel mesh as a template collector, electrospun nanofiber meshes with well-tailored architectures and patterns were successfully prepared from biodegradable poly(ε-caprolactone) (PCL). It was found that the resulting PCL nanofiber (NF) meshes had similar topological structures to that of the template stainless steel mesh. Such PCL nanofiber meshes (NF meshes) had improved the tensile strength with Young’s modulus of 62.7 ± 5.3 MPa, which is >40% higher than the modulus of 44 ± 5.7 MPa as measured with the corresponding randomly oriented PCL nanofiber mats (RNF mat). On the other hand, the ultimate strain (87.30%) of the PCL NF meshes was distinctly lower than that of the PCL RNF mats (146.46%). To the best of our knowledge, this is the first time that the mechanical properties of nanofiber meshes with tailored architectures and patterns were studied and reported. When cultured with a mouse osteoblastic cell line (MC3T3-E1), the electrospun PCL NF meshes gave a much higher proliferation rate as compared with the randomly oriented PCL RNF mats. More importantly, it was found that the cells grew and elongated along the fiber orientation directions, and the resulted cellular organization and distribution mimicked the topological structures of the PCL NF meshes. These results indicated that the electrospun nanofiber scaffolds with tailored architectures and patterns hold potential for engineering functional tissues or organs, where an ordered cellular organization is essential.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Since it was introduced in 1930 with an electrospray, electrospinning has proved to be a simple, versatile and useful technique for fabricating nanofibers from a rich variety of functional materials [1–5]. As a processing method for tissue-engineering scaffolds, electrospinning offers several advantages, including high surface area to volume ratio (or to mass ratio) of the resulting fibers, formation of interconnected porous networks, small diameter fibers that mimic the fibrous architecture of the extracellular matrix (ECM) of soft tissue and multiple possibilities for surface functionalization and biomimicking, etc. These characteristics in combination are critical in the success of tissue engineering because they promote cell adhesion and migration, and enable the transport of nutrients throughout a scaffold. Investigation of electrospun fibrous materials in biomedical applications has become more and more important in the areas such as wound healing [6, 7], tissue engineering [8–10] and drug delivery [11–14].
In a typical electrospinning process, a high voltage is applied to a metallic capillary, which is connected to a reservoir holding a polymer solution with proper viscosity, conductivity and surface tension. The electrospun nanofibers are usually collected as nonwoven mats with randomly oriented fibers [15]. In many applications, however, the control of the spatial orientation and the patterning of the resulting fibers are necessary. Recently, many methods have been developed to directly collect electrospun nanofibers as uniaxially aligned arrays [15–18].

With the possibility of fabricating electrospun nanofibers with aligned arrays and with tailored architectures, electrospun nanofiber films can be used as tissue-engineering scaffolds to mimic the extracellular matrix (ECM). Recent researches have shown that the aligned electrospun nanofiber arrays have shown significant difference from random fibers in cell proliferation and morphology for Schwann cells [19], endothelial cells [20], meniscal fibrochondrocytes (MFCs) or mesenchymal stem cells (MSCs) [21, 22], fibroblasts [23], neural stem cells [24] and rabbit conjunctiva fibroblasts (RCFs) [25]. It has also found that the alignment of fibers could not only enhance cell proliferation, but also affect the cell-growing direction and morphologies [26–30].

Very recent research [15, 31] including our most recent study [32] have demonstrated that the electrospinning technique can be used to directly fabricate nanofiber films with various desired patterns and architectural structures by using appropriately designed fiber collectors. We hereby report the direct preparation of electrospun nanofiber films with tailored architectures and patterns by using a stainless steel mesh as a template fiber collector. In this paper, the surface and mechanical properties of such architectural nanofiber films were examined, and the architectural effects of the nanofiber films on cell proliferation and morphology were studied with MC3T3-E1 cells. It is expected that such investigation on electrospun nanofiber films that have sophisticated architectures and patterns will improve our fundamental understanding with respect to their suitability and potential advantages when used as novel tissue-engineering scaffolds.

2. Materials and methods

2.1. Electrospinning

The electrospinning set-up used in our experiments consists of a high-voltage power supply (Model: ES50P-5W/DAM, Gamma High Voltage Research, Ormond Beach, FL, USA), a spinneret and a collector (figure 1(A)). In a procedure, poly(ε-caprolactone) (PCL, molecular weight = 800,000, Aldrich Chemical Co., Milwaukee, WI, USA) was dissolved in a mixture of chloroform and methanol (3:1 by volume) to prepare a 9 wt% solution, which was then loaded into a plastic syringe (10 ml, Exelint International Co., Los Angeles, CA, USA) fitted with a stainless steel needle. This needle was connected to a high-voltage power supply. The solution was continuously supplied using a syringe pump at a rate of 0.01 ml min⁻¹. The voltage used for electrospinning was 20 kV and the collection distance was 22 cm.

A stainless steel mesh (a wire diameter of 0.254 mm, and a wire spacing of 0.381 mm, McNichols Co., Charlotte, NC, USA) was cut into a 15 × 10 cm² coupon (figure 1(B)) and used as a template collector for electrospun nanofibers. A stainless steel sheet with a dimension of 15 cm (L) × 10 cm (W) was used to collect random electrospun PCL nanofiber films. The electrospun PCL nanofiber films with ordered nanofiber alignments collected using the stainless steel mesh were designated as electrospun nanofiber meshes (NF meshes), and electrospun PCL random nanofiber mats collected with the stainless steel sheet was designed as electrospun random fiber mat (RNF mats), respectively.
2.2. Characterization of electrospun nanofibers

2.2.1. Morphology of the electrospun PCL nanofibers. A Nikon optical microscope (Model: Epiphot 200) was first used to examine the as-deposited electrospun PCL nanofibers. The morphology of the electrospun PCL fibers was then studied using a field emission scanning electron microscope (SEM) (S4700, Hitachi Ltd, Tokyo, Japan) with an electron accelerating voltage of 5 kV. Before the SEM measurements, the electrospun PCL NF meshes and RNF mats were coated with platinum using a sputter coating machine (JFC-1200 Fine Coater, JEOL, Tokyo, Japan).

2.2.2. Surface wettability. The surface wettability of the electrospun PCL NF meshes or RNF mats was characterized in terms of water surface contact angle by a sessile drop method using a VCA 2500 XE contact angle measurement system (AST Products Inc., Billerica, MA, USA). Deionized water using a VCA 2500 XE contact angle measurement system terms of water surface contact angle by a sessile drop method was characterized in terms of water surface contact angle by a sessile drop method.

2.2. The test of the electrospun PCL NF meshes or RNF mats was prepared as rectangular coupons with a dimension of 2 cm (L) × 2 cm (W) and then immersed in deionized water for at least 3 days before cell culture studies.

2.3. Tensile test

Mechanical properties of electrospun PCL NF meshes or RNF mats were measured and characterized using a TA-HDi Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA) with a 50 kg load cell and a test speed of 1 mm s⁻¹ in an ambient environment. All test specimens were prepared in the form of rectangular shape with dimensions of 10 cm (L) × 1 cm (W) directly cut from the electrospun PCL NF meshes or RNF mats. The thicknesses of test specimens were 0.1 mm as measured using a digital micrometer. The test specimen was mounted using a thin-film holder provided by the tester vendor. With such a thin-film holder, the fiber specimen was first held in place and then wrapped around the holder for one turn to prevent the slippage of the mounted specimen during the test. At least five specimens were tested for each type of electrospun NF meshes or RNF mats. The test on each test specimen was all extended up to failure, and the ultimate tensile strength (UTS) was determined on the basis of the breaking load directly supplied by the test machine.

When the stress–strain curve of materials is linear, the modulus of elasticity can be calculated by the equation

\[ E \varepsilon = \sigma, \]

where \( E \) is the modulus of elasticity, \( \varepsilon \) is the engineering strain and \( \sigma \) is the engineering stress.

\( E \) is equal to the slope of the stress–strain curve; nevertheless, the tangent or secant modulus is normally used if the stress–strain curve of materials is nonlinear.

2.4. In vitro tests

The electrospun PCL NF meshes or RNF mats were prepared as rectangular coupons with a dimension of 2 cm (L) × 2 cm (W) and then immersed in deionized water for at least 3 days before cell culture studies.

2.4.1. Cell culture. Cell culture was performed with MC3T3-E1 cell line purchased from American Type Culture Collection (ATCC). Cells were grown on the 75 cm² culture flasks at 37 °C in 5% CO₂ modified alpha minimum essential medium (α-MEM) lacking ascorbic acid (AA) (GIBCO), supplemented with 10% fetal bovine serum (FBS) and antibiotics. The culture medium was changed every 3 days until the cells reached a confluence of 90–95%, as determined visually by an inverted light microscope. The cells were then passaged using 0.05 wt% trypsin/EDTA (Invitrogen Corp., Carlsbad, CA, USA). Electrospun PCL NF meshes or RNF mats with sizes of 2 cm × 2 cm were fixed in sterilized petri dishes with a stainless steel wire and sterilized with UV light for 2 h. The cells were then seeded onto the fiber meshes or mats by adding 3 ml cell solution at a concentration of 5 ×10⁴ cells ml⁻¹.

2.4.2. MTT assay. The MTT assay was used for evaluating cell vitality and proliferation. The key component of the MTT assay is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Mitochondrial dehydrogenases of living cells reduce the tetrazolium ring and yield a blue formazan product, which can be measured spectrophotometrically. The amount of formazan produced is proportional to the number of viable cells present. For PCL specimens, after cell cultured for 1 day, 3 days and 7 days, the specimens were moved to other petri dishes and the MTT solution was added to the dishes. After incubation for 4 h at 37 °C, the top liquid was removed and the insoluble remaining was dissolved in DMSO (dimethyl sulfoxide)/ethanol (DMSO: ethanol = 1:1) solution. The optical absorbance at 540 nm of the solution was measured using a Fusion™ microplate analyzer (Packard BioScience, Meriden, CT, USA).

2.4.3. Statistical analysis. The cell proliferation experiments were performed in triplicate and the results were expressed as mean ± standard deviation (SD). A student’s t-test was employed to assess the statistical significant difference of the results. The difference was considered statistically significant at \( p < 0.05 \).

2.5. Cell proliferation, morphology and distribution

Cell morphology was examined using laser scanning confocal microscopy (LSCM) and environmental scanning electron microscopy (ESEM) (Quanta 600 F, FEI, Hillsboro, OR, USA). For LSCM measurements, the cells were fixed with Carnoy’s fixative (one part glacial acetic to three parts absolute methanol), then stained with Hoechst (H 6024) and then examined using the LSCM. For ESEM measurements, the samples were fixed with 2% glutaraldehyde and 2%...
Figure 2. A digital pictorial view and an optical microscopic image (×5) of a typical electrospun PCL nanofiber mesh obtained with a stainless steel mesh as a template collector with a collection time of 60 min. The scale bar is 600 μm.

paraformaldehyde in a 0.1 M cacodylate buffer solution and then investigated by the ESEM directly.

3. Results

3.1. Surface structures and properties of electrospun PCL NF meshes

The electrospun PCL NF meshes with dimensions of 15 cm (L) × 10 cm (W) were obtained using a stainless steel mesh (shown in figure 1(B)) as a template collector. The resulting PCL NF meshes could easily be lifted off from the stainless steel mesh collector, and free-standing nanofiber meshes with the same size as the collector were obtained. As shown in figure 2, the collected free-standing PCL NF meshes showed a similar topological structure to that of the template collector of the stainless steel mesh. Figure 3 shows the optical images of the resulted PCL NF meshes collected with various electospinning durations ranging from 5 min to 60 min. It was found that a better alignment of nanofibers was obtained with longer collection time.

Figure 4 shows the SEM images of the PCL NF meshes and electrospun PCL random nanofiber mats (RNF mats). It can be seen that, in comparison with randomly oriented nanofibers in RNF mats (figure 4(d)), the NF meshes (figures 4(a)–(c)) showed a well-organized topological structure and repeated patterns with the nanofibers aligned in two directions. In the open area of the stainless steel mesh collector, the nanofibers tend to form a multi-layer hierarchical structure with fibers aligned along the diagonal direction of the open squares (figure 4(c)).

The surface wettability of the electrospun PCL NF meshes and RNF mats were evaluated with water contact angle measurements and the results are shown in figure 5. As seen in figure 5, the PCL NF meshes had a water contact angle of 140.1° ± 1.2°, which is essentially superhydrophobic, while the PCL RNF mats showed a slightly lower water contact angle 134.9° ± 2.1°. In contrast, the continuous PCL thin films prepared by solution casting had a much lower water contact angle (86° ± 2.7°), which is in good agreement with the number of 85 ± 3°C as reported in the literature [20].

Figure 3. Optical microscopic images (×20) of electospinning PCL nanofibers meshes on a stainless steel template collector with a collection time of (a) and (b) 5 min; (c) and (d) 15 min; (e) and (f) 30 min and (g) and (h) 60 min.

3.2. Mechanical properties of electrospun PCL NF meshes

Figure 6 shows that the typical stress–strain curves of electospun PCL NF meshes and RNF mats are nonlinear.
Table 1. Tensile testing results of the electrospun PCL nanofiber meshes (PCL NF meshes) and PCL random nanofiber mats (PCL RNF mats).

<table>
<thead>
<tr>
<th></th>
<th>Tensile tangent modulus (MPa)</th>
<th>Ultimate tensile stress (MPa)</th>
<th>Ultimate strain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF mesh</td>
<td>62.7 ± 5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.6 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>−87.30</td>
</tr>
<tr>
<td>RNF mat</td>
<td>44 ± 5.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.5 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>146.46</td>
</tr>
</tbody>
</table>

<sup>a</sup> The tensile modulus of state A shown in figure 6.
<sup>b</sup> The tensile modulus of state B shown in figure 6.

Data are representative of five independent experiments and represented as mean ± SD (n = 5).

Figure 4. SEM images of a typical electrospinning PCL nanofiber mesh collected after 15 min on a stainless steel mesh template collector obtained at various locations with (a) cross fiber bundles (×150); (b) the fibers on the fiber bundles (×2 k) and (c) the fibers in an open area of the mesh (×500) as compared with (d) a typical SEM image (×500) of PCL randomly arranged nanofiber mats collected on a stainless steel sheet with collection time of 60 min.

Figure 5. The water contact angles of electrospun PCL nanofiber meshes (PCL NF meshes) and PCL random nanofiber mats (PCL RNF mats) as compared with PCL continuous films (PCL CN films). ** shows a significant difference with p < 0.01 and # shows a significant difference with p < 0.001.

Figure 6. The typical stress–strain curves obtained with PCL NF meshes (the blue/black line) and PCL RNF mats (the pink/grey line) by tensile tests.

On the stress–strain curves of both PCL NF meshes and RNF mats, as seen in figure 6, there are two distinguished regions including state A with strain <0.1% and state B with strain >0.1. Because these two regimes showed distinctly different mechanical behaviors, their tangent modulus should be calculated and analyzed, respectively. Table 1 summarizes the detailed tensile test results. In state A with strain <0.1%, as noted from table 1, the PCL NF meshes showed a much...
higher tensile modulus (62.7 ± 5.3 MPa) than PCL RNF mats (44 ± 5.7 MPa). In state B, a similar trend to that noticed in state A was observed, i.e., the PCL NF meshes gave a higher modulus (28.6 ± 0.6 MPa) than the PCL RNF mats (20.5 ± 0.6 MPa). It was noted that, however, the ultimate strain of the PCL NF meshes (87.30%) was distinctly lower than that of the PCL RNF mats (146.46%), and the corresponding ultimate tensile stresses were 28.6 ± 0.4 MPa and 33.2 ± 0.9 MPa for PCL NF meshes and RNF mats, respectively.

3.3. Cell proliferation, morphology and distribution

The proliferation behaviors of MC3T3-E1 cells on the PCL NF meshes and RNF mats were studied with cell culture durations of 1, 3 and 7 days. The cell viability and proliferation properties were then evaluated and analyzed using the MTT assay and environmental scanning electron microspectroscopy (ESEM). The MTT analysis data are shown in figure 7 and the assay and environmental scanning electron microspectroscopy durations of 1, 3 and 7 days. The cell viability and proliferation behaviors of MC3T3-E1 cells on the PCL NF meshes and RNF mats were studied with cell culture.

The proliferation behaviors of MC3T3-E1 cells on the PCL NF meshes and RNF mats, respectively. In state A, the PCL NF meshes gave a higher tensile modulus (62.7 ± 5.3 MPa) than the PCL RNF mats (44 ± 5.7 MPa). In state B, a similar trend to that noticed in state A was observed, i.e., the PCL NF meshes gave a higher modulus (28.6 ± 0.6 MPa) than the PCL RNF mats (20.5 ± 0.6 MPa). It was noted that, however, the ultimate strain of the PCL NF meshes (87.30%) was distinctly lower than that of the PCL RNF mats (146.46%), and the corresponding ultimate tensile stresses were 28.6 ± 0.4 MPa and 33.2 ± 0.9 MPa for PCL NF meshes and RNF mats, respectively.

The cell morphology and distribution of MC3T3-E1 cells after being cultured for 1 day, 3 days and 7 days in the three representative areas on the PCL NF meshes were examined using ESEM and the images are shown in figures 8(a)–(c). The corresponding cell morphology and distribution on the PCL RNF mats are shown in figure 8(d). From figures 8(a)–(c), it can be seen that the cell morphologies and distributions on different representative areas of the NF meshes showed significant differences, although the cells almost completely covered the mesh surfaces after 7 days cell culture. As compared with the aligned nanofibers shown in figures 8(a) and (b), the random arrangement (shown in figure 8(c)) showed a much less quantity of cells after being cultured for 3 and 7 days (figures 8(c2) and (c3)). From figures 8(a) and (b), it is found that all the cells on the PCL NF meshes elongated along the alignment direction of the electrospun nanofibers. In contrast, the cells on the PCL RNF mats developed in all different directions (figures 8(d)).

The cell morphologies and distributions of MC3T3-E1 cells on the PCL NF meshes and RNF mats were also studied using laser scanning confocal microspectroscopy (LSCM). As seen from the LSCM images shown in figure 9, results that are consistent with ESEM investigations have been obtained. From figures 8 and 9, it should be noted that the cells proliferation and distribution mimicked the topological structures of the PCL NF meshes and the cells elongated along the alignment directions of the PCL nanofibers that constructed the NF meshes. It is understandable, therefore, that randomly distributed cells were observed with the PCL RNF mats, in which the electrospun nanofibers are randomly oriented.

4. Discussion

The results shown in figures 2 and 3 clearly indicate that, during the electrospinning process, the selection of a collector with a well-defined topological structure could be used to modify the fiber deposition behaviors, the fiber orientations and the topological structures of the collected nanofiber films. In this study, when a stainless steel mesh (figure 1(B)) was used as a template collector, PCL nanofiber meshes with fiber orientations and ordered patterns that simulated the topological structure of the collector have been obtained.

In the electrospinning process, the protrusions as well as the diameter of the electroconductive wires in the stainless steel collector play a key role in the control of the alignment of the nanofibers and the ordered architectures of the resulted PCL NF meshes [31]. In fact, the space between the overlap of any two wires (protrusions) with the middle of wires (low-point) shown in figure 1(B) can be regarded as a void gap model, which is generally recognized as a gap method used to collect aligned electrospun nanofiber arrays [33]. It was reported that the orientation of any forthcoming nanofiber could greatly be altered due to the electrostatic repulsion caused by the previously deposited fibers on a collector [5]. This explains well why the nanofibers shown in figure 4 became more aligned and better organized with longer collection time.

It has been recognized that the surface microscopic roughness or surface micro-structure in micrometer scale down to sub-micrometer scale could significantly affect the surface wettability of materials [34, 35]. In the case of hydrophobic PCL polymers, as shown in figure 5, the micro-structures constructed with the electrospun PCL nanofibers have made both NF meshes and RNF mats much more hydrophobic with water contact angle as high as 135°–140°, which is very close to the so-called ultrahydrophobic surfaces.

In figures 2–4, the PCL NF meshes show repeated and well-organized patterns with a topological structure similar to the stainless steel mesh collector. In other words, the PCL NF meshes were composed of PCL nanofibers with well-organized fiber alignment arrays/patterns and ordered structures. In the early stretching stage with strain <0.1% (state A shown in figure 6), because of the presence of networks simulating
<table>
<thead>
<tr>
<th>Culture period</th>
<th>1 day</th>
<th>3 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="https://example.com/image1.png" alt="Image" /></td>
<td><img src="https://example.com/image2.png" alt="Image" /></td>
<td><img src="https://example.com/image3.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td><img src="https://example.com/image4.png" alt="Image" /></td>
<td><img src="https://example.com/image5.png" alt="Image" /></td>
<td><img src="https://example.com/image6.png" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td><img src="https://example.com/image7.png" alt="Image" /></td>
<td><img src="https://example.com/image8.png" alt="Image" /></td>
<td><img src="https://example.com/image9.png" alt="Image" /></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 8.** ESEM micrographs (scale bar = 100 μm) of MC3T3-E1 cells on (a)–(c) PCL nanofiber meshes (PCL NF meshes) and (d) PCL random nanofiber mats (PCL RNF mats) of the electrospun PCL mat after 1, 3 and 7 days of cell culture. The white squares show the areas observed on the PCL NF meshes with ESEM; the white arrows indicate the fiber alignment directions; the darker areas show the existence of transparent cells; the depicted (pink) lines highlight the shape of the cells. The scale bar is 100 μm.

**Figure 9.** LSCM micrographs of MC3T3-E1 cells after 3 days culture on (a) a cross section of a PCL nanofiber mesh (PCL NF mesh) at a corresponding location on the mesh shown in (c), and (b) PCL random nanofiber mats (PCL RNF mats). The white arrow signs show the direction of nanofiber alignment. The scale bar is 50 μm.
to the stainless steel mesh, the PCL NF meshes showed a higher tensile modulus of 62.7 ± 5.3 MPa than the 44 ± 5.7 MPa measured with PCL RNF mats, which are constructed with randomly arranged nanofibers. Because of their random nanofiber orientation in RNF mats, the nanofibers could be drawn out or stretched relatively easier via deformation under the applied stress.

As seen in figures 2 and 4, the PCL nanofibers deposited much more densely on protruded wire overlapping points than that in the other areas of the stainless steel mesh collector. This deposition behavior resulted in non-uniform nanofiber distribution in the PCL NF meshes. This non-uniform fiber distribution in the PCL NF meshes gave a lower ultimate tensile stress of about 28.6 ± 0.4 MPa when compared to that of 33.2 ± 0.9 MPa obtained with PCL RNF mats, which have randomly but uniformly distributed nanofibers. Similarly, the ultimate strain of the PCL NF meshes (87.30%) was significantly lower than that obtained with PCL RNF mats (146.46%).

When used for cell culture study, the well-organized topological structures of PCL NF meshes provided a substratum with distinctly different cellular environments from the PCL RNF mats, in which the nanofibers are randomly arranged. The difference between these two substrates is clearly demonstrated in figure 4. The PCL NF meshes were composed of aligned nanofiber arrays and multi-layer nanofiber meshes. From the results shown in figure 7 obtained with MTT assays, it was noticed that, with 1 day cell culture, a higher attached cell density is found on the PCL RNF mats than that on organized the PCL NF meshes. This result suggests that the isotropic and random structure of the PCL RNF mats could provide more direction guide for cell attachment. At the initial stage, therefore, cells could be easier to adhere to the surface of substrata than the anisotropic PCL NF meshes. On the other hand, after 3 and 7 days cell culture, however, there was no difference in the attached cell density between these two substrata. This result suggests that the cell proliferation rate on the PCL NF meshes must be higher than that on the PCL RNF mats so that the cell density on the former substratum could catch up with that on the latter ones. Some research [21, 24, 25] revealed that the regular alignment of electrospun nanofibers could improve cell proliferation capability. It is believed that the directional alignment and ordered multi-layer structure of nanofibers in PCL NF meshes must be the reason that resulted in a higher cell proliferation rate than that in the randomly arranged PCL RNF mats. This has been further verified by less cell numbers observed on the protrusion areas of the PCL NF meshes (figures 8(c2) and (c3)) as compared with the other areas of this substratum shown in figures 8(a) and (b) after 3 and 7 days cell culture.

It was also noted that, as seen in figures 8 and 9, the cells seeded on the PCL NF meshes elongated and orientated along the nanofiber alignment directions, and the organization of the cultured cells mimicked the underlying organization of the substrata. This result evidently indicated that the topological structures of the PCL NF mesh scaffold could guide the cell growing and spreading directions. In other words, electrospun fibrous films with tailored architectures and patterns could be used for tissue-engineering scaffolds in order to control cell alignment, cell morphology and multi-cellular organization, which are the central goals of tissue engineering many functional tissues and organs such as native cardiac tissues and ventricles.

5. Conclusions

Electrospun nanofiber meshes with well-tailored architectures and patterns were successfully prepared in large sizes from biodegradable poly (ε-caprolactone) (PCL) by using a stainless steel mesh as a template collector. It was found that the resulting PCL nanofiber meshes had similar topological structures to that of the stainless steel mesh template collector. This method enabled direct fabrication of PCL nanofibers’ meshes that have well-organized topological structures and patterns with aligned nanofibers as required by many tissue-engineering scaffolds. In comparison with the randomly arranged electrospun nanofiber mats, these PCL nanofiber meshes with tailored architectures and patterns showed improved tensile strength with tensile modulus when compared with corresponding randomly arranged PCL nanofiber mats. On the other hand, the ultimate strain of the PCL nanofiber NF meshes (87.30%) was distinctly lower than PCL RNF mats (146.46%)

When cultured with a mouse osteoblastic cell line (MC3T3-E1), the electrospun PCL nanofiber meshes that had the tailored architectures and patterns gave a much higher proliferation rate than the randomly arranged PCL nanofiber mat. More importantly, it was found that the cells grew along the fiber orientation directions, and showed elongated morphology along the aligned PCL nanofibers. As a result, cellular organizations that mimicked the topological structures of the PCL nanofiber meshes were obtained. These results indicated that the electrospun nanofibrous scaffold with tailored architectures and patterns hold potential for engineering functional tissues or organs where an ordered cellular organization is essential.

Acknowledgments

This study was supported by grants from the Bioprocessing and Biosensing Center at University of Missouri, Columbia, MI, USA, and China Scholarship Council, China, as well as the Chinese Ministry of Science and Technology (2004DFA06400) and the Chongqing Municipality, China (CSTC2006AA5014-3). The authors are grateful to Professor Robert Guidoin for his helpful discussion of the work, to Mr Young Jo Kim and Mr Andrew Ritts for their assistance in experimental works.

References

[1] Reneker D H and Yarin A L 2008 Electrospinning jets and polymer nanofibers Polymer 49 2387–425
[2] Fong H and Reneker D H 2001 Electrospinning and the formation of nanofibers Structure Formation in Polymeric
Fibers ed D R Salem (Munich, Cincinnati, OH: Hanser Publishing) pp 225–46


[5] Li D and Xia Y 2004 Electrospinning of nanofibers: reinventing the wheel Adv. Mater. 16 1151–70


[9] Sombatmankhong K, Sanchavanakit N, Pavasant P and Supapohl P 2007 Bone scaffolds from electrospun fiber mats of poly (3-hydroxybutyrate), poly (3-hydroxybutyrate-co-3-hydroxyvalerate) and their blend Polymer 48 1419–27


[14] Tungrapra S, Janchud I and Supapohl P 2007 Release characteristics of four model drugs from drug-loaded electrospun cellulose acetate fiber mats Polymer 48 5030–41


[16] Li D, Wang Y and Xia Y 2004 Electrospinning nanofibers as uniaxially aligned arrays and layer-by-layer stacked films Adv. Mater. 16 361–4


[20] Ma Z, He W, Yong T and Ramakrishna S 2005 Grafting of gelatin on electrospun poly(caprolactone) nanofibers to improve endothelial cell spreading and proliferation and to control cell orientation Tissue Eng. 11 1149–58


[23] Bashur C A, Dahlgren L A and Goldstein A S 2006 Effect of fiber diameter and orientation on fibroblast morphology and proliferation on electrospun poly (l-lactico-glycolic acid) meshes Biomaterials 27 5681–8


[33] Li D, Wang Y and Xia Y 2003 Electrospinning of polymeric and ceramic nanofibers as uniaxially aligned arrays Nano Lett. 3 1167–71

[34] Barthlott W and Neinhuis C 1997 Purity of the sacred lotus, or escape from contamination in biological surfaces Condens. Matter. 9 1–8