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Short communication

Fabrication of aligned, porous and conductive fibers and their effects on cell adhesion and guidance

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ABSTRACT

The potential applications of aligned, conductive electrospun fibers have been widely studied in anisotropic tissue regeneration. In this study, aligned porous poly L-lactic acid fibers were obtained with electrospinning, then polypyrrole nanoparticles (PPy NPs) were coated onto the porous fibers with oxidation polymerization to prepare electrically conductive fibers with about 1.24 μm of diameter, and their surface conductivity was about 50 mS. The results of L929 cell test showed that more than 55% of cells grew along the aligned porous fiber axis, confirming that the cell guidance of aligned porous fibers was better than that of non-porous fibers. The results of differentiated PC12 cells on porous fibers showed that the alignment degree of neurite outgrowth and average neurite length of the cells were 84% and 111 μm, respectively, which were larger than those on the non-porous fibers. A primary mechanism was proposed to explain effect of these pores on cell/neurite adhesion and orientation along the aligned porous fibers.

1. Introduction

A bioactive 3D scaffold has been introduced into injury site to bridge nerve gap and to guide axon growth after peripheral nerve defect [1]. The scaffold for nerve regeneration should be capable of delivering appropriate bioactive molecules to regenerate nerve [2,3] . Topographic cue from electrospun scaffolds plays a key role in guiding axon growth [4], and aligned electrospun fibers, a good material with anisotropic structure to guide nerve growth [5,6], can be fabricated with some collectors [7,8]. In addition, RGD modification of polymer [9,10], employing of gelators [11,12] or altering of chirality [13] can increase cell/nerve adhesion and guide axonal growth on fibers, and pores produced during electrospinning can also increase surface area of fibers and promote cell adhesion [14–16]. Karimi and Xia et al. [17,18] found that the proper solvent system and humidity environment could adjust the formation of pores in the fibers.

Polypyrrole (PPy), a biocompatible conductive polymer [19], can be prepared into composite conductive fiber via *in situ* chemical oxidation for the use of tissue scaffolds [20] . Lee and Xie et al. [8,21] fabricated aligned conductive fibers to guide neurite growth, however, aligned conductive fibers with porous structure have not been used in nerve regeneration.

In this work, PLLA was first electrospun into aligned porous fibers [22]. Then PPy nanoparticles (NPs) were coated onto the porous fibers via the oxidation polymerization. Finally, the effects of pores of PPy-coated fibers on cell adhesion and neurite alignment were analyzed via the tests of L929 cells and PC12 cells.

2. Materials and methods

2.1. Preparation of conductive porous fibers

The aligned porous PLLA fibers were prepared via electrospinning (See the Supplementary material), then these fibers were coated with PPy NPs by chemical oxidation of pyrrole. In brief, the fiber film of $20 \times 20\,mm^2$ was immersed in 20 mL of aqueous solution with pyrrole (Py, 14 mM) and sodium dodecylbenzene sulfonate (DBS, 14 mM) in a 50 mL beaker, then PPy was obtained in the mixed solution of Py and $FeCl_3$ (38 mM) on PLLA fibers surface at 4 °C for 4–24 h, to form conductive fibers. The obtained PLLA-PPy fiber films were washed with large amounts of deionized water and alcohol, respectively, then the films were dried in a vacuum oven at RT for further use.

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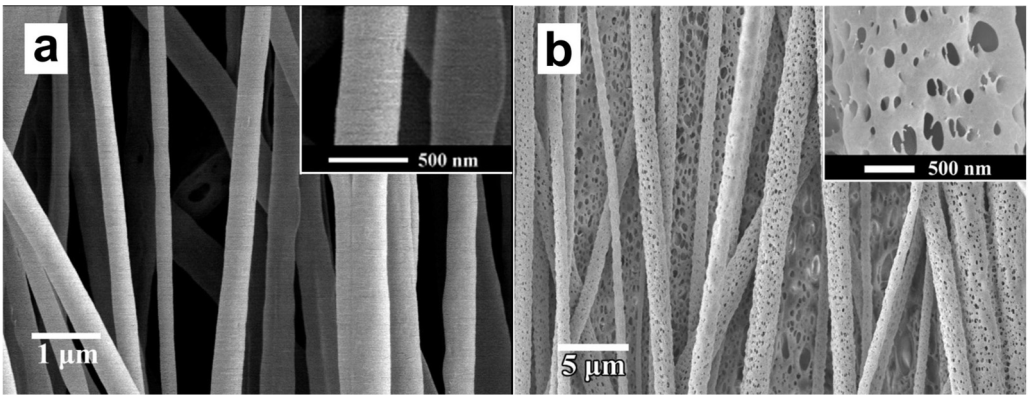

Fig. 1. SEM images of electrospun aligned fibers without pores (a) and with pores (b). The upper right corner inset for the corresponding enlargements.

2.2. Morphology observation and conductivity measurement

Scanning electron microscopy (SEM, HITACHI S4800, Japan) was used to observe the surface morphology of the fibers. Surface conductivity of PLLA-PPy fibers was measured by four-point probe (Qianfeng Electric Co., Shanghai, China) method. The surface resistivity was calculated as follow: $\rho_s = \frac{\pi}{\ln 2} \times \frac{V}{I}$ [23] , where I is the applied current by outer couple probes and V is the voltage measured by inner couple probes. So conductivity can be calculated using $\sigma_s = 1/r_s$.

2.3. Cell experiments

2.3.1. L929 cell adhesion and growth on PPy-coated porous fibers

L929 cells were used to check the contact guidance of aligned porous PLLA-PPy fibers. The cells were cultured in RPMI 1640 medium complemented with 10% newborn calf serum and 1% Penicillin/Streptomycin solution at 37 °C in a humidified 5% CO_2. The culture media was changed every other day. When the cultures reached to 90% confluence, the cells were detached from the flasks using 0.25% trypsin for seeding.

Prior to seeding, porous PLLA-PPy fiber films were stuck on 24 -well plate and were sterilized by UV radiation overnight. Subsequently, the cell suspension was seeded onto each PPy-coated fiber film at a density of 5×10^4 cells/well.

The cells were immunostained after 48 h of culture as follows: first, the cells were fixed with 4% paraformaldehyde for 20 min; then were extracted with 0.1% Triton X-100 for 5 min and rinsed with PBS containing 1% bolvine serum albumin (BSA); subsequently, the cells were stained with DAPI and Rhodamine-labeled phalloidin, respectively; finally, the stained cells were observed and photographed using inverted fluorescence microscope (Olympus IX71, Japan). Alignment of L929 cells on the fiber films was quantified by measuring the angle between nuclear major axis of cells and fiber axis, and all cells within less then 10° were considered to align along the fiber axis [24].

Cell samples were fixed with 4% glutaraldehyde in PBS (pH 7.4) for 45 min. Subsequently, the fixed cells were dehydrated with a series of ethanol/water solutions followed by hexamethyldisilizane drying. Prior to SEM observation, the samples were sputter-coated with platinum coating.

2.3.2. Orientation of PC12 cell neurites on aligned porous PLLA-PPy fibers

PC12 cells were cultured in Ham's F-12K medium containing 10% horse serum, 5% fetal bovine serum and 1% penicillin–streptomycin solution at 37 °C in 5% CO_2. The medium was changed every other day. As a non-adherent cell line, PC12 cells were sub-cultured by centrifuging cells and suspending the pellet at a density of 1×10^5 cells/mL. Prior to seeding, the cells were primed with the differentiation medium including 50 ng/mL NGF for 3 days.

PC12 cells were seeded on disinfected PLLA-PPy fiber films at a density of 1×10^4 cells/well and cultured for 48 h in the medium supplemented with 50 ng/mL NGF. The cell immunostaining and fixation protocols followed above mentioned. Two parameters were examined to assess outgrowth of PC12 cells: (i) the average neurite length; (ii) cell alignment. The neurite length was measured from the tip of the neurite to the cell body, and only the neurites longer than the cell body were analyzed. Alignment of PC12 cells on aligned fibers was quantified by measuring the angle between neurite major axis of cells and fiber axis, and all neurites within less then 10° were considered to align along the fiber axis. The bipolar morphology of PC12 cells cultured on aligned fibers was also observed by SEM.

Data were presented as mean ± standard deviation and were calculated from at least three samples for each condition. The statistical analysis was performed using single factor ANOVA ($p < 0.05$).

3. Results and discussion

3.1. Electrospinning aligned porous fibers

As shown in Fig. 1a, no pores were found on aligned fibers electrospun at 4.5% (w/v) of PLLA solution and diameter of the fiber was about 0.4 μm. However, when fibers were spun at 7.5% (w/v) of PLLA solution, there were abundant pores in the fibers (Fig. 1b). The diameter of porous fibers was ca. 0.82 μm and the size of pore was about 200 nm. If the concentration of PLLA solution was further increased, the solution viscosity was too high to electrospin PLLA into fibers. When the fibers were spun at 6 % (w/v) of PLLA solution, only a few of pores were seen in some fibers (Fig. S1d). Random fibers were also electrospun with these concentrations of PLLA solution, and the pores in random fibers were similar to those in aligned fibers (Fig. S1a–c). Because the rotation of drum could lead to fast phase separation in the PLLA solution [16], there were more uniform pores in the aligned fibers than those in random fibers [25]. The micro polymer-rich regions were solidified into matrix and the micro solvent-rich regions were turned into pores through the solvent evaporation during the jet traveling to the collector. However, there were no pores in Fig. 1a, because high proportional solvent inhibited phase separation during the electrospinning [26]. For further investigation, the fibers in Fig. 1a and b were named as aligned fibers (AFs) and aligned porous fibers (APFs), respectively. Random fibers (RFs) were also prepared as control.