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Aligned natural–synthetic polyblend nanofibers for peripheral nerve regeneration

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ABSTRACT

Peripheral nerve regeneration remains a significant clinical challenge to researchers. Progress in the design of tissue engineering scaffolds provides an alternative approach for neural regeneration. In this study aligned silk fibroin (SF) blended poly(L-lactic acid-co-ε-caprolactone) (P(LLA-CL)) nanofibrous scaffolds were fabricated by electrospinning methods and then reeled into aligned nerve guidance conduits (NGC) to promote nerve regeneration. The aligned SF/P(LLA-CL) NGC was used as a bridge implanted across a 10 mm defect in the sciatic nerve of rats and the outcome in terms of of regenerated nerve at 4 and 8 weeks was evaluated by a combination of electrophysiological assessment and histological and immunohistological analysis, as well as electron microscopy. The electrophysiological examination showed that functional recovery of the regenerated nerve in the SF/P(LLA-CL) NGC group was superior to that in the P(LLA-CL) NGC group. The morphological analysis also indicated that the regenerated nerve in the SF/P(LLA-CL) NGC was more mature. All the results demonstrated that the aligned SF/P(LLA-CL) NGC promoted peripheral nerve regeneration significantly better in comparison with the aligned P(LLA-CL) NGC, thus suggesting a potential application in nerve regeneration.

1. Introduction

Peripheral nerve injury that cannot be directly repaired by end to end sutures remains a significant clinical problem. The current gold standard for treating large nerve defects involves nerve autograft transfers, but they are limited by donor site morbidity, shortage of donor nerve and inadequate functional recovery [1,2]. An alternative approach is the use of a nerve guidance conduit (NGC) that could provide a pathway for nerve out-growth and also promote nerve regeneration. A number of synthetic or natural biopolymers, such as poly(L-lactic acid) (PLLA), poly(lactic–co-glycolic acid) (PLGA), poly(caprolactone) (PCL), collagen and chitosan, have been utilized to construct NGCs for nerve repair [3–7]. Although the advances are encouraging, neural regeneration achieved through these degradable NGCs is still unsatisfactory as a result of their biological or mechanical properties [8].

Nanofibrous scaffolds generated by electrospinning have gained increasing popularity in the field of tissue engineering over the past few years [9–14]. They are characterized by an extremely high porosity and surface area to volume ratio mimicking the features of the extracellular matrix (ECM) that is critical for tissue regeneration. Various synthetic or natural polymers and even mixed solutions of these synthetic and natural polymers can be electrospun into nanofibers, to be used in tissue engineering applications. Moreover, aligned nanofibers can be obtained by correct choice of the collector in the electrospinning equipment. Aligned nanofibers have been shown to direct cell migration, which plays a critical role in nerve regeneration [15–17].

Recently our group has reported the development and characterization of natural–synthetic polymeric nanofibers comprised of well-blended silk fibroin (SF) and poly(L-lactic acid-co-ε-caprolactone) (P(LLA-CL)) by electrospinning [18]. Blending SF into P(LLA-CL) has proved to greatly improve the cell affinity of P(LLA-CL). In this study aligned SF/P(LLA-CL) nanofibers were fabricated by electrospinning with a rotating drum collector and then reeled on a stainless steel bar to fabricate an aligned NGC. The effect of the aligned SF/P(LLA-CL) NGC on nerve regeneration was assessed in a rat sciatic nerve injury model using electrophysiological, histological and immunohistological techniques and electron microscopy.

2. Materials and methods

2.1. Electrospinning materials

Bombyx mori silkworm cocoons were kindly supplied by Jiaxing Silk Co. Ltd. (China). A co-polymer of P(LLA-CL) (50:50) (M_w =

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34.5×10^4 g mol^{-1}), which has a composition of 50 mol.% L-lactide, was provided by Nara Medical University (Japan). 1,1,1,3,3,3,-Hexafluoro-2-propanol (HFIP) was purchased from Daikin Industries Ltd. (Japan).

2.2. Preparation of regenerated SF

Raw silk was degummed three times with 0.5 wt.% Na_2CO_3 solution at 100 °C for 30 min each and then washed with distilled water. Degummed silk was dissolved in a ternary solvent system of $CaCl_2/H_2O$/ethanol solution (mole ratio 1/8/2) for 1 h at 70 °C. After dialysis through cellulose tubular membrane (250-7u, Sigma) in distilled water for 3 days at room temperature the SF solution was filtered and lyophilized to obtain regenerated SF sponges.

2.3. Preparation of aligned SF/P(LLA-CL) nanofibrous scaffolds and NGCs

Polymer solution (8% (w/v)) was prepared by dissolving SF/P(LLA-CL) blends at a weight ratio of 25:75 in hexafluoroisopropanol and stirred at room temperature for 6 h. Pure 8% (w/v) and 4% (w/v) P(LLA-CL) solutions were prepared by dissolving P(LLA-CL) in hexafluoroisopropanol and stirred at room temperature for 6 h. In previous experiments thick fibers (>1000 nm) were obtained by electrospinning the pure 8% (w/v) P(LLA-CL) solution, so we selected the 4% (w/v) P(LLA-CL) solution as a control electrospinning solution. The solutions were placed in a 2.5 ml plastic syringe with a blunt-ended needle (inner diameter 0.21 mm). The syringe was placed in a syringe pump (789100C, Cole-Pamer Instrument Co., Vernon Hills, IL) operating at a flow rate of 1.2 ml h. A voltage of 12 kV was generated by a high voltage power supply (BGG6-358, BMEI Co. Ltd., Beijing, China). To obtain aligned nanofibers a rotating drum collector was used at a speed of 4000 r.p.m. The distance between the needle and the collector was 12–15 cm (Fig. 1). The SF/P(LLA-CL) nanofibrous scaffolds obtained were placed in a desiccator saturated with 75% ethanol vapor at 25 °C for 6 h, making the scaffolds insoluble in water.

The fabrication process for the aligned NGC is outlined in Fig. 2. The aligned nanofibrous scaffold was reeled onto a stainless steel bar with a diameter of 1.4 mm and sealed with 8–0 nylon monofilament suture stitches (Shanghai Pudong Jinhuan Medical Products Co. Ltd., Shanghai, China), ensuring that the orientation of the nanofibers was parallel to the axis of the bar. The length of the NGC was 12 mm and the inner diameter and wall thickness were 1.4 and 0.3 mm, respectively.

2.4. Structural morphology of the aligned nanofibrous scaffolds

The morphology of the aligned SF/P(LLA-CL) and P(LLA-CL) nanofibers was observed by scanning electron microscopy (SEM) (JSM-5600, Japan) at an accelerating voltage of 15 keV. The mean fiber diameters were estimated using ImageJ image analysis software (National Institutes of Health, Bethesda, MD).

2.5. In vivo nerve regeneration studies

2.5.1. Surgical procedure

In this study all experimental procedures involving animals were conducted under Institutional Animal Care guidelines and approved ethically by the Administration Committee on Experimental Animals (Shanghai, China).

Thirty-six adult male Sprague–Dawley rats weighing 200–250 g were randomly divided into three groups of 12 animals each. These

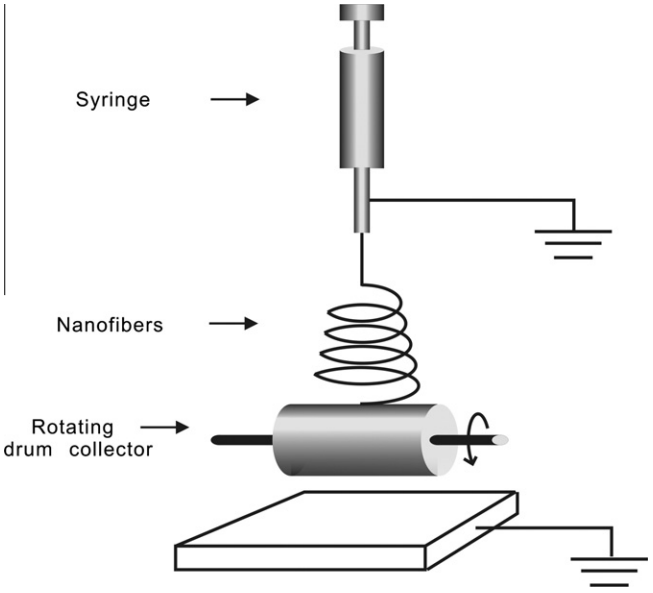

Fig. 1. A schematic illustration of the experimental set-up for fabricating aligned nanofibers.

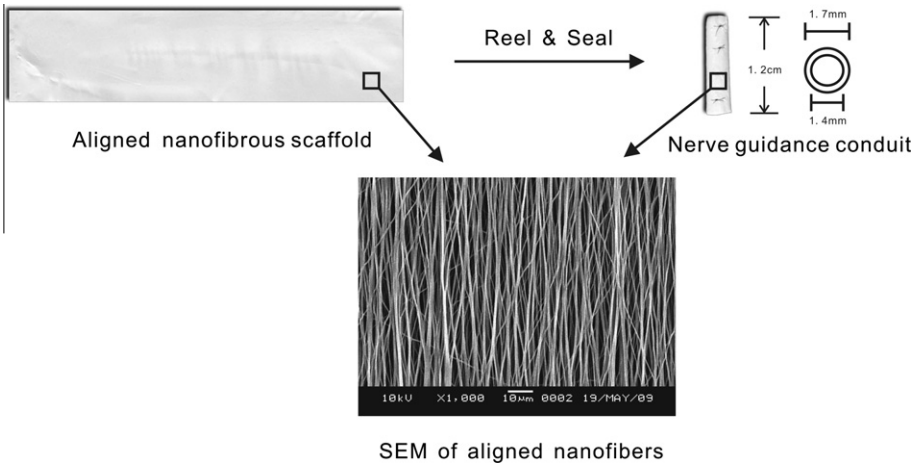

Fig. 2. A schematic illustration of the design of the aligned nerve guidance conduit (NGC). The aligned silk fibroin (SF)/P(LLA-CL) and P(LLA-CL) nanofibrous scaffolds were reeled onto a stainless steel bar and sealed with nylon monofilament suture stitches. The orientation of the nanofibers was parallel to the axis of the bar.

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