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Electrospun silk fibroin nanofibers promote Schwann cell adhesion, growth and proliferation

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Abstract

In this study, Schwann cells, at a density of 1 × 10^5 cells/well, were cultured on regenerated silk fibroin nanofibers (305 ± 84 nm) prepared using the electrospinning method. Schwann cells cultured on the silk fibroin nanofibers appeared more ordered, their processes extended further, and they formed more extensive and complex interconnections. In addition, the silk fibroin nanofibers had no impact on the proliferation of Schwann cells or on the secretion of ciliary neurotrophic factor, brain-derived neurotrophic factor or nerve growth factor. These findings indicate that regenerated electrospun silk fibroin nanofibers can promote Schwann cell adhesion, growth and proliferation, and have excellent biocompatibility.

Key Words

peripheral nerve regeneration; nerve tissue engineering; Schwann cells; silk fibroin; electrospinning; neural regeneration

Abbreviations

SCs, Schwann cells; DRG, dorsal root ganglia

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INTRODUCTION

Schwann cells (SCs) play an essential role in peripheral nerve regeneration by releasing bioactive molecules and providing support for axonal growth and migration. SCs not only provide the Bands of Büngner, which serve as scaffolds for regenerating axons, but they also express adhesion molecules on the surface of their plasma membrane and produce trophic factors, such as brain derived neurotrophic factor, glial-derived neurotrophic factor, as well as extracellular matrix molecules and growth factors[1-5]. Therefore, SCs should be taken into consideration when formulating clinical treatments for peripheral nerve injury. Nerve tissue engineering is a rapidly expanding area of research, which provides a promising approach for nerve repair and regeneration[6]. Scaffold surfaces play a vital role in tissue engineering as the interaction between cells and scaffolds is critical for regeneration[7]. However, due to the unavailability of ideal scaffolding materials and limited knowledge of the interactions between cells and scaffold materials, there have been few breakthroughs in nerve tissue engineering[8]. Cell affinity towards synthetic hydrophobic polymers is poor, as a consequence of their low hydrophilicity and lack of surface cell recognition sites[9]. Fibroin from *Bombyx mori* silkworm has been the dominant source of silk-based biomaterials. Silk fibroin in various forms (films, fibers, nets, meshes, membranes, yarns and sponges) has been shown to

support stem cell adhesion, proliferation and differentiation *in vitro*, and promote tissue repair *in vivo* [10-17]. However, there have been few reports on silk-based materials for peripheral nerve repair. Scaffolds suitable for nerve tissue engineering should mimic the structural and biological functions of native extracellular matrix. Extracellular matrix has a fibrous structure, with fiber bundles varying in diameter (50–500 nm)[18-21]. Electrospinning is an easy and efficient technique used for the fabrication of scaffolds with nanoscale dimensions and high porosity. These features allow it to provide a large surface area for the attachment of cells, and act as a bridge for regenerating axons to traverse large nerve defects. Similar to the structure and function of the extracellular matrix, electrospun silk fibroin nanofibers both support and regulate cell activities[22-23].

In this study, we aimed to examine the biocompatibility and cytotoxicity of electrospun silk fibroin fibers *in vitro*, using cultured SCs from rat dorsal root ganglia (DRG).

RESULTS

Morphology of electrospun silk fibroin nanofibers

Scanning electron micrographs showed that the nanofibers had a smooth surface and formed an interconnected porous network, forming a fibriform three-dimensional structure. The average diameter of the fibers was 305 ± 84 nm (Figure 1).

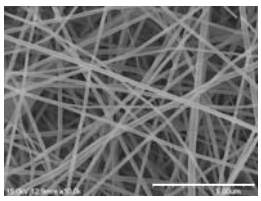

Figure 1 Scanning electron micrograph of electrospun silk fibroin nanofibers (scale bar: 10 μm).

SC morphology on silk fibroin nanofibers

Inverted phase contrast microscopy

The growth of DRG-derived cells on silk fibroin fibers was observed using inverted light microscopy (Figure 2). Cells began to attach on the polylysine and silk fibroin surfaces 20–30 minutes after seeding. Cell attachment on silk films was compared with that on polylysine at different time points. The speed of cell attachment on silk fibroin was similar to that on polylysine. This pattern continued and was more obvious 4 hours after seeding. The majority of SCs were spherical in shape and adhered to silk fibroin, although some cells exhibited a spindle shape, and a small number of SCs died and detached, which were suspended in the culture medium.

Significant changes in cell morphology were observed on polylysine and silk fibroin after 15 hours of culture. A large number of the cells attached on polylysine surfaces acquired a spindle-like morphology, displaying a random orientation with various shapes. In contrast, a number of cells had migrated out of the DRGs on silk fibroin and commenced forming well-ordered connections. At 48 hours, with the elongation and spreading of SCs on silk fibroin, the cells acquired a bipolar or tripolar shape, and spindle-shaped cell bodies were more evident. Some SCs formed connections with each other *via* neurites which were observed to elongate from neurons in the DRG and extend along the matrix. After 2–3 days, a large number of cells migrated out of the DRG, gradually forming bipolar and tripolar extensions. At 72 hours, cell protrusions became long and thin, forming long cell chains and complex networks, especially on the silk fibroin, while SCs cultured on polylysine were randomly distributed.

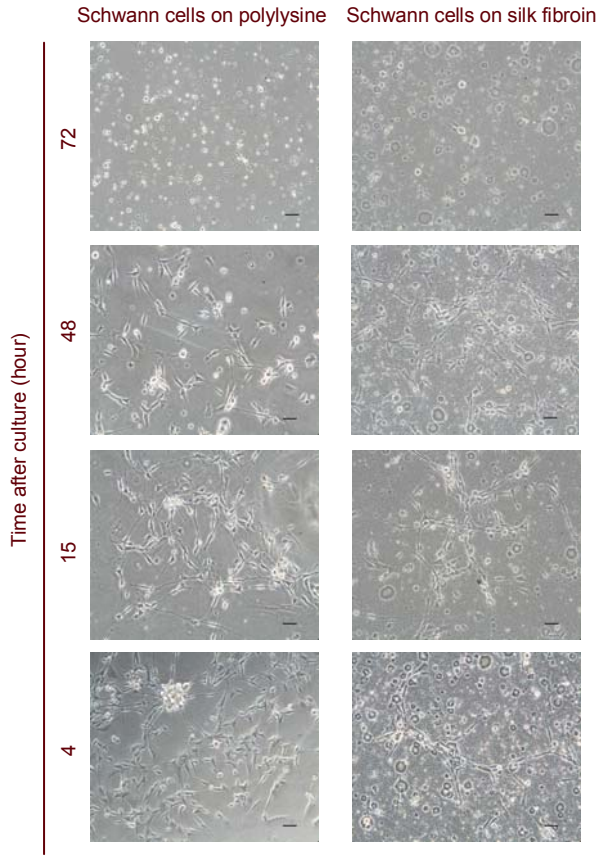

Figure 2 Schwann cells from the dorsal root ganglia cultured on polylysine and electrospun silk fibroin nanofibers (light microscopy, scale bars: 40 μm).

Immunocytochemistry

In the present study, we observed that SCs formed a network on the electrospun silk fibroin using confocal microscopy (Figure 3). The cells, which attached to and encircled the silk fibroin material, were S-100-positive,

which indicated that cells on the silk fibroin surface could maintain their characteristic SC phenotype. Although there was no significant difference between polylysine and electrospun silk fibroin in terms of cell morphology, cell protrusions on silk fibroin were longer and thinner than on polylysine, and the morphology of SCs migrating on silk fibroin appeared ordered in comparison with that on polylysine. Furthermore, cells on silk fibroin formed a more effective and complex interconnecting network, *via* the longer neurites, compared with polylysine (Figure 3). In the absence of the topographical guidance provided by the scaffold, SCs cultured on polylysine adopted an unorganized and disordered morphology, with a random orientation (Figure 3).

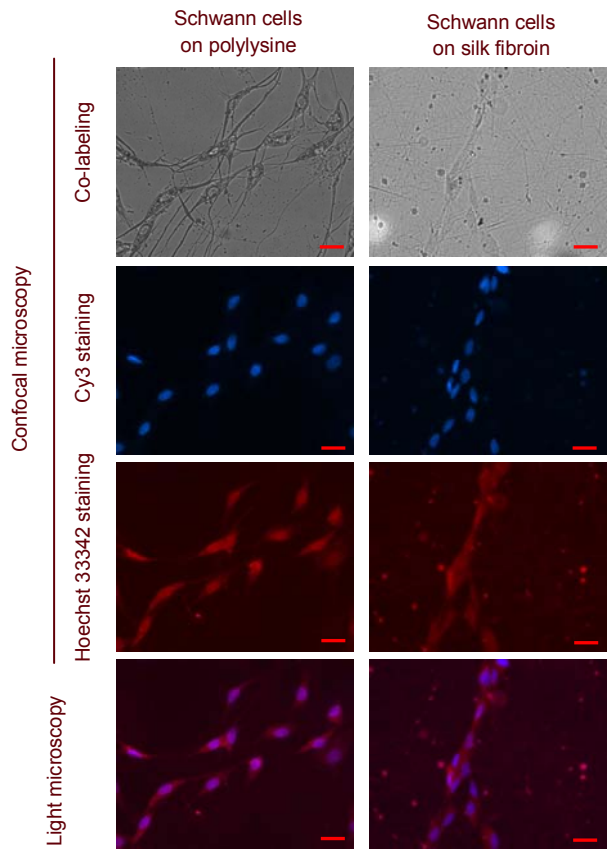

Figure 3 Immunocytochemistry of dorsal root ganglia cultured for 2 days (scale bars: 20 μm).

Red: S-100 immunopositive cells, that might be considered Schwann cells; blue: nuclei labeled with Hoechst 33342.

Scanning electron microscopy

Micrographs of SCs on electrospun nanofiber scaffolds showed normal cell morphology by scanning electron microscopy (Figure 4). Cells attached tightly to the silk fibroin fibers, and exhibited either a spherical or spindle shape. SCs on the silk fibroin surface possessed bipolar and tripolar extensions, with a spindle-shaped morphology. Neurites elongated from neurons within the DRG and extended along the material in an interconnected manner. Cell protrusions became long and thin, and formed a complex network. A large number of cells formed compact arrangements, of an either side-by-side or end-to-end configuration, forming a single or multi-layered structure along the silk fibroin fibers.

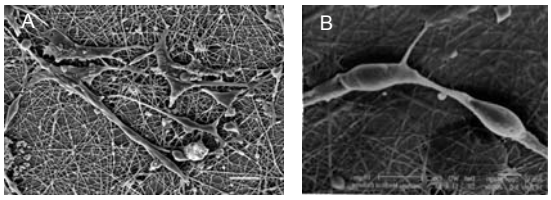

Figure 4 Scanning electron micrographs of dorsal root ganglia-derived cells cultured on electrospun silk fibroin nanofibers after 2 days of culture (scale bar: 20 μm in A, 5 μm in B).

Cell proliferation assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay showed that cell numbers increased with increasing culture duration. Similar numbers of SCs were present on silk fibroin and polylysine between day 1 and 5 ($P > 0.05$; Figure 5), indicating similar proliferation rates.

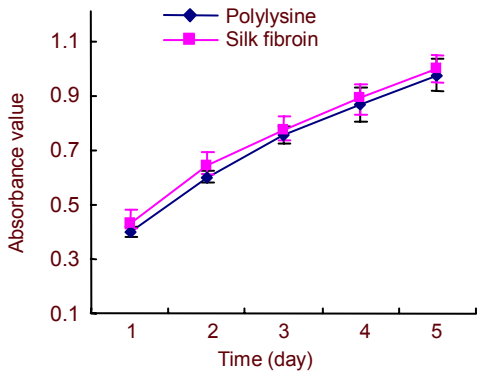

Figure 5 MTT assay for cell proliferation.

At least three repeat tests were performed. Data were expressed as mean ± SD. One-way analysis of variance, followed by Student-Newman-Keuls test, was performed to analyze the difference between groups.

The absorbance values for cells grown on silk fibroin and polylysine showed no significant difference during 1–5 days of culture ($P > 0.05$).

Secretion of neurotrophic factors by SCs

SCs can synthesize and secrete a cocktail of neurotrophic molecules, including nerve growth factor, brain-derived neurotrophic factor and ciliary neurotrophic factor, which act directly and indirectly to promote survival of regenerating neurons[24-29]. The levels of neurotrophic factors in the culture medium were analyzed using enzyme linked immunosorbent assay. The expression of these factors increased with culture

duration. The levels of nerve growth factor, ciliary neurotrophic factor and brain derived neurotrophic factor were similar between the electrospun silk fibroin surface and polylysine ($P > 0.05$; Figure 6). Our results demonstrate that the electrospun silk fibroin surface did not affect the expression of neurotrophic factors associated with SCs, indicating that electrospun silk fibroin is not cytotoxic towards SCs.

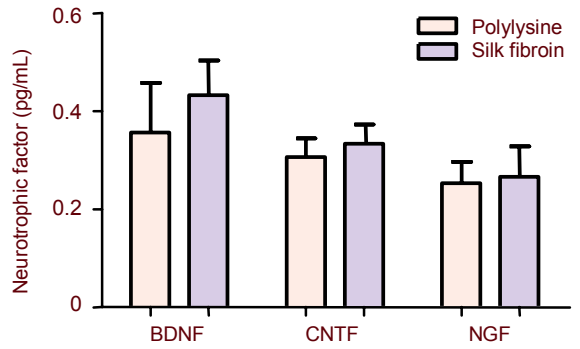

Figure 6 Quantification of NGF, CNTF, BDNF levels in culture mediums secreted by SCs after 4 days of culture using ELISA analysis.

Data were expressed with mean ± SD. One-way analysis of variance followed by Student-Newman-Keuls test was performed to analyze the difference between groups. There was no significance of variance difference at NGF, CNTF and BDNF levels between silk fibroin and polylysine.

NGF: Nerve growth factor; CNTF: ciliary neurotrophic factor; BDNF: brain-derived neurotrophic factor; SCs: Schwann cells.

DISCUSSION

In this study, the feasibility of utilizing electrospun silk fibroin scaffolds for nerve tissue engineering was assessed by scanning electron microscopy, light and fluorescence microscopy, MTT and ELISA assays. The results suggest that electrospun silk fibroin nanofibers promote the adhesion, proliferation and migration of SCs. SCs on nanofibers formed a complex interconnecting network *via* the longer neurites, and mimiced the Bands of Büngner to help guide regenerating axons. Thus, our results indicate that an electrospun silk fibroin surface supports SC viability and maintains cellular phenotype, without any negative effect on the expression of neurotrophic factors *in vitro*.

Electrospinning is a novel technique for producing nanofiber scaffolds which mimic the structural and functional morphology of the extracellular matrix[23]. Nanoscale dimensions have been shown by various researchers to influence cell behavior. Cells attach and organize around fibers with diameters smaller than those of the cells[18]. In our experiment, the average diameter of the fibers was 305 ± 84 nm, with randomly oriented fibers. Nanoscale features have the potential to improve the specificity and utility of materials for a number of neural engineering applications, ranging from neural probes to guidance channels for neural regeneration[30].

An ideal scaffold should be biodegradable, biocompatible and mechanically robust, with a large surface area, high porosity and interconnected pores[18, 23]. Silk fibroin comprises a high ratio of Ala, Asp and Arg residues, and less Gly. In addition, it contains more non-polar amino acids, with a ratio of polar to non-polar amino acids of 0.27[31]. Moreover, it is well known that the presence of the Arg-Gly-Asp (RGD) tripeptide sequence may act as a biological recognition signal to promote cell adhesion[32]. Ren *et al* [33] showed that amino groups are more effective than hydroxyl and carboxyl groups in promoting nerve stem cell proliferation and migration. Thus, the hydrophilicity of a scaffold likely affects the binding of serum proteins, thereby influencing biological responses, such as cell adhesion and proliferation[34-35].

Based on cell morphology under the inverted light microscope, there was no difference between polylysine and silk fibroin in terms of attachment and migration out of the DRG. However, cell protrusions on silk fibroin were longer and thinner than on polylysine, and SCs migrating on silk fibroin had a more well-organized and complex morphology than on polylysine. Thus, silk fibroin is suitable for SC adhesion, growth and migration, helping them to mimic the Bands of Büngner.

SCs respond to topographic cues from the randomly oriented fibers by stretching across multiple fibers. The contact guidance provided by silk fibroin appears to be stronger than that provided by polylysine. This results in a “hill and valley” distribution of SCs as soon as they become confluent[17]. Electrospun silk fibroin is being assessed as a biomaterial scaffold due to its unique mechanical properties, and recent studies have confirmed its biodegradability, biocompatibility and non-cytotoxicity[17, 36-37].

The effect of different nanofiber scaffolds with different diameters and different structures are as yet unknown. Many studies have compared organized scaffolds with randomly oriented scaffolds[38-41]. Some researchers regard aligned fibers as more suitable to cell growth than random scaffolds, because contact guidance provided by the aligned fibers appears to be stronger than that provided by randomly oriented fibers. When cultured on aligned fibers, cells aligned and elongated on the fiber axes, emulating the structure of the Bands of Büngner[42]. Ghasemi-Mobarakeh *et al* [35] studied SC migration and orientation on both aligned and random nanofibrous polycaprolactone/gelatin scaffolds, and suggested that fiber surface hydrophilicity plays a critical role in cell adhesion, proliferation and morphology. It has also been observed that the proliferation of SCs is greater on

random nanofibers than on aligned fibers; a possible reason for this is that random fibers contain many interconnected pores and rough surfaces that assist in adhesion and proliferation of a greater number of cells[30]. In our study, random electrospun silk fibroin nanofibers were able to provide contact guidance for SCs, and with the migration of cells from the DRG, a greater number of cells extended tightly along the silk scaffolds forming longer cell chains than on polylysine. Consequently, silk fibroin nanofibers are likely to promote the regeneration of axons across a nerve gap, such as that caused by a lesion.

Multiple protrusions formed a complex interconnecting network, identified by scanning electron microscopy starting on day 2, similar to previously published reports[43-44]. Therefore, nanofiber scaffolds can serve as substrates for cell attachment, migration and proliferation better than traditional scaffolds[30-33]. In our study, silk fibroin nanofibers provided contact guidance for SCs and were beneficial to the adherence, proliferation and migration of SCs. Similar effects have been observed with other cell types[45-47]. Thus, electrospun silk fibroin may provide enhanced biocompatibility and bioactivity for neural tissue engineering.

Although it is still not known whether the level of neurotrophins expressed by seeded SCs and endogenous SCs are comparable, we believe that the possible enhancement in the maturation of SCs in contact with electrospun fibers as compared with a flat surface may be one of the reasons for the enhanced sciatic nerve regeneration observed in a previous study[48]. Findings from a number of studies have shown that some scaffolds may mimic the structure and function of the extracellular matrix.

Although the mechanisms underlying the ability of the extracellular matrix to promote myelination is still not fully known, it is possible that integrins may interact with the cytoskeleton or regulate myelin-related gene expression[49]. Chew *et al* [41] showed that human SCs were induced into the myelinating state by topographic cues from electrospun fibers, and that electrospun scaffolds had the potential to promote the maturation of SCs. The electrospun fibrous scaffolds may, therefore, be a better microenvironment for SC attachment and growth than a two-dimensional film because they help these cells maintain a more physiological phenotype[40, 50].

In this study, the feasibility of using electrospun silk fibroin scaffolds for nerve tissue engineering was assessed by scanning electron microscopy, light and fluorescence microscopy, MTT and ELISA assays. The electrospun silk fibroin scaffolds supported the growth and expansion of SCs, based on cell proliferation, and morphological and phenotypic assessment *in vitro*. These results suggest that an electrospun silk fibroin surface is a potential candidate for a bioscaffold, since it supports SC viability, maintains their phenotype, and promotes cellular reorganization without negatively impacting neurotrophin expression. Our findings could encourage the development of novel biomaterials that mimic the chemical and physical microenvironment of neural tissues. Thus, future studies should be aimed at developing an ideal functional tissue-engineered graft based on the results of the present study.

MATERIALS AND METHODS

Design

A bioengineering tissue culture study.

Time and setting

This experiment was performed at Jiangsu Province Key Laboratory of Stem Cell Research and National Engineering Laboratory for Modern Silk of Soochow University, China, from November 2009 to May 2010.

Materials

Animals

Neonatal Sprague-Dawley rats, 1–2 days old, were obtained from the Experimental Animal Centre of Soochow University (license No. SYXK2002-0037). The rats were fed with sterilized water and food in a laminar flow room at 18–25°C and 55–58% humidity. All experimental procedures were in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of China[51].

Main materials

Bombyx mori silk was obtained from the College of Textiles and Clothing Engineering of Soochow University in China. Raw silk fibers were degummed twice with 0.5% (w/w) $NaHCO_3$ solution at 100°C for 30 minutes and then rinsed thoroughly with deionized water to remove glue-like sericin proteins. The extracted silk was then dissolved in 9.3 M LiBr solution at 60°C, followed by dialysis using cellulose tubular membranes (molecular weight cut-off of 8 000–14 000 Da) against distilled water for 3 days. The silk fibroin membrane was prepared by spreading silk fibroin solution on the polyethylene plastic board and dried at room temperature. Silk fibroin solutions, 10% concentration, were prepared by dissolving the regenerated silk fibroin membranes in 98% formic acid for 3 hours. In the electrospinning process, a voltage of 10 kV was applied to the stainless steel needle and a distance of 10 cm between the syringe tip and the collecting target were employed. In the electrospinning process, different fiber diameters

were obtained by adjusting solution concentration, spinning voltage and distance. Silk fibroin fiber mats electrospun with different solvent were immersed in 75/25 (v/v) ethanol/water for 30 minutes to induce crystallization of silk fibroin, and then dried in vacuum at room temperature for 24 hours. We obtained silk fibroin with an average diameter of 305 nm. The resulting silk fibroin was sterilized by radiation prior to use[52-53].

Methods

SC culture with silk fibroin

Primary cultures of SCs were prepared from rat DRGs of neonatal Sprague-Dawley rats[37]. After the epineurium and perineurium were removed using dura forceps (Dumont, Switzerland), the DRGs were detached and placed in Hank's balanced salt solution and connective tissues were stripped carefully from their surface. After dissection, the DRGs were placed in 2 mL of chilled serum-free DMEM/F-12 (Gibco, Grand Island, NY, USA) medium and centrifuged at 1 000 r/min for 5 minutes. After discarding the supernatant, the tissue was trypsinized with 2.5% trypsin (Sigma, St. Louis, MO, USA) for 15 minutes, at 37°C, 5% CO_2. Trypsinization was terminated using a complete culture medium consisting of DMEM/F-12 with 20% fetal bovine serum (Gibco). The cells were then suspended in complete culture medium and seeded at a density of 1.0×10^5 cells/well on pre-wetted cover slips coated with silk fibroin or 0.01% polylysine (Sigma) in 35 mm Petri dishes. One milliliter of cell suspension was placed in each dish (only on the scaffolds) and incubated at 37.1°C in a humidified 5% CO_2 chamber. Half of the medium was replaced twice over 7 days. At different times of culture, SC morphological changes were observed under an inverted light microscope (Olympus CKX41, Tokyo, Japan).

Identification of SCs by immunocytochemistry

After days 2, 4, 6 and 7 of culture, cells on electrospun silk fibroin were fixed with freshly-prepared 4% paraformaldehyde solution for 30 minutes at room temperature. The fixed samples were blocked for 30 minutes in phosphate-buffered saline containing 3% bovine serum albumin, 0.2% Triton X-100 and 0.02% NaN_3 (v/v) at room temperature. Cells were then incubated with mouse monoclonal anti-S-100 antibody (1:500; Sigma) overnight at 4°C in a humidified chamber. After washing three times with phosphate-buffered saline, the samples were incubated with Cy3-labeled goat anti-mouse IgG (1:100; Boster, Wuhan, China) for 2 hours at room temperature. After washing three times with phosphate-buffered saline, cell nuclei were stained with Hoechst 33258 (1:100; Sigma) at 37°C for 1 hour. Cover slips were washed and mounted with 50% glycerin in phosphate-buffered saline before imaging (fluorescence microscope AF6000, Leica, Germany).

Observation of SC microstructure by scanning electron microscopy

Cells were washed twice with phosphate-buffered saline and fixed in 2.5% glutaraldehyde on days 2, 4 or 6 of culture on the 305-nm silk fibroin fibers. Cells were then post-fixed with 1% OsO_4, dehydrated stepwise in increasing concentrations of ethanol, and dried in a critical point dryer (Hitachi, Tokyo, Japan). Afterwards, the samples were coated with gold in a JFC-1100 unit (Jeol Inc., Tokyo, Japan) and scanned with a scanning electron microscope (Hitachi S-520, Tokyo, Japan).

MTT cell proliferation assay

After SCs were seeded at a density of 1×10^4 cells/well in a 96-well culture plate, their viability was examined using MTT at 1, 2, 3, 4 and 5 days of co-culture. A volume of 20 μL of MTT (Sigma), dissolved in phosphate-buffered saline at 5 mg/mL, was added to each well, and incubation was continued at 37°C for 4 hours. The reaction solution was carefully removed from each well and 200 μL of dimethyl sulfoxide (Sigma) was added to each well to dissolve the dark blue crystals. The absorbance was measured at 490 nm with a reference wavelength of 570 nm in an EIX-800 microplate reader (Bio-Rad, Hercules, USA). Cell number was correlated to absorbance.

Quantification of nerve growth factor, brain derived neurotrophic factor and ciliary neurotrophic factor levels by ELISA analysis

After 4 days of culture, the culture medium was collected for analysis of neurotrophic factor levels. The levels of nerve growth factor, brain-derived neurotrophic factor and ciliary neurotrophic factor were analyzed using ELISA Kits (Promega, Madison, USA). The assays were performed following the manufacturer's instructions. Standard and test samples were incubated for 90 minutes at 37°C in 96-well plates. The following antibodies were used: rabbit anti-rat nerve growth factor polyclonal antibody (1:500), rabbit anti-rat brain derived neurotrophic factor polyclonal antibody (1:1 000) and rabbit anti-rat ciliary neurotrophic factor polyclonal antibody (1:800). After incubation for 90 minutes at 37°C, goat anti-rabbit IgG conjugated to HRP (1:100) was added and incubated for 90 minutes at 37°C. The tetramethyl benzidine chromogenic substrate solution was added to the plates, and the reaction was stopped by adding stop buffer. Absorbance was measured at 450 nm in a microplate reader (Bio-Rad). Standard curves were generated using known amounts of growth factors, and diluents were used as controls. The standard curves for nerve growth factor, brain derived

neurotrophic factor and ciliary neurotrophic factor provided linear plots of absorbance *versus* concentration. The linear plots were used to determine the concentration of nerve growth factor, brain derived neurotrophic factor and ciliary neurotrophic factor in the test samples. All experiments were performed in triplicate.

Statistical analysis

At least three repeats were performed and all data were expressed as mean ± SD. One-way analysis of variance, followed by Student-Newman-Keuls test, was performed to analyze differences between groups. The differences were considered significant when *P* was less than 0.05.

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Author contributions: Aijun Hu conceived and designed this study, analyzed data and wrote the draft of the manuscript. Feng Zhang was in charge of data integration and support. Qing Lan and Huangxing Zhang authorized the manuscript and guided the study. Baoqi Zuo provided technical support.

Conflicts of interest: None declared.

Ethical approval: All animal experimental procedures conformed to the regulations of the Soochow University Committee on Animal Research in China.

REFERENCES

[1] Jessen KR, Mirsky R. Schwann cells and their precursors emerge as major regulators of nerve development. Trends Neurosci. 1999;22(9):402-410.

[2] Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med. 1999;341(10):738-746.

[3] Sendtner M, Holtmann B, Kolbeck R, et al. Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. Nature. 1992;360(6406):757-759.

[4] Höke A, Cheng C, Zochodne DW. Expression of glial cell line-derived neurotrophic factor family of growth factors in peripheral nerve injury in rats. Neuroreport. 2000;11(8):1651-1654.

[5] Meyer M, Matsuoka I, Wetmore C, et al. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. J Cell Biol. 1992;119(1):45-54.

[6] Prabhakaran MP, Venugopal J, Chan CK, et al. Surface modified electrospun nanofibrous scaffolds for nerve tissue engineering. Nanotechnology. 2008;19(45):455102.

[7] Vasita R, Shanmugam IK, Katt DS. Improved biomaterials for tissue engineering applications: surface modification of polymers. Curr Top Med Chem. 2008;8(4):341-353.

[8] Evans GR. Challenges to nerve regeneration. Semin Surg Oncol. 2000;19(3):312-318.

[9] Ciardelli G, Chiono V, Vozzi G, et al. Blends of poly-(epsilon-caprolactone) and polysaccharides in tissue engineering applications. Biomacromolecules. 2005;6(4):1961-1976.

[10] Wang Y, Kim HJ, Vunjak-Novakovic G, et al. Stem cell-based tissue engineering with silk biomaterials. Biomaterials. 2006;27(36):6064-6082.

[11] Meinel L, Fajardo R, Hofmann S, et al. Silk implants for the healing of critical size bone defects. Bone. 2005;37(5):688-698.

[12] Meinel L, Hofmann S, Betz O, et al. Osteogenesis by human mesenchymal stem cells cultured on silk biomaterials: comparison of adenovirus mediated gene transfer and protein delivery of BMP-2. Biomaterials. 2006;27(28):4993-5002.

[13] Meinel L, Betz O, Fajardo R, et al. Silk based biomaterials to heal critical sized femur defects. Bone. 2006;39(4):922-931.

[14] Karageorgiou V, Tomkins M, Fajardo R, et al. Porous silk fibroin 3-D scaffolds for delivery of bone morphogenetic protein-2 in vitro and in vivo. J Biomed Mater Res A. 2006;78(2):324-334.

[15] Li C, Vepari C, Jin HJ, et al. Electrospun silk-BMP-2 scaffolds for bone tissue engineering. Biomaterials. 2006;27(16):3115-3124.

[16] Zhang X, Baughman CB, Kaplan DL. In vitro evaluation of electrospun silk fibroin scaffolds for vascular cell growth. Biomaterials. 2008;29(14):2217-2227.

[17] Acharya C, Ghosh SK, Kundu SC. Silk fibroin film from non-mulberry tropical tasar silkworms: A novel substrate for in vitro fibroblast culture. Acta Biomater. 2009;5(1):429-437.

[18] Ma Z, Kotaki M, Inai R, et al. Potential of nanofiber matrix as tissue-engineering scaffolds. Tissue Eng. 2005;11(1-2):101-109.

[19] Ma PX. Scaffolds for tissue fabrication. Mater Today. 2004;7(5):30-40.

[20] Ashammakhi N, Ndreu A, Yang Y, et al. Nanofiber-based scaffolds for tissue engineering. Eur J Plast Surg. 2012;35(2):135-149.

[21] Smith LA, Ma PX. Nano-fibrous scaffolds for tissue engineering. Colloids Surf B Biointerfaces. 2004;39(3):125-131.

[22] Murugan R, Ramakrishna S. Nano-featured scaffolds for tissue engineering: a review of spinning methodologies. Tissue Eng. 2006;12(3):435-447.

[23] Pham QP, Sharma U, Mikos AG. Electrospinning of polymeric nanofibers for tissue engineering applications: a review. Tissue Eng. 2006;12(5):1197-1211.

[24] Klein R. Role of neurotrophins in mouse neuronal development. FASEB J. 1994;8(10):738-744.
[25] Yin Q, Kemp GJ, Frostick SP. Neurotrophins, neurones and peripheral nerve regeneration. J Hand Surg Br. 1998; 23(4):433-437.
[26] Frostick SP, Yin Q, Kemp GJ. Schwann cells, neurotrophic factors, and peripheral nerve regeneration. Microsurgery. 1998;18(7):397-405.
[27] Anand U, Otto WR, Casula MA, et al. The effect of neurotrophic factors on morphology, TRPV1 expression and capsaicin responses of cultured human DRG sensory neurons. Neurosci Lett. 2006;399(1-2):51-56.
[28] Moore K, MacSween M, Shoichet M. Immobilized concentration gradients of neurotrophic factors guide neurite outgrowth of primary neurons in macroporous scaffolds. Tissue Eng. 2006;12(2):267-278.
[29] Hadlock T, Elisseeff J, Langer R, et al. A tissue-engineered conduit for peripheral nerve repair. Arch Otolaryngol Head Neck Surg. 1998;124(10):1081-1086.
[30] Seidlits SK, Lee JY, Schmidt CE. Nanostructured scaffolds for neural applications. Nanomedicine (Lond). 2008;3(2): 183-199.
[31] Freddi G, Gotoh Y, Tsutsui T, et al. Chemical structure and physical properties of antheraea assama silk. J Appl Polym Sci. 1994;52(6):775-781.
[32] Pierschbacher MD, Ruoslahti E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature. 1984;309(5963): 30-33.
[33] Ren YJ, Zhang H, Huang H, et al. In vitro behavior of neural stem cells in response to different chemical functional groups. Biomaterials. 2009;30(6):1036-1044.
[34] Cai K, Yao K, Cui Y, et al. Influence of different surface modification treatments on poly(D, L-lactic acid) with silk fibroin and their effects on the culture of osteoblast in vitro. Biomaterials. 2002;23(7):1603-1611.
[35] Ghasemi-Mobarakeh L, Prabhakarana MP, Morshed M, et al. Bio-functionalized PCL nanofibrous scaffolds for nerve tissue engineering. Mater Sci Eng C. 2010;30(8): 1129-1136.
[36] Acharya C, Ghosh SK, Kundu SC. Silk fibroin film from non-mulberry tropical tasar silkworms: A novel substrate for in vitro fibroblast culture. Acta Biomater. 2009;5(1): 429-437.
[37] Yang Y, Chen X, Ding F, et al. Biocompatibility evaluation of silk fibroin with peripheral nerve tissues and cells in vitro. Biomaterials. 2007;28(9):1643-1652.
[38] Yang F, Murugan R, Wang S, et al. Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. Biomaterials. 2005;26(15):2603-2610.
[39] Lee CH, Shin HJ, Cho IH, et al. Nanofiber alignment and direction of mechanical strain affect the ECM production of human ACL fibroblast. Biomaterials. 2005;26(11): 1261-1270.
[40] Zong X, Bien H, Chung CY, et al. Electrospun fine-textured scaffolds for heart tissue constructs. Biomaterials. 2005;26(26):5330-5338.
[41] Chew SY, Mi R, Hoke A, et al. The effect of the alignment of electrospun fibrous scaffolds on Schwann cell maturation. Biomaterials. 2008;29(6):653-661.
[42] Gupta D, Venugopal J, Prabhakaran MP, et al. Aligned and random nanofibrous substrate for the in vitro culture of Schwann cells for neural tissue engineering. Acta Biomater. 2009;5(7):2560-2569.
[43] Yang F, Murugan R, Ramakrishna S, et al. Fabrication of nanostructured porous PLLA scaffold intended for nerve tissue engineering. Biomaterials. 2004;25(10): 1891-1900.
[44] Wen X, Tresco PA. Effect of filament diameter and extracellular matrix molecule precoating on neurite outgrowth and Schwann cell behavior on multifilament entubulation bridging device in vitro. J Biomed Mater Res A. 2006;76(3):626-637.
[45] Yim EK, Leong KW. Significance of synthetic nanostructures in dictating cellular response. Nanomedicine. 2005;1(1):10-21.
[46] Chen CS, Mrksich M, Huang S, et al. Micropatterned surfaces for control of cell shape, position, and function. Biotechnol Prog. 1998;14(3):356-363.
[47] Dalby MJ, Riehle MO, Yarwood SJ, et al. Nucleus alignment and cell signaling in fibroblasts: response to a micro-grooved topography. Exp Cell Res. 2003;284(2): 274-282.
[48] Chew SY, Mi R, Hoke A, et al. Aligned protein-polymer composite fibers enhance nerve regeneration: a potential tissue-engineering platform. Adv Funct Mater. 2007;17(8): 1288-1296.
[49] Ogata T, Yamamoto S, Nakamura K, et al. Signaling axis in schwann cell proliferation and differentiation. Mol Neurobiol. 2006;33(1):51-62.
[50] Tofaris GK, Patterson PH, Jessen KR, et al. Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. J Neurosci. 2002;22(15):6696-6703.
[51] The ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.
[52] Fini M, Motta A, Torricelli P, et al. The healing of confined critical size cancellous defects in the presence of silk fibroin hydrogel. Biomaterials. 2005;26(17):3527-3536.
[53] Meinel L, Hofmann S, Karageorgiou V, et al. The inflammatory responses to silk films in vitro and in vivo. Biomaterials. 2005;26(2):147-155.

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