Laminin-coated nerve guidance conduits based on poly(l-lactide-co-glycolide) fibers and yarns for promoting Schwann cells’ proliferation and migration†

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To develop an effective nerve guidance conduit with cooperative effects of topological structure and biological cues for promoting Schwann cells’ (SCs) proliferation and migration, a laminin-coated and yarn-encapsulated poly(l-lactide-co-glycolide) (PLGA) nerve guidance conduit (LC-YE-PLGA NGC) was fabricated in this study. The PLGA fiber yarns were fabricated through a double-nozzle electrospinning system and then the PLGA fibrous outer layer was collected using a general electrospinning method. Subsequently, laminin was coated on the yarn-encapsulated PLGA NGC through covalent binding. The results showed satisfactory tensile mechanical strength of the laminin-coated PLGA fibers/yarns and good compressive mechanical support of the LC-YE-PLGA NGC. SCs proliferation was significantly superior (\(p<0.05\)) on the PLGA and laminin-coated PLGA yarns than the PLGA fibers. Furthermore, the LC-YE-PLGA NGC performed much better in SCs migration compared with the NGCs without yarn-encapsulation or laminin-coating, indicating the synergistic effect of the three-dimensional yarn structure (topological structure) and the laminin-coating (biological cues) for SCs proliferation and migration. Therefore, the LC-YE-PLGA NGC demonstrated promising potential in promoting SCs proliferation and inducing SCs migration in nerve tissue engineering.

1. Introduction

Tissue engineered nerve conduits serve as alternatives to supplement or even substitute autologous nerves. The latest advances in neural tissue engineering for peripheral nerve regeneration have focused on designing new materials, different cues, innovative techniques and original concepts. The challenge is disruption of the naturally resident unidirectional aligned architecture, which directs and guides axons toward their appropriate targets within normal tissue. Hence, it is imperative to build a bridge that spans the lesion gap with all the morphological, chemical and biological cues that mimic the normal tissue.1,2

Scaffolds designed to bridge peripheral nerve defects have evolved from simple hollow tubes to complex microstructured conduits, presenting both physical and chemical cues that are important for oriented glial cell migration and axonal growth.3 These scaffolds are called nerve guidance conduits (NGCs). For example, one or more intraluminal channels can be introduced to construct a multichannel NGC to mimic the architecture of nerve fascicles, and therefore the dispersion of regenerating axons within the NGC lumen could be reduced.4–6 Dinis et al. fabricated electrospun multichannel silk conduits, bi-functionalized with nerve growth factor (NGF) and ciliary neurotropic factor (CNTF) to enhance peripheral nerve regeneration. The study demonstrated that the bioactive guides consisting of longitudinally oriented channels and aligned nanofibers imitated the fascicular architecture and fibrous extracellular matrix in native nerves.7 Jeffries et al. reported a nerve guide that incorporated parallel microfibers into 3D constructs while retaining an open architecture. The nerve
guide consisted of many microchannels lined with a thin layer of longitudinally-aligned microfibers, aiming to maximize the benefits of topographical cues without inhibiting cellular infiltration.\(^8\) The overall results demonstrated that the longitudinally aligned multi-channels simulated the natural nerve structure and supported the nerve cells' adhesion and migration. Another modification of neural scaffolds is the incorporation of physical fillers into the NGC lumen. An array of biomaterial-based fillers in the form of fibers, filaments, gels or sponges in the lumen of NGCs have been reported to provide topological cues for improving nerve regeneration.\(^6,9–11\) Nowadays, some modified electrospinning methods have been developed to fabricate nano-fiber yarns, and the yarns induced cells' infiltration and migration when applied for tissue engineering.\(^12–16\) Li et al. preliminarily studied the applicability of nanofiber yarns as filaments in the nerve conduits.\(^12\) They constructed a nerve conduit that incorporated aligned poly(\(\varepsilon\)-lactide) (PLLA) nanofiber yarns into a hollow poly(\(\varepsilon\)-lactide-co-caprolactone) (P(LLA-CL)) tube. The results demonstrated the feasibility of the interior yarns for Schwann cells' (SCs) proliferation and distribution. It showed that the nanofiber yarns served as longitudinally aligned filaments inside the lumen and provided fibrous surface for cells.

Different classes of biodegradable synthetic polymers like poly(glycolic acid) (PGA), PLLA, poly(caprolactone) (PCL) and their copolymers have been extensively used to produce random or aligned nanofibers via electrospinning methods for nerve tissue applications.\(^17–20\) Among these materials, PLGA is one of the top biodegradable synthetic polymers used for tissue engineering due to the ease of controlling its mechanical properties and biodegradation rate.\(^21\) Besides this, efforts have concentrated on the optimal incorporation of biochemical cues within a tissue engineered NGC to increase neurite extension and axonal regeneration. Natural materials like collagen, laminin, silk fibroin and chitosan have been explored to mimic the biomolecular recognition of native soft tissues.\(^1,17,21–23\) Laminin-coated conduits have been reported to improve nerve regeneration by promoting Schwann cell regeneration.\(^18,22,24\) Furthermore, laminin served as a major glycoprotein of the basement membrane of nerve tissue, possessing the main function of stimulating neurite outgrowth associated with axonal guidance.\(^20\) He et al. electrospun PLLA nanofibers and modified their surfaces using laminin/chitosan polyelectrolyte multilayers.\(^18\) The laminin modified PLLA scaffolds significantly promoted cell proliferation and induced the neurite outgrowth of DRG neurons compared to the pure PLLA nanofibrous scaffolds. Junka et al. fabricated laminin functionalized PCL/chitosan nanofibrous scaffolds, which showed significantly higher proliferation rates of SCs than the PCL/chitosan scaffolds without laminin.\(^24\) It can be seen that laminin is an effective biological cue for promoting nerve cells' proliferation and inducing nerve axon extension.

Although the past several decades have witnessed great advances in the modification of topological structure, biochemistry cues and other interactions on nerve conduits, neural tissue engineering needs further significant progress towards the development of ideal NGCs with comprehensive properties for translation to clinical applications. To address this issue, a novel yarn-encapsulated NGC based on PLGA fibers and PLGA fiber yarns was developed via a double-nozzle electrospinning system and a subsequent common electrospinning method. The PLGA yarns filled in the lumen of the PLGA fibrous tube, serving as the filaments. Then laminin was coated on the yarn-encapsulated PLGA NGC, aiming to promote Schwann cells' (SCs) proliferation and migration. The mechanical properties and SCs proliferation, distribution and migration on laminin-coated PLGA fibers, PLGA yarns or yarn-encapsulated PLGA NGC were evaluated to assess the potential of the laminin-coated and yarn-encapsulated PLGA NGC in nerve tissue engineering.

### 2. Materials and methods

#### 2.1. Materials

Poly(\(\varepsilon\)-lactide-co-glycolide) (\(\varepsilon\)-PLGA, LA : GA = 82 : 18; IV(dL g\(^{-1}\)) : 1.9) was supplied by Jinan Daigang Biomaterial Co., Ltd (Jinan, China). Natural mouse laminin isolated from the Engelbreth-Holm-Swarm sarcoma was purchased from Invitrogen by Life Technologies Co., (USA). 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP) was obtained from Shanghai Darui Co. Ltd (China). Rat Schwann cells (SCs) were purchased from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, China). Cell culture medium and reagents were provided by Gibco Life Technologies Co., (USA) unless specified.

#### 2.2. Fabrication of PLGA membranes and yarns

PLGA was dissolved in HFIP at a concentration of 15% and stirred well with a magnetic stirrer overnight. To fabricate the PLGA nanofibrous membranes, a common single-nozzle electrospinning method was performed using a high voltage of 12 kV, a flow rate of 1.0 mL h\(^{-1}\) and a collect distance of 12 cm. The PLGA fibers could be continuously and stably obtained under these conditions and the electrospinning process was sustained for 2 hours to collect the PLGA fibers. For preparing the PLGA nanofiber yarns, electrospinning equipment for fiber yarns (TFS-700, Beijing Technova Technology Co., Ltd, China) was utilized with a rotatable funnel to twist the nanofibers to yarns and a rotatable rod to collect the yarns. Two spinnersets in the opposite directions were used with flow rates of 0.02 mL min\(^{-1}\) and 0.032 mL min\(^{-1}\), respectively. A positive high voltage at +10 kV and a relative negative high voltage at –10 kV was connected to the needles. A rotating rate of the funnel at 450 rpm was used to twist the fibers to fiber yarns. A collecting roller with a rotating rate of 10 rpm and horizontal movement of 10 mm min\(^{-1}\) was utilized to gather the PLGA fiber yarns steadily. These parameters were selected because PLGA yarns could be continuously collected under these conditions. The electrospinning process lasted for 2 hours to obtain the PLGA yarns.

#### 2.3. Laminin coating on the PLGA fibers and yarns

The PLGA fibrous membranes were punched into discs with a 14 mm diameter, and the PLGA yarns were wrapped on the 14 mm-diameter cover slips. The prepared samples were placed in 24-well tissue culture plates (TCPs) and secured using
stainless rings. All of the samples were immersed in 0.01 M NaOH for 20 minutes at room temperature and then in 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES) solution with 1 mL per well for 30 min at 4 °C. N-Hydroxysuccinimide (NHS) and ethylene diaminecarbodiimide (EDC) were dissolved in MES solution with a concentration of 6 mg mL⁻¹ and 4 mg mL⁻¹, respectively, at 4 °C. The MES solutions in the 24-wells were removed and 1 mL of the MES/EDC/NHS solution was added per well for 1 hour at room temperature. The samples were rinsed with 4 °C MES solution three times, followed by immersing in 20 µg mL⁻¹ laminin solution with gentle shaking at 4 °C. After overnight reaction, the laminin-coated PLGA (LC-PLGA) fibers and yarns were washed with phosphate-buffered saline solution (PBS) solution and stored at 4 °C for use.

### 2.4. Fabrication of yarn-encapsulated PLGA NGCs and laminin coating

The schematic diagram of fabricating the laminin-coated and yarn-encapsulated PLGA (LC-YE-PLGA) NGC is shown in Fig. 1. To begin with, the PLGA fiber yarns were pre-fabricated as mentioned above. Then, the pre-fabricated PLGA yarns were cut to 5 cm in length and fixed around a 2 mm-diameter stainless steel rod in a parallel sequence and along the axial direction. Then the rod with the PLGA yarns on it was utilized as a collector with a rotating rate of 100 rpm to collect electrospun PLGA fibers as the tube wall. The voltage, flow rate and collect distance was 12 kV, 1.0 mL h⁻¹ and 12 cm, respectively. The electrospinning process for collecting the PLGA fibers was maintained for 2 hours. Then, the stainless steel rod was removed and the yarn-encapsulated PLGA (YE-PLGA) NGC was obtained. After incubating in a vacuum oven overnight, the YE-PLGA NGC was cut into a 1 cm length and placed in the 5 mL vial. The laminin coating process was performed as mentioned above. The obtained laminin-coated and yarn-encapsulated PLGA (LC-YE-PLGA) NGCs were stored at 4 °C for use as well. The bare PLGA (B-PLGA) NGCs without encapsulated yarns were electrospun as a comparison. The electrospinning voltage, flow rate and collect distance was 12 kV, 1.0 mL h⁻¹ and 12 cm, respectively. A stainless steel rod with a rotating rate of 100 rpm was used as the collector. The electrospinning process was maintained for 2 hours to obtain B-PLGA NGCs.

![Fig. 1 A schematic diagram of the fabrication of the laminin-coated and yarn-encapsulated PLGA (LC-YE-PLGA) NGC (YE NGC: yarn-encapsulated nerve guidance conduit; LC-YE NGC: laminin-coated and yarn-encapsulated nerve guidance conduit).](image)

### 2.5. Characterization

The structure and morphology of LC-YE-PLGA NGC was investigated using a scanning electron microscope (SEM) (Phenom XL, Phenom-World B.V., Netherlands). X-ray photoelectron spectrometry (XPS) full spectrum and atomic ratios of carbon, oxygen and nitrogen on the surface of different scaffolds were investigated using an Axis Ultra spectrometer (Kratos Analytical, UK). The coating amounts of laminin on LC-PLGA fibers, LC-PLGA yarns and LC-YE-PLGA NGCs were detected using a Micro BCA™ Protein Assay Kit (Thermo Fisher Scientific, USA) according to the manufacturer’s test protocol. The laminin coating amounts were calculated from a laminin standard curve. The hydrophilicity of the PLGA fibers and laminin-coated PLGA (LC-PLGA) fibers was measured using a contact angle measurement instrument (OCA40, Dataphysics, Germany). The tensile mechanical properties of the PLGA fibers, LC-PLGA fibers, PLGA yarns and LC-PLGA yarns were tested using a tensile and compression testing machine (HY-940FS, Hengyu Instrument Co., Ltd, China). Stress–strain curves were obtained using the Origin 9.0 software. The axial and radial compress mechanical properties of B-PLGA NGC, YE-PLGA NGC and LC-YE-PLGA NGC were tested as well. Force–time curves and force–strain curves were acquired using the Origin 9.0 software.

### 2.6. SCs proliferation and morphology on laminin-coated PLGA fibers and yarns

The PLGA fibers, LC-PLGA fibers, PLGA yarns and LC-PLGA yarns on slides were all placed in 24-well plates and secured by stainless rings. TCPs were processed using the same procedure for comparison. Before seeding the SCs on the fibers and yarns, the samples were immersed in 75% ethanol solution overnight for sterilization and followed by rinsing with PBS solution three times. Then the samples were soaked in Dulbecco’s modified Eagle’s medium (DMEM) under conditions of 37 °C, 5% CO₂ and 95% humidity.

The SCs were cultured in DMEM medium with 10% fetal bovine serum and 1% antibiotic–antimycotic under the same conditions. The culture medium was replenished every two days. After the SCs in the culture flasks were expanded to almost 80% confluence, the cells were digested and seeded on the samples at a concentration of 1 × 10⁵ cells per mL, and the medium was changed every two days as well. The SCs proliferation was evaluated on PLGA fibers, LC-PLGA fibers, PLGA yarns and LC-PLGA yarns using cell counting kit-8 (CCK-8, Dojindo Lab., Japan) after culturing for 1, 4 and 7 days. Briefly, the medium in each 24-well plate was pipetted out and substituted with the fresh medium with 10% CCK-8. After incubating for 3 hours at 37 °C, the aliquots were extracted into a 96-well plate and the absorbance was measured at 450 nm using an Enzyme-labeled Instrument (Multiskan MK3, Thermo, USA). All of the measurements were performed with duplicate specimens (n = 6).

When the SCs were incubated on samples for 4 days, the samples were washed with PBS and processed with 4% paraformaldehyde for fixing cells. After rinsing the samples with
PBS again, the samples were dehydrated with gradient ethanol (30%, 50%, 70%, 80%, 90%, 95% and 100%) and dried in a vacuum oven. Then, the samples were coated with gold sputter and observed under the SEM. When the SCs were incubated for 1, 4 and 7 days, additional samples were permeabilized in 0.1% Triton X-100 (Sigma, USA) at room temperature for 5 min and rinsed again with PBS three times. Then, 4’,6’-diamidino-2-phenylindole hydrochloride (DAPI, Invitrogen, USA) and rhodamine-conjugated phalloidin (Invitrogen, USA) were used to stain the nuclei and cytoskeletons of the cells. The samples were observed under an inverted fluorescence microscope (IFM, Olympus IX71, Japan).

2.7. SCs growth in NGCs

SCs were seeded into B-PLGA NGCs, YE-PLGA NGCs and LC-YE-PLGA NGCs to assess the SCs distribution and migration. Briefly, the NGCs were cut into 1 cm lengths and placed into 24-well plates. Then, the samples were sterilized in 75% ethanol and rinsed with PBS solution. After the samples were soaked in DMEM medium for 2 hours at 37 °C, the samples were placed in 1 × 10^5 cells per mL were seeded in one end of the NGCs lumen. The NGCs with SCs were incubated on a 50 rpm shaker in the incubator after the SCs adhesion for 3 hours. The medium was refreshed every two days. After 7 days of culturing, the NGCs with SCs were fixed with 4% paraformaldehyde overnight at 4 °C. Then, the samples were processed using conventional paraffin embedding hematoxylin–eosin (H&E) staining, Cross and longitudinal sections of the NGC–cell composites were obtained after dehydration and embedding in paraffin blocks. Next, the specimens were stained with H&E and observed under an optical microscope (Nikon Eclipse, Japan). The NGC–cell composites were also used to perform an immunofluorescence assay with rabbit anti-rat S-100 antibody (1:100, Thermo Fisher Scientific, USA) overnight and then stained with fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG (1:200, Thermo Fisher Scientific, USA) and DAPI (1:200) for 30 min and 5 min, respectively. The samples were observed under a histological section scanner (Pannoramic MIDI, 3D HISTECH, Hungary) to detect the SCs distribution and migration.

2.8. Statistics

Statistics analysis was carried out using origin 9.0 (Origin Lab Inc., USA). All the values were averaged in duplicate specimens and presented as means ± standard deviation (SD). Significant differences were determined using One-Way ANOVA analysis and it was considered to have statistical significance at p < 0.05.

3. Results and discussion

3.1. Structure of the laminin-coated and yarn-encapsulated PLGA NGC

The overall structure and fiber/yarn morphology of the laminin-coated and yarn-encapsulated PLGA (LC-YE-PLGA) NGCs is presented in Fig. 2. The LC-YE-PLGA has a diameter of 2 mm with dozens of PLGA yarns in the lumen (Fig. 2A and B). SEM images of the laminin-coated PLGA (LC-PLGA) fibers and yarns in the LC-YE-PLGA NGC are shown in Fig. 2C and D. This demonstrated that laminin was coated on the surface or at the joints of the PLGA fibers (Fig. 2C), and wrapped around the fibers in the PLGA yarns as well (Fig. 2D). Fig. 2E illustrates the schematic of covalent coupling of laminin using EDC/NHS chemistry onto PLGA fibers and yarns. Covalent binding was achieved using water-soluble carbodiimide (EDC) and N-hydroxy-succinimide (NHS) as the coupling reagents. It has been reported that the covalent coupling of laminin on electrospun polymer fibers by EDC/NHS crosslinking could bind a greater amount of laminin than physical absorption.26

### Table 1

<table>
<thead>
<tr>
<th>Samples</th>
<th>LC-PLGA fibers</th>
<th>LC-PLGA yarns</th>
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</thead>
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<tr>
<td>C atomic concentration (%)</td>
<td>64.84</td>
<td>75.15</td>
</tr>
<tr>
<td>N atomic concentration (%)</td>
<td>4.71</td>
<td>2.51</td>
</tr>
<tr>
<td>O atomic concentration (%)</td>
<td>30.45</td>
<td>22.34</td>
</tr>
</tbody>
</table>

3.2. Characterization

The presence of laminin coupled onto PLGA fibers and yarns was shown and verified using XPS and MicroBCA protein assay. XPS is commonly performed to provide both qualitative and quantitative information of the surface chemistry of a material after a certain treatment. XPS full spectra demonstrated that laminin was successfully added on the surface of fibers/yarns as indicated by the presence of N1s peaks (Fig. S1, ESI†), which showed the same results as a previously reported study.25 Analysis of atomic compositions showed that the N atomic percentage of the covalently bound laminin–PLGA fibers/yarns (4.71% of fibers and 2.51% of yarns, Table 1) was significantly higher than the physically adsorbed counterparts (0.67% of fibers and 0.36% of yarns, Table S1, ESI†), indicating that the amount of covalently bound laminin was significantly greater than that incorporated via physical adsorption.

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Fig. 2 (A) An optical image of laminin-coated and yarn-encapsulated PLGA (LC-YE-PLGA) NGC; (B–D) SEM images of (B) the cross section of LC-YE-PLGA NGC, (C) laminin-coated PLGA (LC-PLGA) fibers in the tube wall and (D) intraluminal LC-PLGA yarns; (E) a schematic of the covalent coupling of laminin onto PLGA fibers and yarns using the EDC/NHS method.
Furthermore, the quantification of laminin coupled onto fibers/yarns was achieved using a MicroBCA protein assay. The amounts of covalent coating of laminin on LC-PLGA fibers, LC-PLGA yarns and LC-YE PLGA NGCs are presented in Table 2. The amounts of covalently bound laminin on LC-PLGA fibers, LC-PLGA yarns and LC-YE PLGA NGCs were 1.96 ± 0.42 μg, 1.33 ± 0.25 μg and 1.64 ± 0.33 μg, respectively. Besides, the amounts of covalently bound laminin on PLGA fibers/yarns were greater than their physically adsorbed counterparts (1.61 ± 0.39 μg of fibers and 1.01 ± 0.24 μg of yarns, Table S2, ESI†), demonstrating the same results as the XPS measurements. The overall results showed that laminin could be effectively covalently bound onto the electrospun polymer scaffolds through EDC/NHS methods, and the laminin coating amounts could be increased compared with the physically adsorbed method.25

The hydrophilicity of the PLGA and LC-PLGA fibers was tested using water contact angles measurement. The results in Fig. 3A show that the PLGA fibers were hydrophobic with a water contact angle of 135.4 ± 3.58° and the angle remained unchanged during the measurement period. However, the surface of the LC-PLGA fibers became hydrophilic after laminin coating and the water contact angle was reduced from 87.75 ± 7.24° to 0° after contacting for 30 seconds. This confirmed that coating laminin protein on the surface contributed to enhancing the hydrophilicity of the electrospun fibers.26

3.3. Mechanical properties

The tensile stress–strain curves of PLGA fibers, LC-PLGA fibers, PLGA yarns and LC-PLGA yarns are displayed in Fig. 3B and C, and the corresponding tensile stress, strain and modulus are presented in Table S3 (ESI†). The ultimate tensile stress of LC-PLGA fibers was 6.52 ± 0.81 MPa, which was greater than that of the PLGA fibers (5.25 ± 0.90 MPa). The elongation of the LC-PLGA fibers was decreased to 174.38 ± 22.79% compared to that of the PLGA fibers (292.07 ± 10.50%). This was mainly due to the increased stiffness of the PLGA fibers after the laminin coating, which significantly affected the mechanical properties of PLGA fiber and yarns. Similarly, the tensile stress of a single LC-PLGA yarn was increased to 24.08 ± 5.01 MPa, which was better than that of the untreated single PLGA yarn (18.76 ± 3.70 MPa). The elongation at break of the LC-PLGA yarn showed a slight decrease compared with the PLGA yarn (from 233.62 ± 26.48% to 183.13 ± 20.44%).

The compressive behavior of the conduits is usually measured before in vivo implantation as well. The compressive mechanical behavior of bare PLGA (B-PLGA) NGCs, yarn-encapsulated PLGA (YE-PLGA) NGCs and LC-YE-PLGA NGCs was assessed both in radial and axial directions. From the force–time curves and force–strain curves in the radial direction (Fig. 4), the LC-YE-PLGA NGCs showed better performance in compression resistance than the B-PLGA and YE-PLGA NGCs when elastic deformation was 50%. The encapsulated PLGA yarns in the lumen provided compressive support and the coated laminin also took effect. Compared with the B-PLGA NGCs, the YE-PLGA and LC-YE-PLGA NGCs exhibited a highly nonlinear and closed hysteresis. After 5 compression cycles, the YE-PLGA and LC-YE-PLGA NGCs still performed better than the B-PLGA NGCs in elastic recovery,
indicating the improvement of the encapsulated yarns in the NGCs lumen for resisting the compression. As the force–time curves and force–strain curves show in the axial direction (Fig. 5), the YE-PLGA and LC-YE-PLGA NGCs showed better performance in compressive resistance than the B-PLGA NGCs as well, regardless of the cycle times. Hence, the results showed that the YE-PLGA and LC-YE-PLGA NGCs presented better resilience properties than the B-PLGA NGCs both in the radial and axial directions.

The compressive force of the LC-YE-PLGA NGCs showed matched performance when compared with the previously reported study. Jiang et al. fabricated fibrous conduits comprised of aligned electrospun PCL microfibers or nanofibers and evaluated their compressive behavior before in vivo evaluation.27 The results showed that the nanofibrous and microfibrous PCL conduits showed resistance towards compression at 1.97 ± 0.14 N vs. 1.37 ± 0.23 N, respectively, when 50% deformation of the longitudinal axis direction was performed. In comparison, the LC-YE-PLGA NGCs in this study showed better pressure resistance even after 5 compression cycles (4.90 ± 0.12 N after 1 compression and 2.18 ± 0.19 N after 5 compression cycles) than the reported conduits. The overall mechanical results indicated that the LC-PLGA fibers and yarns had good tensile mechanical support, and the LC-YE-PLGA NGCs performed satisfactorily in the resistance to mechanical compression as well. The encapsulated PLGA yarns and coated laminin provided enhanced tensile and compressive mechanical support to the LC-YE-PLGA NGCs.

3.4. SCs proliferation and morphology on the LC-PLGA fibers and yarns

Fig. 6A showed the SCs proliferation on PLGA fibers, LC-PLGA fibers, PLGA yarns and LC-PLGA yarns after culturing the SCs for 1, 4 and 7 days. The SCs proliferation on TCP had a gradient increase from day 1 to day 7 and showed significantly better (p < 0.05) performance than that of pure PLGA fibers after culturing for 7 days, indicating that pure synthetic polymer (PLGA fibers) was insufficient for cells’ proliferation. At day 1, there was no significant difference in SCs growth among different samples, while SCs production on LC-PLGA yarns showed a significant difference (p < 0.05) compared with the PLGA fibers after culturing for 4 days, indicating the synergistic effect of topological structure (three-dimensional yarns) and biological cues (laminin-coating). After culturing SCs on the samples for 7 days, SCs proliferation on PLGA yarns and LC-PLGA yarns was significantly better (p < 0.05) than the PLGA and LC-PLGA fibers, demonstrating the more remarkable influence of the topological structure (three-dimensional yarns) than the biological cues (laminin-coating) on SCs proliferation.

SEM images of the SCs growth on PLGA fibers, LC-PLGA fibers, PLGA yarns and LC-PLGA yarns are displayed in Fig. 6B–E. On the PLGA fibers, SCs only extended on the fiber surface (Fig. 6B). For the LC-PLGA fibers, it was found that SCs spread not only on the surface but also inside the LC-PLGA fibers (Fig. 6C). Similarly, SCs proliferated to a cell sheet on the surface of one single PLGA yarn (Fig. 6D), while the cells on LC-PLGA yarns preferred to grow on the yarn surface and inside the yarn (Fig. 6E). SCs linkage between the neighboring yarns was also observed on the LC-PLGA yarns. The evaluation of SCs proliferation and morphology indicated the better performance of laminin-coated fibers/yarns than the untreated ones. The SCs morphologies were also observed using fluorescence staining after culturing for 1, 4 and 7 days (Fig. 7). The SCs proliferation on the scaffolds and TCP presented a considerable increase all through day 1 to day 7. For PLGA fibers, LC-PLGA fibers and TCP, the SCs proliferated with the interaction between cells and cells, and cells linked to an integral SCs layer after 7 days. As for the PLGA and LC-PLGA yarns, SCs proliferated along the single yarn initially from day 1 to day 4, while SCs tended to wrap the yarns and link the closely neighboring yarns through cell–cell interaction at day 7. Better cell linkage between adjoining yarns was observed in the LC-PLGA yarns than the untreated PLGA yarns.
yarns, showing improved cell-cell interaction on the laminin-coated yarns.

Normally, combining synthetic polymers with natural proteins or polysaccharides will enhance cell adhesion and proliferation in comparison to pure polymers.\textsuperscript{22,28,29} SCs proliferation on laminin-coated PLGA fibers and yarns were better than their untreated counterparts after culturing for 7 days. However, the scaffold structure was also an influencing factor on cells' proliferation. It has been reported that the nanofiber yarn structures significantly promoted cells' proliferation and infiltration when compared with the dense nanofibers.\textsuperscript{12,15,30} In this study, cells' proliferation on LC-PLGA and PLGA yarns was significantly better ($p < 0.05$) than the PLGA and LC-PLGA fibers at day 7, indicating that the scaffold structure had a greater effect on cells' proliferation than laminin-coating treatment. Moreover, the nanofiber yarns provided organized directions to induce cells spread along the yarns.\textsuperscript{13,30,31} The LC-PLGA yarns' cooperative yarn structure with laminin-coating cues presented better cells' proliferation and interactions than the other groups. The overall results indicated the advantages of combining topological structure with biological cues on SCs behavior.

### 3.5. SCs distribution and migration in NGCs

S100 protein expression was detected using immunocytochemical staining to observe SCs distribution and migration in different NGCs after culturing for 7 days. The S100 protein has been identified as a glial marker that is expressed in Schwann cells.\textsuperscript{32} Although a previous study has reported that S100 is not fully expressed in the early stage of culturing SCs, and a variety of other cells, such as chondrocytes, adipocytes, cardiomyocytes, skeletal muscle cells, and melanoma cells also express S100 \textit{in vivo},\textsuperscript{33–35} it could be used to observe SCs growth morphology on tissue engineered scaffolds \textit{in vitro} using immunocytochemical staining.\textsuperscript{32,36,37}

Fig. 8 shows the H&E images (Fig. 8A and B) and immunofluorescence images with anti-S100 antibody (Fig. 8C and D) of the cross sections after SCs were seeded in bare PLGA (B-PLGA) NGCs, yarn-encapsulated PLGA (YE-PLGA) NGCs and laminin-coated and yarn-encapsulated PLGA (LC-YE-PLGA) NGCs for 7 days. From the H&E and immunofluorescence images, it was observed that SCs only proliferated on the lumen surface or the outer surface of the B-PLGA NGCs and the SCs were partially piled at some areas. However, SCs grew around every single yarn in the lumen of YE-PLGA and LC-YE-PLGA NGCs, and more sufficient SCs filled the spaces between the adjacent yarns in the LC-YE-PLGA NGCs. Fig. 9 correspondingly presents the H&E images (Fig. 9A and B) and immunofluorescence images with anti-S100 antibody (Fig. 9C and D) of the longitudinal sections after the SCs were cultured for 7 days. It was also found that SCs were produced on the surface of the lumen in B-PLGA NGCs and partly stacked SCs were observed as well. Similarly, no significant SCs migration was detected in the lumen of the YE-PLGA NGCs. However, both SCs production and migration were observed on the LC-YE-PLGA NGCs, and SCs migration from the cell-seeding end in the lumen was apparent and is marked by white lines and arrows. This demonstrated the cooperative effect of the topological structure (three-dimensional yarns) and the biological cues (laminin coating), where the yarn structure significantly promoted SCs proliferation and the laminin-coating cues induced effective SCs migration.
Nerve conduits with multi-channels or intra-filled filaments were considered to imitate the natural architecture of nerve fascicles. Several techniques based on electrospinning methods have been conducted to fabricate multi-channel nerve conduits for inducing cells’ survival and migration. Dinis et al. firstly fabricated electrospun silk sheet with aligned nanofibers. Then, the silk sheet was rolled up at 360° using a Teflon stick, and this process was repeated for 50 sticks. Next, the rest of the silk sheet was rolled up around the structure created. After removing the Teflon sticks, the conduit with multi-channel was obtained.7 Nerve conduits with multi-channels were successfully fabricated but the cells’ organization along the channels was not presented. Jenkins et al. designed a nerve guidance conduit with topographical (multi-channels) and biochemical cues (RGD conjugation) in a previous study.39 The multi-channels were formed by rolling the electrospun scaffolds into a tubular conduit and dissolving out the bridged sucrose fibers. The nerve guidance conduit was shown to enhance cell survival, migration and guide neurite extension. Compared with the reported NGCs with multi-channels, the LC-YE-PLGA NGCs in this study showed advantages in the manufacturing process. Fabrication of the LC-YE-PLGA NGCs avoided the post-processing steps of rolling or dissolving the template fibers after electrospinning. Besides, the covalent coating of laminin was easily performed without destroying the yarns-encapsulated and tubular structure of the as-spun NGCs. Furthermore, the results in Fig. 8 and 9 show that SCs proliferation surrounding the intraluminal yarns and SCs migration was enhanced by the laminin coating on the encapsulated yarns. The overall results demonstrated that the incorporation of topological structure and biological cues was important for promoting SCs proliferation and inducing SCs migration.

4. Conclusions

The laminin-coated and yarn-encapsulated PLGA nerve guidance conduits (LC-YE-PLGA NGCs) were fabricated using electrospinning technologies and subsequent covalent binding of laminin. The LC-YE-PLGA NGCs consisted of PLGA yarns with axial alignments as the filaments in the lumen and PLGA fibers as the external tube wall. The laminin-coated PLGA (LC-PLGA) fibers and yarns had sufficient tensile mechanical properties and the LC-YE-PLGA NGC possessed suitable compressive mechanical support for nerve tissue engineering. The LC-PLGA and PLGA yarns had significant better SCs proliferation than the PLGA fibers, indicating the effective promotion of topological cues (yarns structure) for SCs proliferation. Furthermore, SCs migration was much better in the LC-YE-PLGA NGCs than the ones without yarn-encapsulation or laminin-coating, demonstrating better guidance of laminin-coating to SCs migration. Therefore, the LC-YE-PLGA NGCs with the synergistic effects of topological structure (yarns structure) and biological cues (laminin coating) provided a promising alternative for regulating SCs proliferation and migration in nerve tissue engineering.

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