Development of Multi-channel Electro-conductive Nanofibrous Conduits for Peripheral Nerve Regeneration

Niloofar Nazeri

Tehran University of Medical Sciences

Mohammad Ali Derakhshan

Shiraz University of Medical Sciences

Korosh Mansoori

Tehran University of Medical Sciences

Hossein Ghanbari (✉ hghanbari@tums.ac.ir)

Tehran University of Medical Sciences <https://orcid.org/0000-0001-7639-995X>

Research Article

Keywords: Align nanofibers, Electrospinning, Laminin, MWCNT, Sciatic nerve

Posted Date: April 14th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-367393/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Multichannel structures in the design of nerve conduits offer potential advantages for regeneration of damaged nerves due to their bio-mimicking architecture. However, lack of biochemical cues and electrical stimulation could hamper satisfactory nerve regeneration. The aim of this study was to simultaneously evaluate the effects of topographical, biological and electrical cues on sciatic nerve regeneration in a rat model. Accordingly, a series of multichannel nanofibrous nerve conduit was made using longitudinally-aligned laminin-coated electrospun PLGA/CNT nanofibers (NF, mean diameter: 455 ± 362 nm) in the lumen and randomly-oriented PCL NF (mean diameter: 340 ± 200 nm) on the outer surface. *In vitro* studies revealed that both materials were nontoxic to Schwann cells and able to promote cell attachment and proliferation. To determine the influence of topographical, biological and electrical cues on nerve regeneration, either of hollow PCL conduits, PLGA NF-embedded, PLGA/CNT NF-embedded or laminin-coated PLGA/CNT NF-embedded PCL conduits were implanted in rats. A new surgery method was utilized and results were compared with an autograft. After animal treatments, motor and sensory tests showed significant improvement in the rats treated with NF-embedded PCL conduits. H&E images obtained from cross-sectional and, longitudinal-sections of the regenerated nerves demonstrated the formation of regenerative nerve fibers and also, angiogenesis in laminin-coated PLGA/CNT NF-embedded PCL conduits. Results suggested that these conduits have the potential for clinical application to reconstruct peripheral nerve defects.

Introduction

Nerve injuries impose a huge burden on the healthcare systems all over the world [1]. Although some of the nerve gaps become repaired through the natural repair mechanisms of the peripheral nervous system (PNS), extensive damages need to be reconstructed surgically [1]. If regeneration does not occur within a certain period, the communications with the brain and spinal cord become blocked and therefore, proximal stump forms a neuroma and related muscle becomes atrophic [1,2]. Even though, the methods of treatment seem to depend on the size of injury. When end-to-end tensionless neurorrhaphy is not possible, autologous nerve grafts are the best standard options for repair [3]. Nevertheless, it causes some problems in donor-site such as tissue morbidity. As an alternative, development of nerve conduits has been considered. These structures are used to provide a similar native extracellular matrix (ECM) environment, bridge the gap and direct the regenerating axons of proximal stump towards the distal one [4,5]. However, none of the commercially-available nerve conduits act better than standard technique of autologous nerve grafting [6,7,4], thus, it has prompted designing of new synthetic scaffolds to treat nerve injuries.

The ECM of the PNS exhibits special architecture and microenvironment to guide neurite regeneration, which are not found in the currently available nerve conduits [5]. Recent developments aim to design the scaffolds that mimic the native ECM by presenting appropriate biochemical and physical cues to improve nerve regeneration [7]. As the natural nerve is composed of tubular fascicles, a simple tube cannot replicate the intrinsic characteristics of the natural nerve [8]. Designing the lumen with a natural

framework, such as longitudinally-oriented channels associated with aligned NF, not only modifies the physical properties of the conduit but also helps in the fibrin matrix stabilization. It also would mimic the bands of Büngner, where Schwann cells (SCs) form aligned bands between damaged nerve ends to guide axons [5, 9].

In addition to architectural design, taking the electrophysiological properties of nerve tissues into account can significantly promote the cellular responses of nerve-derived cells in nerve regeneration [9]. A conductive neural interfacing material can remodel the passive and active bioelectric properties of neural stem cells and their progeny, which is followed by increased differentiation of these stem cells into the neural lineage [10]. Recent studies revealed that compared to the non-conductive conduits, the conduits filled with parallel-aligned conductive fibers facilitated the functional recovery of regenerating nerves [11]. Also, these conductive structures were able to achieve significant results over the autologous nerve grafts [11,9]. Thus, incorporation of electric stimulation to the nerve conduits facilitates regeneration over long gaps. In order to take advantages of electrical stimulation, scaffolds with different levels of electrical conductance have been designed [12,9,11]. Multi-walled carbon nanotube (MWCNT) is an excellent choice to be used as filler in the scaffolds to improve their mechanical and electrical properties [13,14].

It is previously suggested that extra pharmacological factors could be incorporated into the conductive conduits to further improve the nerve regeneration [9,15]. Laminin, as a primary component of basal lamina in nerve tissue, is continuously synthesized after nerve injury and has been shown to serve as an ideal biochemical cue for promoting the differentiation of neuronal cells [16,2]. Adhesive properties of this protein can encourage cells migration and proliferation during nerve regeneration [16,17]. Moreover, recent studies have shown that neurite outgrowth was increased on scaffolds that were physically or covalently bound with laminin [18]. To functionalize the nanofibrous scaffolds with laminin, dopamine chemistry has been used [19].

In the present study, a series of multi-channel nanofibrous nerve conduits was fabricated. The outer layer was made of random electrospun polycaprolactone (PCL) NF to provide physical support. Previous studies showed that PCL conduits could provide ideal support for axonal regeneration both in wider gaps and longer periods of time that are needed for nerve regeneration in human [20-22]. The channels were comprised of aligned poly lactic-co-glycolic acid (PLGA) NF as their degradation rate can be engineered to fit particular needs [17]. However, the hydrophobicity of PLGA NF and the absence of cell recognition sites and electrical conductivity were compensated by adding laminin and MWCNTs, respectively. At present, a majority of studies examined the effect of conductive CNT-containing scaffolds only *in vitro* on different cells such as PC12 or SCs [23-25]. In this study, *in vivo* experiments were performed to further explain the role of CNT-containing scaffolds along with biochemical cues in nerve regeneration. To the best of our knowledge, it is the first report that presents multi-channel nerve conduits, which combine electro-conductive aligned NF and ECM protein to integrate electrical, topographical and biochemical cues for nerve regeneration. To evaluate the cyto-compatibility of the conduit materials and cell infiltration, SCs were cultured on the scaffolds and in the fibrin gels loaded in the conduits. Finally, to examine the

efficacy of the nerve conduits *in vivo*, comprehensive evaluations were carried out using the new and efficient surgery method to implant designed conduits in a rat model of sciatic injuries.

Materials And Methods

2.1. Materials

MWCNT (-COOH functionalized, diameter of 20-30 nm with a length of 10-30 μ m) was purchased from US Research Nanomaterials, USA. PLGA with a copolymer ratio 50:50 (Mw= 50kDa), PCL (Mw=70kDa) were purchased from Esun Industrial Company, China. Dulbecco’s Modified Eagle’s medium (DMEM), fetal bovine serum (FBS) and penicillin–streptomycin were obtained from Gibco, USA. 3-hydroxytyramine (dopamine) hydrochloride, Engelberth-Holm-Swarm murine sarcoma laminin-1, anti-rabbit IgG-FITC, anti-S100 antibody, bovine serum albumin (BSA), diacetate (4,6-diamidino-2-phenylinolole) (DAPI), MTT reagent, Tris (hydroxymethyl) amino methane and all solvents such as methanol, ethanol and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) were obtained from Sigma Aldrich, USA and used as received without further purification. Rat SCs were purchased from the Pasteur Institute, Iran.

2.2. Fabrication of PLGA/CNT intra-luminal nanofibrous guidance channels

CNT solution (0.5 %, w/v) in ethanol and PLGA solution (17%, w/v) in HFIP were separately prepared. The solutions were loaded into two separate plastic syringes. Then, two distinct syringe pumps were utilized to feed the solutions into two 21G nozzles placed in opposite sides of the rotating drum (2500rpm) and then, parameters of PLGA electrospinning and CNT electro-spraying were set according to the Table 1. The PLGA/CNT NF were collected on the aluminum foil wrapped around a rotating drum.

Table1: Parameters of PLGA electrospinning and CNT electro-spraying.

Parameters	PLGA Syringe	CNT syringe
Feeding rate (ml/h)	0.7	1.4
Distance from drum (cm)	17	10
Voltage (kV)	17	14

2.3. Laminin-Coating on Intra-luminal Nanofibrous Guidance Channels

The scaffolds were coated with laminin based on our previous report [12]. In brief, PLGA/CNT scaffolds were immersed in dopamine (2 mg/ml) solution prepared from 10 mM Tris-HCl buffer, pH 8.5, and shaken for 1 h at room temperature [26]. Then polydopamine- coated PLGA/CNT scaffolds were washed with DI

water three times to remove the unbound dopamine. The scaffolds were incubated with laminin (100 µg/mL) overnight at 4°C, followed by washing with DI water [27,28].

2.4. Fabrication of PCL Nerve Conduits with Intra-luminal Nanofibrous Channels

A 12 % w/v solution of PCL was prepared in chloroform/methanol (70/30, v/v) with stirring for 4 hours. Solution was electrospun by loading into a 5 ml syringe equipped with a blunt-end 21G needle. Fibers were electrospun onto a rotating mandrel (2 mm diameter) with the rotation rate of 1000 rpm for conduit manufacture. The spinning conditions for polymer solution were set according to the Table 2.

Table 2: Parameters of PCL conduit electrospinning

NO.	Voltage (kV)	Tip-Drum Distance (cm)	Feed rate (ml/h)
1	17	10	1
2	17	13	1
3	17	16	1
4	17	16	0.7
5	14	13	1
6	11	13	1

Electrospun PCL fibers on a rotating mandrel were collected as a tube wall. Then, the stainless steel rod was removed and the PCL nanofibrous tube obtained. After incubating in a vacuum oven overnight, the laminin-coated (PLGA/CNT-lam) and un-coated PLGA/CNT membranes were cut into 1 cm², rolled and placed in the PCL nanofibrous tube.

2.5. Characterization of Surface Morphology

The surface morphology of the PLGA/CNT-lam scaffolds, PCL conduits and the whole structure of conduits were analyzed by scanning electron microscopy (SEM, XL 30, Philips, USA). DiameterJ and OrientationJ, which are ImageJ plugins were used to estimate the diameter and orientation of the PLGA/CNT-lam NF.

2.6. Analyzing Surface Electrical Resistance

The electrical resistance of PLGA/CNT and PLGA/CNT-lam scaffolds were analyzed by a digital four point probe apparatus (5450/5451, ADCMT, japan). In this method, a corresponding electrical current was obtained when providing a voltage for samples cut in square shape. One pair of probes was used for the current injection while the other probes were used for the voltage measurement.

2.7. Cell-scaffold interaction Studies

2.7.1. *In vitro* SC Culture

To study cells viability and their behavior on scaffolds, SCs were used. The cells were cultured in a 25 mL cell culture flask in DMEM, supplemented with 10% FBS and 1% penicillin/streptomycin antibiotics at 37°C in a humidified 5% CO₂ incubator.

2.7.2. Cell Proliferation Assay

The PLGA/CNT-lam and PCL scaffolds were placed in a 24-well plate and fixed with plastic rings. Then they were sterilized by adding 70% ethanol and exposing to ultraviolet light for 30 min, followed by washing with PBS three times [29]. After the confluence was sufficient, SCs were detached by trypsin/EDTA, counted and seeded on the scaffolds with a density of 1.0×10^4 cells/well. Cell proliferation on the scaffolds was analyzed using MTT assay after 1, 3 and 5 days of cell culture and evaluated in comparison with control well (TCP) containing only SCs lack of NF. At mentioned time points, MTT solution was added, incubated in darkness for 4 h at 37°C, and then, replaced by dimethyl sulfoxide (DMSO) to dissolve the formazan crystals and finally absorbance was read at 570 nm by a plate reader.

2.7.3. Cell Attachment and Morphology Analysis of SCs on the Scaffolds

After 5 days of SCs incubation on the scaffolds, they were washed with PBS and processed with 4% paraformaldehyde to fix the cells on the scaffolds. After rinsing the samples with PBS, the samples were dehydrated using gradient ethanol (30% to 100%) and then, dried at room temperature. To evaluate the cells morphology and orientation, samples were gold sputtered and observed by SEM. In addition, the cell-scaffold constructs were characterized by antibodies against S100 protein specific marker for SCs. The samples were rinsed with PBS and fixed 4% formaldehyde solution for 30 min, followed by permeation with 0.1% TritonX-100 for 1 min. Non-specific labeling was blocked by incubating scaffolds in 2% BSA solution for 1 h and then samples were immersed in mouse anti-S100 (diluted at 1:1000) overnight. Subsequently, specimens were washed with PBS and incubated in FITC-conjugated goat anti-mouse secondary antibody (diluted at 1:100) for 1 h. Furthermore, the cells on samples were counterstained with DNA staining dye (DAPI) for 30 min and immunofluorescence imaging was performed using the fluorescence microscope [30].

2.7.4. SCs Culture in Fibrin Gel

SCs were re-suspended in 3 mgml^{-1} fibrinogen solution at 1×10^4 cells/ml. 90 μl of fibrinogen/cell solution was added to 10 μl thrombin solution (2 mgml^{-1}) and pipetted into the end of each segment of conduits (3 mm length). The fibrin changed into the gel form and then, DMEM with 10% FBS and 1% antibiotic was added to each guide in a 48-well plate. Cells were cultured for 48 h in a 37°C incubator at 5% carbon dioxide.

2.7.5. Cell Infiltration in Nerve Conduits

To evaluate cell infiltration, samples were fixed with a combination of 2 % paraformaldehyde and 2.5 % glutaraldehyde for 90 min. followed by dehydration with gradient ethanol (30% to 100%). Finally, they were placed on SEM stubs, gold sputtered and imaged with FESEM (FESEM, Zeiss SUPRA 55-VP, Oberkochen, Germany).

2.8. *In Vivo* Studies

Thirty healthy adult male Wistar rats (250–270 g) were purchased from Pasteur Institute (Tehran, Iran). Animal experiments were approved by the ethical committee of Tehran University of Medical Sciences and were performed according to the university's guidelines. The rats were randomly divided into 6 groups of five: negative control (with injury but without surgical intervention), hollow PCL conduit, PCL conduit with PLGA membranes (PCL-PLGA), PCL conduit with PLGA/CNT membranes (PCL-PLGA/CNT), PCL conduit with PLGA/CNT-lam membranes (PCL-PLGA/CNT-lam) and autograft. For surgical procedure, the animals were anesthetized by intra-peritoneal injection of ketamine (30 mg/kg) and xylazine (10 mg/kg).

To expose sciatic nerve, a skin incision was made at the left lower limb of the animal and the sciatic nerve was fixed with two sutures (Fig1.a) before severing into proximal and distal segments at the center of the right thigh. The both ends of proximal and distal stumps were fixed with one suture using 6–0 nylon sutures (Fig1.b). The suture strings were transmitted in the conduit channel and pulled out in transverse directions and allowed the nerve stumps to insert into the conduit to a depth of about 3 mm (Fig1.c,d), leaving a 10 mm gap between the stumps. Finally, they were tied together after coming out of the conduit. Then, the skin was closed with 3–0 nylon. In the nerve autograft group, the nerve defect was bridged with the resected nerve segment which was reversed and anastomosed to the proximal and distal stumps. In the negative control group, there was no bridge to connect two stumps. All the implantation procedures were conducted under microscope using microscopy instruments. After surgery, rats were housed and fed regularly and also, their activities were monitored.

2.8.1. Walking-foot-print analysis

Walking track analysis were performed 6 and 12 weeks post-surgery. In this test, the rat footprints were recorded for the analysis of the sciatic functional index (SFI). The rat hind paws were soaked in different color inks and they were placed inside a wooden walking alley (43 cm length, 8.70 cm width and 5.50 cm height) covered with a millimeter paper and ended with a darkened goal box. SFI was calculated by the following equation: **see formula 1 in the supplementary files section.**

Where PL is the distance from the heel to the top of the third toe, TS is the distance between the first and the fifth toe and IT is the distance from the second to the fourth toe. In addition, N and O related to the non-operated and operated foot and index of 0 indicated the normal function while -100 represented total impairment.

2.8.2. Nerve Conduction Test

Twelve weeks post-surgery, to evaluate nerve regeneration, compound muscle action potential (CMAP) amplitudes of the sciatic nerves were evaluated. The animals were anesthetized by injection of ketamine /xylazine and the sciatic nerve proximal to the site of the injury was exposed and stimulated with an electric stimulus (3–5 mA) by means of needle electrodes. To remove any probable interference, a ground electrode was located inside the muscle adjacent to the nerve. The responses were recorded from the needle and cap electrodes (filtering frequency of 10 Hz to 10 kHz, sensitivity of 2 mV/division and sweep speed of 1 ms/division,) by means of an electromyographic recorder (Negarandishegan, Iran).

2.8.3. Gastrocnemius Muscle Wet Weight-loss

Twelve weeks post-surgery, weight-loss of gastrocnemius muscles of the rats were determined. To do this, rats were sacrificed and the posterior gastrocnemius muscles on the injured and uninjured hind limbs were weighed and the wet weight-loss of gastrocnemius muscles calculated by the following equation: **see formula 2 in the supplementary files section.**

2.8.4. Functional Assessment of Sensory Recovery (Hot Plate Latency Test)

Twelve weeks post-surgery, the rats were evaluated for hot plate latency (HPL) by placing their injured limbs on the center of a hot plate (56°C) and recording the time until they reacted (by jumping or licking their paws). The rats were removed from the plate after their reaction. The cut off time for test was set at 12 s.

2.8.5. Histo-pathological Examination

The animals were euthanized 12 weeks post-surgery and the sciatic nerve in the place of the graft was dissected out and fixed with 10% neutral buffered formalin (NBF, PH. 7.26) for 48 h. The harvested tissue samples processed, embedded in paraffin blocks and cut into 5 mm thick sections. For the nerve specimens, transverse (proximal and distal parts) and longitudinal (middle part) sections were obtained from the sciatic nerve (cutting area). All nerve sections were made in duplicate: the first one was stained with haematoxylin and eosin (H&E) and the second one by luxol fast blue (LFB). Finally, histological slides were evaluated by the independent reviewer, using light microscopy (Olympus BX51; Olympus, Tokyo, Japan).

2.9. Statistical methods

All data in this study are reported as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) was conducted and the level of statistical significance is defined as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), **** ($p < 0.0001$). Each parameter was conducted at least with three samples.

Results And Discussion

3.1. Characterizations of the intraluminal PLGA/CNT-lam and PCL Conduit

Electrospun PLGA/CNT scaffolds were prepared as intraluminal guidance channels. Thereafter, our previously-reported method was applied to coat the electrospun PLGA/CNT scaffolds with laminin by means of PD layer [12]. As shown in Fig. 2, PLGA/CNT-lam scaffolds were fabricated as longitudinally aligned nanofibrous guides with the average diameter of 455 ± 362 nm. Previous studies reported that aligned electrospun fibrous scaffolds of 400-600 nm in diameter have been shown to orient dorsal root ganglion (DRG) alignment and SC migration *in vitro* [31]. Another study revealed that nanofibrous scaffolds can support the growth and migration of cultured neural cells by means of increase in cell-spreading on fibers diameters of 400 nm and 800 nm [32,33]. Orientation of the fibers could be modulated by the collector rotation speed and faster rotation speed resulted in more uniform fibers. However, it becomes limited by fiber breakage that could happen during the electrospinning on collector with a very fast rotation speed [34]. The SEM results revealed that the fibers were aligned and continuous without breaking under the rotation speed of 2500 rpm and were covered with MWCNTs partially. According to previous studies, unidirectional fiber alignment can facilitate axon path finding and increasing the speed of nerve tissue regeneration [35,36].

Three electrospinning parameters, namely applied voltage, needle-collector distance and feeding rate have been varied to investigate their influences on the PCL fiber morphology. It was found that when the distance between nozzle and drum was 10 cm, most of the fibers between the nozzle and drum merged (Fig.3.a), increasing the distance to 13 and 16 cm reduced the number of merged fibers and the mean fiber diameter decreased (Fig.3. b, c). Another parameter, the feeding rate, influenced the thickness of channel in a way that reducing the feeding rate to 0.7 ml/h led to the formation of channel with inadequate thickness (50 μ m) (Fig.3.d). When the applied voltage was lower than 17 kV, jet could not form well and the solution sometimes dripped from the syringe tip (Fig.3.e,f) [37]. Eventually, the parameters of sample 3 were selected as the best condition to fabricate channel with relatively uniform NF. Diameters of the obtained NF were 340 ± 200 nm and porosity percentage was approximately 52%. Electrospun PCL fibers which were collected onto a 2 mm-diameter rotating mandrel formed the hollow channel with uniform wall thickness of about 200-220 μ m, length of 17 mm and inner diameter of 1.8 mm. It is previously proved that scaffolds of 100–200 μ m provide sufficient diffusion for nutrient exchange and waste transport for nerve repair [38,39]. In addition, there were some pores among NF (< 10 μ m) which can facilitate the exchange of different nutrients and metabolic products through the conduit wall [40].

As shown in Fig. 4, PLGA/CNT-lam sheets with aligned fibers were rolled and placed in the nanofibrous PCL tube to direct neurite growth during regeneration. PLGA was selected to be the intraluminal guidance material as it has a faster degradation rate. This allows the guidance to degrade after the axons regenerate. As the purpose of the intra-luminal guidance is to direct the axon growth cone across the gap, once the first axons have bridged the gap, the presence of intraluminal guidance is no longer necessary and its degradation provide more space to fill by the native ECM [41].

SEM image analyses of the conduit cross-sections revealed that the aligned functionalized electrospun PLGA/CNT fibers were successfully embedded within the PCL channel with random orientation (Fig. 4). These fibers were used to resemble the natural nerve structure and provide topographical, biological and electrical cues of natural nerve tissues which were expected to play critical roles to guide and improve axonal growth. The complementary effects of these three factors can lead to more complete nerve regeneration by acting on different subcategories of re-growing fibers. Thus, our design provides a three dimensional construct with open channels to guide and stimulate neurons and SCs between the aligned functionalized fibrous substrates.

As electrical cue is one of the important factors for neural regeneration [42], the conductivity of the PLGA/CNT, PLGA/CNT-Lam scaffolds was evaluated by the four point probe method, and the values were measured as 0.17 and 0.11 S/cm. The measurements revealed that a very thin coating layer could not significantly change the surface conductivity of the scaffolds. Previous studies demonstrated that the scaffold with electro-conductivity of 3.90×10^{-4} S/cm could facilitate electrical stimulation to promote axonal regrowth [43]. The conductive scaffolds can transmit the self-originated electric stimulation between the nerve cells which can be the basis for transmission of electrical signals in the nerves and regeneration of the peripheral nerves [44]. According to the existing concerns about toxicity and degradation of CNTs used in biomaterials for neuroengineering, we applied electro-spraying CNT with the possible minimum concentration of CNTs to fabricate electro-conductive scaffolds [43].

3.2. *In vitro* Study of the SCs

3.2.1. Cell Viability Assay

In our previous work, PC12 cells have been used to study the cell viability and cell attachment of PLGA, PLGA/CNT and PLGA/CNT-lam scaffolds. Results revealed that all of the scaffolds increased cell viability as time rises and laminin-coated scaffold could promote neurite outgrowth more than others [12].

As SCs migrate to the injury site after nerve injury and align to form bands of Büngner to support axonal regrowth, the biocompatibility of the conduit with SCs has an important influence on the final result [43]. Here, we evaluated SCs viability on nerve conduits and seeded them onto the intraluminal guides and PCL channel for 1, 3 and 5 days. The proliferation of the SCs on the scaffolds was compared to control group (TCP) in all pre-defined times.

According to the Fig. 5, the cell viability on PLGA/CNT-lam was higher than that on TCP and these modified scaffolds had stronger effect on the improvement in cell proliferation.

3.2.2. Morphology of the SCs

5 days after seeding of the SCs on the aligned PLGA/CN-Lam nanofibrous scaffolds, SEM analyses were done to observe cell attachments and morphologies. According to the Fig. 6-a, b, the SCs excellently adhered and to the scaffolds and extended along the main axis of the fibers which was due to the

significant effects of aligned nano-topography [45]. Furthermore, the morphological evaluation of cells proliferation was also performed by fluorescence staining (Fig. 6.c). As SCs guide regenerating axons, this nano-topography can promote the oriented growth of the regenerating axons. According to our previous study, these aligned scaffolds could guide the growth of new axons in PC12 cells, as well [12].

3.2.3. Cell infiltration

The intraluminal guides were designed to provide cell attachment sites and topographical guidance for the SCs in peripheral nerve regeneration, so it was important to check if the cells could infiltrate in the nerve conduit. Fig. 7 demonstrates the FESEM images of fibrin gel before and after cell culture in the conduit. It should be considered that the gel porosity in Fig. 7a was better maintained due to freeze-drying step. The attachment and cell-scaffold interactions were observed after 48 hours of cell seeding. The porous microstructure of hydrogel in addition to proper conduit wall thickness would provide a convenient system to facilitate the diffusion of sufficient oxygen and nutrients for cultured cells and they could infiltrate inside the fibrin 3D network. Thus, during regeneration, it is anticipated that these biological and topographical cues will increase SCs infiltration in addition to directing neurite.

3.3. *In vivo* Study

All animals tolerated anesthesia without complications. During *in vivo* study different tests were performed to assess functional and sensory properties of rats twelve weeks post-surgery.

3.3.1. Walking-foot-print Analysis (SFI)

Walking-foot-print analysis was used as a gold standard technique to determine functional recovery following injury to the sciatic nerve. Functional nerve regeneration was assessed by SFI. Fig. 8 demonstrates the average SFI values for function of the injured foots in different groups at six and twelve weeks post-surgery. In the PCL-PLGA/CNT-lam group, the SFI value increased from -65.46 ± 4.3 at week 6 to -55 ± 78 at week 12. The analysis showed better functional recovery results in the autograft group that were -60.28 ± 4.2 and -42.43 ± 6.02 at 6 and 12 weeks, respectively. Findings indicated that there was a significant ($p \leq 0.05$) difference between autograft and PCL-PLGA/CNT-lam group and none of the conduits could act as well as the autografts (Fig. 8). Furthermore, the data of conduits with embedded nanofibrous membranes did not differ significantly from each other and they could recover neural functionality to a certain degree. Even though, motor recovery and the SFI value for PCL-PLGA/CNT-lam group was significantly ($p \leq 0.05$) higher than hollow PCL conduits with SFI value of -84.33 ± 5.8 and -76.5 ± 8.7 at week 6 and 12, respectively. These results are in agreement with Jing *et al.* findings about SFI values that the presence of aligned conductive NF in the conduit lumen could increase the performance of the conduit by providing a more effective micro-environment for guiding nerve regeneration [9]. Comparing the SFI values 12 weeks post-operation revealed that the presence of CNT and Laminin coating could simultaneously increase motor recovery of injured sciatic nerve and the presence of each factor alone cannot be sufficient. Salehi *et al.* demonstrated that the presence of protein and MWCNT in the PLA conduit structure as hollow tube cannot improve functional nerve regeneration

notably and the SFI value for the rats with these conduits was -67.86 ± 0.58 at the end of 14th weeks [46]. Altogether, it can be concluded that not only the type of materials used in the conduit is important but also the conduit designing and the positions of the used materials have great importance.

3.3.2. Electrophysiological Analysis

Motor nerve conduction study was performed by electrical stimulation of damaged sciatic nerve and recording CMAP as a response of gastrocnemius muscle supplied by that nerve to evaluate the muscle reinnervation. The peak CMAP value in the PCL-PLGA/CNT-lam group

(10.75 ± 0.88 mV) was much higher than that of the PCL group (6.6 ± 0.47 mV) but recovery of the transected sciatic nerve was still poorer than that of the autograft group (CMAP 13.73 ± 2 mV) at 12 weeks post-surgery. As shown in Fig. 9, other conduits with embedded nanofibrous membranes did not differ significantly from each other but they had higher CMAP amplitude than PCL conduit and negative control. Thus conductivity and laminin coating of conduits could not affect muscle reinnervation as much as embedding nanofibrous membrane in the conduit. There have been many contradictory reports on the effect of electroconductivity of conduits on nerve conduction [47]. Some of them reported that CMAP was significantly increased when the conduit was conductive and the reinnervation of the muscle was improved as a result of the CNT interfacing [47,9]. Others reported that CNTs did not have role in improving and restoring nerve signal conduction [48,46]. Comparing the results of previous studies revealed that in none of the conduits [47,9,46], presence of CNT could not increase the nerve conduction as much as autograft which is consistent with our findings.

3.3.3. Assessment of Gastrocnemius Muscle Wet Weight

Untreated nerve injury can lead to muscle denervation, atrophy and weight loss [49]. Gastrocnemius muscles were innervated by branches of sciatic nerve. Thus, its weight loss is a parameter used to indirectly assess the related motor neuron defects. As shown in the Fig. 10, there were no statistically significant differences between the autograft and groups with nanofibrous embedded conduits or between each two groups ($p > 0.05$), suggesting that the nanofibrous embedded conduits and autograft have similar effects on preventing muscle atrophy. In other words, regaining of muscle weight after implantation of these conduits can be known as indicative of recovery [50]. Comparisons of other groups revealed that even though PCL conduits could increase muscle reinnervation, they could not stop muscle atrophy as efficient as autografts. Our results are in consistence with previous studies which reported that the muscle reinnervation of conduits with intra-luminal guidance channels was similar to that of the autograft group and groups with added biomolecules demonstrated better performance than autograft group. It suggested that introducing intra-luminal guidance channels has potential to encourage muscle regeneration [39].

3.3.4. Hot Plate Latency (HPL) Test

To evaluate the sensory recovery, hot plate test was used to assess the pain responses [51]. The first response, licking as a rapid response to thermal stimuli, is known as a direct indicator of nociceptive threshold. Even though, jumping is a more elaborated response, with a latency, and measuring the HPL is used to evaluate the nociceptive function [52]. As shown in the Fig. 11, twelve weeks after injury, rats in the negative control group could not withdraw their paws from the hot plate within 12s. The withdrawal response displayed much better sensory recovery results in the autograft (5.8 ± 1.8 s) and PCL-PLGA/CNT-lam group (6.81 ± 2.38 s). Furthermore, there were no statistically significant differences between the HPL times of groups with nanofibrous embedded conduits, proposing that these conduits can help in regeneration of the sensory axons across the gap. The latency time for the hollow PCL group was recorded to be 10.2 ± 2.37 s, which was significantly smaller than that of the negative control group (13.6 ± 2.07 s). However, comparing HPL times between hollow PCL conduits and embedded conduits revealed that improved recovery of a heat response could be attributed to the enhanced regeneration of the accurate population of sensory axons by means of inner aligned nanofibrous sheets.

3.3.5. Histological Changes in Injured Sciatic Nerve

The arrangement of sciatic nerve fibers in the negative control group was disrupted and they had swollen or missing axons with various degrees of vacuolation. In addition, multiple signs of nerve damage observed, including irregular distribution and variable thickness of myelin sheath, degeneration of fibers, axonal disintegration and notable edema of the nerve fibers. The number of axons were significantly decreased (especially in distal area) in comparison to other treatments (Fig. 12, a). In other groups with nerve conduits, the tubular PCL structures could be differentiated from regenerated tissues within them. They were shown with arrows both in cross-sectional and, longitudinal-sections of the regenerated nerves. It was in consistent with previous studies that reported PCL conduits conserved their structure 14 weeks post implantation even though breakage might be happen [53]. In the PCL group, the arrangement of the sciatic nerve fibers was acceptable, however, in the middle part, the bridging process was incomplete and the number of axons was significantly decreased compared to the proximal part. Moreover, in distal part, nerves had swollen or missing axons with various degrees of vacuolation (Fig. 12, b). In the PCL-PLGA group, the nerve fibers were symmetrically distributed and thickness of myelin sheath was normal. The biocompatibility of this conduit was completely acceptable and this finding was confirmed in all samples. There was no sign of inflammatory responses in this sample due to the implanted conduit and using nylon suture (Fig. 12, c). Thus incorporating longitudinally aligned nanofibrous intra-luminal guidance channels could improve nerve regeneration and confirmed previous findings that reported topographical cues in multi-channels conduit supported SCs migration and axon extensions *in vivo* [39].

In the PCL-PLGA/CNT group, the histopathological analysis showed uniform myelin sheaths and normal axonal structure (Fig. 12, d). There was no sign of fibrosis and the nerve fibers were free in proximal, middle and distal parts. Our results appeared similar to recent studies investigated the use of CNT incorporated conduits *in vivo* which lead to nerve regeneration promotion by accelerating the electrical signal conduction without any toxic effect [46,43].

In PCL-PLGA/CNT-lam group, there were no significant histopathological changes in sciatic nerve and the bridging process (regeneration) was complete. However, the number of axons was decreased in distal part when compared to the proximal and middle part of sciatic nerve (Fig. 12, e). In addition to the tubular PCL structure, the black remnants of PLGA/CNT-lam intra-luminal nanofibrous scaffolds were evident in this group. Our previous study revealed that after one month of degradation, the PLGA, PLGA/CNT and PLGA/CNT-lam scaffolds were degraded partially and lose about 20 % of their mass [12]. In this study, these scaffold were used as intraluminal guide because they could guide axons during first weeks and then gradually degrade to generate enough space for nerve regeneration and avoid probable nerve compression. These results are in agreement with previously published studies [54]. In the autograft group, although the arrangement of sciatic nerve fibers in proximal parts was disrupted, this nerve was intact in the middle and distal part (Fig. 12, f). The histopathological condition of the PCL-PLGA/CNT/lam group was similar to that of those in the autograft group. It suggests that the PCL-PLGA/CNT/lam conduit could provide the best environment for sciatic nerve regeneration among these four nerve conduits which is in consistence with previous studies [55]. Wu et al, developed multi-channel nerve conduits with three-dimensional laminin coated PLGA yarns and demonstrated that the yarn structure significantly supported SCs proliferation and the laminin-coating cues induced SCs migration [55].

The overall results demonstrated that the incorporation of membranes with topological cues was important for nerve regeneration and presence of nylon suture did not induce inflammatory reaction.

Conclusion

In this study, we successfully examined a simultaneous effect of biological, topographical and electrical cues in the form of PCL-PLGA/CNT-lam conduit for the regeneration of sciatic nerve defect in a rat model. The analyses revealed that embedding nanofibrous membranes as topographical cue inside conduits enhanced the recovery rate of the injured nerve more effective than biological and electrical cues as evidenced by functional recovery tests and study of histological changes. These results could provide useful insights to future conduit designs in repairing the injured peripheral nerve. Based on these approaches, in further studies, electrical stimulation should be considered to further improve the nerve repairing procedure.

Declarations

Funding

This study was financially supported by Tehran University of Medical Sciences (grant No. 94-03-87-28727).

Conflicts of Interest

The authors declare no conflict of interest.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Niloofar Nazeri: Investigation and Writing- Original draft preparation. Mohammad Ali Derakhshan: Supervision. Hossein Ghanbari: Supervision, Reviewing and Editing. Funding acquisition.

Ethics approval

Compliance with Ethical Standards of research involving animals, animal experiments were approved by the ethical committee of Tehran University of Medical Sciences and were performed according to the university's guidelines.

Consent to participate

Not applicable

Consent for publication

Not applicable

References

1. Anderson M, Shelke NB, Manoukian OS, Yu X, McCullough LD, Kumbar SG (2015) Peripheral Nerve Regeneration Strategies: Electrically Stimulating Polymer Based Nerve Growth Conduits. Crit Rev Biomed Eng 43 (2-3):131-159. doi:10.1615/CritRevBiomedEng.2015014015
2. Spearman BS, Desai VH, Mobini S, McDermott MD, Graham JB, Otto KJ, Judy JW, Schmidt CE (2017) Tissue-Engineered Peripheral Nerve Interfaces. Advanced Functional Materials:1701713. doi:10.1002/adfm.201701713
3. Sabongi RG, Fernandes M, dos Santos JBG (2015) Peripheral nerve regeneration with conduits: use of vein tubes. Neural regeneration research 10 (4):529
4. Faroni A, Mobasser SA, Kingham PJ, Reid AJ (2015) Peripheral nerve regeneration: experimental strategies and future perspectives. Adv Drug Deliv Rev 82-83:160-167. doi:10.1016/j.addr.2014.11.010
5. Wieringa PA, Gonçalves de Pinho AR, Micera S, van Wezel RJ, Moroni L (2018) Biomimetic Architectures for Peripheral Nerve Repair: A Review of Biofabrication Strategies. Advanced healthcare materials 7 (8):1701164
6. Dinis TM, Elia R, Vidal G, Dermigny Q, Denoeud C, Kaplan DL, Egles C, Marin F (2015) 3D multi-channel bi-functionalized silk electrospun conduits for peripheral nerve regeneration. J Mech Behav

7. Pabari A, Lloyd-Hughes H, Seifalian AM, Mosahebi A (2014) Nerve conduits for peripheral nerve surgery. *Plast Reconstr Surg* 133 (6):1420-1430. doi:10.1097/PRS.0000000000000226
8. Gao M, Lu P, Lynam D, Bednark B, Campana WM, Sakamoto J, Tuszynski M (2016) BDNF gene delivery within and beyond templated agarose multi-channel guidance scaffolds enhances peripheral nerve regeneration. *J Neural Eng* 13 (6):066011. doi:10.1088/1741-2560/13/6/066011
9. Jing W, Ao Q, Wang L, Huang Z, Cai Q, Chen G, Yang X, Zhong W (2018) Constructing conductive conduit with conductive fibrous infilling for peripheral nerve regeneration. *Chemical Engineering Journal* 345:566-577
10. Guo R, Zhang S, Xiao M, Qian F, He Z, Li D, Zhang X, Li H, Yang X, Wang M (2016) Accelerating bioelectric functional development of neural stem cells by graphene coupling: implications for neural interfacing with conductive materials. *Biomaterials* 106:193-204
11. Fang X, Deng J, Zhang W, Guo H, Yu F, Rao F, Li Q, Zhang P, Bai S, Jiang B (2020) Conductive conduit small gap tubulization for peripheral nerve repair. *RSC Advances* 10 (28):16769-16775
12. Nazeri N, Karimi R, Ghanbari H (2020) The effect of surface modification of poly-lactide-co-glycolide/carbon nanotube nanofibrous scaffolds by laminin protein on nerve tissue engineering. *Journal of Biomedical Materials Research Part A*
13. Jahromi HK, Farzin A, Hasanzadeh E, Barough SE, Mahmoodi N, Najafabadi MRH, Farahani MS, Mansoori K, Shirian S, Ai J (2020) Enhanced sciatic nerve regeneration by poly-L-lactic acid/multi-wall carbon nanotube neural guidance conduit containing Schwann cells and curcumin encapsulated chitosan nanoparticles in rat. *Materials Science and Engineering: C* 109:110564
14. Hasanzadeh E, Ebrahimi-Barough S, Mirzaei E, Azami M, Tavangar SM, Mahmoodi N, Basiri A, Ai J (2019) Preparation of fibrin gel scaffolds containing MWCNT/PU nanofibers for neural tissue engineering. *Journal of Biomedical Materials Research Part A* 107 (4):802-814
15. Afrash H, Nazeri N, Davoudi P, Majidi RF, Ghanbari H (2021) Development of a Bioactive Scaffold based on NGF Containing PCL/Chitosan Nanofibers for Nerve Regeneration.
16. Cho H-j, Madhurakkat Perikamana SK, Lee J-h, Lee J, Lee K-M, Shin CS, Shin H (2014) Effective immobilization of BMP-2 mediated by polydopamine coating on biodegradable nanofibers for enhanced in vivo bone formation. *ACS applied materials & interfaces* 6 (14):11225-11235
17. Sulong AF, Hassan NH, Hwei NM, Lokanathan Y, Naicker AS, Abdullah S, Yusof MR, Htwe O, Idrus R, Haflah N (2014) Collagen-coated polylactic-glycolic acid (PLGA) seeded with neural-differentiated human mesenchymal stem cells as a potential nerve conduit. *Adv Clin Exp Med* 23:353-362
18. Koh HS, Yong T, Chan CK, Ramakrishna S (2008) Enhancement of neurite outgrowth using nano-structured scaffolds coupled with laminin. *Biomaterials* 29 (26):3574-3582. doi:10.1016/j.biomaterials.2008.05.014
19. Gao X, Zhang X, Song J, Xu X, Xu A, Wang M, Xie B, Huang E, Deng F, Wei S (2015) Osteoinductive peptide-functionalized nanofibers with highly ordered structure as biomimetic scaffolds for bone tissue engineering. *International journal of nanomedicine* 10:7109

20. Reid AJ, de Luca AC, Faroni A, Downes S, Sun M, Terenghi G, Kingham PJ (2013) Long term peripheral nerve regeneration using a novel PCL nerve conduit. *Neurosci Lett* 544:125-130. doi:10.1016/j.neulet.2013.04.001
21. Hussain M, Kabir M, Sood A (2009) On the cytotoxicity of carbon nanotubes. *Current Science* (00113891) 96 (5)
22. Pixley SK, Hopkins TM, Little KJ, Hom DB (2016) Evaluation of peripheral nerve regeneration through biomaterial conduits via micro-CT imaging. *Laryngoscope Investig Otolaryngol* 1 (6):185-190. doi:10.1002/lio2.41
23. Zhou Z, Liu X, Wu W, Park S, Miller II AL, Terzic A, Lu L (2018) Effective nerve cell modulation by electrical stimulation of carbon nanotube embedded conductive polymeric scaffolds. *Biomaterials science* 6 (9):2375-2385
24. Nazeri N, Derakhshan MA, Faridi-Majidi R, Ghanbari H (2018) Novel electro-conductive nanocomposites based on electrospun PLGA/CNT for biomedical applications. *Journal of Materials Science: Materials in Medicine* 29 (11):168
25. Ahmadi P, Nazeri N, Derakhshan MA, Ghanbari H (2021) Preparation and characterization of polyurethane/chitosan/CNT nanofibrous scaffold for cardiac tissue engineering. *International Journal of Biological Macromolecules*
26. Rim NG, Kim SJ, Shin YM, Jun I, Lim DW, Park JH, Shin H (2012) Mussel-inspired surface modification of poly(L-lactide) electrospun fibers for modulation of osteogenic differentiation of human mesenchymal stem cells. *Colloids Surf B Biointerfaces* 91:189-197. doi:10.1016/j.colsurfb.2011.10.057
27. Lee YJ, Lee JH, Cho HJ, Kim HK, Yoon TR, Shin H (2013) Electrospun fibers immobilized with bone forming peptide-1 derived from BMP7 for guided bone regeneration. *Biomaterials* 34 (21):5059-5069. doi:10.1016/j.biomaterials.2013.03.051
28. Xie J, Liu W, MacEwan MR, Bridgman PC, Xia Y (2014) Neurite outgrowth on electrospun nanofibers with uniaxial alignment: the effects of fiber density, surface coating, and supporting substrate. *ACS nano* 8 (2):1878-1885
29. Liu J, Li L, Suo H, Yan M, Yin J, Fu J (2019) 3D printing of biomimetic multi-layered GelMA/nHA scaffold for osteochondral defect repair. *Materials & Design* 171:107708
30. Kijeńska E, Prabhakaran MP, Swieszkowski W, Kurzydłowski KJ, Ramakrishna S (2014) Interaction of Schwann cells with laminin encapsulated PLCL core-shell nanofibers for nerve tissue engineering. *European polymer journal* 50:30-38
31. Kim YT, Haftel VK, Kumar S, Bellamkonda RV (2008) The role of aligned polymer fiber-based constructs in the bridging of long peripheral nerve gaps. *Biomaterials* 29 (21):3117-3127. doi:10.1016/j.biomaterials.2008.03.042
32. Dinis T, Elia R, Vidal G, Dermigny Q, Denoëud C, Kaplan D, Egles C, Marin F (2015) 3D multi-channel bi-functionalized silk electrospun conduits for peripheral nerve regeneration. *Journal of the mechanical behavior of biomedical materials* 41:43-55

33. Qu J, Wang D, Wang H, Dong Y, Zhang F, Zuo B, Zhang H (2013) Electrospun silk fibroin nanofibers in different diameters support neurite outgrowth and promote astrocyte migration. *Journal of Biomedical Materials Research Part A* 101 (9):2667-2678
34. Zander NE, Orlicki JA, Rawlett AM, Beebe TP (2010) Surface-modified nanofibrous biomaterial bridge for the enhancement and control of neurite outgrowth. *Biointerphases* 5 (4):149-158
35. Sewell M, Presley L, Holland S, Catalano P (2015) Genetic causes of maturity onset diabetes of the young may be less prevalent in American pregnant women recently diagnosed with diabetes mellitus than in previously studied European populations. *The Journal of Maternal-Fetal & Neonatal Medicine* 28 (10):1113-1115
36. Hoke A, Lim SH, Liu X, Mao H-q (2017) Biodegradable nerve guides. Google Patents,
37. Leach MK, Feng Z-Q, Tuck SJ, Corey JM (2011) Electrospinning fundamentals: optimizing solution and apparatus parameters. *J Vis Exp* 47
38. Stankus JJ, Guan J, Fujimoto K, Wagner WR (2006) Microintegrating smooth muscle cells into a biodegradable, elastomeric fiber matrix. *Biomaterials* 27 (5):735-744
39. Koh HS, Yong T, Teo WE, Chan CK, Puhaindran ME, Tan TC, Lim A, Lim BH, Ramakrishna S (2010) In vivo study of novel nanofibrous intra-luminal guidance channels to promote nerve regeneration. *J Neural Eng* 7 (4):046003. doi:10.1088/1741-2560/7/4/046003
40. Yu W, Zhao W, Zhu C, Zhang X, Ye D, Zhang W, Zhou Y, Jiang X, Zhang Z (2011) Sciatic nerve regeneration in rats by a promising electrospun collagen/poly (ϵ -caprolactone) nerve conduit with tailored degradation rate. *BMC neuroscience* 12 (1):68
41. Waddell R (2003) Using PC12 cells and rat cortical neurons to evaluate poly (caprolactone) and collagenous microcarriers for applications in nerve guide fabrication.
42. Gupta P, Sharan S, Roy P, Lahiri D (2015) Aligned carbon nanotube reinforced polymeric scaffolds with electrical cues for neural tissue regeneration. *Carbon* 95:715-724
43. Li X, Yang W, Xie H, Wang J, Zhang L, Wang Z, Wang L (2020) CNT/Sericin Conductive Nerve Guidance Conduit Promotes Functional Recovery of Transected Peripheral Nerve Injury in a Rat Model. *ACS Applied Materials & Interfaces* 12 (33):36860-36872
44. Zhou Z-F, Zhang F, Wang J-G, Chen Q-C, Yang W-Z, He N, Jiang Y-Y, Chen F, Liu J-J (2016) Electrospinning of PELA/PPY fibrous conduits: promoting peripheral nerve regeneration in rats by self-originated electrical stimulation. *ACS Biomaterials Science & Engineering* 2 (9):1572-1581
45. Mirzaei E, Ai J, Ebrahimi-Barough S, Verdi J, Ghanbari H, Faridi-Majidi R (2016) The differentiation of human endometrial stem cells into neuron-like cells on electrospun PAN-derived carbon nanofibers with random and aligned topographies. *Molecular neurobiology* 53 (7):4798-4808
46. Salehi M, Naseri-Nosar M, Ebrahimi-Barough S, Nourani M, Khojasteh A, Hamidieh AA, Amani A, Farzamfar S, Ai J (2018) Sciatic nerve regeneration by transplantation of Schwann cells via erythropoietin controlled-releasing polylactic acid/multiwalled carbon nanotubes/gelatin nanofibrils neural guidance conduit. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 106 (4):1463-1476

47. Ahn H-S, Hwang J-Y, Kim MS, Lee J-Y, Kim J-W, Kim H-S, Shin US, Knowles JC, Kim H-W, Hyun JK (2015) Carbon-nanotube-interfaced glass fiber scaffold for regeneration of transected sciatic nerve. *Acta biomaterialia* 13:324-334
48. Yu W, Jiang X, Cai M, Zhao W, Ye D, Zhou Y, Zhu C, Zhang X, Lu X, Zhang Z (2014) A novel electrospun nerve conduit enhanced by carbon nanotubes for peripheral nerve regeneration. *Nanotechnology* 25 (16):165102
49. Zhang D, Liu M, Ding F, Gu X (2006) Expression of myostatin RNA transcript and protein in gastrocnemius muscle of rats after sciatic nerve resection. *Journal of Muscle Research & Cell Motility* 27 (1):37-44
50. Clements BA, Bushman J, Murthy NS, Ezra M, Pastore CM, Kohn J (2016) Design of barrier coatings on kink-resistant peripheral nerve conduits. *Journal of tissue engineering* 7:2041731416629471
51. Wong K-H, Kanagasabapathy G, Bakar R, Phan C-W, Sabaratnam V (2015) Restoration of sensory dysfunction following peripheral nerve injury by the polysaccharide from culinary and medicinal mushroom, *Hericius erinaceus* (Bull.: Fr.) Pers. through its neuroregenerative action. *Food Science and Technology* 35 (4):712-721
52. Gunn A, Bobeck EN, Weber C, Morgan MM (2011) The influence of non-nociceptive factors on hot-plate latency in rats. *The Journal of Pain* 12 (2):222-227
53. Pixley SK, Hopkins TM, Little KJ, Hom DB (2016) Evaluation of peripheral nerve regeneration through biomaterial conduits via micro-CT imaging. *Laryngoscope investigative otolaryngology* 1 (6):185-190
54. Peng S-W, Li C-W, Chiu M, Wang G-J (2017) Nerve guidance conduit with a hybrid structure of a PLGA microfibrillar bundle wrapped in a micro/nanostructured membrane. *International journal of nanomedicine* 12:421
55. Wu T, Li D, Wang Y, Sun B, Li D, Morsi Y, El-Hamshary H, Al-Deyab SS, Mo X (2017) Laminin-coated nerve guidance conduits based on poly(l-lactide-co-glycolide) fibers and yarns for promoting Schwann cells' proliferation and migration. *J Mater Chem B* 5 (17):3186-3194.
doi:10.1039/c6tb03330j

Figures

Figure 1

Surgical implantation of a PCL-PLGA/CNT-lam conduit bridging a 10mm sciatic nerve defect in rats (a, b), the sciatic nerve was fixed with two sutures (c, d). The suture strings were transmitted in the conduit and pulled out in transverse directions.

Figure 2

FESEM micrograph of PLGA/CNT-lam scaffolds with magnification of 1 μm (a), 10 μm (b) and 50 μm (c), distribution of fiber orientations (d) and fibers diameters (e). NF of PLGA/CNT-PD-lam scaffolds were aligned with the average diameter of 455 ± 362 nm.

Figure 3

SEM micrograph of nanofibrous PCL conduits with different spinning conditions according to the parameters of Table 2 (1-6) (a-f). The best result is shown in micrograph (c) related to sample 3 in Table 2 could lead to PCL conduit with mean diameter of 340 ± 200 nm.

Figure 4

Cross sections of PCL-PLGA/CNT-lam conduit with different magnifications. Aligned PLGA/CNT-lam fibers were successfully embedded within the PCL channel.

Figure 5

Cell viability of the SCs cultured on PCL and PLGA/CNT-lam scaffolds. The chart illustrates the higher cell viability on PLGA/CNT-lam compared to TCP.

Figure 6

SEM images with different magnifications (a: 50 μm, b: 10 μm) and immunofluorescence staining of the SCs cultured on the aligned PLGA/CNT-lam NF.

Figure 7

FESEM image of fibrin gel before and after SCs culture. The porous microstructure of hydrogel could facilitate SCs infiltration.

Figure 8

Sciatic nerve functions of rats as a function of implantation time. The statistical analyses were done by comparing the SFI values of each group in a specific time-point to the autograft. The SFI value for PCL-PLGA/CNT-lam group was significantly ($p \leq 0.05$) higher than that of the PCL conduits at the end of 12th week.

Figure 9

Electrophysiological analysis of the regenerated nerves 12 weeks post-operation. The statistical analysis was carried out by comparing the amplitude of CMAP values of each group to the autograft. The peak CMAP value in the PCL-PLGA/CNT-lam group was much higher than the values for the PCL group and negative control.

Figure 10

Assessment of gastrocnemius muscle wet weight 12 weeks post-surgery. PCL-PLGA/CNT-lam conduits and autograft have similar effects on preventing muscle atrophy.

Figure 11

Hot plate jumping response of rats 12 weeks post-surgery. The withdrawal response displayed much better sensory recovery results in PCL-PLGA/CNT-lam group than negative control.

Figure 12

Histopathological examination of the sciatic nerve cross-sections stained by hematoxylin-eosin (H&E) and Luxol fast blue (LFB) at the end of 12th week post-surgery. Negative control group (a), PCL group (b), PCL-PLGA group (c), PCL-PLGA/CNT group (d), PCL-PLGA/CNT-lam group (e), and autograft group (f). arrowheads: vacuolation, thin arrows: regeneration of nerve fibers, big arrows: remnant conduit.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [formula.docx](#)