Polydopamine-modified chitin conduits with sustained release of bioactive peptides enhance peripheral nerve regeneration in rats

Abstract
The introduction of neurotrophic factors into injured peripheral nerve sites is beneficial to peripheral nerve regeneration. However, neurotrophic factors are rapidly degraded in vivo and obstruct axonal regeneration when used at a supraphysiological dose, which limits their clinical benefits. Bioactive mimetic peptides have been developed to be used in place of neurotrophic factors because they have a similar mode of action to the original growth factors and can activate the equivalent receptors but have simplified sequences and structures. In this study, we created polydopamine-modified chitin conduits loaded with brain-derived neurotrophic factor mimetic peptides and vascular endothelial growth factor mimetic peptides (Chi/PDA-Ps). We found that the Chi/PDA-Ps conduits were less cytotoxic in vitro than chitin conduits alone and provided sustained release of functional peptides. In this study, we evaluated the biocompatibility of the Chi/PDA-Ps conduits. Brain-derived neurotrophic factor mimetic peptide and vascular endothelial growth factor mimetic peptide synergistically promoted proliferation of Schwann cells and secretion of neurotrophic factors by Schwann cells and attachment and migration of endothelial cells in vitro. The Chi/PDA-Ps conduits were used to bridge a 2 mm gap between the nerve stumps in rat models of sciatic nerve injury. We found that the application of Chi/PDA-Ps conduits could improve the motor function of rats and reduce gastrocnemius atrophy. The electrophysiological results and the microstructure of regenerative nerves showed that the nerve conduction function and remyelination was further restored. These findings suggest that the Chi/PDA-Ps conduits have great potential in peripheral nerve injury repair.

Key Words: angiogenesis; bioactive peptides; nerve repair; neurotrophic factor; peripheral nerve injury; peripheral nerve regeneration; polydopamine; surface modification; synergistic effects; tissue engineering

Introduction
Peripheral nerve injury (PNI) is a global clinical problem characterized by partial or complete loss of sensory, motor, or autonomic functions. Neuropathic pain is a common complication of PNI that adversely affects patients physically and psychologically and creates economic burdens on individuals and society (Jiang et al., 2017). The small gap conduit suture technique can be used to substitute traditional epineurium neurorrhaphy to repair PNI (Rao et al., 2019). However, chitin (Chi) conduits stand out among various types of nerve conduits for use in PNI repair because of their ease of synthesis (Younes and Rinaudo, 2015; Riaz Rajoka et al., 2020). The use of biodegradable Chi conduits combined with tubulization can improve the accuracy of axonal docking, reduce the incidence of neuroma, and shorten operation times (Kou et al., 2013; Zhang et al., 2013).

Brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) provide a microenvironment that is beneficial for peripheral nerve regeneration (Guaiquil et al., 2014; Lopes et al., 2017; Xia and lv, 2018). Recently, certain bioactive peptides have been substituted for growth factors such as BDNF and VEGF. These mimetic peptides are based on the original growth factors, but with simplified sequences and structures, and they retain the ability to activate the equivalent receptors (D’Andrea et al., 2005; Rubert Pérez et al., 2017; Lu et al., 2018). Herein, we used the neurotrophic peptide sequence RGIDKRHWSQ (RGI) to mimic BDNF function and the motif KLTWQELYLQKYG (KLT) as a VEGF mimetic peptide (Rao et al., 2020).

To obtain optimal biological efficacy of the RGI and KLT mimetic peptides, we developed a self-polymerization method to modify Chi conduits and then combined the mimetic peptides into a system that provides sustained release of these peptides. The outstanding properties of polydopamine (PDA),
including remarkable biocompatibility and superior adhesion, have qualified it for a wide range of biomaterial applications (Liu et al., 2014; Cheng et al., 2019). Compared with other physical adsorption strategies, PDA provides an easier and more efficient method of coating Chi conduits.

In this study, we investigated the surface characteristics of Chi conduits coated with PDA (Chi/PDA). The mimetic peptides RGI and KLT were loaded onto the Chi/PDA conduits (Chi/PDA-Ps conduits). Next, we investigated the release efficiency of the mimetic peptides from Chi/PDA-Ps conduits. We studied the effects of sustained release of RGI and KLT on Schwann cells (SCs) and human umbilical vein endothelial cells (HUVECs) in vitro. Finally, the conduits were implanted into rats with 2 mm sciatic nerve defects. The repair performance of these embedded conduits was evaluated in terms of functional recovery and histological effects.

Materials and Methods

Fabrication of the Chi conduits

The Chi conduits were fabricated as described previously (Li et al., 2021). Briefly, 2% glacial acetic acid (MilliporeSigma, Burlington, MA, USA) was used to dissolve chitosan powder (MilliporeSigma). Molds 1.5 mm in diameter were immersed in the chitosan solution to create the conduits. The molds were removed and the semi-finished conduits were left at room temperature for 1 hour. The chitosan solution in the molds was solidified with 5% sodium hydroxide solution and acetylated with acetic anhydride for 30 minutes. After the reaction, the Chi conduits were washed with water to 20 μM and sterilized using a 0.22 μm membrane (MilliporeSigma).

Fourier transform infrared spectrometer analysis

A Fourier transform infrared spectrometer (Nicolet 6700, Thermo Fisher Scientific, Waltham, MA, USA) was used to analyze the surface chemical characteristics of Chi and Chi/PDA. The infrared spectra were recorded from 400 to 6000 cm⁻¹ with a resolution of 4 cm⁻¹.

Water contact angle measurement

A water contact angle measurement platform (OCA 20, Dataphysics Instruments, Germany) was used to measure the contact angle formed by PDA coating on the Chi surface. The contact angle was measured by the angle between the water droplets (n = 3) on the surface of the conduits and the conduits.

Immobilization, observation, and release kinetics of mimetic peptides from Chi/PDA-Ps conduits

To display the binding performances of functional peptides to the various conduits, fluorescein isothiocyanate isomer (FITC) RGI (FITC-RGI) and carboxytetramethylrhodamine (TAMRA) KLT (TAMRA-KLT), as well as RGI and KLT alone, were custom synthesized by Shanghai Apeptotide Co., Ltd., Shanghai, China. Briefly, each of the peptide powders was dissolved in ultrapure water to 20 μM and sterilized using a 0.22 μm membrane (MilliporeSigma). The prepared peptide solutions were combined (1 v/v) to form RGI/KLT and FITC-RGI/TAMRA-KLT. Sterilized Chi/PDA conduits were placed in a 24-well culture plate with 500 μL of mimetic peptides or fluorescently labeled mimetic peptides for 24 hours to load the peptides.

To visualize the conduits, Chi/PDA-Ps were washed with ultrapure water three times and then viewed under a fluorescence microscope (Leica, Wetzlar, Germany).

For the sustained-release experiment, Chi/PDA conduits loaded with RGI or KLT were immersed in 1 ml phosphate buffered saline (PBS; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and gently stirred at 37°C. Twenty-four hours later, 1 mM cytarabine (MilliporeSigma) was used to eliminate fibroblasts. The purified SCs were then cultured in a 37°C incubator (Thermo Fisher Scientific) in a 5% CO₂ atmosphere.

HUVECs were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco’s Modified Eagle medium (Thermo Fisher Scientific) containing 10% fetal bovine serum (Thermo Fisher Scientific) and 1% penicillin/streptomycin solution (Beijing Solarbio Science & Technology Co., Ltd.).

Live/dead staining

Because it is difficult to directly verify the biocompatibility of the surface of conduits, we seeded SCs on Chi, Chi/PDA, or Chi/PDA-Ps films and used the fluorescent live/dead staining kit (Beijing Solarbio Science & Technology Co., Ltd.) to evaluate the biocompatibility of the films. After 3 days, the SCs in each group were washed with PBS three times, the cells were stained with calcine-AM (a green fluorescent dye for live cells) and propidium iodide (a red fluorescent dye for dead cells) for 15 minutes at 37°C, and live and dead cells were observed in 6-well plates by fluorescence microscopy (Leica).

Cellular immunofluorescence

SCs and HUVECs were seeded in 6-well plates at a density of 3 × 10⁵ cells per well. One Chi, Chi/PDA, or Chi/PDA-Ps conduit was placed into each well, and the cells were cultured for 5 days. The SCs and HUVECs were then gently rinsed with 1% PBS and fixed with 4% paraformaldehyde in PBS at 4°C for 30 minutes. The cell samples were permeabilized with 0.5% Triton X-100 (MilliporeSigma) at room temperature for 5 minutes. The SCs were incubated with rabbit anti-S100 antibody (1:400, MilliporeSigma, Cat#: S2644, RRID: AB_477501) at 4°C overnight, and the HUVECs were incubated with fluorescein phallolidin (1:1000, MilliporeSigma) at room temperature for 30 minutes. Finally, a fluorescence microscope (Leica) was used to image the cell samples.

Cell proliferation

SCs and HUVECs were seeded in 12-well plates at a density of 2 × 10⁵ cells per well, and the Chi, Chi/PDA, or Chi/PDA-Ps conduits were added for 1 or 5 days. Then, the cells were incubated with the relevant solution from the Cell Counting kit-8 (Dojindo Laboratories, Kumamoto, Japan) at 37°C for 2 hours. The absorbance of the medium at 450 nm was read using a microplate reader (Bio-Rad Laboratories).

Enzyme-linked immunosorbent assay

SC secretion ability was assessed by enzyme-linked immunosorbent assay. Briefly, the supernatants of each SC group were collected at 1 and 5 days after the conduit treatment. Enzyme-linked immunosorbent assay kits (Jiangsu Meinian Industrial Co., Yancheng, China) were used in accordance with the manufacturer’s instructions to evaluate the levels of BDNF, a major neurotrophic factor (CNTF), nerve growth factor (NGF), and VEGF in the supernatants. The absorbance at 450 nm was measured using a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA), and each sample was measured three times.

Human umbilical vein endothelial cell migration assay

HUVECs were seeded in 12-well plates at a density of 2 × 10⁵ cells per well (three replicates per group) and incubated until they reached confluence. A sterile 100 μL tip was used to create a scratch across the surface of each cell layer. Then, the cell layers were washed with PBS to remove detached cells. Chi, Chi/PDA, or Chi/PDA-Ps conduits were placed on the bottom of each well. HUVECs were cultured in Dulbecco's Modified Eagle medium (Thermo Fisher Scientific) containing 2% fetal bovine serum (Thermo Fisher Scientific) for 24 hours and then observed and photographed using an optical microscope (Leica).

The rate of wound closure was calculated as follows: Rate of wound closure (%) = (M₀ - Mₜ)/M₀ × 100, where M₀ represents the area of the initial wound, and Mₜ represents the area of the wound 24 hours postwounding.

Surgical procedures and conduit implantation

Animal experiments were performed in accordance with the ethical principles of the Institutional Animal Care and Use Committee of the Peking University People's Hospital (Approval No. PUPH20170907, December 10, 2020). All experiments were designed and reported according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (Percue di Sert et al., 2020). After PNI, there were sex-related differences in collateral sprouting of axons and pain sensitivity (Kovacic et al., 2003; Stephens et al., 2019). In addition, because female rats are less aggressive than male rats, we chose to use female rats as our experimental animals to eliminate sex-related differences as much as possible. Twenty-four specific-pathogen-free female Sprague–Dawley rats (weight 150–200 g; sterilization rate > 95%, purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd.) were used as the experimental animals. The rats were kept in a specific-pathogen-free laboratory at
**Figure 2B**

P, the water contact angle of the PDA-modified conduits, was significantly higher than that in the Chi and Chi/PDA groups (P < 0.05). After 5 days of treatment, the proliferation ability of the SCs in the Chi/PDA-Ps groups was significantly greater than that in the other groups (P < 0.05). The release curves of RGI and KLT release from Chi/PDA conduits at 37°C in PBS showed that FITC-RGI and TAMRA-KLT were immobilized on the surface of the Chi/PDA conduits (Figure 2B). There was no significant fluorescence in Chi/PDA conduits (image not shown).

**Results**

**Surface characterization of chitin conduits and polydopamine-modified chitin conduits**

The Chi conduit was nearly completely transparent, but the Chi/PDA conduit had low transparency and appeared black (Figure 1A and B). The scanning electron microscope images of the microstructures of the two conduits showed that there were a large number of uniformly distributed PDA particles on the surface of Chi/PDA conduits (Figure 1C and D). Fourier transform infrared spectrometer spectra of the Chi and Chi/PDA conduits are shown in Figure 1E. Both Chi and Chi/PDA conduits had a broad spectrum corresponding to the -OH group at 3000–3500 cm⁻¹. The Chi/PDA conduits had different -OH and -NH stretching vibrations between 3400–2800 cm⁻¹ due to inter- or intramolecular bonds between Chi and PDA. The Chi/PDA conduits had C=O bonds at 1600 cm⁻¹ and C-C bonds at 1500 cm⁻¹. These data suggest that PDA particles had been deposited on the Chi surface.

**Behavioral analysis**

We analyzed the recovery of motor function 12 weeks after surgery using walking tracks recorded by the CatWalk XT 10.6 gait analysis system (Noldus, Wageningen, Netherlands). Briefly, a high-speed camera (Noldus) automatically recorded the rats' paw prints as they crossed a walkway with an illuminated glass floor. Outcome measurements included the paw print area, paw intensity, and the sagittal functional index (SFI). The SFI was measured using the following formula (Rao et al., 2020):

$$ SFI = \frac{SFT - STS}{STS} \times 100\% $$

An SFI of 0 represents normal nerve function, whereas an SFI of -100 represents total nerve dysfunction. ETS indicates the experimental toe spread (the distance from the first to the fifth toe), NTS indicates the normal toe spread (the distance from the first to the fifth toe minus the distance from the top to the third toe), NPL indicates the normal paw length, EIT indicates the experimental intermediary toe spread (the distance from the second to the fourth toe), and NIT indicates the normal intermediary toe spread.

**Electrophysiological examination**

At 12 weeks after surgery, all rats were anesthetized by 3% isoflurane inhalation (RWD Life Science Co., Ltd.). Sciatic nerves were exposed by a small incision 5 mm from the proximal end of the implanted nerve conduit, and we applied an electrical signal with a rectangular pulse (stimulus intensity 0.9 mA, pulse duration 0.1 ms). The latencies and amplitudes of the compound muscle action potential (CMAP) were recorded for the targeted gastrocnemius muscle with an electrophysiological instrument (Oxford Instruments, Oxford, UK).

**Gastrocnemius muscle wet weight analysis**

At 12 weeks after surgery, the rats were euthanized by carbon dioxide inhalation with a filling rate of 30–70% per minute. Then, the gastrocnemius muscles of both hind limbs were surgically removed and immediately weighed with an electronic balance (Metler Toledo, Greifensee, Switzerland). The gastrocnemius muscle wet weight rate was calculated using the following equation:

$$ \text{Wet weight rate} = \frac{W - W_0}{W} \times 100\% $$

where W indicates the wet weight of the operated muscle, and W₀ indicates the wet weight of the nonoperated muscle.

**Tissue analysis**

The gastrocnemius muscle specimens were fixed with 4% paraformaldehyde at 4°C for 24 h. The middle gastrocnemius muscle, in which the sciatic nerve had been dehydrated through a graded ethanol series, the specimens were embedded in paraffin and sliced into 5 μm thick cross-sections. For Masson’s trichrome staining, the muscle samples were deparaffinized and hydrated. The samples were then stained in hematoxylin for 5 minutes to stain cell nuclei, and the samples were rinsed with distilled water. The specimens were then stained with Mayer’s hematoxylin, acid ponceau (Beijing Solarbio Science & Technology Co., Ltd.). The specimens were then rinsed, and stained with 1% osmic acid in PBS for 2 hours. The specimens were dehydrated through a graded ethanol series, the specimens were embedded in epoxy resin. An ultramicrotome (Leica) was used to cut the samples into 700 nm semithin sections. Then, the 700 nm semithin sections were stained with 1% toluidine blue and photographed using a microscope (Olympus Corporation, Tokyo, Japan). For each specimen, photographs were taken from three random fields, and the density of regenerated axons was analyzed.

**Cytotoxicity of Chi/PDA-Ps conduits**

As shown in Figure 3A, the SCs were seeded on films with Chi, Chi/PDA, or Chi/PDA-Ps. After live/dead staining, the live cells appeared green by calcein-AM staining, and the dead cells were stained red by propidium iodide (Figure 3B). There were fewer live cells and more dead cells in the Chi group than in the other two groups. The SCs cultured on Chi/PDA-Ps conduits were fusiform or polygonal in shape, indicating that the SCs were more biocompatible than the Chi substrate.

**Chi/PDA-Ps conduits enhance Schwann cells proliferation and levels of secreted neurotrophic factors in vitro**

SCs were treated with Chi, Chi/PDA, or Chi/PDA-Ps for 5 days to evaluate functional alterations. On the 5th day, immunofluorescence results showed that the SCs were fusiform or polygonal in shape, indicating that the cells were healthy. The number of SCs in the Chi/PDA-Ps groups was significantly higher than that in the Chi and Chi/PDA groups (Figure 4A). Moreover, after 1 day of treatment, there was no difference in the proliferation ability among the three groups of SCs (P > 0.05). After 5 days of treatment, the proliferation ability of SCs in the Chi/PDA-Ps groups was significantly greater than that in the other groups (P < 0.05; Figure 4B). The levels of the neurotrophic factors CTNF, BDNF, NGF, and VEGF that were released from Chi/PDA-Ps conduits were determined using enzyme-linked immunosorbent assay. In Figure 4C–F, it was found that CTNF, BDNF, NGF, and VEGF were released from SCs at higher levels at 1 and 5 days after Chi/PDA-Ps conduits treatment compared with Chi and Chi/PDA treatment.

Chi/PDA-Ps conduits promote attachment, proliferation, and migration of HUVECs in vitro

The SCs were stained positive for a vascular endothelial cell marker, CD31 (Additional file 1). As shown in Figure 5A, HUVECs exhibited a more extended spreading of the cytoskeleton after treatment with Chi/PDA-Ps. The HUVEC proliferation assay was used to evaluate the proangiogenic effects of the functional peptides released from Chi/PDA-Ps conduits. The results showed that Chi/PDA-Ps conduits could greatly improve the HUVEC proliferation on the 5th day (Figure 5B). A scratch test was performed to assess cell migration (Figure 5C and D). After 24 hours of incubation, HUVECs treated with Chi and KLT migrated the most among the three groups, covering more than 77.1% of the initial wounded area. The rate of wound closure was greater in the Chi/PDA-Ps group than in the Chi group.

**Additional file 1**

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Chi/PDA-Ps conduits improve sciatic nerve function and reduces gastrocnemius atrophy in rats with sciatic nerve injury

The three-dimensional pressure diagram of paw prints of each group presented that the spread of each toe and the toe pressure of the right foot in the Chi/PDA-Ps group were greater than those in the Chi and Chi/PDA groups (Figure 6A). The SFI of the Chi/PDA group was higher than the Chi group (P < 0.01). In addition, the SFI was significantly improved in the Chi/PDA group compared with that in the Chi group (P < 0.01) and Chi/PDA group (P < 0.01) (Figure 6B).

The degree of gastrocnemius muscle atrophy induced by PNI was evaluated by the wet weight of the right hind leg. As shown in Figure 6C, Chi/PDA-Ps treatment improved the gastrocnemius muscle atrophy more than Chi and Chi/PDA conduit treatment.

Masson’s trichrome staining of the cross-sectional area of the gastrocnemius muscles showed no obvious fibrosis or inflammatory cell infiltration in the gastrocnemius muscles among the three treatment groups (Figure 6D). The mean value of the cross-sectional areas of the muscle fibers was highest in the Chi/PDA-Ps group and lowest in the Chi group (Figure 6E).

Chi/PDA-Ps conduits improve the recovery of electrical conduction of nerves in rats with sciatic nerve injury

Electrophysiology experiments examined the recovery of electrical conduction of nerves. The representative CMAP images in each group are shown in Figure 7A. The quantitations of CMAP latency and amplitude are shown in Figure 7B and C. There was no significant difference in proximal CMAP latency between the Chi/PDA-Ps group (P > 0.05), but proximal CMAP latency was significantly higher in the Chi and Chi/PDA groups than in the Chi/PDA-Ps group (P < 0.05). In addition, the amplitude of CMAP was significantly higher in the Chi/PDA-Ps group than in the Chi and Chi/PDA groups (P < 0.05).

Chi/PDA-Ps conduits improve axonal regeneration and remyelination in rats with sciatic nerve injury

At 12 weeks after surgery, the regenerated sciatic nerve was exposed and examined. We observed that new connections had formed at both ends of the nerve defect. The gross morphology of nerves treated with Chi, PDA, or Chi/PDA conduits showed no neuroma formation at the suture site in all groups, and there was no apparent inflammation or tissue adhesion around the conduits (Figure 8A).

To evaluate the neuroregenerative effect of mimetic peptide-loaded conduits in vivo, we isolated the regenerated sciatic nerves of all rats. According to toluidine blue staining (Figure 8B), the number of regenerated nerves was greater in the Chi/PDA-Ps group than in the Chi and Chi/PDA groups (P < 0.05). The results of transmission electron microscopy revealed that the diameters of regenerated nerve fibers and the thicknesses of myelin sheaths were greatest in the Chi/PDA-Ps group (P < 0.05). However, there were no significant differences in the diameters of regenerated nerve fibers or the thicknesses of myelin sheaths between the Chi/PDA and Chi groups (P > 0.05; Figure 8C–F).

Discussion

Inspired by the nerve-selective regeneration theory, our team has confirmed that repair of PNI is improved by the application of small gap tubulization technology (Zhang et al., 2015, 2018). The small gap formed between the conduit and the stumps can reduce internal tension, provide a stable conduit structure, and accelerate the repair of nerve fibers. The Chi/PDA-Ps conduits, which have a larger diameter of myelinated nerve fibers in the Chi/PDA-Ps group compared with those in the Chi and Chi/PDA groups. These findings indicate that functional peptide-based sustained-release conduits were effective in enhancing axonal regeneration, remyelination, and functional recovery. Our results are expected to provide practical experimental and theoretical basis for further improvement of peripheral nerve conduit design and use.

In this study, we prepared a Chi nerve conduit using PDA as a peptide carrier. Our in vitro studies indicate that Chi/PDA-Ps conduits successfully supplied functional activators to promote the proliferation of SCs and endothelial cells. In addition, the secretion of neurotrophic factors from SCs and the migration of endothelial cells were improved by sustained release of the RGI and KLT mimetic peptides. In vivo analysis revealed that the PNI rats treated with Chi/PDA-Ps conduits and the KLT mimetic peptides exhibited significantly improved axonal regeneration, remyelination, and functional recovery. Our research is expected to provide practical experimental and theoretical basis for further improvement of peripheral nerve conduit design and use.

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Conflicts of interest: The authors declare that they have no conflict of interest.

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.
Cytotoxicity of Chi/PDA-Ps conduits.

Chi/PDA-Ps: polydopamine-coated chitin conduits loaded with functional peptides; SCs: Schwann cells.

Figure 1 | Chitin (Chi) surface properties after polydopamine (PDA) modification. (A, B) Visualization of the Chi conduits without (A) or with (B) PDA under general observation. The Chi conduit was nearly transparent, while the Chi/PDA conduit appeared black with low transparency. Scale bars: 1 mm. (C, D) Microstructure by scanning electron microscope of Chi surface without (C) or with (D) PDA. There were a large number of uniformly distributed PDA particles on the surface of Chi/PDA conduits. Scale bars: 5 μm. (E) Element composition of Chi and Chi/PDA surfaces were evaluated by Fourier transform infrared spectrometer spectra. Arrows indicate characteristic peaks. (F, G) Water drops on Chi (F) and Chi/PDA (G) surfaces. PDA greatly reduced the water contact angle of the Chi conduits. (H) Quantitative results of water contact angles for the Chi and Chi/PDA surfaces. Data are expressed as the mean ± SEM. The experiments were repeated three times. **P<0.01, vs. Chi group (Student’s t-test). Chi: Chitin; Chi/PDA: polydopamine-coated chitin conduits.

Figure 2 | Observation and release kinetics of mimetic peptides from Chi/PDA-Ps conduits. (A) Schematic diagram of Chi/PDA-Ps conduit preparation. (B) Side and cross-section view after functional peptides were immobilized on Chi/PDA conduits. RGI was modified with FITC (green fluorescence) and KLT was modified with TAMRA (orange fluorescence). (C) Release curves of RGI and KLT from Chi/PDA-Ps conduits. Data are expressed as mean ± SEM. The experiments were repeated three times. Chi/PDA-Ps: polydopamine-coated chitin conduits; Chi: chitin; Chi/PDA: polydopamine-coated chitin conduits; Chi/PDA-Ps: polydopamine-coated chitin conduits loaded with functional peptides; FITC: fluorescein isothiocyanate isomer; RGI: RGIDKRHWNSQ; KLT: KLTWQELYQLKYKGI; TAMRA: carboxytetramethylrhodamine.

Figure 3 | Cytotoxicity of Chi/PDA-Ps conduits. (A) Schematic representation of SCs seeded onto Chi/PDA-Ps films. (B) The survival ability of SCs seeded on different films and analyzed with a live/dead staining kit. The number of live cells (green, calcein-AM) was lower in the Chi group than in the other two groups, and the number of dead cells (red, propidium iodide) was greater in the Chi group than in the other two groups. Scale bars: 50 μm. (C) Quantitative results of the percent of dead/live cells. All data are represented as the mean ± SEM. The experiments were repeated three times. *P<0.05, **P<0.01, vs. Chi group (Student’s t-test). Chi: Chitin; Chi/PDA: polydopamine-coated chitin conduits.

Figure 4 | Chi/PDA-Ps conduits promote SCs proliferation and secretion. (A) SCs were identified by S100 staining (red, Alexa Fluor 594) and nucleus staining (blue, DAPI). The number of SCs in the Chi/PDA-Ps group was larger than those in the Chi and Chi/PDA groups. Scale bars: 50 μm. (B) SCs proliferation analysis was performed using a Cell Counting Kit-8 (Dojindo Laboratories). (C-F) Protein levels of CNTF, BDNF, NGF, and VEGF in supernatant by enzyme-linked immunosorbent assay. All data are represented as the mean ± SEM (n = 3 for each group). **P<0.05, ***P<0.01, vs. Chi group; #P<0.05, ##P<0.01, vs. Chi/PDA group (one-way analysis of variance followed by Tukey’s post hoc test). Chi: Chitin; Chi/PDA: polydopamine-coated chitin conduits; Chi/PDA-Ps: polydopamine-coated chitin conduits loaded with functional peptides; CNTF: ciliary neurotrophic factor; BDNF: brain-derived neurotrophic factor; NGF: nerve growth factor; VEGF: vascular endothelial growth factor.

Research Article
The continuous release of mimetic peptides from Chi/PDA-Ps conduits enhances conduction function of the regenerated sciatic nerve.

Chi/PDA-Ps conduits improve motor functional recovery in PNI rats 12 weeks after surgery. 3D: Three-dimensional; Chi: Chitin; Chi/PDA: polydopamine-coated chitin conduits; Chi/PDA-Ps: polydopamine-coated chitin conduits loaded with functional peptides; DAPI: 4′,6-diamidino-2-phenylindole; FITC: fluorescein isothiocyanate isomer; HUVECs: human umbical vein endothelial cells; OD: optical density.

Figure 5 | Chi/PDA-Ps conduits accelerate the attachment, proliferation, and migration of HUVECs.

(A) HUVECs were identified by phallolidin staining (green, FITC) and nucleus staining (blue, DAPI). The HUVECs in the Chi/PDA-Ps group had more extended morphology than those in the other groups. Scale bars: 75 µm. (B) The Cell Counting Kit-8 (Dojindo Laboratories) determined HUVECs proliferation after treatment with the various conduits. (C) A representative photograph of HUVECs migration in the wound healing assay. The migration distance was longer in the Chi/PDA-Ps group than in the other groups. Scale bars: 100 µm. (D) Quantitative results for HUVECs migration in the wound healing assay. All data are represented as the mean ± SEM (n = 3 for each group). *P < 0.05, **P < 0.01, vs. Chi group; #P < 0.01, vs. Chi/PDA group (one-way analysis of variance followed by Tukey’s post hoc test). Chi: Chitin; Chi/PDA: polydopamine-coated chitin conduits; Chi/PDA-Ps: polydopamine-coated chitin conduits loaded with functional peptides; DAPI: 4′,6-diamidino-2-phenylindole; FITC: fluorescein isothiocyanate isomer; HUVECs: human umbilical vein endothelial cells; OD: optical density.

Figure 6 | Chi/PDA-Ps conduits improve motor functional recovery in PNI rats 12 weeks after surgery.

(A) Representative 3D plantar pressure distribution of affected hind limbs and healthy hind limbs for each group. The spread and the toe stress of the right hind limb were greater in the Chi/PDA-Ps group than in the other groups. (B) Quantitative results of the SFI values (n = 3 for each group). (C) Quantitative results of the gastrocnemius muscle wet weight rate (n = 3 for each group). (D) Cross-sectional area of gastrocnemius muscle fibers 12 weeks after surgery. The cross-sectional area of muscle fibers was greater in the Chi/PDA-Ps group than in the other groups. Scale bars: 50 µm. (E) Quantitative results of the cross-section of the gastrocnemius muscle fiber (n = 8 for each group). All data are represented as the mean ± SEM. *P < 0.05, **P < 0.01, vs. Chi group; #P < 0.05, ##P < 0.01, vs. Chi/PDA group (one-way analysis of variance followed by Tukey’s post hoc test). Chi: Chitin; Chi/PDA: polydopamine-coated chitin conduits; Chi/PDA-Ps: polydopamine-coated chitin conduits loaded with functional peptides; PNI: peripheral nerve injury; SFI: sciatric functional index.

Figure 7 | The continuous release of mimetic peptides from Chi/PDA-Ps conduits enhances conduction function of the regenerated sciatic nerve.

(A) Typical electromyography 12 weeks after surgery. The rats in the Chi/PDA-Ps group had shorter CMAP latency and higher CMAP amplitude than those in the other groups. Abscissa, 50 ms/lattice; ordinate, 10 mV/lattice. (B) The latency of CMAPs 12 weeks after surgery. (C) The amplitude of CMAPs 12 weeks after surgery. All data are represented as the mean ± SEM (n = 3 for each group). *P < 0.05, **P < 0.01, vs. Chi group; #P < 0.05, ##P < 0.01, vs. Chi/PDA group (one-way analysis of variance followed by Tukey’s post hoc test). Chi: Chitin; Chi/PDA: polydopamine-coated chitin conduits; Chi/PDA-Ps: polydopamine-coated chitin conduits loaded with functional peptides; CMAP: compound motor action potential.

Figure 8 | Chi/PDA-Ps conduits facilitate axonal regeneration and remyelination.

(A) General view of Chi, Chi/PDA, and Chi/PDA-Ps conduits 12 weeks after surgery. We observed no neuroma, inflammation, or tissue adhesion around the conduits. (B) The regenerated nerves were subjected to transmission electron microscope observation. Regenerated axons with thick myelin sheaths (red arrows) were more common in the Chi/PDA-Ps group. Scale bars: 5 µm. (D) Quantification of the density of myelinated nerve fibers. (E) Quantification of the myelin sheath thickness. (F) Quantification of the diameter of myelinated nerve fibers. All data are represented as the mean ± SEM (n = 8 for each group). **P < 0.01, vs. Chi group; #P < 0.05, ##P < 0.01, vs. Chi/PDA group (one-way analysis of variance followed by Tukey’s post hoc test). Chi: Chitin; Chi/PDA: polydopamine-coated chitin conduits; Chi/PDA-Ps: polydopamine-coated chitin conduits loaded with functional peptides.
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