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Constructing conductive conduit with conductive fibrous infilling for peripheral nerve regeneration

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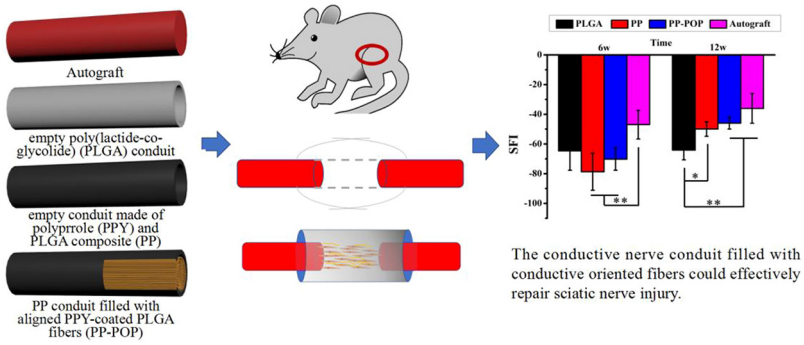
HIGHLIGHTS

- Degradable polypyrrole-containing conduit and oriented fibers were prepared.
- Conduit with fibrous infilling displayed good dimension stability.
- Conductive substrates favored the proliferation and differentiation of PC12 cells.
- Conductive fiber-filled conduit regenerated the rat sciatic nerve efficiently.

GRAPHICAL ABSTRACT



The conductive nerve conduit filled with conductive oriented fibers could effectively repair sciatic nerve injury.

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ABSTRACT

Nerve conduits are essential for guiding the regeneration of injured peripheral nerves, and empty conduits usually cannot perform satisfactorily due to problems with cell migration and nutrient transportation. Taking the electrophysiological features of nervous tissues into consideration, in this study, a conductive conduit filled with parallel-aligned conductive fibers was constructed, and its potential for enhancing peripheral nerve regeneration was evaluated *in vivo*. The conductive fibers were prepared via depositing a polypyrrole (PPY) shell onto electrospun parallel-aligned poly(lactide-co-glycolide) (PLGA) fibers. The conductive conduit was prepared from PLGA/PPY emulsion via dip-coating on a mandrel. Both materials were non-cytotoxic to PC12 cells and were able to promote cell proliferation and differentiation. Moreover, the aligned fibers provided strong orientation guidance for nerve fibers. Sciatic nerve defects were created in Sprague-Dawley rats, and empty or fiber-filled conduits were sutured into the defects. Meanwhile, the control groups received PLGA conduits or autografts. Twelve weeks post-operation, the fiber-filled conductive conduit showed much better nerve regeneration outcomes than both the PLGA conduit and the empty conductive conduit and showed comparable results to the autograft in terms of electrophysiological properties, sciatic function indices, and regenerated myelinated nerve fibers as well as axon diameter and myelin thickness. It is possible that the oriented conductive fibers in the conductive conduit provide a favorable micro-environment for nerve growth due to their capacity to transmit

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self-originated electrical stimulation between cells. The results of animal testing confirmed the feasibility of using combined conductive conduits for guiding nerve regeneration.

1. Introduction

The repair of peripheral nerve injuries remains a challenging clinical issue. A promising approach in nerve tissue engineering includes the use of nerve guidance conduits (NGCs), in which a conduit is sutured between the two stumps of a transected nerve, providing temporary mechanical support and a favorable micro-environment for nerve regeneration [1,2]. Conventional NGCs are made of silicone rubber, which is bioinert, elastic, and nondegradable [3]. Some degradable NGCs, primarily composed of collagen or degradable aliphatic polyester, have been approved by the US Food and Drug Administration (FDA) and are commercially available and clinically used [4]. They have met the requirements for clinical therapy of peripheral nerve regeneration to some extent, but are still inferior to the existing gold standard of using autologous nerve grafts for nerve reconstruction [5–7]. Autologous nerve grafting also has obstacles, however, including donor site morbidity, mismatches between the donor nerve and the recipient site, etc. [8,9]. Many efforts have been made to achieve nerve regeneration with enhanced functional recovery, including seeding of neural precursor cells [10,11], loading of bioactive factors [12,13], and controlling conduit topography [14]. The geometry inside nerve conduits is normally created in an axially oriented morphology to guide the extension of axons and the growth of nerve fibers by taking advantage of the contact guidance effect. Parallel-aligned fiber bundles have been identified as good infillings for NGCs [15–18].

It is also beneficial to take the electrophysiological properties of nerve tissues/cells into account when NGCs are designed and fabricated. Several studies have shown that electrical stimulation can significantly promote the cellular responses of nerve-derived cells and the regeneration of peripheral nerve injuries [19,20]. Recent research has revealed that electronegative nerve cells can generate a weak internal electric field, and these local internal electric fields can be greatly enhanced if the cells are grown on conductive substrates, which helps to stabilize the bioelectric properties of the cell membrane [21].

Conductive materials have since been welcomed in the field of nerve tissue engineering. For instance, carbon nanomaterials including carbon nanotubes (CNTs), carbon nanofibers (CNFs) and graphene have been tested for their potential to regulate the biological behavior of neurons and to stimulate nerve regeneration on account of their conductive features [22–24]. Conductive polymers, such as polyaniline (PANI), polypyrrole (PPY) and poly[3,4-ethylene dioxythiophene-co-1,3,5-tri[2-(3,4-ethylene dioxythienyl)]-benzene] (EPh), have been synthesized and used to stimulate the proliferation and neurite outgrowth of nerve-derived cells, such as PC12 and Schwann cells [25–27]. Among these conductive materials, PPY is a good candidate for conduit design on account of its good biocompatibility, cell affinity, and high electrical conductivity as well as its cheap price and flexible preparation [28,29].

At present, most studies about conductive biomaterials in nerve tissue engineering have mainly focused on the stimulation effect of electrical conduction on nerve-derived cells *in vitro* [30,31]. To evaluate the feasibility of using conductive conduits for guiding the regeneration of peripheral nerves, *in vivo* tests must be used; however, to our knowledge, *in vivo* studies are presently limited. Recently, two studies reported the successful regeneration of injured peripheral nerves in rats by using PPY [32] or PANI-based [33] conduits without introducing extra stimulation. In another report, the authors repaired long-range peripheral nerve injuries in a rat model by combining electrical stimulation with PPY-coated conduits [34]. These approaches revealed that conductive polymers are promising for both *in vitro* and *in vivo* studies. In these studies, empty conduits were sutured between the stumps of injured nerves, but it has been proposed that NGCs with oriented infillings could promote nerve regeneration more efficiently [16,17,35]. Taking the electrophysiological properties of nerve tissues into account, satisfactory peripheral nerve regeneration outcomes can be expected if the oriented infillings are also conductive.

To this end, a degradable conductive tube was fabricated from an emulsion containing poly(lactide-co-glycolide) (PLGA) and PPY via the dip-coating method and was filled with parallel-aligned conductive PLGA/PPY core/shell fibers, which were produced via PLGA solution electrospinning and subsequent surface PPY coating. PLGA was selected because of its nontoxicity, biodegradability and good processability; furthermore, its degradation rate can be engineered to fit particular needs [36]. The conduit demonstrated sufficient mechanical stability to be sutured between the two stumps of sectioned sciatic nerves in Sprague-Dawley (SD) rats. The axially patterned fibers inside the conduit lumen act as bridges between the two stumps to facilitate cell migration and the prolongation and orientation of neurites. The control groups received either PLGA conduits, non-filled conductive conduits or autologous nerve grafts. Comprehensive evaluations, including the examination of electrophysiological properties, the sciatic function index (SFI), regenerated myelinated nerve fibers, axon diameter and myelin thickness, were conducted. The positive hypothesis for the present study was that electroactive conduits with multiple stimuli are proper choices for neural tissue regeneration.

2. Materials and methods

2.1. Electrospinning of parallel-aligned PLGA fibers

PLGA (lactide/glycolide: 75/25 in molar ratio; M_w: 100000) was purchased from Shandong Pharmaceutical Sciences Pilot Plant (China) and used for electrospinning directly. PLGA solution (20 wt%) was prepared by dissolving the polymer in trifluoroethanol (Sigma-Aldrich), and was then transferred into a syringe with a steel needle (inner diameter = 0.5 mm). The solution was electrospun at fixed parameters (flow rate: 0.6 mL/h; voltage: 20 kV) using a rotating cylinder, which was placed 20 cm away from the needle tip, as a collector. The produced parallel-aligned fibrous mesh was then thoroughly vacuum-dried to a constant weight at room temperature to remove any residual solvent.

2.2. Coating PPY onto PLGA fibers

The PLGA fibrous mesh was immersed into an aqueous solution containing 14 mM pyrrole (Aladdin, USA) and 7 mM paratoluene sulfonic acid sodium salt (pTs, Aldrich). The system was incubated at 4 °C for 1 h, followed by the addition of 100 mL $FeCl_3$ aqueous solution (38 mM). Then, the system was incubated at 4 °C for another 12 h under continuous shaking to carry out the polymerization of pyrrole, allowing the deposition of PPY onto the PLGA fibers. After the reaction, the obtained black mesh was retrieved and washed with deionized water and acetone and then vacuum-dried at room temperature to a constant weight. The obtained PPY-coated PLGA fibrous mesh was termed POP. For comparison, PPY films were fabricated by submerging coverslips in pTs aqueous solution containing 5% (v/v) pyrrole and 10% (w/v) $FeCl_3$ at 4 °C, in which PPY deposits onto coverslips to form the PPY films.