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Bridging peripheral nerves using a deacetyl chitin conduit combined with short-term electrical stimulation

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Abstract

Previous studies have demonstrated that deacetyl chitin conduit nerve bridging or electrical stimulation can effectively promote the regeneration of the injured peripheral nerve. We hypothesized that the combination of these two approaches could result in enhanced regeneration. Rats with right sciatic nerve injury were subjected to deacetyl chitin conduit bridging combined with electrical stimulation (0.1 ms, 3 V, 20 Hz, for 1 hour). At 6 and 12 weeks after treatment, nerve conduction velocity, myelinated axon number, fiber diameter, axon diameter and the thickness of the myelin sheath in the stimulation group were better than in the non-stimulation group. The results indicate that deacetyl chitin conduit bridging combined with temporary electrical stimulation can promote peripheral nerve repair.

Key Words: *nerve regeneration; peripheral nerve injury; deacetyl chitin conduit; electrical stimulation; NSFC grant; neural regeneration*

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Introduction

Peripheral nerve injury is a major trauma. The unfavorable prognosis of peripheral nerve injury leads to a high probability of disability. At present, epineurial or perineurial suture is a common method for repairing the injured peripheral nerve. Accumulating research (Jiang et al., 1994, 2006; Jiang and Yoshida, 1998; Jiang and Li, 2003; Zhang et al., 2008, 2009; Huang et al., 2010b) indicates that selective regeneration of different types of axons can occur following injury. Previous studies (Jiang et al., 1994; Jiang and Yoshida, 1998; Jiang and Li, 2003) demonstrated that a deacetyl chitin nerve conduit can promote nerve regeneration after nerve injury. Moreover, a number of researchers (Jiang et al., 2006; Zhang et al., 2008, 2009) found that a deacetyl chitin conduit could provide a suitable microenvironment for nerve regeneration, and that it was better than the traditional perineurial suture repair method. Huang et al. (2010b) showed that temporary electrical stimulation could increase the rate of regeneration of the injured peripheral nerve after delayed repair. We hypothesized that a deacetyl chitin conduit combined with electrical stimulation might be an effective strategy for promoting the regeneration of the injured peripheral nerve after a delayed repair.

Materials and Methods

Establishment of the sciatic nerve transection injury model

A total of 32 male specific-pathogen-free Sprague-Dawley rats aged 3 months and weighting 200–250 g were provided by the Experimental Animal Center of Shandong University School of Medicine, China (license No. SCXK (Lu) 2013 0009). The rats were intraperitoneally anesthetized with phenobarbital sodium 30 mg/kg. Disinfection of the skin of the right leg was preformed, and the right sciatic nerve was exposed and cut. The distance from the transection to the bifurcation of the sciatic nerve was 1 cm. The study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Weifang Medical University in China (permit number: 3461).

Deacetyl chitin conduit suturing

Deacetyl (deacetylated) chitin conduit was invented by People's Hospital of Peking University and the Chinese Textile Academy, in China (patent No. 01136314.2). The length was 6 mm, the inner diameter was 2–4 mm, and the wall thickness was 1 mm. The conduit was positioned between the proximal and distal ends of the nerve under a 4 × operating microscope (Moller Hi-900, Wedel, Germany). Both the distal and proximal ends of the nerve were inserted into the conduit about 2 mm. A needle was inserted through the surface of the chitin tube and into the epineurium to suture the bridge in place at each end. The distance between the two ends of the nerve was 2 mm.

Electric pulse stimulation

A total of 32 rats with deacetyl chitin conduit were equally and randomly divided into non-stimulation and stimulation groups. After surgery, the rats in the stimulation group were subjected to electrical stimulation, applied with the Trimix Linus apparatus (Nihon Medix SD-5101, Matsudo, Japan). Two copper wires were coiled as stimulation electrodes. About 3–5 mm of the insulation on the end of the wires was removed. The tip of the two wires was twisted to form a loop that was secured into the proximal end. The other end of the wires was connected to the Trimix Linus. Weak square 0.1-ms electrical pulses (3 V, 20 Hz) were then applied to the proximal nerve stumps for 1 hour. The electrodes were hooked onto the proximal end of the nerve in the non-stimulation group for 1 hour without electrical stimulation. The wounds were covered with wet gauze to prevent dehydration. After stimulation, the wires were removed and the wounds were closed by suture. After the procedure, the rats were put back into their cages.

Electrophysiological study

At 6 and 12 weeks after operation, 8 rats in each group were subjected to electrophysiological study. The rats were intraperitoneally anesthetized with phenobarbital sodium (30 mg/kg). The sciatic nerve of the right leg was exposed for recording with an EMG recorder (Keypoint 4C, Dantec, Skovlunde, Denmark). Recording electrodes were inserted into the proximal and distal surfaces of the gastrocnemius. Stimulation electrodes were put on the nerve stem of both the proximal and distal surfaces of the conduit. Comparison electrodes were inserted subcutaneously between the recording electrodes and the stimulation electrodes. Pulse stimulation (0.1 ms, 0.9 mA, 10 Hz, 6 times) was cut-in and electric signals were recorded. We determined the motor nerve conduction velocity (m/s) of the sciatic nerves by measuring the distance between the electrodes and the latency.

Histological study

After measuring motor nerve conduction velocity, rats were given an overdose of phenobarbital sodium (50 mg/kg). Tissue perfusion (0.9% NaCl 250 mL and 4% paraformaldehyde in 0.1 mol/L phosphate buffer 250 mL (pH 7.4)) was preformed through the left ventricle. The sciatic nerve was cut off. The specimen was fixed with 4% paraformaldehyde for 24 hours, stained with osmic acid for 12 hours, dehydrated with a graded alcohol series, immersed in clearing liquid, imbedded in paraffin, and sliced into 2-μm sections. Morphometric measurements were performed using Adobe Photoshop CS3 software. The shortest lengths of the outer and inner margins of the myelin sheath were measured to determine fiber diameter and axon diameter. After obtaining the fiber and axon diameter, myelin thickness was calculated as (fiber diameter–axon diameter)/2. The number of myelinated nerve fibers was counted using an Olympus BX51 microscope (Olympus, Tokyo, Japan). The thickness of the myelin sheath was calculated by measuring the diameters of the outer and inner edges of the myelin sheath with a DP70 image acquisition system (Olympus).

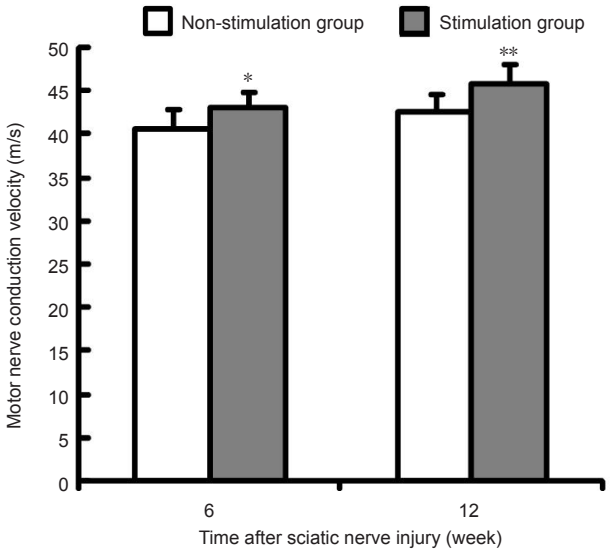

Figure 1 Effect of deacetyl chitin conduit combined with electrical stimulation on motor nerve conduction velocity in rats with peripheral nerve injury (electrophysiological study).
Data are presented as mean ± SD of eight rats in each group. Statistical analyses were performed with paired *t*-test. $*P < 0.05$, $**P < 0.01$, *vs.* non-stimulation group.

Statistical analysis

All data were analyzed using SPSS 15.0 software (SPSS, Chicago, IL, USA). The data are presented as mean ± SD. Statistical analyses were performed using paired *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

Deacetyl chitin conduit combined with electrical stimulation increased motor nerve conduction velocity in rats with peripheral nerve injury

Results of electrophysiological study showed that at 6 and 12 weeks after peripheral nerve injury, motor nerve conduction velocity in the stimulation group was higher than in the non-stimulation group ($P < 0.05$ or $P < 0.01$; **Figure 1**).

Deacetyl chitin conduit combined with electrical stimulation improved peripheral nerve morphology in rats with peripheral nerve injury

Histological examination showed that at 6 and 12 weeks,

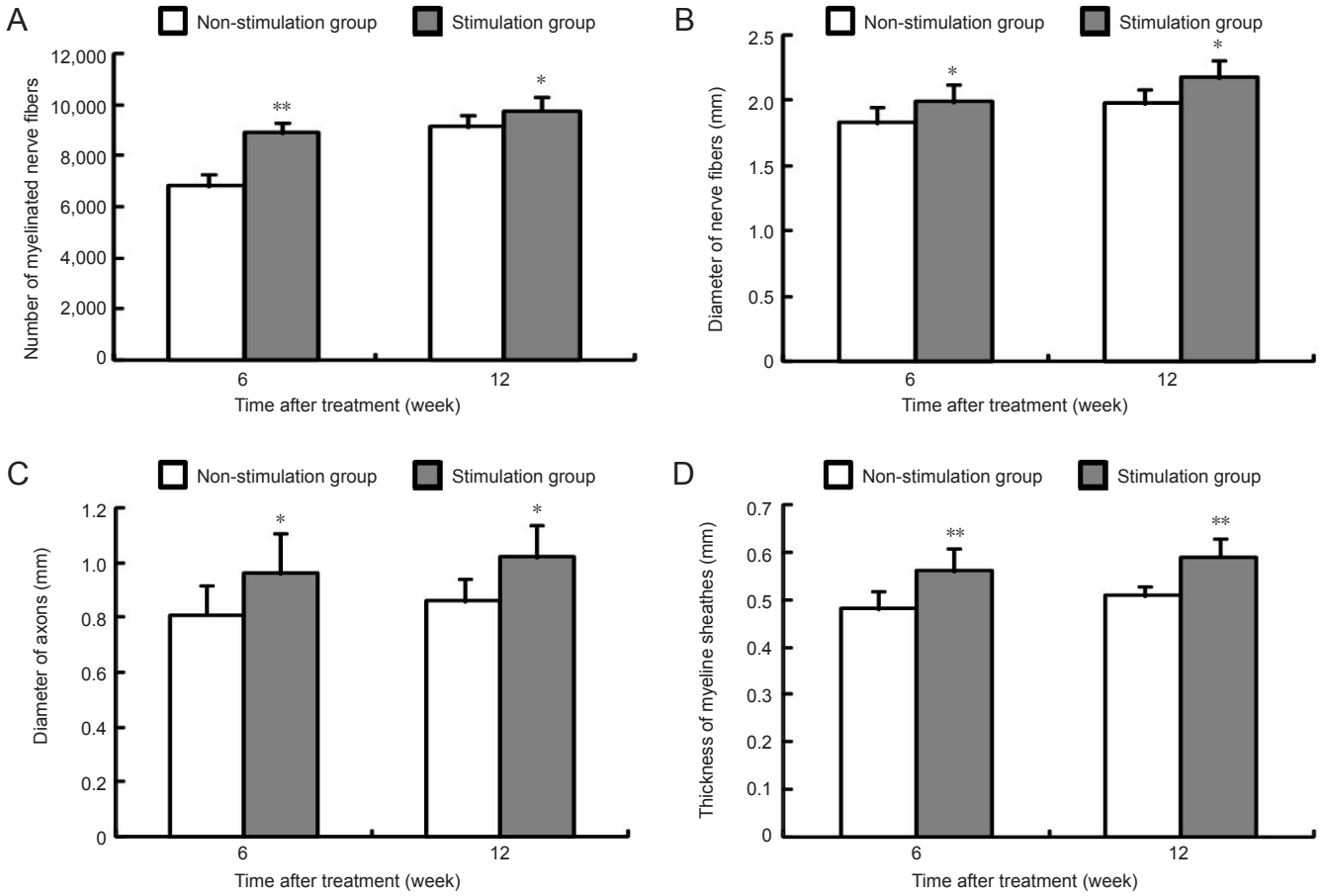

Figure 2 Effect of deacetyl chitin conduit combined with electrical stimulation on myelinated nerve fiber counts (A), fiber diameter (B), axon diameter (C) and myelin thickness (D) in the distal stump in rats with peripheral nerve injury (electrophysiological study).
The shortest lengths of the outer and inner margins of the myelin sheath were measured to determine the fiber diameter and axon diameter. Myelin thickness was calculated as (fiber diameter–axon diameter)/2. Data are presented as mean ± SD of eight rats in each group. Statistical analyses were performed with paired *t*-test. $*P < 0.05$, $**P < 0.01$, *vs.* non-stimulation group.

peripheral nerve regeneration was found in both groups. However, the number of myelinated nerve fibers, fiber diameter, axon diameter and myelin thickness were greater in the stimulation group than in the non-stimulation group at both 6 and 12 weeks ($P < 0.01$ or $P < 0.05$; **Figure 2**).

Discussion

Nerves have the capacity to regenerate spontaneously and establish synaptic connections with target tissue. However, the clinical prognosis of peripheral nerve injuries is still poor. Functional recovery mainly depends on the accuracy and efficiency of reconnecting different types of nerve fibers (Alluin et al., 2009). Numerous researchers are focused on improving the accuracy of the new connections (Lundborg, 2000). Several studies (Jiang and Yoshida, 1998; Zhang et al., 2009; Huang et al., 2010b) demonstrated a novel method of repairing peripheral nerves using deacetylated biological chitin conduits. Experiments conducted by Sun et al. (2002a, b, c, d) have shown that the biocompatibility and safety of these biological conduits are acceptable. These conduits promote a better joining of nerves and make it possible for nerve fibers to regenerate in the shortest path into the distal segment of the nerve. The microenvironment provided by the conduit is suitable for nerve regeneration and is advantageous for incorporating exogenous and endogenous factors. The conduit itself can inhibit the formation of scar tissue and provide mechanical support to limit compression, thereby enhancing the speed and fidelity of regeneration. Thus, deacetyl biological chitin conduit bridging has the potential to replace traditional epineurial suture for repairing the injured peripheral nerve. After conduit bridging, distal target tissue physiology and function can be restored to a satisfactory level.

Schmidt et al. (1997) showed that electrical stimulation promotes the growth of axons. Li et al. (2007) applied percutaneous electrical stimulation to 23 patients suffering from peripheral nerve injuries and found that electrical stimulation can promote the recovery of motor nerves. Numerous studies showed that electrical stimulation regulates the function of Schwann cells (Gigo-Benato et al., 2010; Huang et al., 2010a; Singh et al., 2012). Appropriate stimulation can regulate the proliferation, adhesion and apoptosis of Schwann cells and promote the secretion of nerve growth factor and brain-derived neurotrophic factor.

In our study, we used deacetyl biological chitin conduits to bridge nerves with a 2-mm gap, and combined this with

direct electrical stimulation. Motor nerve conduction velocity, diameter of nerve fibers, diameter of axons and thickness of the myelin sheath in the electrically stimulated rats at 6 and 12 weeks were improved compared with rats that did not receive electrical stimulation. These results indicate that conduit bridging combined with electrical stimulation efficiently promotes the regeneration of peripheral nerves.

In summary, biological chitin conduit nerve bridging combined with short-term electrical stimulation results in better regeneration compared with conduit bridging alone. Short-term electrical pulse stimulation improves motor nerve conduction velocity, increases the diameter of nerve fibers and axons, and increases the thickness of myelin sheaths. Our research provides a novel, flexible and effective strategy for promoting peripheral nerve regeneration following injury.

Author contributions: *Zhang ZL was responsible for data intergration and wrote the final version of the manuscript. Li X and Xin J were responsible for experiment implementation and animal treatment. Zuo SJ was responsible for data collection and analysis. Zhang PX provided information and technical support. All authors approved the final version of the paper.*
Conflicts of interest: *None declared.*

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