NEURAL REGENERATION RESEARCH
January 2015, Volume 10, Issue 1
www.nrronline.org

SPECIAL ISSUE

Biological conduit small gap sleeve bridging method for peripheral nerve injury: regeneration law of nerve fibers in the conduit

Pei-xun Zhang[#], Li-ya A[#], Yu-hui Kou[*], Xiao-feng Yin[*], Feng Xue[#], Na Han[*], Tian-bing Wang, Bao-guo Jiang

Department of Trauma and Orthopedics, Peking University People's Hospital, Beijing, China

Correspondence to:
Na Han, Ph.D., 876804725@qq.com.
Yu-hou Kou, Ph.D., kouyuhui@163.com.
Xiao-feng Yin, Ph.D., M.D., xiaofengyin@bjmu.edu.cn.

 # These authors contributed equally to this work.

doi:*10.4103/1673-5374.150709*
http:*//www.nrronline.org/*

Accepted: *2014-08-14*

Abstract

The clinical effects of 2-mm small gap sleeve bridging of the biological conduit to repair peripheral nerve injury are better than in the traditional epineurium suture, so it is possible to replace the epineurium suture in the treatment of peripheral nerve injury. This study sought to identify the regeneration law of nerve fibers in the biological conduit. A nerve regeneration chamber was constructed in models of sciatic nerve injury using 2-mm small gap sleeve bridging of a biodegradable biological conduit. The results showed that the biological conduit had good histocompatibility. Tissue and cell apoptosis in the conduit apparently lessened, and regenerating nerve fibers were common. The degeneration regeneration law of Schwann cells and axons in the conduit was quite different from that in traditional epineurium suture. During the prime period for nerve fiber regeneration (2–8 weeks), the number of Schwann cells and nerve fibers was higher in both proximal and distal ends, and the effects of the small gap sleeve bridging method were better than those of the traditional epineurium suture. The above results provide an objective and reliable theoretical basis for the clinical application of the biological conduit small gap sleeve bridging method to repair peripheral nerve injury.

Key Words: *nerve regeneration; peripheral nerve; small gap; axons; Schwann cells; repair; injury; biological conduit; NSFC grants; neural regeneration*

Funding: *This study was supported by grants from the National Program on Key Basic Research Project of China (973 Program), No. 2014CB542200; Program for Innovative Research Team in University of Ministry of Education of China, No. IRT1201; the National Natural Science Foundation of China, No. 31271284, 31171150, 81171146, 30971526, 31100860, 31040043, 31371210; and Program for New Century Excellent Talents in University of Ministry of Education of China, No. BMU20110270.*

Zhang PX, A LY, Kou YH, Yin XF, Xue F, Han N, Wang TB, Jiang BG (2015) Biological conduit small gap sleeve bridging method for peripheral nerve injury: regeneration law of nerve fibers in the conduit. Neural Regen Res 10(1):71-78.

Introduction

The effective repair of peripheral nerve injury depends on the accurate abutment of nerve fibers with different natures at the proximal and distal ends (Li et al., 2007; Huang et al., 2010a). Epineurium and perineurium sutures commonly used in the clinic cannot achieve the effective accurate abutment of nerve fibers with different natures (Jiang et al., 1994; Jiang and Yoshida, 1998; Jiang and Li, 2003; Mohammadi et al., 2014). The selective regeneration of the peripheral nerve provides some theoretical basis for a biological conduit small gap sleeve bridging method in the repair of peripheral nerve injury (Lundborg, 2000; Jiang et al., 2006, 2010). Previous animal experiments and multi-center clinical trials have suggested that the repair effects of the 2-mm small gap sleeve bridging of the biological conduit in the repair of peripheral nerve injury are better than those in the commonly-used epineurium suture (Zhang et al., 2008, 2009, 2011, 2013). The deacetylated chitin biological conduit used in these studies not only provides a suitable microenvironment for nerve regeneration, but is also non-toxic to organisms, can be completely degradated and absorbed *in vivo*, and has good biocompatibility (Yu et al., 2009; Zhang et al., 2010). The corresponding production process and repair methods have been granted patents (Yu et al., 2009; Zhang et al., 2010). However, the regeneration law of nerve fibers in the biological conduit is not clear. Thus, this study investigated the changes in the microenvieronment of the nerve stump, and the change trend of the nerve fibers and the myelin sheath in the biological conduit.

Materials and Methods

Animals

108 male specific-pathogen-free Sprague-Dawley rats, weighing 250 ± 4.5 g and aged 3 months old, were purchased from the Beijing Institute of Xieerxin Biology Resource, China (Animal license No. SCXK (Jing) 2013-0001) and included in this

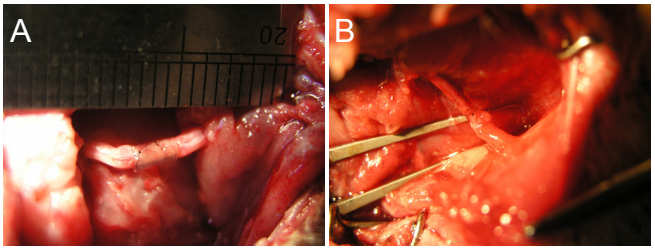


Figure 1 Repair of sciatic nerve transection.
(A) 2-mm small gap sleeve bridging of the biological conduit; (B) epineurium suture.

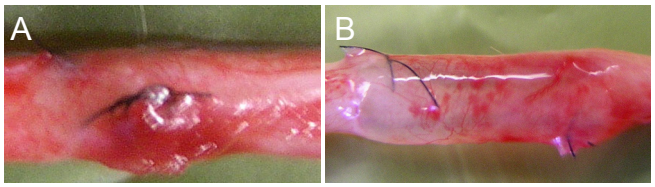


Figure 3 Morphology of sciatic nerve at 28 days after repair of sciatic nerve transection.
(A) 2-mm small gap sleeve bridging of the biological conduit; (B) epineurium suture (× 4). Trace of the conduit was observed below the black suture.

study. This study was approved by the Ethics Committee of the People's Hospital of Peking University in China (Approval No. 2013-059). The rats were equally and randomly assigned to a sleeve bridging group and a control group.

Preparation of models of sciatic nerve injury
The rats were intraperitoneally anesthetized with pentobarbital (30 mg/kg). The right sciatic nerve was transected 1 cm from the bifurcation. Rats in the sleeve bridging group were subjected to biological conduit small gap sleeve bridging. Rats in the control group received conventional epineurium suture. One rat was randomly selected and its sciatic nerve was left normal to serve as a normal group.

Sciatic nerve repair
2-mm small gap sleeve bridging of biological conduit
In the sleeve bridging group, under a 4× operating microscope (CX22; Olympus, Tokyo, Japan), a 6-mm-long biodegradable chitin biological conduit (3-mm inside diameter, 1-mm thickness; purchased from China Textile Academy) was placed between two nerve stumps, and sutured with a 10-0 nylon suture. Approximately 1-mm nerves at both distal and proximal ends were inserted in the conduit, and the distance between nerve stumps in the conduit was 2 mm (**Figure 1A**).

Epineurium suture
In the control group, under a 4 × operating microscope, the epineurium was sutured with a 10-0 nylon suture, with two stitches on each stump (**Figure 1B**).

Sample collection
At 3, 5, 7, 14, 28 and 56 days after injury, nine rats were

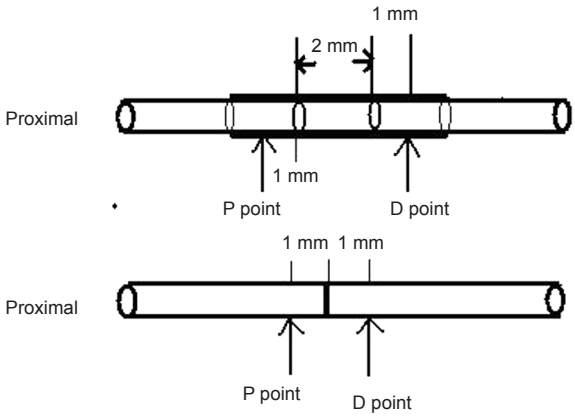


Figure 2 Position of sciatic nerve collection in rats.
P point: 1 mm proximal to proximal broken end of the conduit/1 mm proximal to proximal epineurium suture point; D point: 1 mm distal to distal broken end of the conduit/1 mm distal to distal epineurium suture point.

obtained from each group. Rats were intraperitoneally anesthetized with 2% sodium pentobarbital, and fixed in the supine position. An incision was made in the middle of the chest to expose the thoracic cavity. An infusion needle was inserted in the cardiac apex. After turning on the saline side of the tee pipe and cutting the right auricle, perfusion was conducted until the liver became white. Subsequently, the specimen was perfused with 4% paraformaldehyde for 30 minutes. The right sciatic nerve was obtained after repair, and post-fixed in paraformaldehyde for 4–5 hours. The position of sample collection is shown in **Figure 2**. The sciatic nerve was divided into three parts: injured segment, proximal injured segment and distal injured segment. Specimens after postfixation were immersed in sucrose solution overnight at 4°C, embedded in an optimal cutting temperature embedding medium, and sliced into 7-μm frozen sections. These sections were dried at room temperature for 24 hours and stored in a refrigerator.

Hematoxylin-eosin staining
As shown in **Figure 2**, paraffin sections of rat sciatic nerve were dewaxed, hydrated, and stained with hematoxylin and eosin, and then observed using a light microscope (Olympus).

Immunofluorescence staining
As displayed in **Figure 2**, frozen sections of rat sciatic nerve were fixed in acetone at –20°C for 20 minutes, and washed three times with 0.3% Triton X-100/PBS, each for 5 minutes. These sections were then blocked with 10% normal goat serum for 1 hour, incubated with mouse anti-rat glial fibrillary acidic protein monoclonal antibody (1:200; Sigma-Aldrich, St. Louis, MO, USA) and mouse anti-rat NF200 monoclonal antibody (1:200; Sigma-Aldrich) at room temperature overnight, washed three times with 0.3% Triton X-100/PBS, each for 5 minutes. The sections were incubated with Cy2 and Cy3 conjugated rabbit anti-mouse IgG (1:200; Friendship Biotechnology, Beijing, China) at room temperature for 1 hour, and washed three times with 0.3% Triton X-100/PBS,

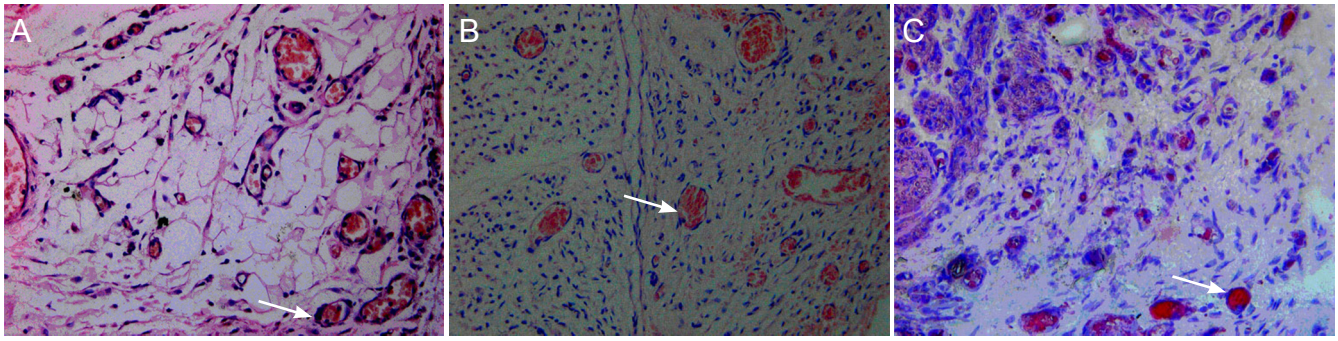


Figure 4 Microvascular changes in the sciatic nerve bundle in the biological conduit in rats after repair by 2-mm small gap sleeve bridging method (hematoxylin-eosin staining, × 200).
(A–C) At 7, 28 and 56 days after model establishment, interstitial edema between the sciatic nerve disappeared and the number of capillary vessels increased with prolonged time. Arrows show capillary vessels.

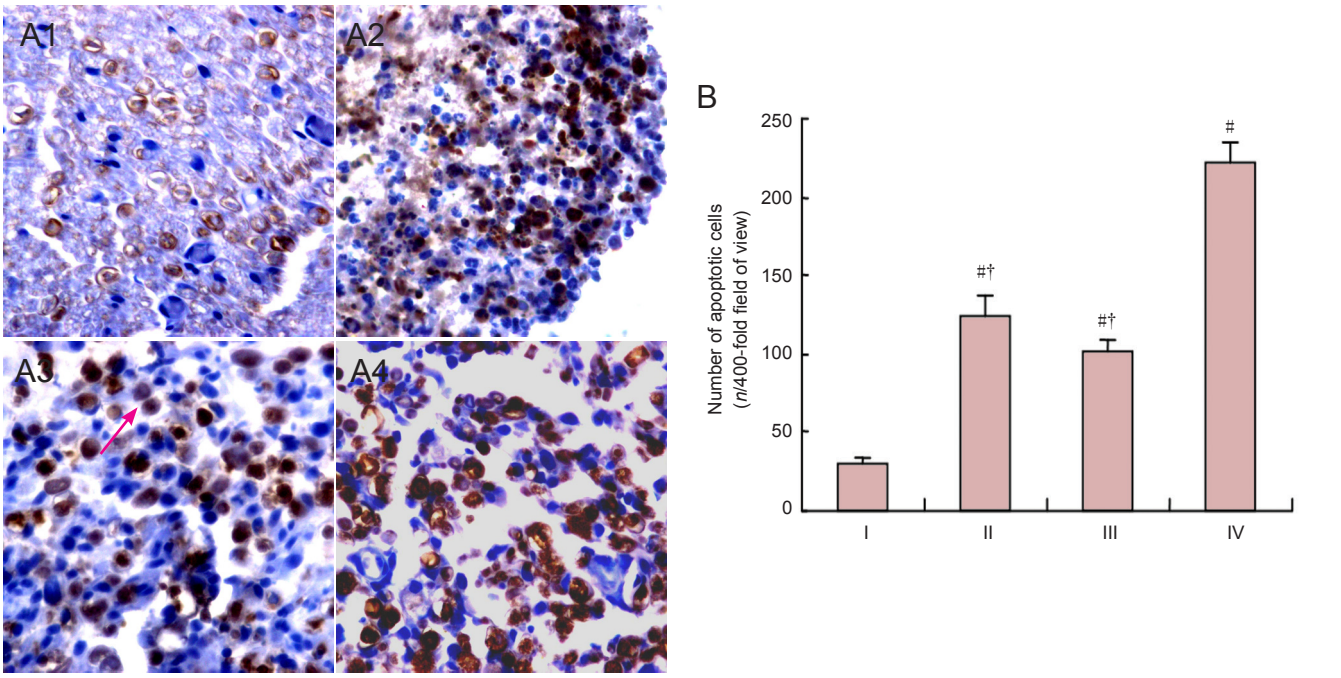


Figure 6 Cell apoptotis inside and outside the biological conduit for repairing sciatic nerve injury by the 2-mm small gap sleeve bridging method at 3 days after model establishment (× 400).
(A) Morphology of apoptotic cells inside and outside the biological conduit (TUNEL staining). (A1–4) Normal sciatic nerve, proximal end of the conduit, proximal end of the injured sciatic nerve, and distal end of the injured sciatic nerve. Arrow shows apoptotic cells. (B) Number of apoptotic cells. Data are expressed as the mean ± SD. One rat was used in the normal group, and six rats were used in the other groups. The difference was compared using one-way analysis of variance and an independent-sample *t*-test. ♯$P < 0.05$, *vs.* normal sciatic nerve; †$P < 0.05$, *vs.* distal end of injured sciatic nerve. I: Normal sciatic nerve; II: Proximal end of the conduit: III: Proximal end of the injured sciatic nerve; IV: Distal end of the injured sciatic nerve.

each for 5 minutes. These sections were then mounted with a fluorescent mounting medium (Fluoro-Gel; Head (Beijing) Biotechnology Co., Ltd., Beijing, China) and observed with a fluorescence microscope (Olympus). Immunofluorescence images were overlayed using Photoshop software CS2 V9.0 (Adobe, San Jose, CA, USA) (superposition of red and green). Red fluorescence represents axons: the primary antibody is NF, and the secondary antibody is Cy2 conjugated rabbit anti-mouse IgG; green fluorescence represents Schwann cells: the primary antibody is glial fibrillary acidic protein, and the secondary antibody is Cy3-conjugated rabbit anti-mouse IgG. Schwann cells and axons were quantified in nerve specimens within the cross-section range using Image-pro Plus 6.0 software (Media Cybernetics, Bethesda, MD, USA).

Cell apoptosis

Cell apoptosis was measured with a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) apoptosis

detection kit (Head (Beijing) Biotechnology Co., Ltd.). As shown in **Figure 2**, frozen sections of rat sciatic nerve were pretreated and washed with PBS. Two drops of TdT enzyme buffer were dripped on each section at room temperature for 5 minutes. TdT enzyme reaction mixture was added in a wet box at 37°C for 1 hour. The reaction-terminated liquid was added on sections in a dye vat. After washing with PBS, sections were treated with peroxidase-labeled digoxin antibody at room temperature for 30 minutes, and visualized with 0.05% 3,3′-diaminobenzidine at room temperature. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole. Results were observed under a light microscope. The number of apoptotic cells was calculated with Image-pro Plus 6.0 software at 400× magnification.

Statistical analysis

The data were expressed as the mean ± SD, and analyzed using SPSS 17.0 software (SPSS, Chicago, IL, USA). The differences at different time points were compared using one-way analysis of variance and independent-sample *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

General conditions of models of sciatic nerve injury repaired by 2-mm small gap sleeve bridging of biological conduit

At 28 days after injury, localized swelling was visible at sciatic nerve suture sites in rats of the control group. The wall of the biological conduit was transparent in rats of the sleeve bridging group, with an evident vascular network on its surface, which showed that the biological conduit had good biocompatibility. The conduit did not apparently adhere to surrounding tissues. No obvious neuroma formed (**Figure 3**).

Histomorphology of rat models of sciatic nerve injury repaired by 2-mm small gap sleeve bridging of biological conduit

Blood vessels

Hematoxylin-eosin staining revealed that edema below the sciatic nerve epineurium and telangiectasia were observed in the conduit at 7 days after injury (**Figure 4A**). At 28 days after injury, interstitial edema between the sciatic nerve disappeared and the number of capillary vessels was increased (**Figure 4B**). At 56 days, abundant newly born small capillaries were observed, as shown in **Figure 4C**.

Nerve fibers

Hematoxylin-eosin staining revealed that at 7 days after injury, Wallerian degeneration was visible in nerve fibers in the distal conduit after repair with 2-mm small gap sleeve bridging of the biological conduit. Nerve fibers were distorted and disorganized, and the axons and myelin sheath disintegrated. A small section of nerve fibers extended to the distal end across the gap in the conduit (**Figure 5**).

Cell apoptosis inside and outside the biological conduit in rat models after repair by sleeve bridging method

At 3 days after injury, compared with the control group, the number of apoptotic cells was significantly higher in the sites proximal and distal to the injured sciatic nerve and the region proximal to the conduit in the sleeve bridging group ($P < 0.05$). In the sleeve bridging group, compared with the distal end of the injured site, the number of apoptotic cells was significantly less in the sites proximal to the injured sciatic nerve and the conduit ($P < 0.05$; **Figure 6**).

Changes in Schwann cells and axons in the region with sciatic nerve injury in rat models after repair by biological conduit small gap sleeve bridging method

Proximal end of injured sciatic nerve

Immunofluorescence staining demonstrated that the morphology of Schwann cells and axons was similar at the proximal end of the injured nerve in the control group and sleeve bridging group, with uniform size, regular arrangement and high density at the time of nerve injury. As time increased, the number of Schwann cells and axons gradually decreased at the proximal end of the injured sciatic nerve in the sleeve bridging group, and was significantly different from the control group at 5 and 7 days ($P < 0.01$). The number of Schwann cells and axons in the proximal end of the injured sciatic nerve of rats from the control group was increased at 3–5 days, peaked at 5 days, and then decreased, reached its lowest point at 7 days, peaked at 14 days, and then reduced, but it was significantly lower than the sleeve bridging group at 28 days ($P < 0.01$). The difference in the change curves of the number of Schwann cells and axons in the proximal end of the injured sciatic nerve is possibly one factor in regeneration differences between the conduit suture and the epineurium suture (**Figure 7**).

Distal end of injured sciatic nerve

Immunofluorescence staining performed at 5 days after injury revealed that the axons and myelin were scattered, and the nerve fibers were in the disintegration stage. The number of complete nerve fibers was few, and nerve fibers were irregular in the distal end of the injured nerve in both the control and sleeve bridging groups. At 28 days, the number of Schwann cells and axons increased and were tightly distributed, and abundant newly born tiny myelinated nerve fibers were observed in the distal end of the injured sciatic nerve in both groups. The number of axons in the sleeve bridging group gradually diminished with prolonged time, reached its lowest point at 5 days, and then increased gradually, and became significantly greater than the control group at 28 days ($P < 0.01$). Nevertheless, the number of Schwann cells was lower in the sleeve bridging group than that in the control group at 5 days after injury ($P < 0.01$), gradually increased, and was significantly higher than the control group at 28 days ($P < 0.01$; **Figure 8**).

Discussion

Common methods for repairing peripheral nerve injury use epineurium or perineurium suture (Schmidt et al., 1997; Alluin et al., 2009; Singh et al., 2012; Farjah et al., 2013). Functional recovery after peripheral nerve repair mainly depends

on two factors: (1) effective abutment of the proximal and distal ends of the nerve fibers with different natures (Cheng and Zochodne, 2002; Biazar and Keshel, 2013; Pabari et al., 2014; Sachanandani et al., 2014). (2) Effective reinnervation of distal target organs (Gigo-Benato et al., 2010; Zhao et al., 2013). The outcomes of biological conduit small gap sleeve bridging have been shown to be better than those using epineurium suture (Zhang et al., 2008, 2009, 2011, 2013). This study demonstrated that histocompatibility was satisfactory, and no common neuroma was detected at stumps after repair with the biological conduit. The biological conduit probably plays a mechanical guiding role, guiding the growth of regenerating axons at the proximal end to the distal end, and preventing the escape of regenerating axons. In the sleeve bridging and control groups, the regeneration laws of Schwann cells and axons were slightly different. During the prime nerve fiber regeneration time (2–8 weeks) after repair, the number of Schwann cells and nerve fibers was higher in the sleeve bridging group than in the control group. At 3 days, the number of apoptotic cells was higher at the distal and proximal ends of the conduit and within the conduit than that in the control group, suggesting that the microenvironment of the conduit effectively promoted the removal of necrotic cells and tissues in the early stage of nerve injury. Additionally, the above local microenvironment is more conducive to regenerating the peripheral nerve.

Peripheral nerve injury causes complicated changes in the proximal and distal ends of the nerve. Wallerian degeneration was observed at the distal end. Schwann cell proliferation formed Büngner's band, provided channel for the axonal growth of regenerating motor neurons, and secrected various extracellular matrix components related to nerve regeneration (Huang et al., 2010b; Wang et al., 2010). In the control group (traditional epineurium suture), the proliferation time of Schwann cells in the proximal and distal ends of the conduit appeared early (day 5), and the number of Schwann cells was noticeably greater than in the sleeve bridging group. Nevertheless, 2 weeks later, the number of Schwann cells at the proximal and distal ends of the conduit was higher than in the epineurium suture group. Based on previous research findings and clinical experience analysis, there is a significant delay of about 1 week in early regeneration, and ≥ 2 weeks is the prime regeneration time after peripheral nerve repair. The number of Schwann cells and axons was significantly higher in the biological conduit group than in the epineurium suture group within 3–8 weeks of repair (Sun et al., 2002a, b, c, d), which may be the reason why the effects of nerve regeneration at the distal end in the biological conduit group was better than that in the control group (Jiang et al., 2007).

The results of this study confirmed that during the prime time of regeneration (2–8 weeks), not only the number of Schwann cells, but the number of regenerating axons was significantly higher in the biological conduit group than in the epineurium suture group. Previous studies verified that the number of axons and the axonal cross-sectional area were greater than that at the proximal end of the injured nerve, which has also been called multiple-bud regeneration, *i.e.*, multiple amplification, in peripheral nerve regeneration (Jiang et al., 2007; Wang et al., 2014; Zhang et al., 2014). The relatively closed regenerative microenvironment constructed by the 2-mm small gap sleeve bridging of the biological conduit was adequate to allow multiple-bud axonal regeneration (Liang et al., 2014; Yan et al., 2014), which may be a reason why repair effects are better than when using the traditional epineurium suture.

In summary, this study first compared the change trends of the number of Schwann cells and axons at the distal and proximal ends after peripheral nerve repair using a biological conduit sleeve bridging method, which provides a solid theoretical foundation for the use of biological conduit small gap sleeve bridging to treat peripheral nerve injury.

Author contributions: *NH and YHK participated in study concept and design. PXZ and BGJ were responsible for fund-raising. LYA provided the data and performed experiments. PXZ wrote the manuscript. FX was in charge of manuscript authorization. XFY and TBW anticipated in statistical analysis. All authors approved the final version of the paper.*
Conflicts of interest: *None declared.*

References

Alluin O, Wittmann C, Marqueste T, Chabas JF, Garcia S, Lavaut MN, Guinard D, Feron F, Decherchi P (2009) Functional recovery after peripheral nerve injury and implantation of a collagen guide. Biomaterials 30:363-373.

Biazar E, Keshel SH (2013) Chitosan-cross-linked nanofibrous PHBV nerve guide for rat sciatic nerve regeneration across a defect bridge. ASAIO J 59:651-659.

Cheng C, Zochodne DW (2002) In vivo proliferation, migration and phenotypic changes of Schwann cells in the presence of myelinated fibers. Neuroscience 115:321-329.

Farjah GH, Heshmatian B, Karimipour M, Saberi A (2013) Using eggshell membrane as nerve guide channels in peripheral nerve regeneration. Iran J Basic Med Sci 16:901-905.

Gigo-Benato D, Russo TL, Geuna S, Domingues NRSR, Salvini TF, Parizotto NA (2010) Electrical stimulation impairs early functional recovery and accentuates skeletal muscle atrophy after sciatic nerve crush injury in rats. Muscle Nerve 41:685-693.

Huang J, Ye Z, Hu X, Lu L, Luo Z (2010a) Electrical stimulation induces calcium-dependent release of NGF from cultured Schwann cells. Glia 58:622-631.

Huang J, Lu L, Hu X, Ye Z, Peng Y, Yan X, Geng D, Luo Z (2010b) Electrical stimulation accelerates motor functional recovery in the rat model of 15-mm sciatic nerve gap bridged by scaffolds with longitudinally oriented microchannels. Neurorehabil Neural Repair 24:736-745.

Jiang B, Zhang P, Jiang B (2010) Advances in small gap sleeve bridging peripheral nerve injury. Artif Cells Blood Substit Immobil Biotechnol 38:1-4.

Jiang B, Zhang P, Zhang D, Fu Z, Yin X, Zhang H (2006) Study on small gap sleeve bridging peripheral nerve injury. Artif Cells Blood Substit Immobil Biotechnol 34:55-74.

Jiang BG, Yoshida Y (1998) The histological observation after small gap artery bridging inperipheral nerve. Zhonghua Shou Waike Zazhi 14:50-52.

Jiang BG, Li J (2003) Replacing the traditional neural epineurium suture-study on small gap bridging peripheral nerve injury. Zhongguo Jiaoxing Waike Zazhi 11:544-546.

Jiang BG, Wang SH, Feng CH (1994) The comparison of small gap artery bridging and epineural neurorrhaphy in peripheral nerve. Beijing Yike Daxue Xuebao 26:249-250.

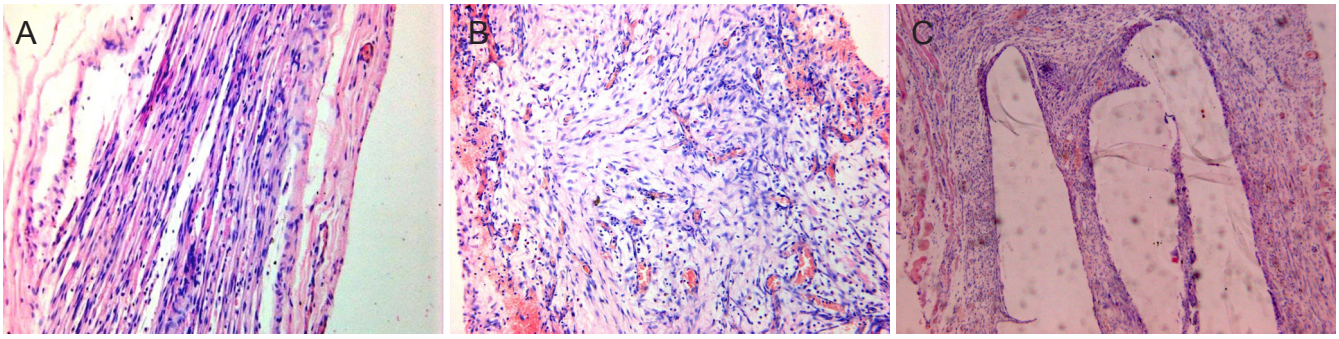


Figure 5 Alterations in sciatic nerve in the rat sciatic nerve bundle at 7 days after repair using 2-mm small gap sleeve bridging of the biological conduit (hematoxylin-eosin staining).
(A, B) Proximal and distal ends of sciatic nerve (× 100); (C) nerve in the conduit (× 50). A few nerve fibers extended to the distal end across the biological conduit.

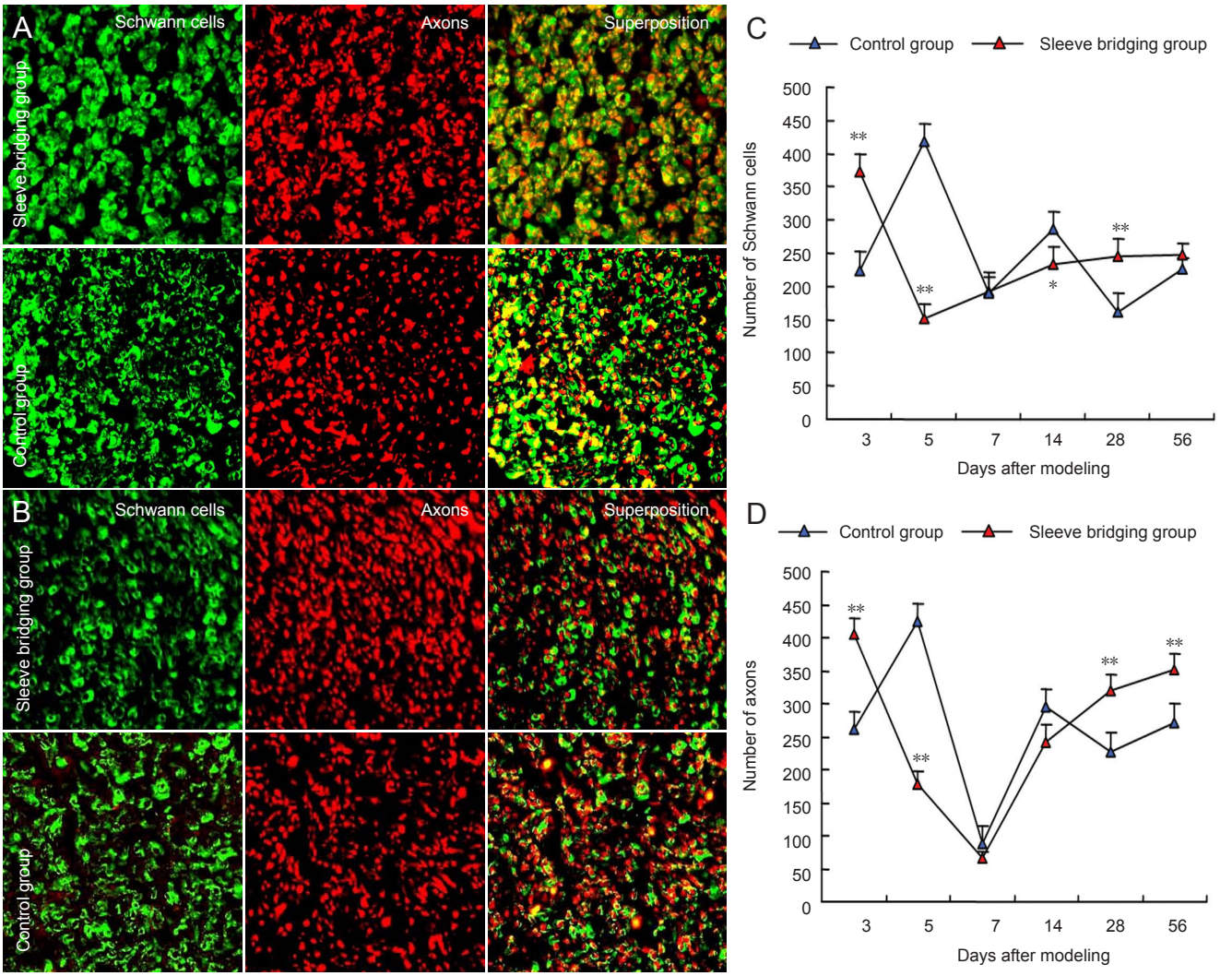


Figure 7 Effects of 2-mm small gap sleeve bridging of the biological conduit on Schwann cells and axons in the proximal end of the rat models of sciatic nerve injury.
(A, B) Morphology of Schwann cells and axons at the proximal end of the injured nerve in rats at 5 and 14 days after model establishment (immunofluorescence staining, × 400). Positive expression of neurofilament shows red fluorescence, stain is Cy2, representing axons; positive expression of glial fibrillary acidic protein shows green fluorescence, stain is Cy3, representing Schwann cells. (C, D) Alterations in the number of Schwann cells and axons in the proximal end of the injured nerve of rats. Data are expressed as the mean ± SD. Six rats were used in each group. The difference was compared using one-way analysis of variance and independent-sample *t*-test. $*P < 0.05$, $**P < 0.01$, *vs.* control group.

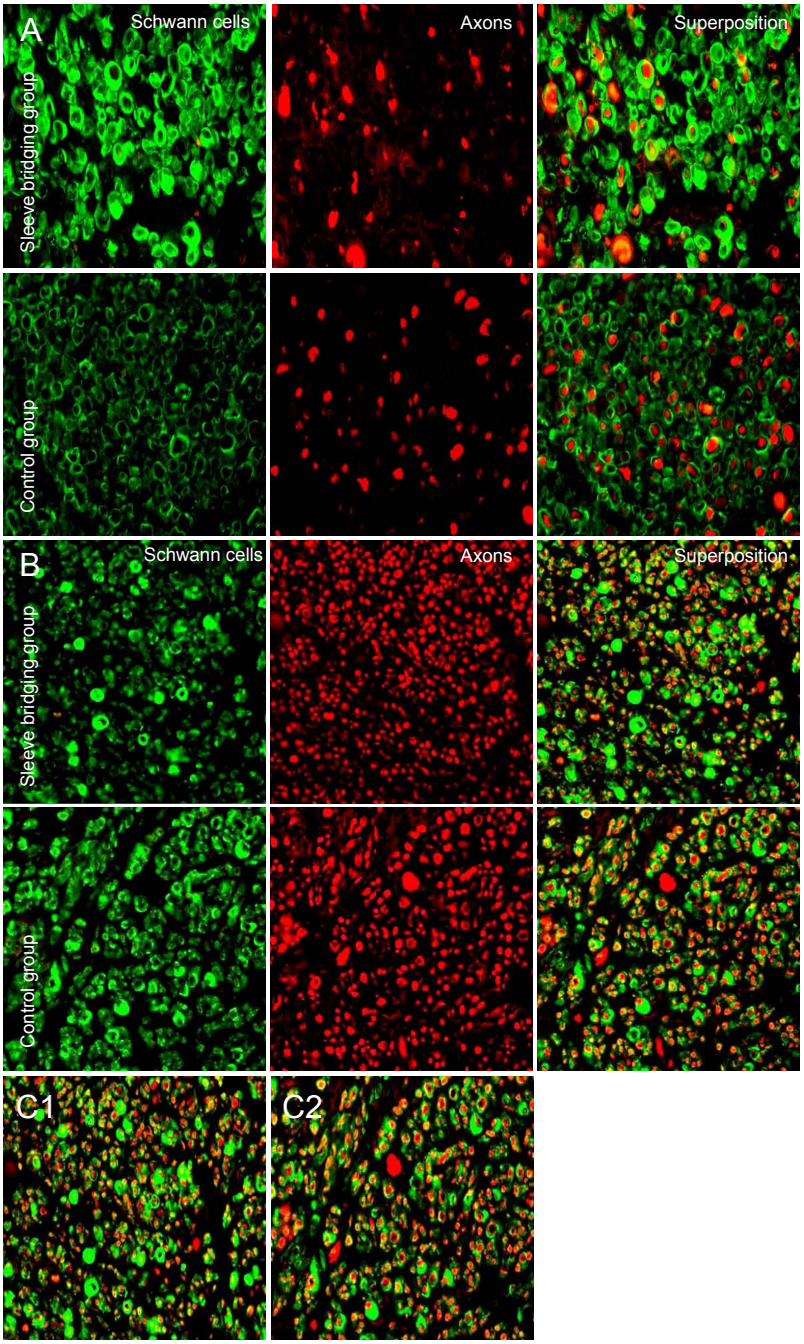
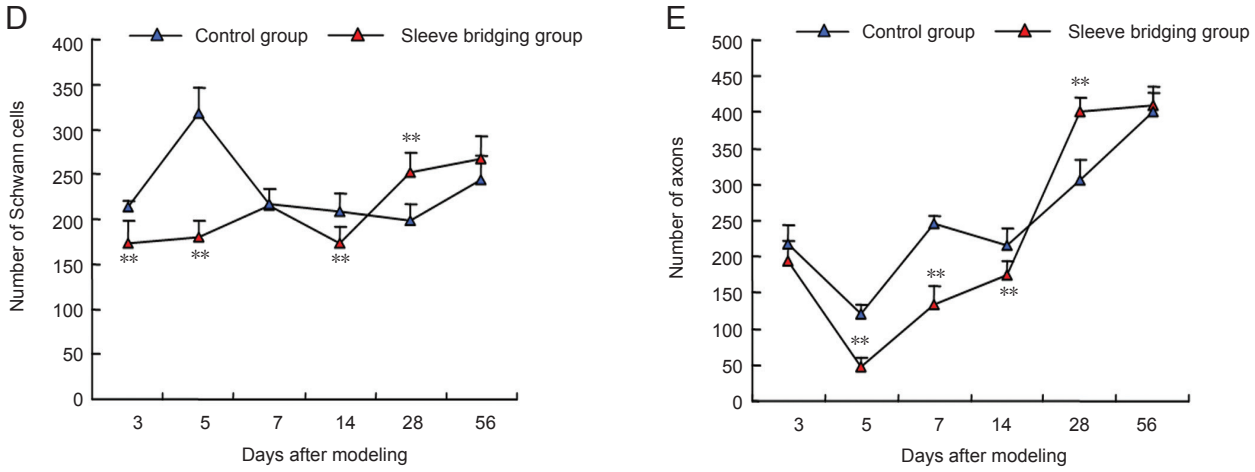


Figure 8 Effects of 2-mm small gap sleeve bridging of the biological conduit on Schwann cells and axons in the distal end of the rat models of sciatic nerve injury.

(A, B) Morphology of Schwann cells and axons in the distal end of injured nerve in rats at 5 and 28 days after model establishment (immunofluorescence staining, × 400). (C1, 2) Superposition of Schwann cells and axons in the distal end of injured nerve in sleeve bridging group and control group rats at 56 days after modeling (immunofluorescence staining, × 400). Positive expression of neurofilament shows red fluorescence, stain is Cy2, representing axons; positive expression of glial fibrillary acidic protein shows green fluorescence, stain is Cy3, representing Schwann cells. (D, E) Alterations in the number of Schwann cells and axons in the distal end of injured nerve of rats. Data are expressed as the mean ± SD. Six rats were used in each group. The difference was compared using one-way analysis of variance and independent-sample *t*-test. $^{**}P < 0.01$, *vs.* control group.



Jiang BG, Yin XF, Zhang DY, Fu ZG, Zhang HB (2007) Maximum number of collaterals developed by one axon during peripheral nerve regeneration and the influence of that number on reinnervation effects. Eur Neurol 58:12-20.

Li Q, Wang JW, Zeng BF (2007) Progress of neuromuscular electrical stimulation on denervated muscle atrophy. Zhongguo Kangfu Yixue Zazhi 22:478-480.

Liang XD, Cai HF, Hao YY, Sun G, Song YY, Chen W (2014) Sciatic nerve repair using adhesive bonding and a modified conduit. Neural Regen Res 9:594-601.

Lundborg G (2000) A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. J Hand Surg Am 25:391-414.

Mohammadi R, Heydarian H, Amini K (2014) Effect of local administration of cyclosporine A on peripheral nerve regeneration in a rat sciatic nerve transection model. Chin J Traumatol 17:12-18.

Pabari A, Lloyd-Hughes H, Seifalian AM, Mosahebi A (2014) Nerve conduits for peripheral nerve surgery. Plast Reconstr Surg 133:1420-1430

Sachanandani NF, Pothula A, Tung TH (2014) Nerve gaps. Plast Reconstr Surg 133:313-319

Schmidt CE, Shastri VR, Vacanti JP, Langer R (1997) Stimulation of neurite outgrowth using an electrically conducting polymer. Proc Natl Acad Sci U S A 94:8948-8953.

Singh B, Xu QG, Franz CK, Zhang R, Dalton C, Gordon T, Verge VMK, Midha R, Zochodne DW (2012) Accelerated axon outgrowth, guidance, and target reinnervation across nerve transection gaps following a brief electrical stimulation paradigm. J Neurosurg 116:498-512.

Sun YS, Jiang BG, Zhu QS, Fu ZG, Lin HT, Zhang DY, Wang Y (2002a) Experimental study of functional bio-medical materials in animals: resorbable research in vivo. Fangzhi Kexue Yanjiu 13:7-10,14.

Sun YS, Jiang BG, Zhu QS, Fu ZG, Lin HT, Zhang DY, Wang Y (2002b) Experimental study of functional bio-medical materials in animals: hemostatic effect research in vivo. Fangzhi Kexue Yanjiu 13:11-14.

Sun YS, Jiang BG, Zhu QS, Fu ZG, Lin HT, Zhang DY, Wang Y (2002c) Experimental study of functional bio-medical materials in animals: anti-tendon adhesion membrane research. Fangzhi Kexue Yanjiu 13:15-19.

Sun YS, Jiang BG, Zhu QS, Fu ZG, Lin HT, Zhang DY, Wang Y (2002d) Experimental study of functional biomedical materials in animals: anti-tendon adhesion research. Fangzhi Kexue Yanjiu 13:20-22.

Wang J, Zhang P, Wang Y, Kou Y, Zhang H, Jiang B (2010) The observation of phenotypic changes of Schwann cells after rat sciatic nerve injury. Artif Cells Blood Substit Immobil Biotechnol 38:24-28.

Wang Z, Han N, Wang J, Zheng H, Peng J, Kou Y, Xu C, An S, Yin X, Zhang P, Jiang B (2014) Improved peripheral nerve regeneration with sustained release nerve growth factor microspheres in small gap tubulization. Am J Transl Res 18:413-421.

Yan H, Zhang F, Kolkin J, Wang C, Xia Z, Fan C (2014) Mechanisms of nerve capping technique in prevention of painful neuroma formation. PLoS One 9:e93973.

Yu K, Zhang C, Wang Y, Zhang P, Zhang D, Zhang H, Jiang B (2009) The protective effects of small gap sleeve in bridging peripheral nerve mutilation. Artif Cells Blood Substit Immobil Biotechnol 37:257-264.

Zhang C, Zhang P, Wang Y, Yu K, Kou Y, Jiang B (2010) Early spatiotemporal progress of myelinated nerve fiber regenerating through biological chitin conduit after injury. Artif Cells Blood Substit Immobil Biotechnol 38:103-108.

Zhang P, Yin X, Kou Y, Wang Y, Zhang H, Jiang B (2008) The electrophysiology analysis of biological conduit sleeve bridging rhesus monkey median nerve injury with small gap. Artif Cells Blood Substit Immobil Biotechnol 36:457-463.

Zhang P, Zhang C, Kou Y, Yin X, Zhang H, Jiang B (2009) The histological analysis of biological conduit sleeve bridging rhesus monkey median nerve injury with small gap. Artif Cells Blood Substit Immobil Biotechnol 37:101-104.

Zhang P, Kou Y, Yin X, Wang Y, Zhang H, Jiang B (2011) The experimental research of nerve fibers compensation amplification innervation of ulnar nerve and musculocutaneous nerve in rhesus monkeys. Artif Cells Blood Substit Immobil Biotechnol 39:39-43.

Zhang P, Han N, Wang T, Xue F, Kou Y, Wang Y, Yin X, Lu L, Tian G, Gong X, Chen S, Dang Y, Peng J, Jiang B (2013) Biodegradable conduit small gap tubulization for peripheral nerve mutilation: a substitute for traditional epineurial neurorrhaphy. Int J Med Sci 10:171-175.

Zhang ZL, Li X, Zuo SJ, Xin J, Zhang PX (2014) Bridging peripheral nerves using a deacetyl chitin conduit combined with short-term electrical stimulation. Neural Regen Res 9:1075-1078.

Zhao F, He W, Zhang YZ, Tian DH, Zhao HF, Yu KL, Bai JB (2013) Electric stimulation and decimeter wave therapy improve the recovery of injured sciatic nerves. Neural Regen Res 8:1974-1984.

Copyedited by Jackson C, Frenchman B, Yu J, Qiu Y, Li CH, Song LP, Zhao M