Neural Stem Cells Enhance Nerve Regeneration after Sciatic Nerve Injury in Rats

Lin Xu · Shuai Zhou · Guo-Ying Feng · Lu-Ping Zhang · Dong-Mei Zhao · Yi Sun · Qian Liu · Fei Huang

Abstract With the development of tissue engineering and the shortage of autologous nerve grafts in nerve reconstruction, cell transplantation in a conduit is an alternative strategy to improve nerve regeneration. The present study evaluated the effects and mechanism of brain-derived neural stem cells (NSCs) on sciatic nerve injury in rats. At the transection of the sciatic nerve, a 10-mm gap between the nerve stumps was bridged with a silicon conduit filled with 5×10^5 NSCs. In control experiments, the conduit was filled with nerve growth factor (NGF) or normal saline (NS). The functional and morphological properties of regenerated nerves were investigated, and expression of hepatocyte growth factor (HGF) and NGF was measured. One week later, there was no connection through the conduit. Four or eight weeks later, fibrous connections were evident between the proximal and distal segments. Motor function was revealed by measurement of the sciatic functional index (SFI) and sciatic nerve conduction velocity (NCV). Functional recovery in the NSC and NGF groups was significantly more advanced than that in the NS group. NSCs showed significant improvement in axon myelination of the regenerated nerves. Expression of NGF and HGF in the injured sciatic nerve was significantly lower in the NS group than in the NSCs and NGF groups. These results and other advantages of NSCs, such as ease of harvest and relative abundance, suggest that NSCs could be used clinically to enhance peripheral nerve repair.

Keywords Sciatic nerve · Neural stem cells · Nerve repair · Rats

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Introduction

Peripheral nerve injury is an important medical condition because patients are often at the peak of their employment productivity and any loss or decrease of function is particularly devastating [1]. As a result of huge clinical demand, peripheral nerve regeneration has become a prime focus of research. Accelerating axonal regeneration to promote reinnervation and improve functional recovery after peripheral nerve injury is a clinical necessity and an experimental challenge.

After nerve injury, peripheral axons have the ability to regenerate and, given a proper pathway, reconnect with their targets. Despite this capacity, the functional outcome is often poor mainly because severed peripheral nerves may be too distant from their targets to re-establish a connection [2]. Functional recovery depends on the slow process of regeneration and on correct placement of injured axons [3]. In clinical practice, the ideal repair of nerve injury is to perform an end-to-end coaptation of proximal and distal stumps to achieve a tension-free repair. Autologous donor nerves are the gold standard to bridge peripheral defects by producing a guideline and biological environment for nerve regeneration [4]. However, autograft harvest requires a second operative site, with sacrifice of a functional nerve, resulting in donor sensory loss, potential formation of neuroma, and neuropathic pain [5, 6]. Limited

sources of nerve grafts, mismatches between host and donor nerves, morbidity of donor area, and neuroma formation as complications of nerve graft procedure have prompted evaluation of alternative methods for nerve gap reconstruction [7]. When a nerve graft bridges a nerve defect, the graft functions as a conduit for regenerating axons. Hence, the development of tissue-engineered alternatives: nerve conduits are tubular structures designed to bridge the gap of a sectioned nerve, protect the nerve from scar formation, and guide regenerating fibers into the distal nerve stump [5, 8]. Attempts to develop conduits of synthetic or biodegradable materials, which could guide regeneration of axons without inhibiting growth and maturation [9], have not yet been effective clinical alternatives to autograft.

The regenerative potential of the peripheral nervous system (PNS) results from interplay between growth factors, cellular elements (Schwann cells) and their basal lamina, and extracellular matrix proteins [10–12]. NSCs produce many neurotrophic factors [13, 14] that can improve peripheral regeneration in vivo [15–17]. With growing applicability of stem cells in all avenues of medicine, new opportunities have become available for treating degenerative and traumatic nerve injuries [18, 19].

This study investigated the effects of NSCs on hindlimb motor function recovery and the morphological parameters of nerves in rats subjected to sciatic nerve transection. The results provide an experimental and theoretical basis for using NSCs for clinical treatment of peripheral nerve injury.

Materials and Methods

Experimental Animals

We used adult male Sprague–Dawley rats weighing 200–220 g, provided by the Experimental Animal Center of Yantai Greenery (ACYG; Yantai, China). All rats were fed a normal diet and given water freely, and were kept in cages at least 2 days prior to the experiments. They were fasted for 12 h before the experiments, but were given free access to water. All the experiments were conducted in compliance with the animal welfare guidelines of the ethical committee of ACYG.

Cell Culture and Conduit Preparation

NSCs were isolated from embryonic rats at gestational day 15 and maintained in Dulbecco's modified Eagle's medium: Nutrient Mixture F-12 (DMEM/F-12; Invitrogen, UK) plus GlutaMAX (Invitrogen) containing 1% penicillin–streptomycin and supplemented with basic fibroblast growth factor (bFGF; 20 ng/ml; Sigma–Aldrich, St. Louis, MO, USA), epidermal growth factor (EGF; 20 ng/ml; Sigma–Aldrich), and 2% Gibco B-27 Supplement (Invitrogen). NSCs were passaged to the third generation. The character of the NSCs was confirmed by testing for nestin expression, followed by culture with 10% fetal bovine serum and withdrawal of bFGF, EGF, and B-27, using β-tubulin to test differentiation capability. The conduits were immersed in 75% alcohol for 30 min and then sterilized under a Vita-light lamp for 30 min before utilization.

Experimental Design and Surgical Procedure

Three experimental groups were included: NS group, conduit filled with NS; NSCs group, conduit seeded with NSCs; and NGF group, conduit seeded with NGF. In all groups (18 animals in each), the conduits were left in place for 1, 4, or 8 weeks, and subsequently, conduits were harvested together with the proximal and distal nerve stumps. A few hours before implantation in the rats, NSCs were trypsinized and, after centrifugation, 5×10^5 cells were suspended in 20 μl growth medium and injected into the tubes. The conduit with NGF (Sigma–Aldrich) contained 20 μl NGF (1 ng/μl). The conduit seeded with NS contained 20 μl sterile NS (0.9%). A microinjection pump (L0107-1A; Huaibei Zhenghua Bioinstrumentation Co., Ltd., China) was used to seed cells in the silicon conduit (length 10 mm, inner diameter 0.7 mm). The entire procedure was performed under sterile conditions at room temperature (25°C).

The operation was performed on the right sciatic nerve under aseptic conditions using a power-focus surgical microscope (LZL-6A; Zhenjiang Zhongtian Optical Instrument Co., Ltd., China). A skin incision from the right knee to the hip was made for exposure of the underlying muscles, which were then retracted to reveal the sciatic nerve, as described previously [20]. The sciatic nerve was transected and nerve ends were fixed to the conduit by a single epineural suture (9/0). After meticulous dissection of the sciatic nerve, a 1-cm segment was excised and proximal and distal nerve stumps were inserted into the tube, thus leaving a 10-mm gap. Muscles and fascia layers were closed with single resorbable stitches (4/0) and the skin was closed by a continuous running suture (4/0). All experimental groups were housed on sawdust, one animal per cage, with a 12-h light/dark cycle (lights on at 06.00 h) and received food and water ad libitum.

Functional Analysis: Sciatic Nerve Function Index (SFI)

Four weeks following sciatic nerve transection, all animals were subjected to a series of weekly motor activity assessments. Recovery of activity was considered proof of adequate post-nerve crush reinnervation of the right hindlimb, and functional recovery was monitored by analysis of the free-walking pattern. This method [21] describes an index based on measurements of the footprints of walking rats, which provides a reliable and easily quantifiable method of evaluating the functional condition of the sciatic nerve. For this test, the rats were trained to walk over a white sheet of paper covering the bottom of a 100-cm-long, 8.5-cm-wide track, which ended in a dark box.

Afterwards, the animals had their plantar hind feet painted with dark dye and were placed on the track to walk.

The rat footprints were used to determine the following measurements: distance from the heel to the third toe [print length (PL)], distance from the first to the fifth toe [toe spread (TS)], and distance from the second to the fourth toe [intermediary toe spread (ITS)]. These three measurements were obtained from both the experimental (E) and normal (N) sides of the animal. Several prints of each foot were obtained on each track, but only three prints of each foot were used to determine the mean measurements in the E and N sides. These mean measurements were then included in the SFI formula: $SFI = -38.3 \ (EPL-NPL)/NPL + 109.5 \ (ETS-NTS)/NTS + 13.3 \ (EIT-NIT)/NIT - 8.8$. The result obtained was considered a functional index of the sciatic nerve, where 0 to −12 represented excellent function, −13 to −99 indicated partial recovery of neurological function, and −100 represented complete deficit of nerve function [22].

Functional Analysis: Nerve Conduction Velocity (NCV)

The length of the sciatic nerve reached about 4 cm once the rats were euthanized. The sciatic NCV was detected by stimulating both proximally and distally to the regenerated nerve polygraph (ASB240U; Chengdu Aosheng Electronics Co., Ltd., China). The NCV record was obtained as described by Yao Hong-ping [23].

Tissue Harvesting and Histological Observation

After 4 or 8 weeks of conduit implantation, rats were anesthetized with chloral hydrate solution (3.5%, 1 ml/100 g, i.p.). The regenerated right sciatic nerves were harvested under an operating microscope (SZX7; Olympus, Japan) together with proximal and distal stumps. Nerve samples were soaked in 4% paraformaldehyde for 12 h, rinsed under running water for 12 h, dehydrated in gradient alcohol, paraffin embedded, cut into consecutive 4-μm-thick slices, stained with hematoxylin and eosin (H&E), and observed under a light microscope (BX51; Olympus).

Transmission Electron Microscopy (TEM)

The right sciatic nerve was isolated and excised. The proximal segments and regenerated nerves were immersed in fixative solution containing 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) at room temperature for 2 h, then post-fixed in 1% OsO_4 in PB, dehydrated in a graded series of alcohol and acetone, infiltrated in Poly/Bed 812 resin, and polymerized at 60°C for 72 h. Semithin cross-sections for light microscopy were cut at a thickness of 500 nm and stained in toluidine blue. Ultrathin cross-sections for TEM (GEM1400, JEOL Ltd.) were cut at a thickness of 60–70 nm, collected on copper
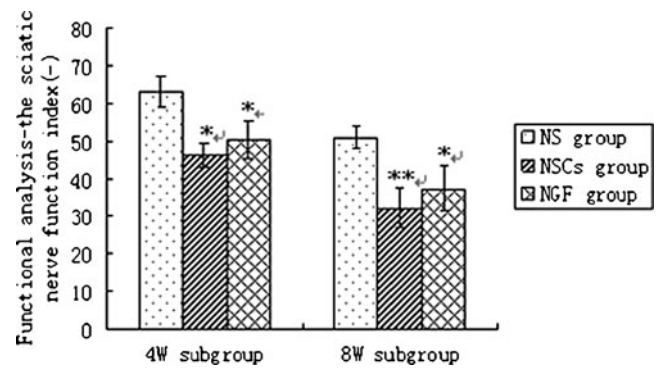

Fig. 1 Functional analysis of the sciatic nerve. SFI was calculated for each animal at 4 weeks (*4W*) and 8 weeks (*8W*) following nerve transection (six animals per group at each time point). The mean SFI±SD (*error bars*) of each group at each time point is displayed in the graph. Groups are indicated according to the treatment received via conduit implant after nerve transection. Note that the units on the *y* axis represent negative values, with −100 representing complete loss of function and 0 representing full normal function. *$P < 0.05$ versus NS group; **$P < 0.01$ versus NS group

grids, and contrast-stained in uranyl acetate and lead citrate. Images of the proximal and regenerated portions of the right sciatic nerve from six rats in each group were captured.

Immunohistochemical Staining

PV-6001 Two-Step Immunohistochemistry (Zhongshan Golden Bridge Biotechnology Co., Ltd., China) was used after deparaffinizing samples. The sections were treated with 3% H_2O_2 and antigen retrieval by microwave, submerged in rabbit NGF and HGF antiserum at 4°C for 12 h, goat anti-rabbit IgG antibody–horseradish peroxidase polymer at 37°C for 2 h, stained by diaminobenzidine, and sealed by neutral gum. Negative controls for immunostaining were included by
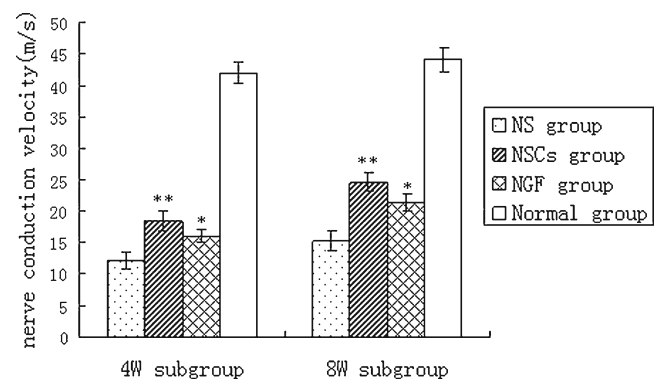

Fig. 2 NCV analysis of the sciatic nerve. NCV was calculated for each animal at 4 weeks (*4W*) and 8 weeks (*8W*) following nerve transection (six animals per group at each time point). The mean NCV±SD (*error bars*) of each group at each time point is displayed in the graph. Groups are indicated according to the legend for Fig. 1, with the addition of a normal group, which gave values for normal, unmanipulated control mice. Units on the *y* axis represent meters per second. *$P < 0.05$ versus NS group; **$P < 0.01$ versus NS group

Fig. 3 Representative photos at 8 weeks following transection of a regenerated nerve in the conduit from the NS group (**a**), NSCs group (**b**), and NGF group (**c**). The images show the connecting fibers in the conduit as indicated
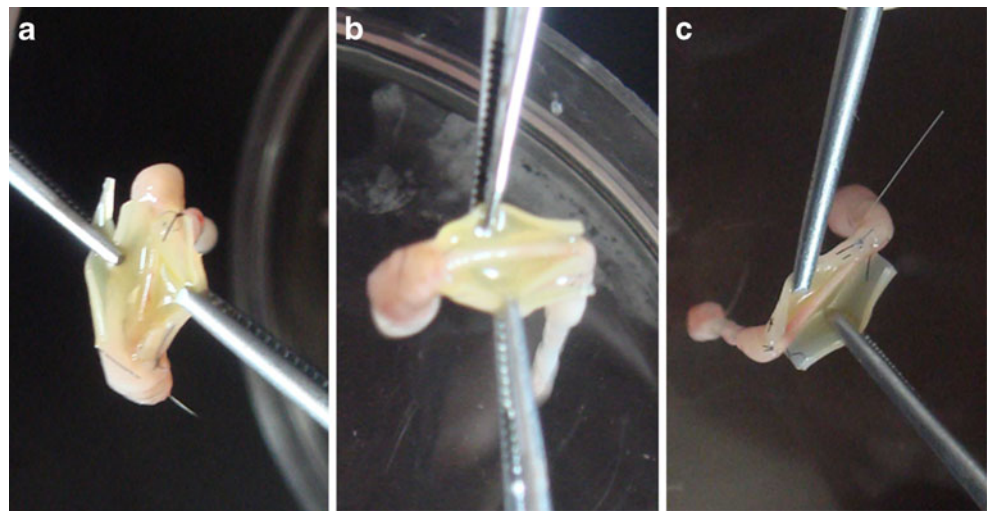

omitting the primary antibody and replacing this step with phosphate-buffered saline. The distribution of NGF and HGF was observed on each section by viewing five different visual fields, randomly selected on each slice, and NGF and HGF expression was analyzed by Image-Pro Plus 6.0 (Media Cybernetics Inc., Bethesda, MD, USA). The data were expressed as mean optical density values.

Statistical Analysis

SPSS (version 13.0) statistical software and Microsoft Office Excel 2003 were used for data analysis. One-way analysis of variance was used for statistical analysis. Differences between two groups were compared with the q test and t test for paired data. Significance was accepted at $P < 0.05$.

Fig. 4 Histopathological changes of the injured sciatic nerve (H&E staining, ×400). Digitized images of cross-sections obtained from the proximal portions of the sciatic nerve. **a** NS group; *A1* representative section at 4 weeks, showing many proliferated Schwann cells. *A2* representative section at 8 weeks shows that Schwann cell proliferation occurred persistently. **b** NSCs group; *B1* representative section at 4 weeks showing many proliferated Schwann cells; *B2* at 8 weeks, minimal Schwann cell proliferation was observed. **c** NGF group; *C1* representative section at 4 weeks shows minimal proliferation; *C2* at 8 weeks, there was no Schwann cell proliferation
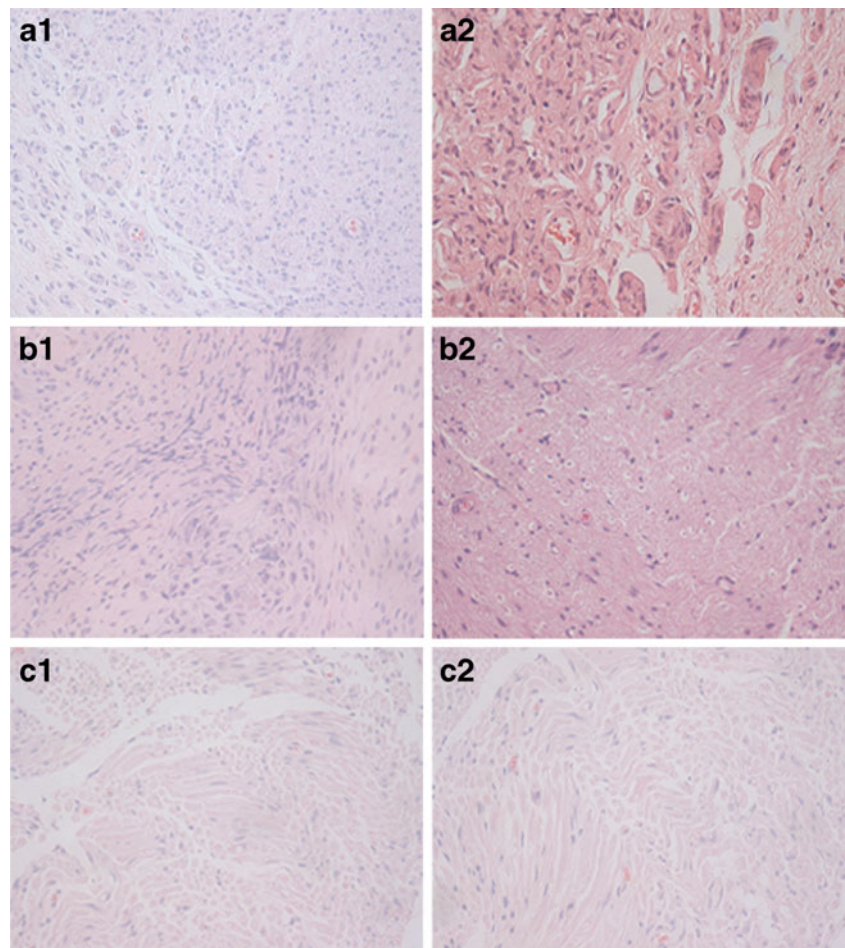
## Results

Behavioral Observation

All animals survived and no trophic ulcerations were observed on the operated leg over the 8-week regeneration period. After transection of the right sciatic nerve, motor function of the right hindlimb was lost, the toes were kept tight, and a dragging walk was observed. Acupuncture of the right foot with a pin elicited no pain reaction. There was no wound infection, and there was ankle tumescence in all groups. Four weeks after surgery, the activity of the ankle joint gradually recovered, the tumescence had not dissipated, and the toes began to separate. There was a shrink-and-

escape response upon acupuncture of the right foot, but the injured side was obviously limping. Eight weeks after the operation, the tumescence had dissipated, the toes were separated, and limping had improved.

SFI

Two weeks after sciatic nerve transection, the right hindlimb was obviously dragging and the footprint was unclear. Therefore, a proper measure of SFI could not be achieved. Four weeks after the operation, SFI was measured. There was a weekly increase in SFI values, showing gradual improvement of hindlimb motor function. Four weeks after sciatic nerve transection, rats in the NS group showed

Fig. 5 Ultrathin cross-sections under TEM from the proximal segments of the injured sciatic nerve. **a** NS group; *A1* representative section at 4 weeks showing small and poorly developed regenerating clusters exhibiting fibers with a thin myelin sheath (*arrows*); *A2* representative section at 8 weeks showing further developed, regenerating clusters exhibiting fibers with a thin myelin sheath (*arrows*). **b** NSCs group; *B1* representative section at 4 weeks showing many thickly myelinated (*arrows*) nerve fibers; *B2* representative section at 8 weeks showing more and thicker regenerated myelinated (*arrows*) nerve fibers than at 4 weeks. A Schwann-cell nucleus is observed (*asterisk*). **c** NGF group; *C1* representative section at 4 weeks showing many and thick myelinated (*arrows*) nerve fibers; *C2* representative section at 8 weeks showing a regenerating cluster of myelinated (arrows) nerve fibers. Many myelinated nerve fibers with well-preserved axoplasm and a proper myelin sheath are seen
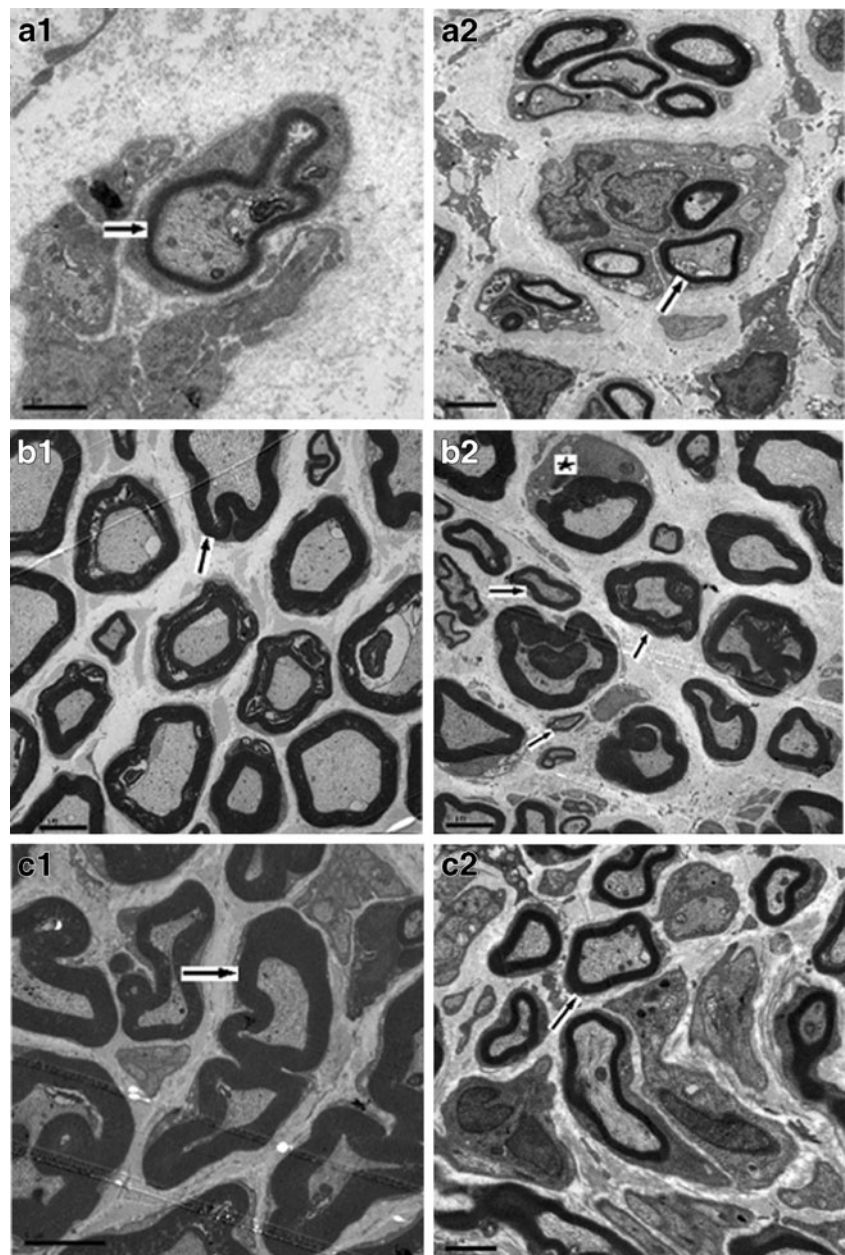
significantly lower SFI values when compared with rats in the NSCs and NGF groups (Fig. 1).

NCV

Rats were euthanized by cervical dislocation. The sciatic nerves from both hindlimbs (transected on the right, undisturbed control on the left) were quickly excised to test the conduction velocity of the sciatic nerve. One week after sciatic nerve transection, there was no connection between the proximal and distal nerve stumps, but 4 weeks following transection, slim connecting fibers could be seen in the conduit and NCV was measurable. At week 8, there were obvious fibers connecting the proximal and distal stumps in

the conduit, and NCV was substantially increased compared with that at 4 weeks after the operation. The mean NCVs of the NSCs and NGF groups were each significantly faster than that in the NS group (Fig. 2).

Histological Alteration of Sciatic Nerve

Upon gross examination of the injured sciatic nerve, there was no connection in the conduit in any of the three groups at 1 week after transection. At week 4, the fragile connection in the conduit was visible and appeared thicker by 8 weeks (Fig. 3). H&E staining of cross-sections from the proximal portion of the transected sciatic nerve showed Schwann cell proliferation in all three groups at week 4,

Fig. 6 Ultrathin cross-sections from the segments of the regenerating sciatic nerve. **a** NS group; *A1* representative section at 4 weeks showing no fiber formation; *A2* representative section at 8 weeks showing small and poorly developed regenerating clusters exhibiting fibers with a thin myelin sheath (*arrows*). **b** NSCs group; *B1* representative section at 4 weeks showing many thinly myelinated (*arrows*) nerve fibers; *B2* representative section at 8 weeks showing a regenerating cluster consisting of myelinated (*arrows*) and non-myelinated nerve fibers (*arrowheads*) surrounded by the processes of perineurium-like cells. A Schwann-cell nucleus is observed (*asterisk*). Many myelinated nerve fibers with a well-preserved axoplasm and a proper myelin sheath are seen. **c** NGF group; *C1* representative section at 4 weeks showing many thinly myelinated (*arrows*) and non-myelinated (*arrowheads*) nerve fibers; *C2* representative section at 8 weeks showing a regenerating cluster of myelinated nerve fibers (*arrows*)
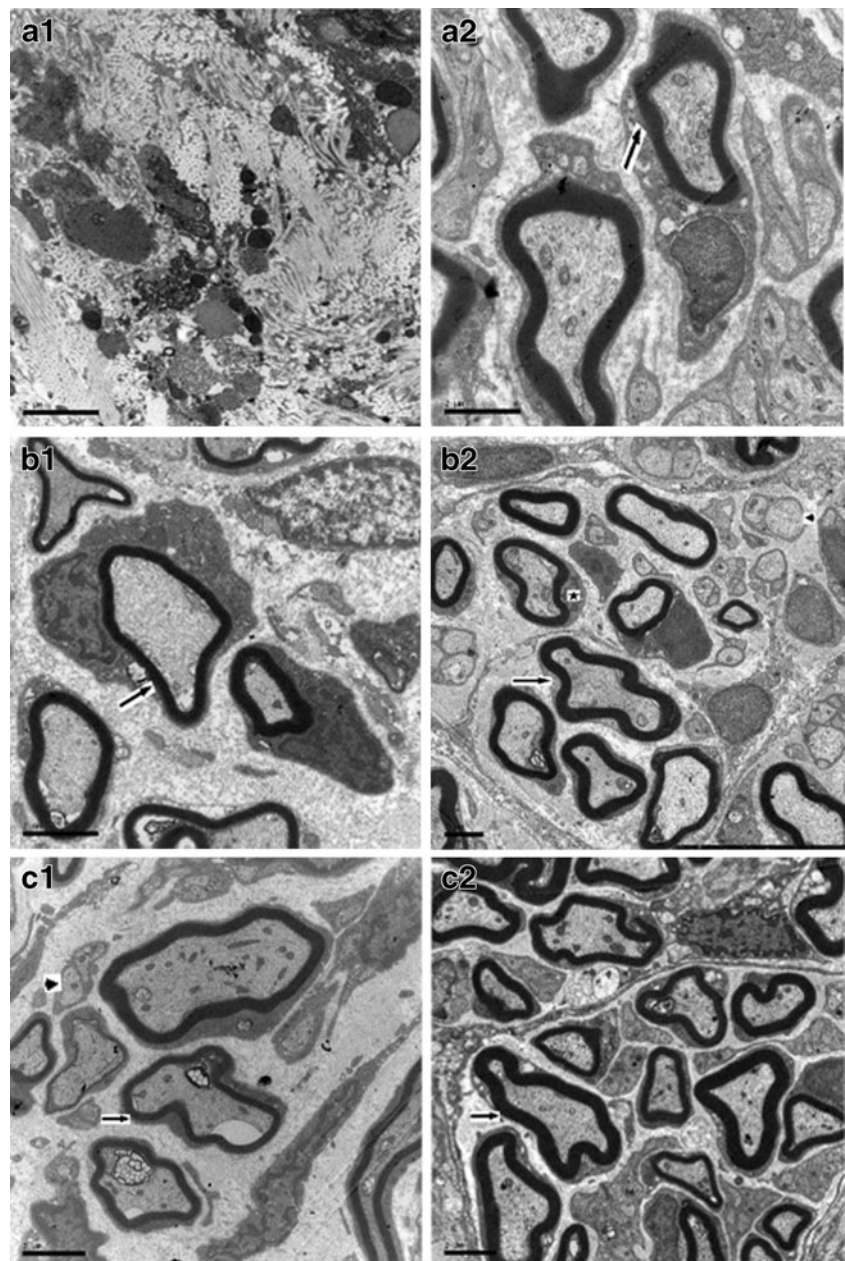
although to a markedly lesser degree in the NGF and NSCs groups. Furthermore, at week 8, Schwann cell proliferation was persistent in the NS group, minimal in the NSC group, and there was no obvious proliferation in the NGF group (Fig. 4).

TEM

TEM revealed regenerating nerve fibers in the NSCs and NGF groups at 4 weeks after transection (Figs. 5 and 6). In contrast, few regenerating nerve fibers were observed in the NS group (Figs. 5 and 6). Ultrastructural analysis showed that the growing nerves in the NSCs and NGF groups exhibited many regenerating clusters of preserved nerve fibers, indicating appropriate tissue organization. Conversely, the NS group showed growing nerves with comparatively fewer and smaller regenerating clusters, smaller-diameter fibers, and thinner myelin sheaths. At week 8, the NSCs and NGF groups continued to exhibit more regenerated nerve fibers with a greater number and diameter of myelin sheaths compared with those of the NS group.

Immunohistology of NGF and HGF

NGF is known to play a role in nerve growth and regeneration. We therefore examined its expression in the recovering nerve tissue as an indicator of active regeneration. NGF-immunoreactive signals were mainly distributed in the cytoplasm of Schwann cells and excluded from the nucleus. At week 4, NGF expression levels were significantly higher in the NSCs and NGF groups than in the NS group. At week 8, expression levels of NGF were low in all groups (Fig. 7).

HGF has been shown to play a role in regeneration and is broadly distributed throughout the nervous system; therefore, we examined its expression in recovering injured nerves. HGF-immunoreactive signals were also mainly distributed in the cytoplasm of Schwann cells and excluded from the nucleus. At week 4, HGF expression levels were significantly higher in the NSCs and NGF groups than in the NS group, and at week 8, HGF expression level was higher in the NSCs group than in the NGF and NS groups (Fig. 8).

Discussion

The generally poor and variable outcome after traumatic peripheral nerve lesions [24–26] has stimulated much research to evaluate alternative methods for nerve gap reconstruction and functional recovery. In this study, we used an adult rat peripheral-nerve transection and a surgical repair model to demonstrate that NSCs implanted into a silicone tube were able to increase the number of nerve fibers and improve the function of the recovering sciatic nerve. We further observed the expression of NGF and HGF in the injured sciatic nerve during recovery.

The PNS has great regeneration potential, particularly when an appropriate microenvironment is provided [27, 28]. When a graft bridge is introduced in the context of a nerve defect, the graft functions as a conduit for regenerating axons. The graft produces a guideline and a biological environment for nerve regeneration [4]. Different conduits have been used to bridge nerve gaps. With the progress in tissue engineering, one of the main strategies for repairing peripheral-nerve defects has focused on creating biological and non-biological tubular nerve guides. During the past few years, studies have concentrated on various conduit materials, particularly biodegradable polymers such as polyglycolic acid, polylactic acid, polyphosphoester, and also silicone [29]. These studies have indicated that the conduit itself does not have a profound effect on the outcome of nerve repair. Thus, approaches to nerve repair are now

Fig. 7 Immunohistological staining to detect NGF expression. **a** NS group; **b** NSC group; **c** NGF group. NGF expression appears as a brown immunoreactive substance and was detected in the cytoplasm, but not in the nucleus. NGF expression was significantly higher in the NSCs and NGF than the NS groups (P<0.05). **d** Immunohistochemical staining for NGF immunoreactivity in the sciatic nerve. **P<0.01 versus NS group

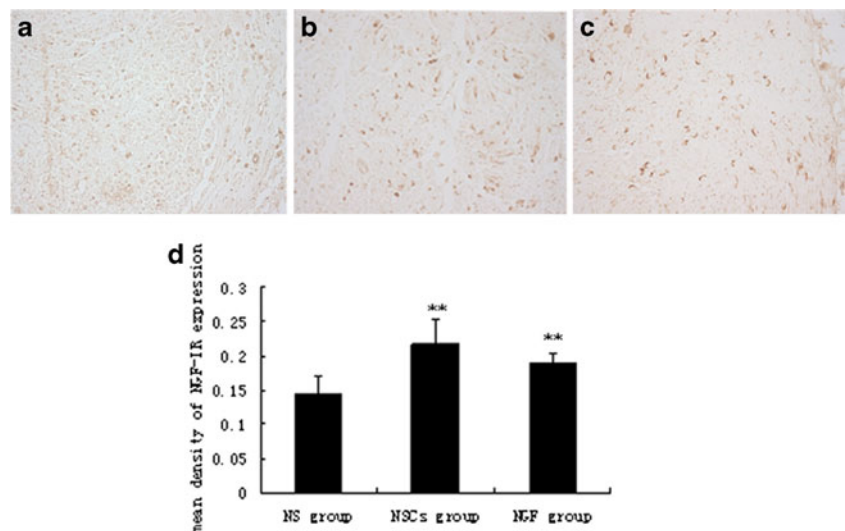**Fig. 8** Immunohistological staining to detect HGF expression. **a** NS group; **b** NSCs group; **c** NGF group. HGF expression appears as a brown immunoreactive substance and was detected in the cytoplasm, but not in the nucleus. HGF expression was significantly higher in the NSCs and NGF group than NS groups (*P*<0.05). Expression of HGF was significantly increased in the NSCs group in the 4-week compared with the 8-week sub-group, and the difference was not observed in other groups. **d** Immunohistochemical staining for HGF immunoreactivity in the sciatic nerve. *P<0.05 ver-sus NS group; **P<0.01 versus NS group
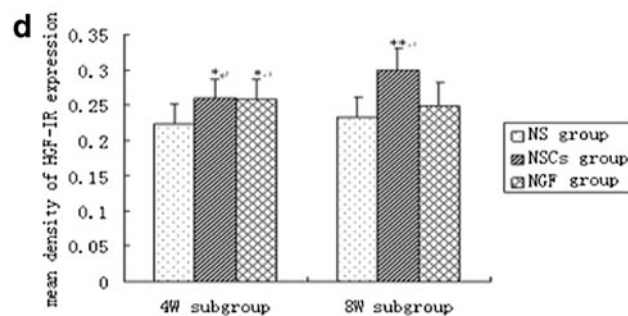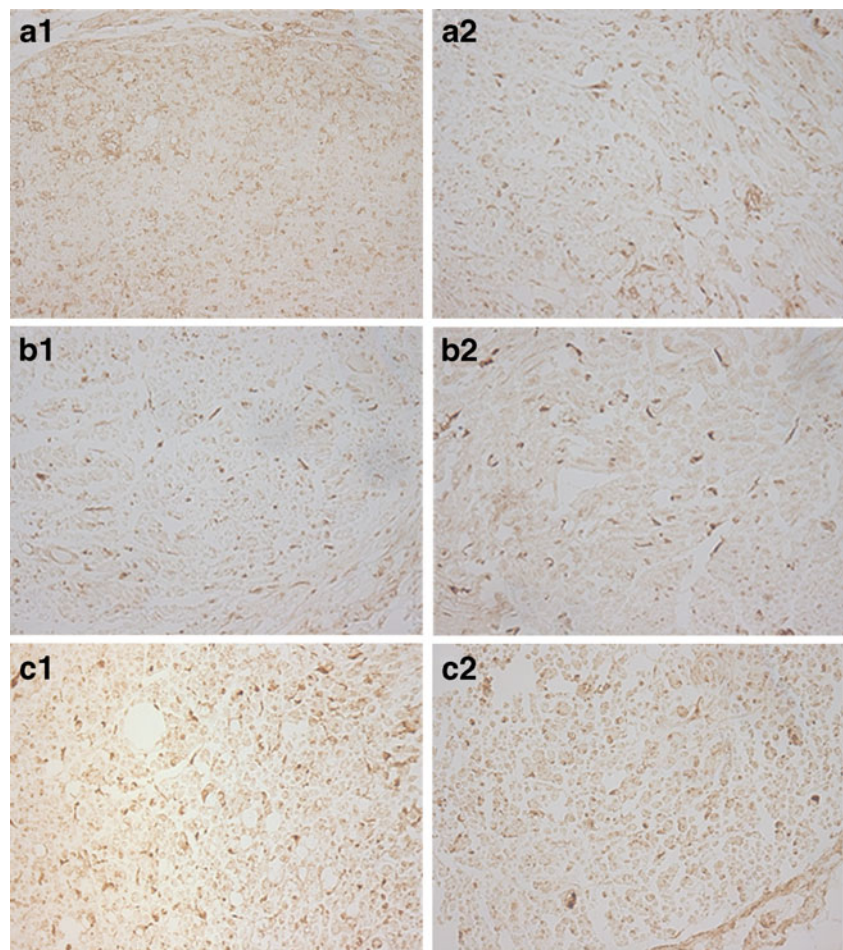
focused on molecular biological manipulation of the internal features of the conduit, to optimize the combination of scaffold tissue, trophic factors, and stem cells derived from various sources [26, 30]. As the silicone tube has properties of strength, biological inertness, elasticity, transparency, and malleability, silicone is ideal for bridging nerve regenera-tion. Its use allows nerve regeneration to be easily observed; the tube does not easily collapse, and it can be shaped as needed. Thus, silicone is the most frequently used synthetic conduit material.

Cell transplantation strategies offer great potential for enhancement of nerve regeneration because of their trophic and anti-inflammatory effects to the damaged nerves,

combined with remyelination of regenerating axons. Stem cells are totipotent; they can self-replicate and differentiate into various cell types of particular tissues. Recent experi-mental studies have demonstrated the use of different types of stem cells in the process of nerve regeneration. Cui and colleagues [31] have shown that after in vitro induction of neural differentiation, transplanted embryonic stem cells can differentiate into myelin-forming cells and promote nerve regeneration. Moreover, neuroprotective effects may result from anti-apoptotic activity, free radical scavenging, and anti-glutamate excitotoxicity [32]. NSCs are found in the central nervous system and can differentiate into neurons, astrocytes, and oligodendrocytes in vitro [33]. In this work,

we implanted NSCs into a silicone tube that was inserted between the two stumps of a transected sciatic nerve, and confirmed that NSCs could increase the number of nerve fibers and improve the functional recovery of the injured sciatic nerve.

Peripheral nerve regeneration occurs mainly through a series of reactions produced by activated Schwann cells so that the axon of the proximal nerve stump grows through the distal stump that is in close contact with the Schwann cell bands [34]. These findings suggest that, when available, Schwann cell transplantation may be a useful substitute for autografting to repair nerve injury. Studies have shown in various animal models that artificial nerve grafts (synthetic guidance channels) made with Schwann cells have the potential for axonal regeneration and functional recovery [35]. In our study, 4 weeks after sciatic nerve injury, proliferation of Schwann cells occurred mainly in the NS group. At week 8, proliferation was still evident in the NS group, and there was little proliferation in the other two groups. We infer that the NSCs and NGF treatments replaced part of the effect of Schwann cells.

In this study, we further observed that treatment with NSCs significantly improved the recovery of motor function in the reinnervated hindlimbs, as demonstrated by the greater SFI and NCV values, from week 4 to 8 of the nerve repair phase. After conduit delivery of NSCs, they grew and promoted axonal growth and functional recovery of the injured nerve. These results suggest that the transplantation of NSCs has significant clinical potential.

After peripheral nerve injury, nerve regeneration depends largely on whether the surrounding microenvironment is suitable for nerve growth. The regenerating environment includes growth factors, cellular elements (Schwann cells), the basal lamina, extracellular matrix proteins, and other factors [36]. NGF and HGF play important roles in the growth and regeneration of nerves. In the PNS, NGF is mainly produced by non-neuronal cells and has well-known protective effects on the nervous system. Thus, in this work, NGF was used as a positive control. HGF is a pleiotropic cytokine, acting primarily through its high-affinity Met tyrosine kinase receptor (c-Met) to play an important role in organogenesis and regeneration of various tissues [37]. HGF and c-Met are broadly distributed in the nervous system. HGF plays a role in brain and PNS development and shape maintenance. HGF may perform a function similar to neurotrophic factors, which promote neuronal survival and regeneration of injured nerves through binding with specific receptors via autocrine, paracrine, and reverse classic axonal transport. HGF expression increases when the nervous system is injured [38, 39]. In the present study, we observed that expression of NGF and HGF were increased in both the NSCs and NGF groups. These results suggest that NGF and HGF are involved in the regeneration process

of the injured sciatic nerve. One of the mechanisms by which NSCs exert a protective effect on an injured nerve might be through regulation of NGF and HGF expression.

Conclusions

Our findings indicate that NSCs can foster the regeneration of injured sciatic nerve. In addition, NSCs can increase the expression of NGF and HGF in the sciatic nerve. The application of NSCs in clinical trials for patients who have nerve injury may lead to an improved clinical outcome.

Conflict of Interest The authors declare that they have no conflict of interest, personally, professionally, or financially, relating to the publication of this work.

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