ORIGINAL ARTICLE

Poly (dextrogyr-levogyr) lactide acid-triiodothyronine scaffold for peripheral nerve regeneration

Min Wang, Chao Liu, Yong Hao, Yuan Zhang, Xia Zhang

Department of Orthopaedics, Xinqiao Hospital, Third Military Medical University, Chongqing City, P.R. China.

Correspondence to: Min Wang, MD.
Department of Orthopaedics, Xinqiao Hospital, Third Military Medical University
No.2 Xinqiao Street, Shapingba District, Chongqing City, 400037, P.R. China.
TEL: +86-023-68774308; FAX: +86-023-68755608; E-MAIL: 52solar@sina.cn

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Abstract

OBJECTIVES: To evaluate the poly(dextrogyr-levogyr) lactide acid-triiodothyronine (PDLLA-T_3) seeded with Schwann cells conduit for repairing sciatic nerve defect.

MATERIALS & METHODS: The rats were divided into three groups: autologous nerve transplantation (Group A), PDLLA-T_3 + Schwann cells (Group B) and PDLLA + Schwann cells (Group C).

RESULTS: Myelin sheath thickness was significantly greater in Group A compared with Group B and Group C. The regenerated nerves had nearly normal structure in Group A, and in Groups B and C nerve tissues filled in the anastomotic site and angiogenesis was noted. The mean number of myelinated nerve fibers and neurons in Group B was greater than in Group C.

CONCLUSIONS: PDLLA-T_3 is superior to PDLLA alone for repairing nerve defects.

INTRODUCTION

Autologous nerve has been widely accepted as the gold standard for repairing peripheral nerve defects. However, its use in the clinical setting is limited by a limited supply of donor nerves, donor site morbidity, and inadequate recovery of function (Shen *et al.* 2001; Wang *et al.* 2011). Therefore, the repair and reconstruction of peripheral nerve defects remains a challenge in clinical practice. With the development of tissue engineering, an increasing number of studies have been conducted to investigate methods for the repair of peripheral nerve defects.

Various types of materials have been used as scaffolds to bridge the gap between the proximal and distal ends of nerve defects (Giardino *et al.* 1999; Shen *et al.* 2001; Yuan *et al.* 2002). These materials have been recently reviewed (Deumens *et al.* 2010; Siemionow *et al.* 2010). Some conduits have been biodegradable and some non-biodegradable. Recently, more research has been focused on biodegradable type (Wang *et al.* 2011). However, it appears that for optimal recovery, using just a nerve conduit might not be sufficient (Wang *et al.* 2011).

To enhance nerve regeneration, the application of scaffolding materials containing neurotrophic factors has become one of the main strategies (Gordon 2009). There is considerable evidence that in the peripheral nervous system, endogenous neurotrophic factors of axotomized neurons and

denervated Schwann cells play an essential role in axonal regeneration (Voinesco *et al.* 1998). One such neurotrophic factor that has been used is nerve growth factor (Wang *et al.* 2011; Xu *et al.* 2011). Another is thyroid hormone (TH), which is an important neurotrophic factor in both the central nervous system and peripheral nervous system. Studies have found that TH can promote the repair of peripheral nerve defects, and its effectiveness for the repair of peripheral nerve defects is superior to that of extracellular matrix and a single growth factor such as ciliary neurotrophic factor, fibroblast growth factor, and hyaluronic acid (Barakat-Walter 1999; Liu *et al.* 2011). It has also been found that in rats with sciatic nerve injury, TH enhances restoration of the neuromuscular junction as well as axonal regeneration (Panaite and Barakat-Walter 2010). Moreover, at an appropriate concentration TH can stimulate Schwann cells to produce more growth factors. Schwann cells coating the scaffold surface will induce nerve axon regeneration.

To construct an artificial nerve scaffold that contains a neurotrophic factor, in a previous study we combined poly(dextrogyr-levogyr) lactide acid (PDLLA) with triiodothyronine (T_3) (Wang *et al.* 2005c). Poly lactide acid has a tube-like stereostructure (Wang *et al.* 2005b, 2005c). Its degradation time of about 3 months makes it more suitable for clinical use than other kinds of lactide. The PDLLA-T_3 scaffold which could gradually release T_3 during the slow degradation of PDLLA was used to bridge a rat sciatic nerve defect; this scaffold was similar to nerve basal lamina. We found that TH was locally released and had no influence on systemic TH concentration, which avoided the detrimental effect of exogenous TH in rats (Wang *et al.* 2005c).

The aim of the present study was to assess the effectiveness of the PDLLA-T_3 scaffold coated with Schwann cells for repairing sciatic nerve defect in a rat model by using histological evaluation of nerve regeneration in comparison to Schwann cells coating PDLLA alone. It should be noted that some aspects of this study were previously published in a Chinese journal (Wang *et al.* 2005a). However, this paper in English is a much more comprehensive report of the study and includes the results of horseradish peroxidase (HRP) staining and additional statistical analysis as well as photos which were not included in the earlier paper.

MATERIALS AND METHODS

Materials

Neonatal Sprague-Dawley (SD) rats aged 5–6 days (n=5) were purchased from the Experimental Animal Center of Research Institute of Surgery of the Third Military Medical University, Chongqing, China. Animals were sacrificed by decapitation and bilateral sciatic nerves were collected. Tissue culture was performed to acquire Schwann cells and a 2.0×10^6/ml cell suspension was prepared (Wang *et al.* 2005b, 2005c). The cells were incubated with sterilized scaffold (PDLLA or PDLLA-T_3) for 2 weeks (Wang *et al.* 2011) to prepare the artificial nerve with seeding cells.

Animals and grouping

Ninety adult SD rats weighing 300–350 g were purchased from the Experimental Animal Center Institute of Surgery of the Third Military Medical University. Animal care strictly adhered to the guidelines established by the ethic committee of animal study and the study was approved by our institutional review board. A transection defect was created in the left sciatic nerve of each animal and then the animals were divided equally into 3 groups: Group A, autologous nerve transplantation; Group B, PDLLA-T_3 seeded with Schwann cells; Group C, PDLLA seeded with Schwann cells. The right sciatic nerve in Group B and C served as the normal control (Group D).

Preparation of nerve defect and its repair

Animals were intraperitoneally anesthetized with 2.5% amobarbital and then placed in the prone position. The hair around the hip joint was removed and the skin sterilized. A longitudinal incision was made in the lower limb beginning at the left hip joint, and biceps muscle of thigh. About 2.0 cm of left sciatic nerve was exposed and sciatic nerve of 1.0 cm in length was removed at 1–2 cm below the musculus piriformis to create the nerve defect. Autologous nerve (from the contralateral sciatic nerve at the same level) (Group A), PDLLA-T_3 (Group B) and PDLLA (Group C) were used, respectively, to repair the nerve defect. The transplanted nerve was ligated with nerve stumps by tension-free suture, and the anastomotic site was wrapped with poly lactic acid membrane and then fixed (Wang *et al.* 2011). The wound was closed. The right sciatic nerve in Group B and C was left intact as a control.

Sample collection

At 2 weeks, 1 month and 2 months after surgery, 10 rats in each group were sacrificed and the transplanted nerve was exposed. A portion of nerve 2 cm in length was obtained at 1 cm from the anastomotic site and fixed on a plate. Both ends of the nerve were marked and the middle part of the nerve was used for observation. In addition, 5 nerves were separated longitudinally followed by immunohistochemistry for S-100 and Bielschowsky's silver staining and the remaining 5 nerves were subjected to transmission electron microscope (TEM) and histological examination. The right sciatic nerves in Group B and C were also collected as a normal control.

Procedures

Transmission electron microscope: The five nerve samples were fixed in 3% glutaraldehyde, dehydrated and embedded in Epon812 (Hyatt 2002). Ten ultrathin sections were obtained from each sample and 4 fields were randomly selected at a magnification of 2,500–3,000.

Representative photographs were collected and then processed with an image analysis system (Motic Images Advanced 2.0, Xiamen, P.R.China). The mean thickness of myelinated nerve fibers per unit area was calculated.

Histological examination

The five nerve samples were fixed in 4% paraformaldehyde for 2 days, dehydrated in a series of alcohol solutions, embedded in paraffin and cut into sections followed by hematoxylin & eosin (HE) staining (Hyatt 2002).

Bielschowsky's silver staining

Samples were fixed in 4% paraformaldehyde for 2 days and tissues were cut into 10-μm sections. The standard procedures for this staining method were then followed.

Immunohistochemical staining for S-100

Sections were deparaffinized and then treated with 3% H_2O_2 at room temperature for 5–10 minutes. After washing in PBS twice (5 minutes for each), antigen retrieval was carried out. Then these cells were blocked in 10% normal goat serum at room temperature for 10 minutes. The serum was removed and sections were incubated with proportionally diluted primary antibody at 4°C overnight. After washing in PBS 3 times (5 minutes for each), sections were treated with secondary antibody at 37°C for 30 minutes followed by washing in PBS 3 times (5 minutes for each). After treatment with the third antibody at 37°C for 30 minutes, sections were washed in PBS 3 times (5 minutes for each) followed by visualization with DAB. Then, the sections were washed in flowing water followed by counterstaining with hematoxylin and mounted (Hyatt 2002).

Images obtained from immunohistochemical staining for S-100 and Bielschowsky's silver staining were analyzed with Motic Images Advanced 2.0 software.

HRP Retrograde Tracing

At 2 months after surgery, 3 rats were randomly selected and anesthetized. Then, 2–3 μl of 30% HRP was injected at 4 mm away from the distal anastomotic site of the left sciatic nerve and at a similar site for the right sciatic. The injection was done slowly to avoid leakage of the HRP solution. After the injection was completed the wound was closed. At 3 days after surgery, animals were anesthetized and transcardially perfused with 200–250 ml of 0.9% normal saline (37 °C), 4% paraformaldehyde (4 °C), 1.25% glutaraldehyde in 0.1 mol/L PBS (pH=7.4) for 30 minutes and then 200 ml of 10% sucrose in 0.1 mol/L PBS was given. The L4–5 spinal cord segment was collected and 40-μm frozen sections were obtained. Tretramethylbenzidine (TMB) staining was performed according to the standard procedure described by Brushart and Mesulam (Brushart & Mesulam 1980).

Data Analysis

Continuous variables were presented as mean ± SD in each group across different time points (2 weeks, 1 month, 2 months post-operation), respectively. The differences between groups and over time were analyzed by two-way analysis of variance (ANOVA) with interaction of group and time. The pairwise post-hoc tests using Bonferroni correction was applied when there were significant findings with ANOVA. The statistical analyses were performed with SAS software version 9.2 (SAS Institute Inc., Cary, NC, U.S.A.). A two-tailed $p<0.05$ indicated statistical significance.

RESULTS

Ultrastructure of Transmission Electron Microscopy

TEM showed many myelinated nerve fibers and the myelin sheath was thick in normal sciatic nerve (Group D, data not shown). At 2 weeks after surgery primarily degenerated nerves were found in Groups A, B and C, the myelin had onion-peel like irregular changes, the nerves had insufficient organelles and the number of myelinated nerve fibers was very small (2–5 nerves/field). At 1 month after surgery, there was a reduction in degenerated nerves in Groups A, B and C and some newly generated myelinated and unmyelinated nerve fibers were observed. There was an increase in myelinated nerve fibers (about 7–11 myelinated nerve fibers/field) but the myelin sheath was thin. At 2 months after surgery, the structure of nerves in Group A was clear and the thickness of the myelin sheath was almost normal in a small fraction of regenerated nerves (Figure 1A). The organelles were clear and identifiable. In Groups B and Group C, the regenerated myelin sheath was still thin (Figure 1B&C).

Myelin sheath thickness

The results of two-way ANOVA for myelin sheath thickness showed that the effects of group, time, and their interaction were all significant ($p<0.001$). At 2 weeks post-operation, all 3 experimental groups (A, B, C) had significantly lower mean myelin sheath thickness compared to the normal control group (D), but there was a significant difference among the 3 experimental groups (Figure 2). At 1 month post-operation, myelin sheath thickness was increased for all 3 experimental groups, and all experimental groups still had significantly lower mean myelin sheath thickness compared to the normal control group (D); the difference among the experimental groups was still not significant. At 2 months post-operation, there was no significant difference in myelin sheath thickness between Group A and Group D (2.82±0.21 vs. 2.97±0.12 μm, $p=0.647$). Moreover, Group A had significantly greater mean myelin sheath thickness than that of both Groups B and C (Group A: 2.82±0.21 μm vs. Group B: 2.15±0.27 μm, $p<0.001$; Group A: 2.82±0.21 μm vs. Group C: 1.96±0.20 μm, $p<0.001$), which showed that the recovery of myelinated nerve fibers in group A was better than that in both Groups B and C.

Histological examination

At 2 weeks after surgery, the structure at the anastomotic site was disorderly in Group A. The materials were not completely degraded in Groups B and C, and a cavity was formed into which tissues grew. At 1 month after surgery, the regenerated nerves grew across the anastomotic site in Group A, the structure of the anastomotic site was clear and angiogenesis was also noted at the anastomotic site. In Groups B and C, there were some regenerated tissues but the materials were not completely degraded and several cord-like cavities were observed at the anastomotic site. At 2 months after surgery, the regenerated nerves were nearly normal in structure in Group A (Figure 3A). The materials in Groups B and C were largely degenerated, the cord-like cavity was absent, nerve tissues filled in the anastomotic site and angiogenesis was also noted (Figure 3B&C).

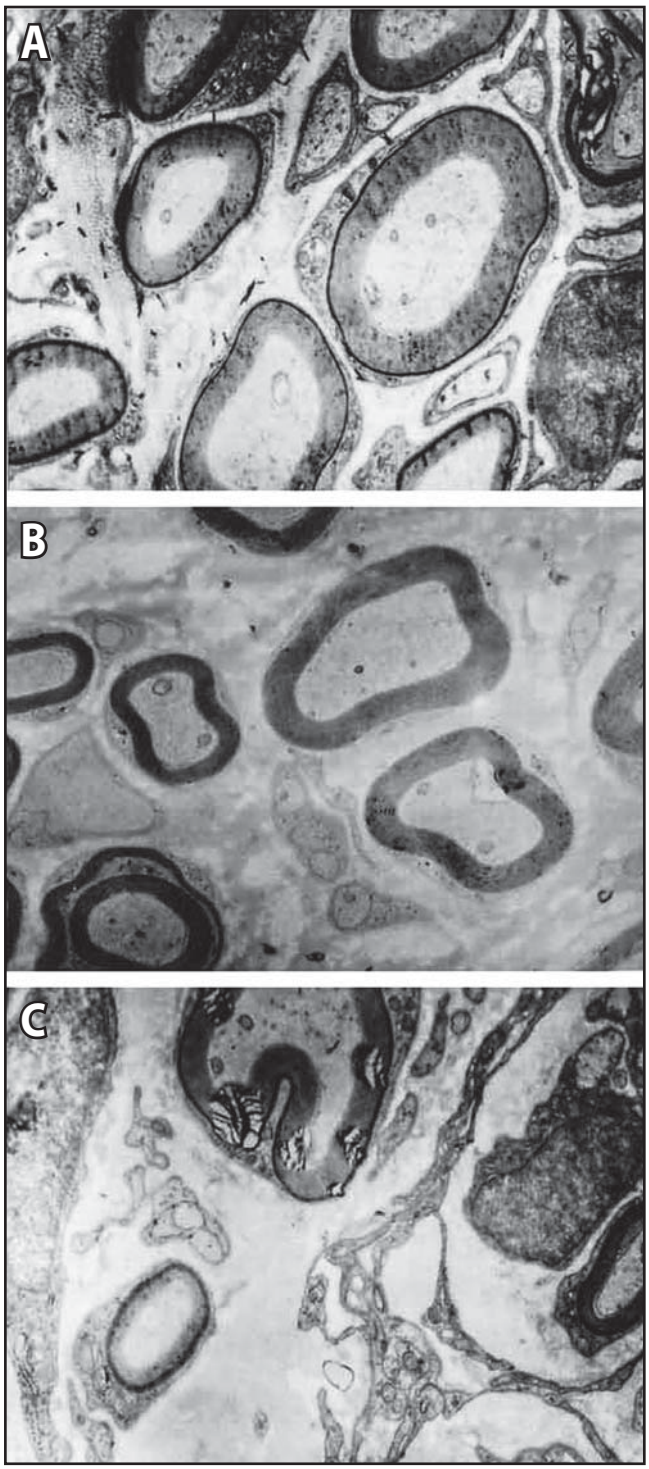

Fig. 1. TEM findings at 2 months after surgery (×3000). **A**) the regenerated myelin sheaths were thin in Group A. **B**) the regenerated myelin sheaths were thinner than normal in Group B. **C**) the regenerated myelin sheaths were thinner than normal in Group C.

Bielschowsky's silver staining of nerve fibers

Myelinated nerve fibers positive for silver staining were black and strand/wave-like, and were in the same direction. The number, length, thickness and density of these nerves varied. When the section was located in the middle of an axon, the black myelinated nerve fiber had a blank line in the center. At 2 months after surgery, many regenerated nerve fibers, especially myelinated nerve fibers, were observed in Group A (Figure 4A). Slightly fewer nerve fibers were seen in Group B, but only a small number of myelinated nerve fibers were noted in Group C (Figure 4B–C).

Number of myelinated nerve fibers with Bielschowsky's silver staining

The results of two-way ANOVA for number of myelinated nerve fibers also showed that the effects of group, time, and their interaction were all significant ($p<0.001$). At 2 weeks post-operation, the mean number of myelinated nerve fibers for all 3 experimental groups was similar and significantly lower than that

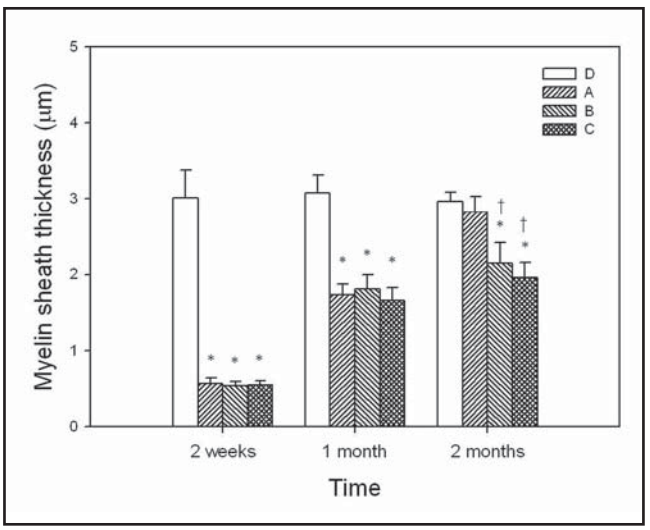

Fig. 2. Bar graph (mean with SD) of myelin sheath thickness in the 4 groups at 2 weeks, 1 month, and 2 months post-operation,. Significant differences were found with post-hoc test using Bonferroni correction: *compared to group D, $p<0.05$; †compared to group A, $p<0.05$. Symbols of significance only showed above the group with lower mean.

of Group D (Figure 5). At 1 month post-operation, the number of myelinated nerve fibers increased in all the experimental groups, but the mean number was still significantly lower compared to Group D (Group A: 51.70±4.14 vs. Group D: 89.00±7.80, $p<0.001$; Group B: 49.20±4.69 vs. Group D: 89.00±7.80, $p<0.001$; Group C: 46.90±3.81 vs. Group D: 89.00±7.80, $p<0.001$). At 2 months post-operation, the mean number of myelinated nerve fibers in Group A were not significantly different from that of Group D, which showed that

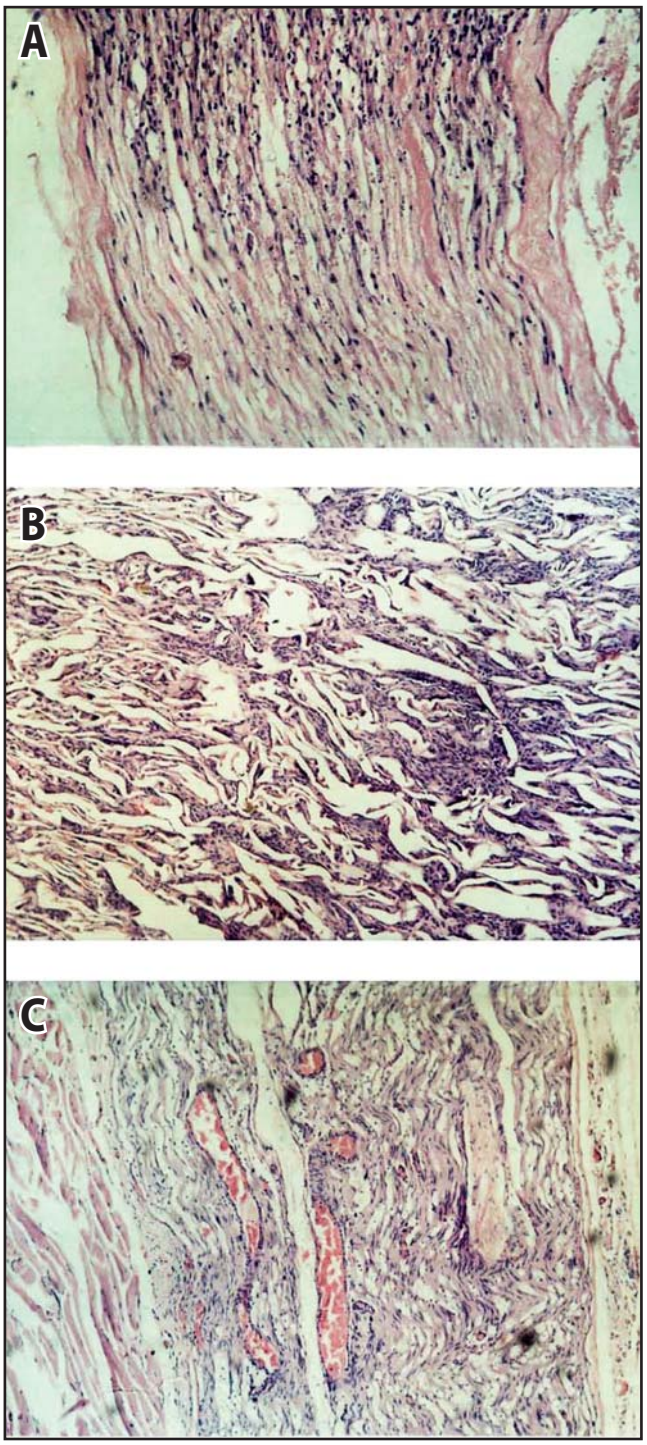

Fig. 3. Histological specimens at 2 months after surgery (HE×40). **A**) The regenerated nerve grew across the anastomotic site in Group A and angiogenesis was also noted . **B**) the materials were not completely degraded in Group B and a cord-like blank region was noted. **C**) the materials in Group C were not completely degraded and a cord-like blank region was observed.

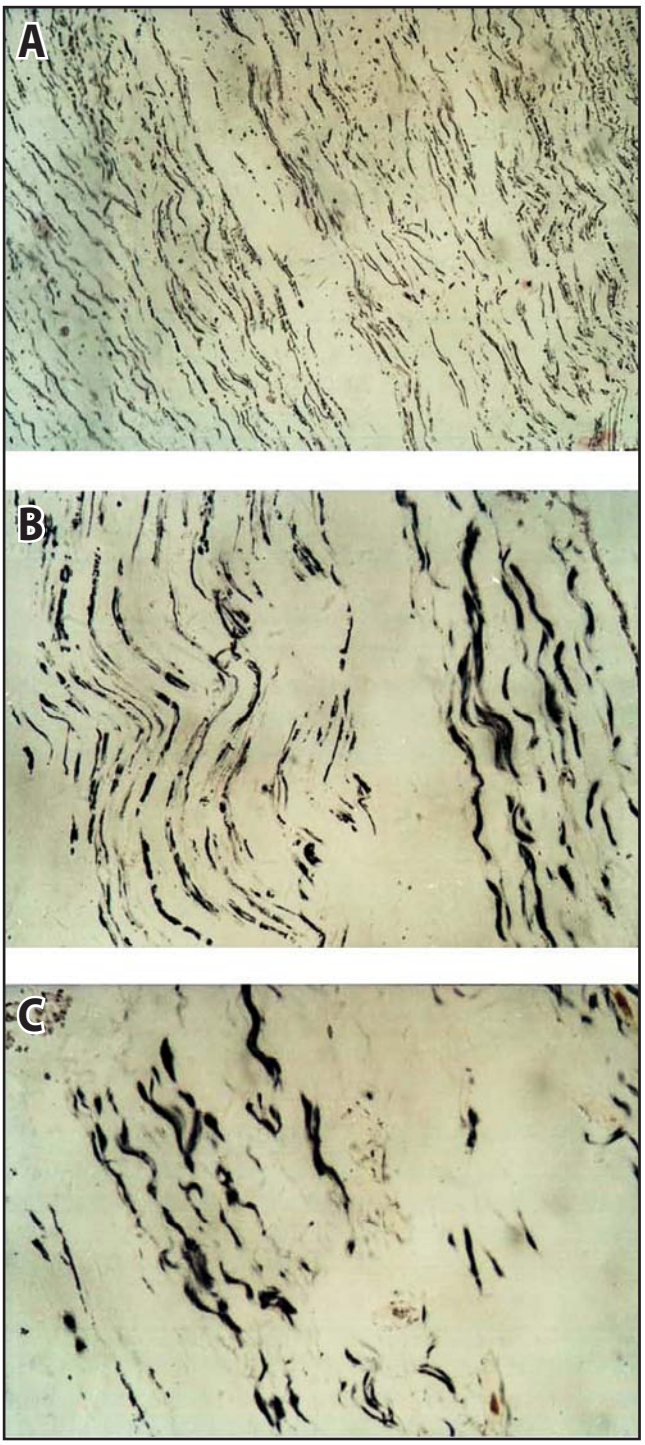

Fig. 4. Bielschowsky's silver staining of nerve fibers at 2 months after surgery. **A**) In Group A, there were many myelinated nerve fibers which were regularly arranged (×100). **B**) the myelinated nerve fibers in Group B grew across the scaffold (×100). **C**) fewer myelinated nerve fibers were found in Group C and the size of these fibers varied (×100)

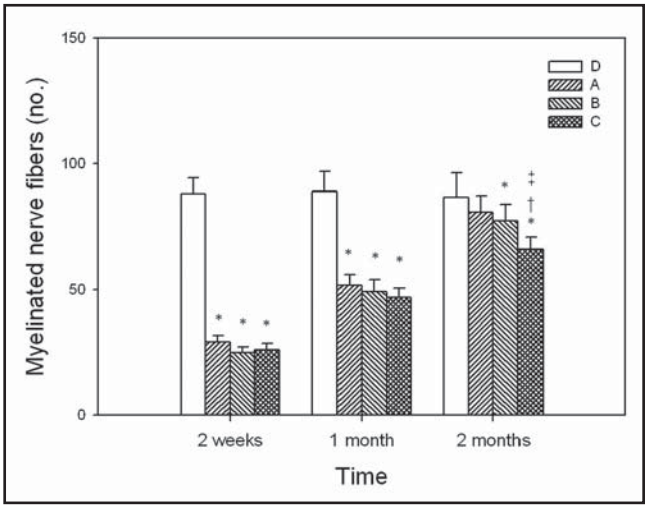

Figure 5. Bar graph (mean with SD) of numbers of myelinated nerve fibers observed with Bielschowsky's silver staining in the 4 groups at 2 weeks, 1 month, and 2 months post-operation,. Significant differences were found with post-hoc test using Bonferroni correction: *compared to group D, $p<0.05$; †compared to group A, $p<0.05$; ‡compared to group B, $p<0.05$. Symbols of significance only showed above the group with lower mean.

good recovery had occurred in Group A (80.90±6.31 vs. 86.60±9.75, $p=0.478$). However, in both Groups B and C, the mean number of myelinated nerve fibers was still significantly lower than in Group D (Group B: 77.50±6.43 vs. Group D: 86.60±9.75, $p=0.040$; Group C: 66.00±4.83 vs. Group D: 86.60±9.75, $p<0.001$). In addition, the mean number of myelinated nerve fibers in Group C was significantly lower than in Group B ($p<0.05$). This indicated that PDLLA-T_3 + Schwann cells conduit was better than PDLLA+Schwann cells conduit for nerve regeneration.

Immunohistochemistry for S-100

In Group D, nerves were positive for S-100 in immunohistochemistry. In the longitudinal sections, dark brown bands were arranged in parallel, suggesting nerve fibers. There were myelinated and unmyelinated nerves which were more regularly arranged and had similar thickness and directions. In the other groups, the size and density of nerve fibers were different at different time points. At 2 weeks after surgery, regenerated nerve fibers grew across the PDLLA-T_3 scaffold in Group B and S-100 positive nerve fibers were noted in Group A and Group C. At 1 month after surgery, the number of S-100 positive nerves in Groups A, B and C were all significantly increased. At 2 months after surgery, a variety of S-100 positive nerve fibers grew across the anastomotic site in Group B, the materials were largely degraded, residual materials were absent and the regenerated nerve fibers were regularly arranged. The number of regenerated nerves in Group B was greater than Group C, but less than in Groups A and D (Figure 6A–C).

Number of nerve fibers with S-100 immunohistochemical staining

With regard to the number of nerve fibers observed with S-100 immunohistochemical staining, the main effects of group and time and their interaction were all significant ($p<0.001$). Similar to findings with Bielschowsky's silver staining, all experimental groups also showed significantly lower mean number of nerve fibers than

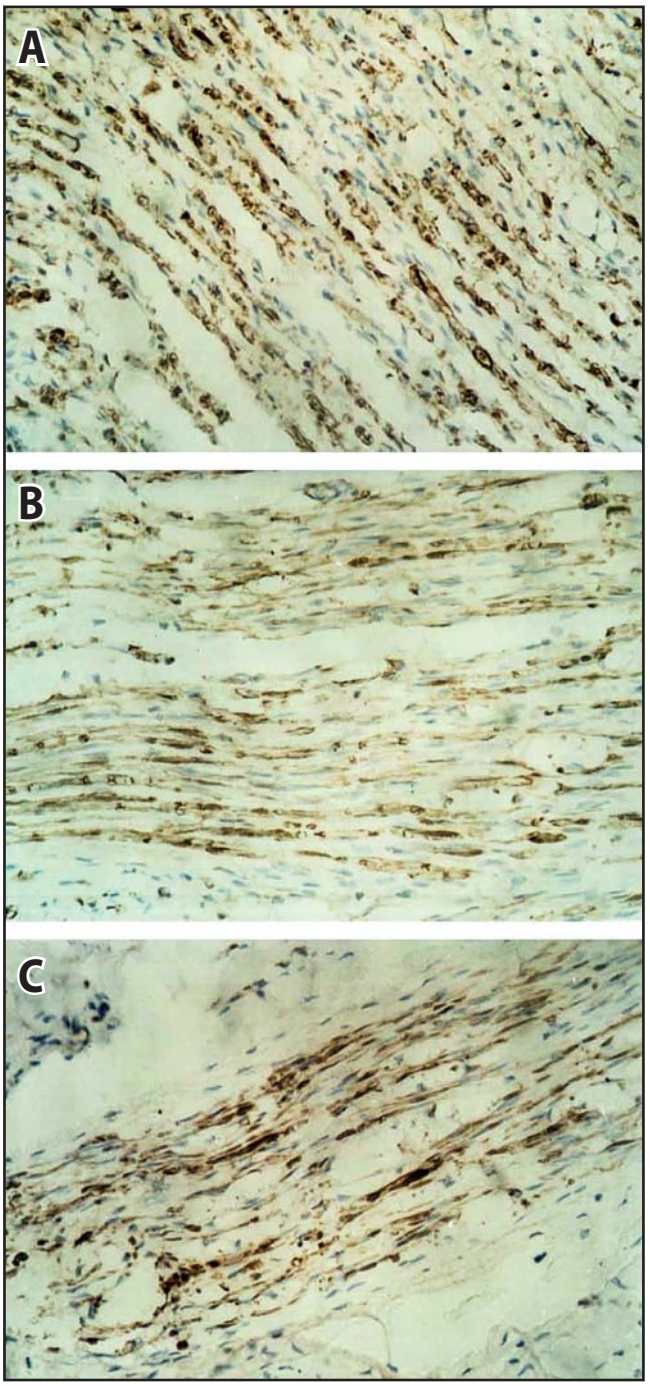

Figure 6. Immunohistochemistry for S-100 at 2 months after surgery (×100). **A**) the regenerated myelinated nerve fibers in Group A were large and densely distributed. **B**) the regenerated nerve fibers grew across the distal anastomotic site in Group B. **C**) fewer nerve fibers were found in Group C

the control group at 2 weeks post-operation (Figure 7). At 1 month and 2 months post-operation, even though number of nerve fibers was increased, all 3 experimental groups still had significantly lower mean number of nerve fibers compared to the control group. Moreover, significant differences among the 3 experimental groups were found only at 2 months post-operation; both Group A and Group B had significantly higher mean number of nerve fibers compared to Group C (group A: 116.00±8.11 vs. group C: 95.60±7.38, p=0.002; group B: 112.90±8.28 vs. group C: 95.60±7.38, p=0.010). The mean number of nerve fibers in Group B was significantly higher than in Group C (p<0.05).

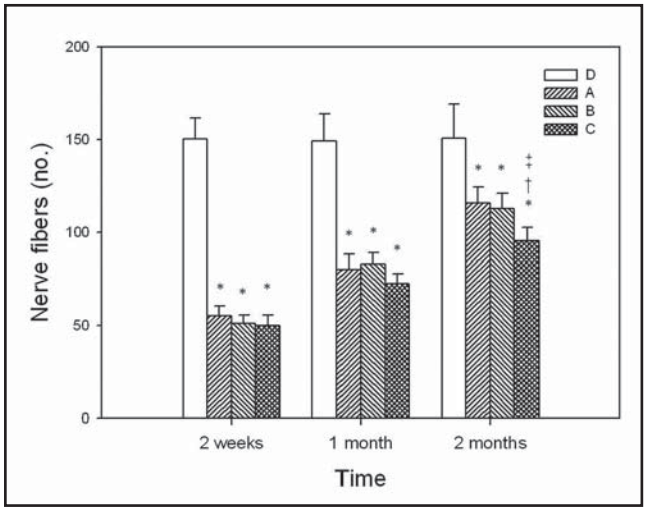

Figure 7. Bar graph (mean with SD) of numbers of nerve fibers observed with S-100 histochemical staining in the 4 groups at 2 weeks, 1 month, and 2 months post-operation,. Significant differences were found with post-hoc test using Bonferroni correction: *compared to group D, p<0.05; †compared to group A, p<0.05; ‡compared to group B, p<0.05. Symbols of significance only showed above the group with lower mean.

HRP Retrograde Tracing

At 2 months after surgery, there were ≈15 cells per section in Group A (Figure 8A) and the size and morphology of these cells varied. They were multipolar, darkly stained and concentrated. In Group B (Figure 8B) and Group C (Figure 8C), the number of positive cells was less than in Group A, but the cell count in Group B was higher than in Group C (3–5 cells per section higher). The cell bodies were large and stained dark. Cell distribution in both Groups B and C was sparser than in Group A. In normal control Group D, HRP staining showed a lot of positive cells (≈30 positive cells/section; Figure 8D).

DISCUSSION

In this study, we compared PDLLA-T_3 with PDLLA and autologous nerve graft for repairing sciatic nerve defect in a rat model. We found that at 2 months after surgery, nerve regeneration was superior in the PDLLA-T_3 group (Group B) compared with the PDLLA group (Group C). Although better results were obtained with autologous graft (Group A) than with PDLLA-T_3 or PDLLA, as was expected, PDLLA-T_3 still might potentially be useful clinically.

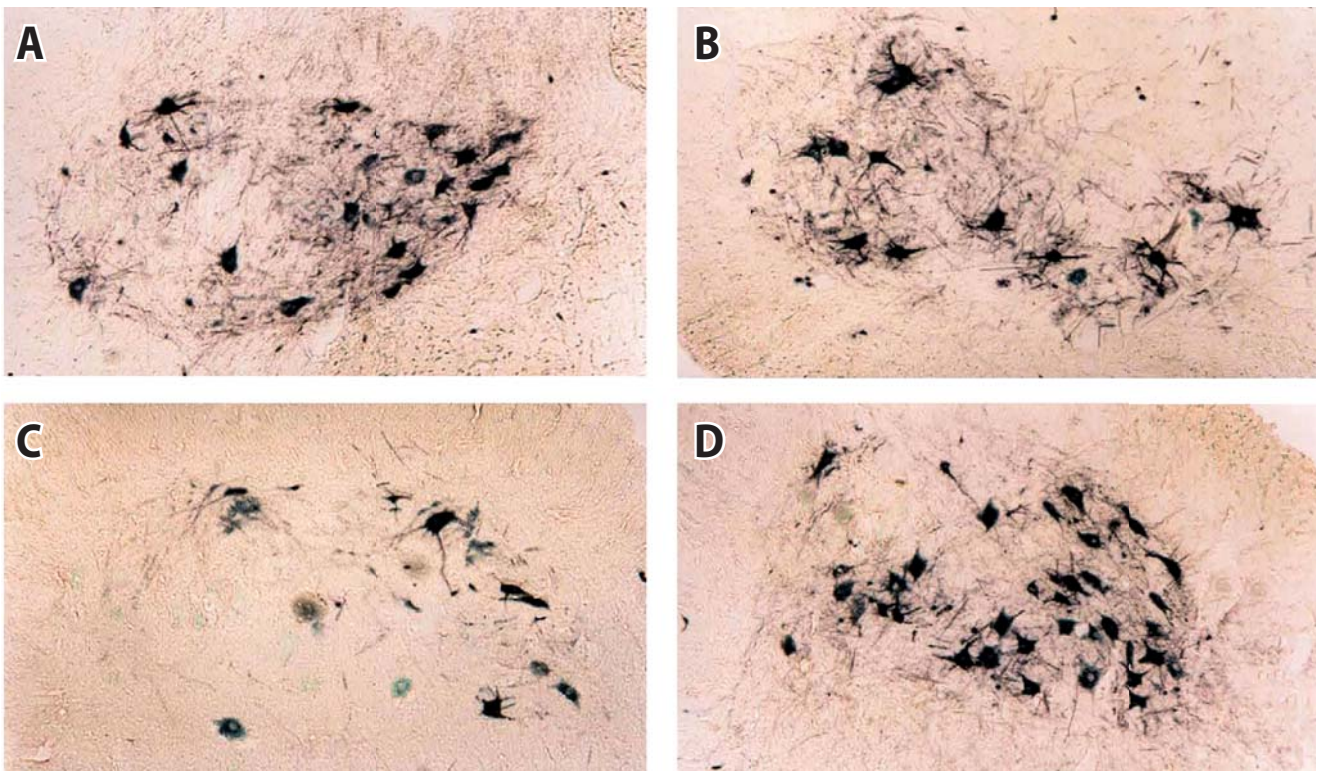

Fig. 8. Horseradish peroxidase retrograde axoplasmic tracing. **A**) Group A, **B**) Group B, **C**) Group C, **D**) Group D.

In nerve tissue engineering there are three key elements: 1) seed cells, 2) biological materials, and 3) formation and regeneration of tissues/organs, in which biological materials play an indispensable role. The rate of degradation of biological materials is a very important consideration for selecting these materials for nerve regeneration. The physical properties of such materials have been studied to determine their potential for use in degradable biomedical systems (Pêgo *et al.* 2002). In previous studies, macromolecular degradable materials have been applied to induce nerve regeneration, but this endeavor is still in its infancy stage (Liu *et al.* 2011) and the reparable length of nerve defect has never exceeded 10 mm (Wang *et al.* 2011). Shen *et al.* (2001) constructed a biological fiber scaffold in animals which was degraded within 6 months, which is a relatively long time. Since the degradability and mechanical properties of these materials have yet to completely match the repair rate of nerves, biologically degradable materials have not been widely used in clinical practice to date. Our findings suggested that PDLLA-T_3 was nearly degraded completely within 2 months after implantation and that nerve regeneration was favorable. When the regenerated nerve grew across the nerve defect, the materials had already almost completely degraded. Therefore, it appears that the rate of material degradation was similar to that of nerve regeneration, which should be beneficial for the reconstruction and remodeling of newly regenerated nerves. At 2 months after surgery, it was also noted that angiogenesis, which is crucial for nerve regeneration, was occurring in the regenerated tissues. This could be attributable to the reconstruction of the local blood circulation.

In the present study, the right sciatic nerve served as a control and the mean thickness of its myelin sheath was 3.01±0.37 μm. Two months after surgery, the myelin sheaths in the PDLLA-T_3 group (Group B) and PDLLA group (Group C) were thinner than that in the autologous nerve group (Group A), but the myelin sheaths in the PDLLA-T_3 group were thicker than those in the PDLLA group, which may be related to the effect of T_3 released by PDLLA-T_3 on Schwann cells. Thyroid has a potent influence on the maturation and myelinization of nerve. Through inducing the proliferation of Schwann cells and release of nerve growth factors, TH can induce nerve regeneration and myelinization. Also, it has been found that sciatic nerve cells have thyroid hormone receptors, which suggests that these cells can respond to TH and this response may play a role in nerve regeneration (Deumens *et al.* 2010). It appears that TH deiodinases play a role in determining T_3 concentration in the central nervous system and peripheral nervous system (Courtin *et al.* 2005). Immunohistochemistry for S-100, a nonspecific marker for Schwann cells, showed the regenerated nerves growing across the scaffold in Group B 2 weeks after surgery, but the size and staining depth of these nerves varied. Image analysis showed that the number of regenerated nerves was larger than in Group C but the difference was not significant. At 2 months after surgery, the number of regenerated nerves in Group B was significantly greater than in Group C. To date, no studies have investigated the effect of TH on peripheral nerve defects over a longer period of time. Our findings suggest that PDLLA-T_3 can promote the regeneration of peripheral nerves through stable release of TH during PDLLA gradual degradation and its effectiveness is superior to that of PDLLA. These findings indicate that during the regeneration of peripheral nerve, local administration of TH at an appropriate dose is effective in promoting the regeneration of peripheral nerves.

We performed silver staining of nerve fibers to demonstrate the influence of PDLLA-T_3 on the myelinization of peripheral nerves. Silver staining revealed that the number of regenerated myelinated nerves in Group B was markedly smaller than in Group A and the control group but was significantly larger than in Group C. The increase in the number and proportion of regenerated myelinated nerves is beneficial for the recovery of lower limb activity in a rat model. Many experiments have utilized toluidine blue staining to observe the cross-section of regenerated nerves and count the number of myelinated nerves, however, this cannot reflect the regeneration of myelinated nerves in the entire segment of transplanted nerve. In the present study, silver staining was done to observe the longitudinal section, which is helpful for observing the growth of myelinated nerves across the scaffold. Of note, the silver staining procedure is more complex than that of toluidine blue staining, and thus there might be some error in the counting. However, application of the same consistent criteria for counting can reduce or even eliminate the error. In our study, the same image analysis system was used and comparisons were done with the same parameters (optical density, etc).

Our results also showed that the regenerated nerves were present at the center and not the periphery of the anastomotic site. Theoretically, at the early stage of repair, the materials acquire the nutrients through penetration, and thus the peripheral nutrients should have been richer and better for substance exchange. Maquet *et al.* (2000) found that a lot of regenerated nerve fibers were at the scaffold periphery, or even under the outer membrane, with fewer present in the center. However, our results were contrary to those previously reported and the specific reasons are unclear and remain to be further studied. We speculate that some T_3 is released at the center of that anastomotic site during the degradation, which then stimulated the proliferation of Schwann cells, regenerated nerves and the myelinization of nerves.

The results of a study by Barakat-Walter *et al.* (2007) corroborate our finding that the addition of T_3 to a scaffold helps facilitate regeneration of the sciatic nerve in a rat model . They added T_3 to a scaffold made of DL-lactide and ε caprolactone and found that T_3 significantly

improves sciatic nerve regeneration. They reported that the results of morphological, morphometric and electrophysiological analysis showed that the addition of T_3 to a conduit produced a significant increase in the number of regenerated myelinated axons.

Our findings suggest that there were regenerated nerve fibers which connected with central nerves in all groups at 2 months after surgery, and that axoplasmic transport had recovered. Horseradish peroxidase staining, which can be used to mark neurons through retrograde axoplasmic transport in regenerated nerves, showed that the number of regenerated nerves in Group B was larger than in Group C, but smaller than in Group A. These findings suggest that the therapeutic effectiveness of using biomaterials for conduits with the addition of TH is still inferior to that of autologous nerves for repairing nerve defects.

Nerve regeneration is a complicated process requiring a good microenvironment (Freitas *et al.* 2010), and the interaction between the microenvironment and modulators, including interleukin-1 (Yuan *et al.* 2002), immunosuppressants, etc., is also important. Our findings show that PDLLA-T_3 can facilitate this process better than PDLLA without T_3 in a sciatic nerve defect rat model, even though the results are not as good as those using autologous nerve. Based on our findings, we believe that further investigation is warranted with the hope that the results of using PDLLA-T_3 can be improved to the point where clinical studies can eventually be initiated.

Competing interests

The authors declare that they have no competing interests.

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