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# Acceleration effect of basic fibroblast growth factor on the regeneration of peripheral nerve through a 15-mm gap

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**Abstract:** In this study, nerve guides composed of poly(D,L-lactide) (PDLLA) were fabricated and used in the repair of transected sciatic nerves (15-mm gaps) of rats. Nerve guides with a two-ply structure (inner layer dense, outer layer microporous) were prepared by controlling the solvent evaporation rate. Then basic fibroblast growth factor (bFGF) was embedded in the inner layer of the nerve guides. Thus the inner dense layer not only could prevent the ingrowth of fibroblast and avoid the outgrowing nerve cable, but it also could retain the released bFGF in the guide lumen. The outer porous layer allowed vascular ingrowth and the diffusion of essential nutrients into the guide lumen. The data show that by using this nerve guide, the transected 15-mm sciatic nerve was regenerated successfully within 4

months. The recovery of function of the regenerated nerves was significantly accelerated by bFGF, as indicated by an electrostimulation test and histologic assays. In addition, the bFGF retained its bioactivity during embedding and continuously was released from the matrix, as confirmed by the results of both the dorsal root ganglia (DRG) and the Schwann cell culture in the presence of PDLLA matrix containing bFGF. The released bFGF enhanced the ability of the nerve fibers to sprout from dorsal root ganglia, and it accelerated the proliferation of Schwann cells. © 2003 Wiley Periodicals, Inc. *J Biomed Mater Res* 66A: 522–531, 2003

**Key words:** poly(D,L-lactide); nerve guide; sciatic nerve; regeneration; basic fibroblast growth factor

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## INTRODUCTION

The repair of transected or damaged peripheral nerves is a common clinical problem because severe traumas often lead to peripheral nerve damage or complete fracture. The usual method of repairing a transected nerve involves mobilization and epineurial suturing of the proximal and distal stumps with coaptation of individual nerve fascicles. When a part of the peripheral nerve is lost, the most widely used technique for reconstruction is suturing of an autologous graft, often one from another nerve of lesser functional importance. But this technique has some disadvantages, such as the need of a second surgical step, causing a sensory deficit at the cutaneous distribution site of the donor nerve, the risk of neuroma formation at the donor site, loss of the donor nerve's function, and a limited supply of donor nerves, as well as a possible mismatch between nerve and graft dimension.

Tubulization, in which nerve guides are used to bridge the nerve gap, is an alternative repair method that aims to eliminate these problems. Bioinert materials, such as silicone rubber, nylon, and porous stainless steel, have ever been used to construct such nerve guides. Although the use of these tubes to bridge nerve gaps has provided an excellent *in vivo* experimental model to study the process of peripheral nerve regeneration, the main objection to using bioinert tubes is that they remain *in situ* after the nerve has regenerated and may cause a chronic foreign-body reaction and, later, nerve compression with secondary complaints and impairment of nerve function.<sup>1,2</sup>

Biodegradable nerve guides could provide a successful alternative. They might overcome these problems because the nerve guide gradually would degrade and disappear after allowing the outgrowth and maturation of the nerve. To date, most studies on the regeneration of peripheral nerves using biodegradable nerve guides have been evaluated in an animal model with favorable results.<sup>3,4</sup> For instance, crystalline and amorphous copolymer of lactide and  $\epsilon$ -caprolactone were used to construct nerve guides and proved to be effective.<sup>5,6</sup> And Schakenraad concluded that nerve guides with an internal diameter of 1.23 mm and a wall thickness of 0.34 mm could ensure nerve regen-

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eration in the case of 10-mm gaps in the sciatic nerve of rats.<sup>7</sup>

More recently, the study of porous nerve guides<sup>8,9</sup> has attracted attention because porous nerve guides have high permeability and can supply the nerve with essential nutrients for revascularization. Nevertheless, gaps that had been bridged in the sciatic nerve of rats generally were 6 mm and seldom were over 10 mm. For a 20≈25-mm sciatic nerve gap in rats, Madison et al. filled silicone tubes with collagen or laminin and compared them with empty control tubes.<sup>10</sup> Although all the tubes with additives demonstrated nerve regeneration extending up to 4≈6 mm, silicone is a biodurable material and gives rise to the problems mentioned above. Kiyotani et al. studied the regeneration of sciatic nerves with gaps of 25 mm in cats using a poly(glycolic acid)/collagen composite tube to bridge the gap.<sup>11</sup> Unfortunately, though a population of myelinated fibers could be observed within the lumen, the guide lumen was small, and, in addition, the mechanical property of this nerve guide was poor.

In previous studies, we successfully used a biodegradable guide composed of poly(D,L-lactide) (PDLLA) for the regeneration in rats of sciatic nerves with gaps of 10 and 20 mm.<sup>12,13</sup> The data showed that PDLLA had good biocompatibility and biodegradability and that it could keep strength, elasticity, and shape until the transected nerve had recovered. However, these researches did not concern themselves with the acceleration effect of growth factor on the nerve regeneration.

It is a common opinion that growth factors can accelerate cellular proliferation and differentiation, offering the possibility that nerve growth factor may have important roles in the regeneration of transected nerves. Growth factors have been reported to promote both the growth and the survival of axons of peripheral and central nervous systems.<sup>14</sup> But growth factors can be exceedingly difficult to administer directly to patients since they typically have short half-lives. They easily can lose their biological activity under body conditions, especially when in contact with enzymes or body liquid. In view of this difficulty, the most promising technique for avoiding the loss of bioactivity of growth factors involves controlled-release devices made of degradable polymers. Such devices can be engineered to provide precisely controlled and prolonged growth factor delivery at a localized sites.<sup>15,16</sup>

In this paper, a method is proposed for embedding basic fibroblast growth factor (bFGF) into nerve guides composed of PDLLA for the regeneration in rats of sciatic nerves with 15-mm gaps. The results are compared by means of electrophysiologic recovery determination and histologic assays with the absence of bFGF. In addition, *in vitro* cultures of dorsal root ganglions (DRG) and Schwann cells in the presence of PDLLA matrixes containing bFGF were performed to

investigate the release behavior of bFGF and its bioactivity.

## MATERIALS AND METHODS

### Preparation of the nerve guide

A complete presentation of the chemical synthesis, purification, and biocompatibility of poly(D,L-lactide) (PDLLA) has been described in previous reports.<sup>12,13</sup> PDLLA with a molecular weight of  $4.67 \times 10^4$  (determined by GPC, Waters510 equipped with Shedox KF-800 columns) was obtained by ring-opening polymerization of D,L-lactide using hexadecanol as an initiator and stannous octoate as the catalyst.

Polymeric solution with a concentration of 5≈10 wt % was prepared by dissolving PDLLA in dichloromethane. A polytetrafluoroethylene (PTFE) mandrel was immersed into the solution for a while and then pulled out to evaporate the solvent. After this process was repeated several times, the outer coatings were prepared by extracting dichloromethane in anhydrous ethanol (instead of evaporating in air) in order to quickly remove solvent. Finally, the prosthesis was stripped off the mandrel and vacuum-dried at room temperature for 48 h.

The final nerve guides had an inner diameter of 2 mm and a wall thickness of 0.1 mm. In the case of nerve guides containing growth factor, bFGF powders were dispersed in the polymeric solution and nerve guides were fabricated as described above. The bFGF was embedded in the inner layers of nerve guides at a concentration of 1 mg of bFGF per gram of polymer. These nerve guides were stored over P<sub>2</sub>O<sub>5</sub> in a desiccator at -20°C and were sterilized with gaseous formaldehyde for 4 h before implantation.

### Surgical procedures

Forty-eight male and female Sprague-Dawley rats, weighing approximately 200 g each, were anesthetized with 3.5% sodium pentobarbital solution (3 mg per 100 g of weight) intraperitoneally. After disinfection with 0.1% benzalkonium bromide solution, the right sciatic nerve was exposed through a gluteal muscle-splitting incision. A 13-mm segment was resected, leaving a gap of about 15 mm caused by the retraction of nerve ends. Twenty-mm nerve guides with or without bFGF were interposed between the proximal and distal stumps. Both the proximal and distal cut ends of the sciatic nerve were telescoped into the tubes and fixed with a single 11-0 nylon suture. The rats were divided into two groups (24 rats each group) stochastically, according to the nerve guides used without (Group A) or with (Group B) bFGF.

After surgery, the animals were placed in separate cages. All animals had free access to standard rat food and water. The right lateral foot was not fixed and no drugs were administered during the postoperative period.

## Function recovery evaluation of regenerated nerves

Four weeks, 10 weeks, and 4 month postoperation, eight rats from each group were killed for each time interval. Then the regeneration and function recovery of the regenerated sciatic nerves were evaluated by macroscopy and microscopy observation and by electrophysiologic and histologic analyses.

### Macroscopy observation

Throughout the 4-month study, the infection, ulceration, and mobility of the right lateral foot were observed. At each time interval (4 weeks, 10 weeks, and 4 month), the right sciatic nerves were re-exposed. The nerve guides then were carefully dissected from surrounding tissues, and the morphologic changes, biodegradation, and absorption of the nerve guides, as well as the inflammation of the tissues surrounding the implant, were evaluated macroscopically.

### Electrostimulation tests

Four month postoperation, the right sciatic nerve tract comprising the guide carefully was re-exposed and dissected from surrounding tissues. After 10 mL of liquid paraffin was poured into the site of the implant, the somatosensory-evoked potentials (SEP) were measured with an electric stimulator (Neuropack-II 5100, Japan). The current and voltage applied in the experiment depended on the responses of the rats; that is, if the rat withdrew its foot and spread its toes when being stimulated, then the current and voltage would not be elevated further. Following, a signal display and process system (BL-310 Intelligent Biosensor Display System, Chengdu Time Electronic Ltd., China) was applied to obtain electromyograph patterns (EMG) and to measure motor nerve conduction velocity (MNCV), amplitude of muscle evoked potentials, and latency. Latency refers to the time between the stimulus and the onset of the first reflex.

### Histologic studies

#### *Preparation for light microscopy*

Histologic evaluation of regenerated nerves was carried out at 4 weeks, 10 weeks, and 4 month after implantation. Nerves were harvested and cut into two pieces of transverse sections at mid-tube and at the distal end. The specimens were fixed in 2.5% glutaraldehyde for 24 h at 4°C, and post-fixed in 1.0% OsO<sub>4</sub> for another 3 h. Subsequently, the specimens were dehydrated in a graded ethanol series and embedded in Epon 812. Transverse sections (1 μm) were cut by using a microtome. Then the sections were washed routinely with supersaturated NaOH ethanol solution to eliminate epoxy resin, cleaned with pure ethanol twice, and stained with 1.0% toluidine blue for observation under an inverted microscope (Olympus IMT-2, A10PL).

Viewed with phase contrast, images of the histologic sections were photomicrographed and subsequently analyzed using

standard image processing and analysis techniques (the software was provided by Yalien Company, China), according to the literature.<sup>2</sup> Images were thresholded and segmented into individual myelinated fiber. The number of axons per three randomly selected fields measuring 220 × 325 μm were counted to give the number of myelinated fibers/mm<sup>2</sup>.

#### *Preparation for electrical microscopy*

At the end of the fourth month following the operation, specimens were harvested at the mid-tube of each group. After being handled as described above, the specimens were cut into transverse slices and then stained with uranyl acetate and lead citrate for observation under scanning electron microscopy (SEM). Subsequently, the diameters of the nerve fibers and the thicknesses of the fiber sheaths were determined by the image analysis system.

### Culture of nerve cells *in vitro*

#### Preparation of the PDLLA films containing bFGF

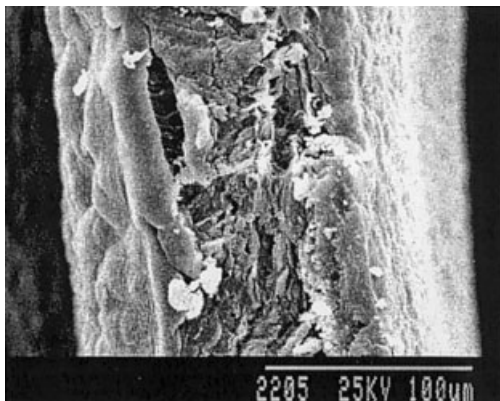
bFGF (Torita, China) powders were dispersed in PDLLA solution in dichloromethane ultrasonically, and subsequently the solution was cast onto a glass plate. After first being air- and then vacuum-dried for 24 h, PDLLA films containing bFGF (1 mg of bFGF per gram of polymer) were obtained. These films were stored at -20°C before use. For comparison, blank PDLLA films also were prepared using a similar method.

#### *In vitro* culture of dorsal root ganglion

Dorsal root ganglions (DRG), obtained and separated from Leghorn eggs, were cultured for 8 days before they were evenly seeded in collagen-coated culture dishes with 1 mL of serum-free culture medium. Ten mg of PDLLA matrix containing or not containing bFGF were placed at one side of the culture dish and immersed in the medium. The system was observed and photos were taken under a microscope (Olympus, IMT-2, A10PL) everyday.

#### *In vitro* culture of Schwann cells

Schwann cells were cultured in 50 mL of cell-culture flasks with Dulbecco's modified Eagle's medium (Gibco) buffered with N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid (HEPES) supplemented with 15% calf serum (Gibco) and 100 U/cm<sup>3</sup> each of penicillin and streptomycin. Cell culture was maintained in a gas-jacket incubator equilibrated with 5% CO<sub>2</sub> at 37°C. When the cells had grown to confluence, they were digested by 1 mL of 0.25% trypsin (Sigma) for 1–2 min. Then 3 mL of culture medium were added to stop digestion, and the culture medium was aspirated to get cell dispersion after the cells counted.



**Figure 1.** The two-ply structure of nerve guide made of poly(D,L-lactide): left, outer layer; right, inner layer. (Original magnification  $\times 500$ )

PDLLA films were cut into small disks (15 mm in diameter) with the aid of a cork borer in order to locate the disks into 24-well tissue-culture plates. All the samples were prewetted and sterilized in 75% ethanol for 0.5 h, and then the ethanol was exchanged with an excess amount of phosphate-buffered saline (PBS).<sup>17</sup> After the PBS in the disks was removed by a pipette, a Schwann cells suspension (0.5 mL) with a density of  $3\sim 4 \times 10^4$  cells/mL was seeded evenly into the wells with a pipette.

The cell-seeded disks were maintained at 37°C under 5% CO<sub>2</sub> conditions for 1, 2, 3, and 4 days. The culture medium was refreshed everyday. At each time interval, residual culture medium and unattached cells were removed by washing the disks three times with PBS after the culture medium was removed. Subsequently, the attached cells on the disks were digested by trypsin and the number of cells remaining in the wells was counted under a microscope.

## RESULTS

### Preparation of the nerve guide

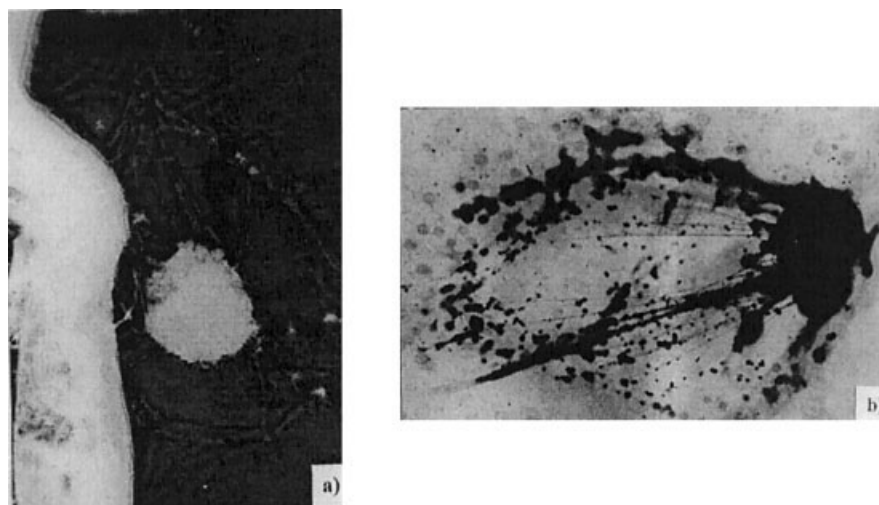
Nerve guides with a length of 20 mm were fabricated using a dip-coating and solvent-evaporation method. Guides with a two-ply structure were obtained, as shown in Figure 1. The inner layer was dense whereas the outer layer was microporous. This two-ply structure was formed as a result of the slow solvent-removing method used, in which the slow solvent evaporation in air lead to a uniform and dense structure; the fast solvent removing by ethanol extraction normally results in a heterogeneous structure.

The inner dense layer could prevent ingrowth of fibroblasts and hamper the outgrowing nerve cable. Furthermore, it could keep the released growth factor in guide lumen for guides containing bFGF since bFGF was embedded in the inner layer of the nerve guides, and thus it could enhance nerve-cell proliferation. On the other hand, the microporous outer layer might enhance nerve regeneration by allowing the revascularization and the diffusion of growth-promoting factors generated in the external environment into the guide lumen.

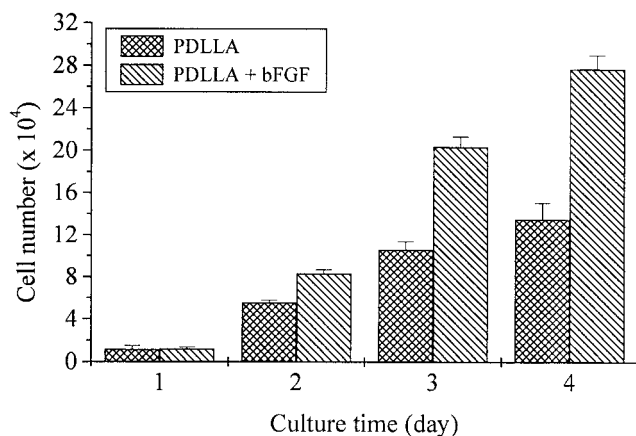
### *In vitro* evaluation of the release and the bioactivity of embedded bFGF

#### *In vitro* culture of dorsal root ganglion

To investigate the controlled release of growth factor from polymeric matrix and the remaining bioactivity, dorsal root ganglions (DRG) were cultured in



**Figure 2.** The inductive growth *in vitro* of DRG cells in the presence of bFGF-embedded PDLLA matrix. These photos were taken after just 1 day of culture (a) near the PDLLA matrix and (b) far from the PDLLA matrix.



**Figure 3.** Comparison (by number of cells as counted under a microscope) of Schwann cells propagated on PDLLA substrates with and without bFGF ( $p < 0.05$ ).

the presence of a PDLLA matrix containing bFGF (and, for comparison, in the absence of bFGF) because DRG has a characteristic that its nerve fibers do not grow within a short time (e.g., 24 h) if no growth factors exist in the medium. Indeed, there was no growth of nerve fibers for DRG observed in the absence of bFGF. In contrast, many nerve fibers sprouted vigorously from dorsal root ganglions in PDLLA matrix containing bFGF, as shown in Figure 2.

Interestingly, the nerve fibers spread around the DRG when DRG was at the site near the PDLLA matrix [Fig.2(a)] whereas the nerve fibers exhibited an orientation toward the PDLLA matrix when the DRG was at a site relatively far from the matrix [Fig.2(b)]. This phenomenon could last for more than 2 weeks.

#### *In vitro* culture of Schwann cells

The *in vitro* culture of Schwann cells was carried out on PDLLA matrices with and without bFGF, and the results of cell propagation are presented in Figure 3.

In comparison to blank PDLLA matrix, the proliferation rate of Schwann cells was significantly accelerated in the presence of PDLLA matrix containing bFGF except on the first day. By days 2, 3, and 4,

vigorous propagation of Schwann cells could be seen in the presence of bFGF, 20–50% higher than that of blank PDLLA matrices.

#### Regeneration of the sciatic nerves of rats with or without bFGF

##### Macroscopy observation

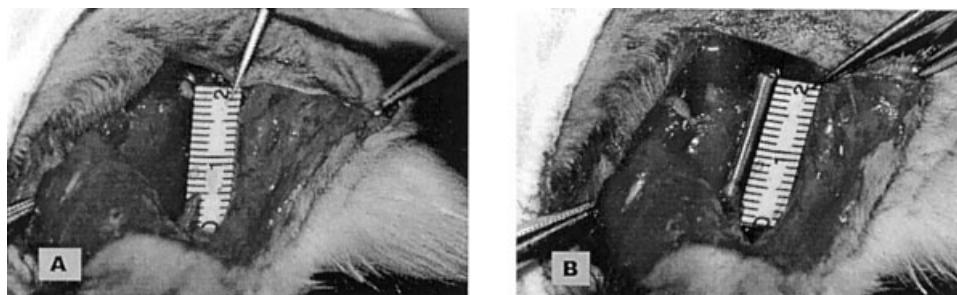
As shown in Figure 4, a 13-mm segment was resected in the right sciatic nerve of rat hind legs, leaving a gap of about 15 mm caused by the retraction of nerve ends. Twenty-mm nerve guides without (Group A) or with (Group B) bFGF were interposed between the proximal and distal stumps. After the surgery, the rats of Group B showed little difference in daily behaviors from those of Group A. Although some of the animals showed ulceration at the second week post-operation, they were self-healed within 3 months.

At each predetermined interval, the guides were carefully re-exposed. As determined by macroscopy observation, all the guides in both groups had attached to surrounding tissues by membranes or fibers 4 weeks postoperation. The transparent guides had changed into an opaque state, as shown in Figure 5(a), but no inflammation could be identified.

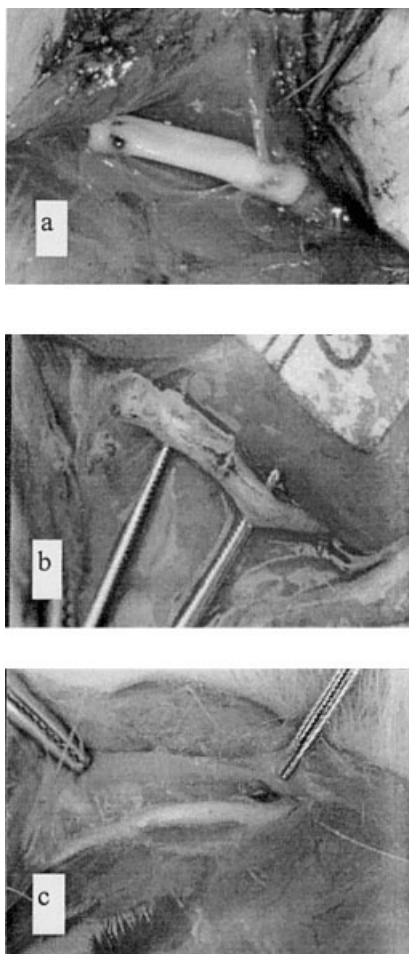
By 10 weeks postoperation, the smooth surfaces of PDLLA nerve guides had become rough, and the guides obviously had broken into fragments [Fig. 5(b)]. By 4 months the regeneration of the transected nerves was quite good from their macroscopic appearance, and the guides had disappeared from the implanting site [Fig. 5(c)]. Little difference could be found by this macroscopic observation between Group A and Group B.

##### Electrostimulation evaluation

The evaluation of the somatosensory-evoked potentials (SEP) of both groups at 4 months postoperation is shown in Figure 6. Obviously, the SEP profiles of



**Figure 4.** Photograph of an (A) 15-mm sciatic nerve gap in a rat and a (B) 20-mm nerve guide made of poly(D,L-lactide) with or without bFGF interposed between the stumps of the transected sciatic nerve.



**Figure 5.** Microscopic observation of the morphologic changes of a nerve guide containing bFGF and the regeneration of the sciatic nerve at different periods postoperation of the rats in Group B: (a) at 4 weeks (b) at 10 weeks (c) at 4 months. (Original magnification  $\times 1$ )

regenerated nerves, for the rats in both Group A and Group B, were not as good as the SEP patterns of the left undamaged lateral feet [Fig. 6(a)]. However, the quantitative determination of electromyograph (EMG) analysis clearly shows the recovery of different functions of the regenerated nerves in different groups, as detailed in Table I. Both the motor nerve conduction velocity (MNCV) and the amplitude of muscle-evoked potentials of the regenerated nerves of rats in Group B were larger than those in Group A, whereas the latency was in the reverse order. The regenerated nerves of rats in Group B had recovered to 70% of the normal nerve.

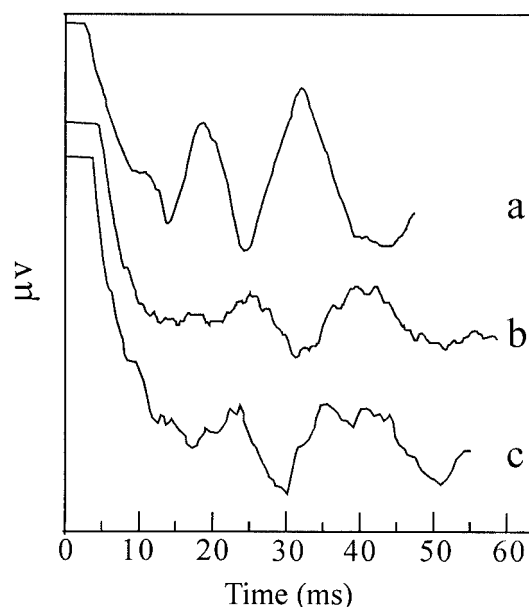
#### Histologic assessment

At the end of 4 months postoperation, the regenerated nerves were harvested, and slices were made from the mid-tube and distal part for histologic analysis. Observed under the phase-contrast microscope,

the number of myelinated nerve fibers could be counted. As seen in Figure 7, the myelinated fibers in three randomly selected fields ( $220 \times 325 \mu\text{m}$ ) was counted to give the number of fibers/ $\text{mm}^2$ . Each of the selected fields should contain more than 500 myelinated fibers. All data are expressed as a mean  $\pm$  SEM (standard error of the mean) and are presented in Table II.

The mean number of myelinated fibers was higher in Group B than it was in Group A both in the guide and at the distal end; however, there was no significant difference between the two groups in the number of myelinated fibers at the distal ends at 4 months postoperation. Obviously, there was a higher number of myelinated fibers at mid-tube and fewer fibers at the distal ends in both groups. Although they all increased with time, the number of the fibers seemed to increase more slowly at the distal end than at mid-tube.

Myelinated and unmyelinated fibers within the regenerated nerve were additionally detected at the same time under SEM observation (Fig. 8). Compared to the regenerated nerve in the absence of bFGF, the number of myelinated nerve fibers was relatively higher and the sheath was thicker if the PDLLA nerve guide contained bFGF (see Fig. 8). The diameter of myelinated nerve fibers and the sheath thickness were calculated using an imaging analysis system, and these are displayed in Table III. The diameter and sheath thickness of myelinated nerve fibers regenerated in the presence of bFGF indeed can be seen to be larger than those nerve fibers regenerated without bFGF.



**Figure 6.** Comparison of averaged patterns of somatosensory-evoked potentials (SEP) among (a) undamaged sciatic nerves and regenerated sciatic nerves (b) without or (c) with bFGF. Time is in milliseconds; amplitudes are in microvolts.

**TABLE I**  
**Electrophysiologic Parameters of the Regenerated Sciatic Nerves in Two Groups**  
**of Rats Studied and of Normal Sciatic Nerves**

	MNCV (m/s)	Amplitude of Evoked Potentials ( $\mu$ v)	Latency (ms)
Normal nerve	47.10 $\pm$ 1.47	16.39 $\pm$ 1.08	2.92 $\pm$ 0.55
Group A*	24.39 $\pm$ 1.45	8.55 $\pm$ 0.73	4.60 $\pm$ 0.92
Group B*	32.42 $\pm$ 1.82	11.69 $\pm$ 1.45	3.22 $\pm$ 0.67

\*Group A and Group B refer to the nerve regeneration performed without and with bFGF, respectively.

Every group has eight rats. Values are expressed as a mean  $\pm$  SEM. All animals were included for NNCV, amplitude of evoked potentials, and latency evaluation ( $p < 0.05$ ).

## DISCUSSION

The aim of this study was to evaluate bFGF relative to nerve function recovery after reconstruction with nerve guides composed of PDLLA of a 15-mm gap in the sciatic nerve of rats. After 4 months, the nerve guides disappeared from the implantation site, and the transected nerve had regenerated. By electrostimulation tests, light microscopy observation, and histologic analysis, it was found that bFGF significantly can enhance the recovery of nerve function.

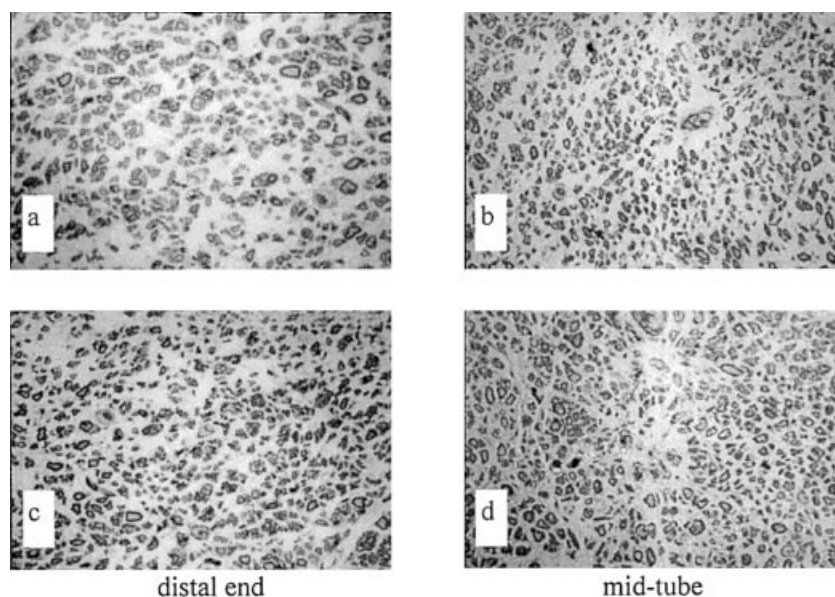
### Degradation and biocompatibility of nerve guide

The practical interest of research on artificial guides centers primarily on procedures for the repair of severe injuries of peripheral nerves. Many reports have shown that resorbable guides allow for better levels of regeneration than do permanent guides.<sup>18,19</sup> It is hypothesized that resorbable tubes allow for better nu-

trient supply to the regenerated nerve, enhance the constitution of the initial matrix and the subsequent nerve cable, and increase their flexibility as they degrade. Thus it is thought that resorbable guides can avoid secondary damage to maturing regenerated nerves.<sup>2</sup>

One of the characteristics of an ideal bioresorbable nerve guide is that it would degrade at a suitable rate relative to the axonal growth rates. A nerve guide should degrade immediately after it has served has provided optimal conditions to the outgrowing and maturing nerve fiber. On the other hand, a nerve guide must not degrade too quickly; otherwise, fibrous tissue could grow into the lumen of the tube and hamper the regeneration and maturation of the nerve fibers.<sup>20</sup>

We have evaluated the degradation of PDLLA films and found that their degradation rates strongly depended on their molecular weight. The molecular weight of this polymer could decrease from  $37.5 \times 10^4$  to  $8 \times 10^4$  after having been implanted *in vivo* for 3 months and the polymer had changed from a trans-



**Figure 7.** Light micrograph demonstrating the regenerated nerves at (right, b and d) mid-tube and (left, a and c) distal end of rats in different groups 4 months postoperation: upper, without bFGF (Group A); lower, with bFGF (Group B). (Original magnification  $\times 20$ )

**TABLE II**  
**Morphologic Parameters of the Regenerated Nerves Mid-tube and at Distal Ends in Two Groups of Rats**

Postoperation Time (Month)	Mid-tube (Number/mm <sup>2</sup> )			Distal End (Number/mm <sup>2</sup> )		
	Group A	Group B	p	Group A	Group B	p
2	8399 ± 4130	30228 ± 9648	<0.05	5622 ± 4364	9328 ± 5119	<0.1
4	11720 ± 5678	34802 ± 13496	<0.05	11146 ± 5301	12993 ± 4699	>0.05

The myelinated fiber density is expressed as a mean ± SEM. Group A and Group B refer to the nerve regeneration performed without and with bFGF, respectively.

parent to an opaque and swollen state but still remained intact.<sup>13</sup>

Pitt et al. showed that the *in vivo* degradation of PDLLA occurs essentially by homogenous bulk erosion over two stages. The first stage takes place by random hydrolytic chain scission of the ester groups, accompanied by a linear loss in Mw. The second stage begins at an average Mw of  $1.5 \times 10^4$ , at which point weight loss occurs.<sup>21</sup> Therefore, in this study, to ensure that the degradation rate of a nerve guide matched the nerve regeneration rate, PDLLA with a lower molecular weight ( $4.67 \times 10^4$ ) was synthesized and used to prepare nerve guides. The reparation experiments of transected sciatic nerves of rats clearly showed these nerve guides could be used as conduits for nerve regeneration. Macroscopy observation showed that the foreign-body reaction was mild and disappeared from the body by 4 months postoperation.

#### Sustained release from PDLLA matrix and bioactivity protection of bFGF

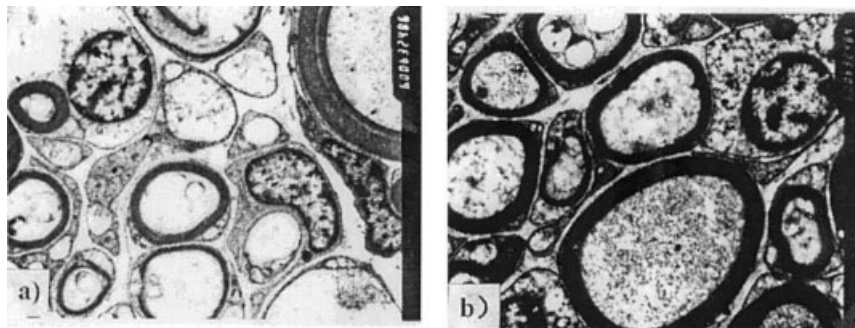
Growth factors are bioactive compounds with short half lives, and they easily can lose their bioactivity by interacting with body fluid, enzymes, etc. Usually polymeric materials are used to embed the growth factors to protect their bioactivity. Many methods have been put forward for embedding growth factors into polymeric matrix; however, the interaction of

growth factor with organic solvents in most cases is inevitable, and therefore the bioactivity of the growth factor is damaged.

Various efforts have been made to overcome this problem, including co-encapsulation of poly(ethyl glycol) (PEG), collagen, or bovine serum album (BSA) with growth factors.<sup>22-24</sup> However, no such protective compounds were used in the embedding of bFGF into PDLLA nerve guides in this study. Although bFGF powders were directly dispersed in the polymeric solution by ultrasonication, it was demonstrated that bFGF had a significant acceleration effects on the proliferation of Schwann cells in comparison with blank PDLLA matrix, as shown in Figure 3. The number of Schwann cells were observed to increase 20~50% in the presence of bFGF after 1 day culture.

In addition, the results of *in vitro* static culture of dorsal root ganglia (DRG) further elucidated that the embedded bFGF could be released continuously from PDLLA matrix and not lose its bioactivity. The DRG have the characteristic that its nerve fiber will not grow if the system contains no growth factor at the early stages of inoculation (e.g., within 24 h). As shown in Figure 2, the nerve fibers could sprout from the DRG within 1 day.

Furthermore, nerve fibers showed an orientation toward the bFGF-embedded PDLLA matrix if the DRG was at a relatively distant site from the matrix. This phenomenon could last for 2 weeks and was thought caused by the different bFGF concentration in the medium. With bFGF being released from the



**Figure 8.** SEM photos indicating the regeneration of myelinated nerve fibers of rats in different groups: left, Group A, PDLLA tube without bFGF; right, Group B, PDLLA tube containing bFGF. (Original magnification  $\times 5000$ )

**TABLE III**  
**Morphologic Parameters of Regenerated Nerve Fibers of Rats**

	Mid-tube		Distal End	
	Group A	Group B	Group A	Group B
Diameter of myelinated fiber ( $\mu\text{m}$ )	$3.28 \pm 1.02$	$4.67 \pm 1.38$	$3.15 \pm 1.23$	$4.56 \pm 1.29$
Thickness of sheath ( $\mu\text{m}$ )	$0.68 \pm 0.11$	$0.83 \pm 0.22$	$0.65 \pm 0.13$	$0.79 \pm 0.18$

Group A and Group B refer to the nerve regeneration performed without and with bFGF, respectively. All animals were included in the diameter of myelinated fibers and the thickness of sheaths ( $p < 0.1$ ).

PDLLA matrix, its concentration certainly should be higher around the matrix and be lower at a site far from the matrix because of its slow diffusion under the static culture condition.

Both the results of Schwann cells and the dorsal root ganglia in *in vitro* culture experiments confirmed that the embedded bFGF could be released continuously from PDLLA matrix and could maintain its bioactivity. Although it is not clear how much bFGF is released and how much its bioactivity is maintained, it can be concluded that using hydrophobic polymers to embed growth factors is a promising method for protecting the bioactivity of growth factors and for obtaining their sustained release behavior.

#### The effects of bFGF on function recovery of regenerated nerve

An important result from our study is that regenerating nerve fibers can cross a 15-mm gap in the sciatic nerve of rats in 10 weeks and that the presence of bFGF can accelerate function recovery of the regenerated nerve significantly.

Successful regeneration after tubularization depends on the formation of a new extracellular matrix scaffold, over which blood vessels, fibroblasts, and later on, Schwann cells migrate and form a new nerve structure.<sup>25,26</sup>

Surviving axons in the proximal stump elongate into the guide along cellular outgrowth that follows the connective strands bridging the gap, and eventually the axons and accompanying Schwann cells progress into the distal nerve stump. If there are not enough regenerative promoting elements inside the guide, this process often fails in long gaps.<sup>27,28</sup> Therefore, the number of myelinated fibers is higher at mid-tube and less so distally in both groups (see Table II).

Although they all increased with time, the growth rates of myelinated fibers were slow 2 month postoperation. This phenomenon demonstrates a delay in axonal elongation and indicates the generation of intratubular neuroma. However, the regeneration of the myelinated fibers is accelerated significantly in the presence of bFGF although the experimental results

did not reach a satisfactory recovery of the transected sciatic nerve.

On the other hand, it was found that nerve cables containing both myelinated and unmyelinated fibers had regenerated through the 15-mm PDLLA tubes in both groups, as shown in Figure 8. But more cables containing myelinated fibers were found, and the fiber diameter and sheath thickness also were larger if the PDLLA guides contained bFGF compared to those (controls) in the absence of bFGF.

After recovery from a sciatic nerve transection lasting 4 months, EMG patterns in hind-leg muscles during locomotion were quite different from those of the unoperated leg whether or not the nerve guides contained bFGF. Gramsbergen et al.<sup>29</sup> concluded that the nerve transection would leave permanent effects on muscle movements and that there would be abnormalities in EMG patterns that would be hard to eliminate even with further recovery. Our quantitative analysis of EMG patterns (Table I) did indicate, however, that the MNCV and amplitude of muscle-evoked potentials were larger in the presence of bFGF than were those in the absence of bFGF. And bFGF also accelerated the regeneration of the injured sciatic nerve, as demonstrated by its shortened latency.

## CONCLUSIONS

Nerve guides with two-ply structure, which makes them especially useful for nerve regeneration, were prepared by a polymeric solution dip-coating and a controlled solvent evaporation rate technique. These guides, composed of PDLLA with a Mw of  $4.76 \times 10^4$ , changed from smooth and transparent to rough and opaque, and they lost their strength 10 weeks postoperation. However, this was long enough to ensure nerve regeneration and maturation across a 15-mm gap in the sciatic nerves of rats. The degradation of the polymer did not appear to inhibit the axonal growth. The electrophysiologic and function recovery of the regenerated nerve was enhanced by embedding bFGF in the inner layer of the nerve guides. The inner dense layer of nerve guides not only prevented the ingrowth of fibroblasts but also retained the growth factor in the

guide lumen long enough to induce nerve regeneration. The results of DRG and of Schwann cell-culture studies *in vitro* further demonstrated that bFGF enhances the regeneration of peripheral nerve, retains its bioactivity after being embedded in PDLA matrix, and can be released continuously from the polymeric matrix for a long time.

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