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Peripheral nerve regeneration through P(DLLA-ε-CL) nerve guides

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P(DLLA-ε-CL) nerve guides can be used perfectly for short nerve gaps in rats, and are even better than short autologous nerve grafts. The tube dimensions, such as the internal diameter and wall thickness, are very important for the final outcome of peripheral nerve regeneration, as well as the recovery of nerve function. Before using biodegradable nerve guides in patients, it will be necessary to control the swelling the biomaterial during degradation. © *1998 Kluwer Academic Publishers*

1. Introduction

When a part of a peripheral nerve is lost, usually an autologous nerve graft is used to bridge the defect. The use of autografts can have disadvantages, such as the loss of donor nerve function and possible (painful) neuroma formation. Owing to these disadvantages, biodegradable nerve guides are developed. The idea behind the use of nerve guides is that outgrowing nerve fibers are directed towards the distal nerve stump, whilst preventing neuroma formation and the ingrowth of obstructing scar tissue. Furthermore, inside the nerve guide, trophic and tropic factors can accumulate, thereby enhancing the regeneration. After serving these functions, the nerve guide may degrade. In this paper, (1) the influence of tube dimensions on the speed and quality of peripheral nerve regeneration, (2) nerve regeneration through biodegradable nerve guides compared with autografts, and (3) return of motor and sensory nerve function after reconstruction using a biodegradable nerve guide, will be presented.

2. Materials and methods

The nerve guides were made of a copolymer of DL-lactide and ε-caprolacone (50/50; D/L 15/85), with a M_w of 1.1×10^6 kg kmol^{-1} and a polydispersity index of 2.5. The copolymer is completely amorphous, which means that it does not contain crystals. Nerve guides were made by a dip-coating technique, which is described in detail by den Dunnen *et al.* [1].

To evaluate the influence of tube dimensions on the speed and quality of peripheral nerve recovery, four types of nerve guides were constructed by variation of the number of dip-coated layers (Table I). As a model, a 1 cm gap in the sciatic nerve of the rat ($n = 24$) was reconstructed with one of the four types of nerve guides. Specimens for histological evaluation were harvested after 4, 8 and 12 wk post-operatively. After each period, eight rats were sacrificed. The histological sections were evaluated for the quality of peripheral nerve regeneration, and the influence of the degrading nerve guides on the regeneration process. Furthermore, the operated paws were evaluated for signs of mutilation. (For further details, see den Dunnen *et al.* [1].)

TABLE I The dimensions of the four types of nerve guides. When the number of dip-coated layers is increased, the internal diameter decreases and the wall thickness increases

	Nerve guide			
	Type 1	Type 2	Type 3	Type 4
No. of dip-coated layers	2	3	4	5
Internal diameter (mm)	1.23	1.18	1.15	1.12
Wall thickness (mm)	0.34	0.43	0.64	0.68

The type of nerve guide which functioned best, was compared with an ideal autologous nerve graft. In group A ($n = 15$), a 10 mm gap was reconstructed using a 12 mm long nerve guide. In group B ($n = 15$), a 7 mm long segment was resected from the sciatic nerve, reversed and finally used as an autologous nerve graft. Specimens for morphometrical analysis were obtained after periods ranging from 2–11 wk post-operatively. The specimens were evaluated for

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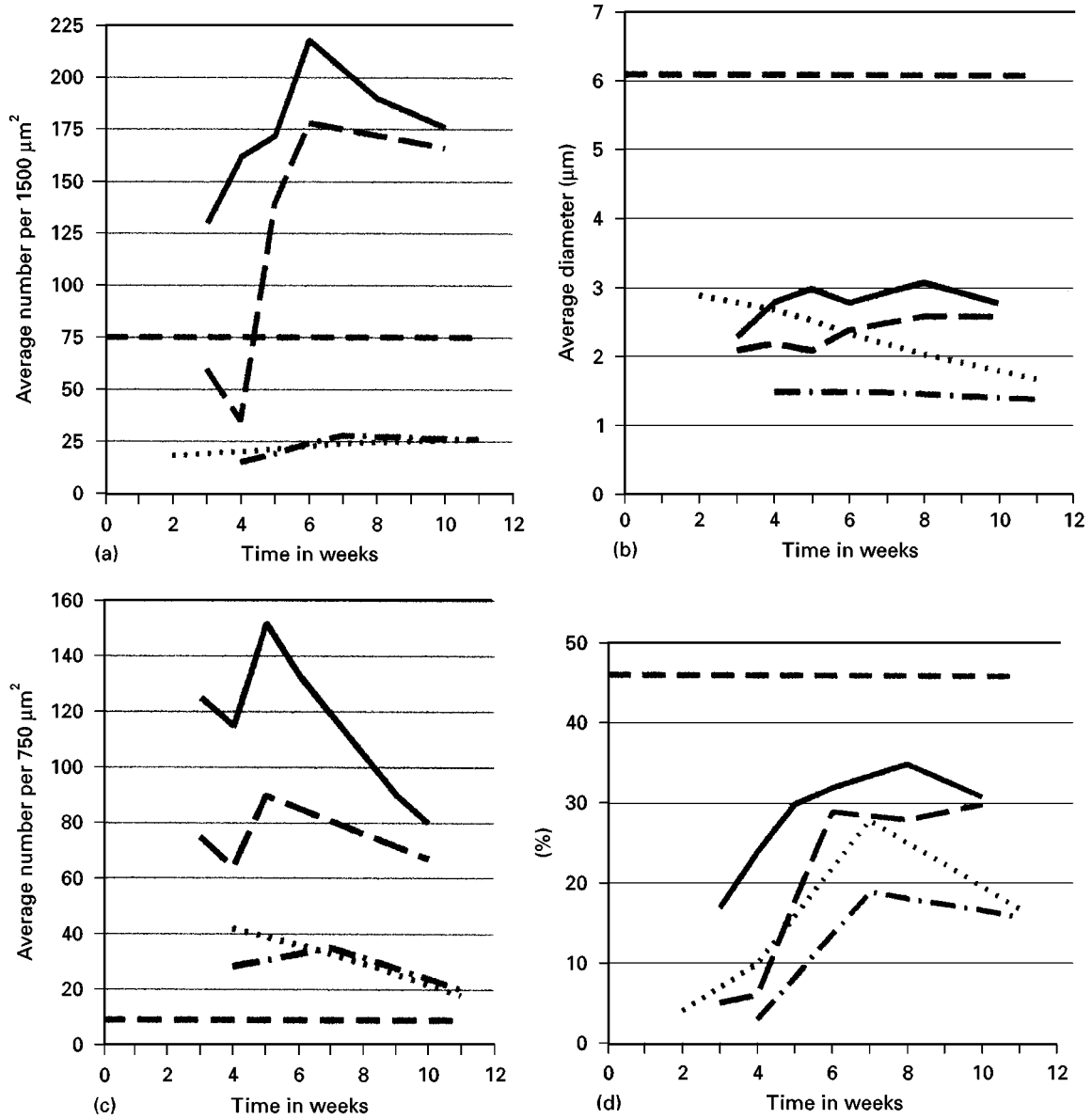

Figure 1 Graphs showing peripheral nerve regeneration through nerve guides (group A) and autologous nerve grafts (group B). The change in the number of (a) myelinated and (c) non-myelinated nerve fibers per standard area with time is shown. (b) The change in the axon diameter of the myelinated nerve fibers with time. (d) The change in the *N*-ratio. The *N*-ratio can be used as a measure for the maturity of the regenerating nerves. Group A: (——) proximal, (– –) distal. Group B: (▪ ▪ ▪) proximal, (▬▪▬) distal. (– – –) Control.

the number of myelinated and non-myelinated nerve fibers, for the average diameter of the myelinated nerve fibers, and for the *N*-ratio, which is the ratio between the area of nervous tissue and the total measured area. (For further details see den Dunnen *et al.* [2].)

A thin-walled nerve guide, with an internal diameter of 1.2 mm and a wall thickness of 0.17 mm, was used for the reconstruction of a nerve gap. In group C ($n = 15$), the nerve guide was only filled with phosphate-buffered saline, and used for the reconstruction of a 10 mm long nerve gap. In group D ($n = 15$), however, the nerve guide was filled with modified denatured muscle tissue, and used for the reconstruction of a 15 mm long nerve gap. After postoperative periods up to 15 wk, recovery of nerve function was evaluated using walking track analysis (motor nerve function) and electrostimulation tests (sensory nerve function). (For further details see Meek *et al.* [3].)

3. Results

A nerve guide constructed of P(DLLA-ε-CL) degrades completely within 1 yr, without the formation of slow degrading crystals [4]. The foreign body reaction is very mild, without the formation of constructing scar tissue [4]. However, owing to swelling of the biomaterial in the early phase of the degradation (up to 3 mon), peripheral nerve regeneration can be hampered. The swelling of nerve guides types 3 and 4 was so severe, that the nerve guides became completely occluded, thereby hampering the nerve regeneration. The hindfeet of the rats in these groups showed signs of severe mutilation. The results obtained from the rats with nerve guide type 2 were better. After 3 mon,

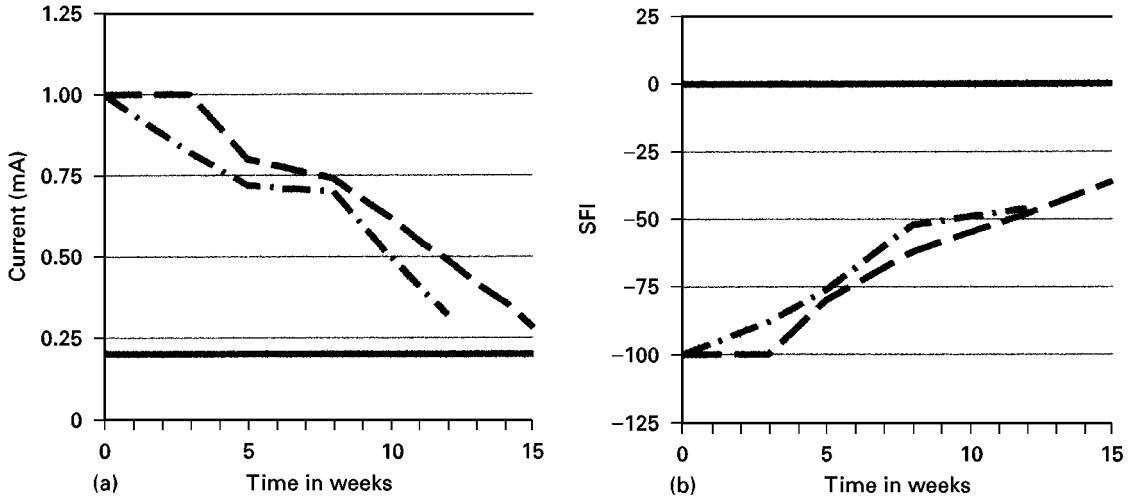

Figure 2 Graphs showing the recovery of nerve function. (a) The recovery of sensory nerve function, i.e. the decrease of the current necessary to cause a withdrawal reflex, with time. Recovery of motor nerve function. An SFI of-100 indicates total impairment, whereas an SFI of 0 indicates no impairment. (——) Control; (– –) group C, (–·–) group D.

however, the regenerating nerves showed signs of compression: axon and myelin sheath degeneration and intraneural fibrosis. At this period, signs of mild multilation could be observed. The hindfeet of the group of rats with nerve guide type 1 showed no signs of multilation at all during the whole observation period. Furthermore, the regenerated nerves had a mature appearance in histological examination.

Because nerve guide type 1 was the best type tested, peripheral nerve regeneration through this nerve guide was compared with regeneration through an autologous nerve graft. The average number of myelinated nerve fibers increased with time in both the nerve guide and nerve graft (Fig. 1a). The number of myelinated nerve fibers in the nerve graft was approximately one-third of the number in the non-operated control nerve, whereas the regenerating nerve in the nerve guide contained more than twice the number of nerve fibers compared with the control nerve. The average diameter of the myelinated axons increased with time in group A (nerve guide), whereas it decreased with time in group B (nerve graft) (Fig. 1b). The regenerating nerve inside the nerve guide also contained far more non-myelinated nerve fibers than the nerve graft of the non-operated control nerve. The numbers decreased with time. The nerve inside the nerve guides contained eight times the number of non-myelinated nerve fibers compared with the control nerve after 10 wk, whereas the nerve graft contained twice the number (Fig. 1c). The *N*-ratio, which is the ratio between the area of nervous tissue and the total measured area, can give information about the maturity of the regenerated nerve. The *N*-ratio is 46% in the non-operated control nerve. With time, the *N*-ratio increases inside the nerve guide. After 10 wk, the *N*-ratio is 30%. In the nerve graft, the *N*-ratio increases with time, but after 7 wk decreases again to 18% (Fig. 1d).

Because the swelling of the degrading biomaterial can negatively influence the regenerating nerves, a thin-walled nerve guide (0.17 mm) was used to reconstruct a 10 mm nerve gap (group C). In group D, a 15 mm long nerve gap was reconstructed with the same thin-walled nerve guide, which was filled with modified denatured muscle tissue (MDMT), in order to function as a three-dimensional regeneration matrix. In group C, almost all the thin-walled nerve guides collapsed. In contrast to these results, nerve regeneration through the thin-walled nerve guides filled with MDMT (group D) was faster and better. In group C, the first signs of sensory nerve function could be observed after 3 wk, whereas in group D recovery started earlier (Fig. 2a). In both groups, the threshold-current to cause a withdrawal reflex decreased with time. With time, control values will be reached earlier in group D than in group C. Motor nerve function also started to recover earlier in group D than in group C (Fig. 2b). However, after longer periods, differences in the SFI are only small. After 12 wk the SFI is approximately 46 (e.g. 54% recovery) in both groups. Sensory nerve function, however, has recovered further in group D than in group C, after 12 wk (thresholds of 0.3 and 0.5 mA, respectively).

4. Discussion

A P(DLLA-ε-CL) nerve guide has several characteristics of an ideal nerve guide. The biomaterial is non-cytotoxic [1], transparent, flexible and is easy to apply in microsurgery. Furthermore, the biomaterial degrades completely within 1 y without the formation of slow degrading crystals [4]. The foreign body reaction to this biomaterial is only mild, and constricting scar tissue around this biomaterial was not observed [4].

The presented studies show that the tube dimensions are very important with regard to the functions of a nerve guide. When the internal diameter is made too small, problems quickly occur when telescoping the nerve ends into the nerve guide. When the wall of the nerve guide is made too thick, swelling of the degrading biomaterial will be so severe that the nerve guides will become occluded. On the contrary, when

the wall is made too thin, the nerve guides will collapse unless a stent, such as modified denatured muscle tissue, is used inside the nerve guide.

A nerve guide with the ideal tube dimensions guarantees good quality nerve regeneration. Peripheral nerve regeneration across a 10 mm nerve gap inside a P(DLLA-ε-CL) nerve guide was faster and qualitatively better when compared with nerve regeneration through a 7 mm long autologous nerve graft.

All the studies were performed in rats. The goal of our research was of course, to develop a biodegradable nerve guide which is suited to reconstruct longer nerve gaps in patients. Because swelling of the degrading biomaterial is a negative characteristic, improvements of the polymer itself and/or the construction of the nerve guide might be necessary. In the future, a P(DLLA-ε-CL) network will be evaluated. It is hypothesized that due to the presence of cross-links, surface degradation of the biomaterial will become more important than bulk degradation, in turn leading to less water uptake and swelling. Furthermore, two-ply nerve guides will be tested. Owing to ingrowth of fibrous tissue in the porous outer layer, a stronger construction can be obtained, which is perfectly suited for the reconstruction of longer nerve gaps. It is hypothesized that, after the degradation of the nerve guide, a pseudosynovial sheath is left, which can act in the same way as a nerve guide [5].

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