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THE INFLUENCE OF A FIBRIN-COATING INSIDE A BIODEGRADABLE POLY(DL-LACTIDE-E-CAPROLACTONE) NERVE GUIDE ON PERIPHERAL NERVE REGENERATION

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Abstract

The aim of this study was to evaluate the effect of a fibrin-coating on the inner surface of a biodegradable poly(DL-lactide-ϵ-caprolactone) nerve guide on the speed and quality of the nerve regeneration. The nerve regeneration and orientation of the nerve fibers, as well as the fibrous tissue formation were evaluated. On the short term, nerve regeneration was slightly faster in the non-coated nerve guide. After longer implantation periods ($\geq$ 4 weeks), nerve regeneration in the fibrin-coated nerve guides was characterized by a severe inflammatory response with large numbers of macrophages and polymorphonuclear cells (PMN's). This study clearly demonstrates that nerve regeneration in a fibrin-coated nerve guide is not faster when compared with a non-coated nerve guide, and that nerve regeneration in the fibrin-coated nerve guide is even worse after longer implantation periods.

Key Words: Biodegradable, nerve guide, nerve regeneration, fibrin matrix formation, fibrin matrix replacement, inflammatory response, foreign body reaction, poly(DL-lactide-ϵ-caprolactone).

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Introduction

The most widely used technique for the reconstruction of a peripheral nerve defect is the interfascicular grafting using an autologous nerve graft [8]. However, the disadvantage of this technique is loss of the donor nerve function and possible neuroma formation at the proximal nerve stump of the donor nerve. Therefore, many alternative techniques have been developed. One of these techniques is the use of a polymeric nerve guide to bridge the nerve defect [6].

Polymeric nerve guides, constructed of a biodurable material, such as silicone rubber, guarantee good quality nerve regeneration. However, (biodurable) silicone nerve guides tend to cause compression of the regenerated nerve, followed by a second nerve impairment, as was also clearly shown by Merle *et al.* [12] in three clinical cases.

Therefore, nerve guides constructed of biodegradable polymers were developed [7, 9, 13]. The concept behind the use of a biodegradable nerve guide is to establish a temporary nerve regeneration chamber: guiding of the regenerating nerve fibers towards the distal nerve stump without interference of the surrounding fibrous tissue, and gradually disappearing of the nerve guide after serving this function.

Several growth factors, such as insulin-like growth factor (IGF) [18], ACTH4-9 [16] and nerve growth factor (NGF) [17] can enhance nerve regeneration. Besides growth factors, extracellular matrix proteins [1], such as collagen type IV [15] and laminin [14], positively influence nerve regeneration by decreasing neuronal cell death and by promoting the outgrowth and maturation of axons.

One of the factors that might be of particular importance is fibrin. During the initial phase of nerve regeneration within a nerve guide, first a fibrin bridge is formed in the center of the lumen of the nerve guide, which is completed after approximately one week [6]. Then, fibroblasts migrate along the fibrin bridge, and two weeks after reconstruction axon outgrowth and Schwann cell migration start [6].

The density of the fibrin matrix inside a nerve guide can influence the speed and quality of the nerve regeneration [19]. It is the aim of this study to evaluate the effect of a fibrin coating inside a biodegradable nerve guide on the nerve regeneration. As a model, a 10 mm gap in the sciatic nerve of the rat was created and repaired with a 12 mm long, biodegradable nerve guide, constructed of poly(DL-lactide-ϵ-caprolactone), either coated with fibrin or not coated. Both the non-coated nerve guides (group A) and the fibrin-coated nerve guides (group B) were harvested after implantation times ranging from two to eleven weeks. The regenerating nerves were evaluated for the number and orientation of the regenerating nerve fibers using light microscopy (LM) and transmission electron microscopy (TEM). Furthermore, the fibrous tissue formation inside the regenerating nerve, as well as the foreign body reaction of the p(DLLA-ϵ-CL) (e.g., the amorphous co-polymer is constructed of lactic acid and ϵ-caprolactone (50/50); the lactide component contains 85% L-lactide and 15% D-lactide) were evaluated.

Materials and Methods

Preparation of the nerve guides

The nerve guides are constructed of an amorphous co-polymer of DL-lactide- and ϵ-caprolactone (50:50; the lactide component contains 85% L-lactide and 15% D-lactide). The weight average molecular weight is 1.0 x 10^6 kg/kmol, and the polydispersity index is 2.5. For the preparation of this co-polymer, Sn-2-ethylhexanoate was used as a catalyst. The reaction temperature was 130°C. The nerve guides were made by a dip-coating technique, which is described in detail by Den Dunnen *et al.* [4, 5]. Briefly, a glass mandrel was dipped in a solution of the co-polymer in chloroform. The glass mandrel was pulled out of the solution and the chloroform was left to evaporate while the glass mandrel was rotating. In this manner, a thin co-polymer layer was formed. After air-drying, the next layer could be dip-coated. The nerve guides were stored in 100% ethanol. Before implantation or preparation of the fibrin-coating, the nerve guides were first washed in 0.1 M sterile phosphate buffered saline (PBS) and then filled with 0.1 M sterile PBS.

Preparation of the fibrin-coating

The fibrin-coating on the inner surface of the nerve guides was made by repeatedly injecting fibrinogen solutions through the nerve guides. First, the nerve guides were washed with 0.1 M PBS. Thereafter, a phosphate buffered fibrinogen solution (0.5 mg/ml) was injected into the lumen of the nerve guide. The nerve guides were incubated with the fibrinogen solution for 30 minutes. The nerve guides were washed again with PBS, followed by injection of a phosphate buffered thrombin solution (2 units/ ml), to activate the transition from fibrinogen to fibrin. This sequence of steps was repeated twice. Subsequently, the fibrin-coated nerve guides were stored in sterile 0.1 M PBS at 4°C, and implanted in rats the following day.

All procedures were carried out under sterile conditions in a laminar flow-cabinet (Clean Air F33MVH, Woerden, The Netherlands). Figure 1 shows cryo-scanning electron micrographs of a non-coated nerve guide and a fibrin-coated nerve guide.

Surgical procedures

Male Wistar rats (n = 30) weighing approximately 200 g were premedicated with atropine (0.25 mg/kg body weight) and anesthetized with 1% halothane (Fluothane, Zeneca, Ridderkerk, The Netherlands) + O_2/N_2O. The left sciatic nerve was exposed through a gluteal muscle splitting incision. A 6 mm nerve segment was then resected, leaving a gap of approximately 10 mm due to retraction of the nerve stumps. Continuity was re-established by interposing a 12 mm nerve guide, either fibrin-coated or non-coated. Both the proximal and distal cut ends of the sciatic nerve were telescoped into the lumen of the nerve guides and fixed with a single 10-0 nylon epineural suture (Ethilon, BV-4 needle; Ethicon, Hamburg, Germany).

After the operation, the rats were caged separately, and had access to water and standard rat food *ad libitum*. All procedures were carried out according to the Dutch national guidelines for animal welfare.

Preparation for LM and TEM

Specimens of the regenerating nerves were collected from different positions along the nerve: 1 mm proximal and 1 mm distal to the nerve guide as well as from the middle of the nerve guide. The specimens were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), rinsed for 30 minutes in 6.8% sucrose solution in 0.1 M phosphate buffer, pH 7.4, and postfixed for four hours at 4°C in a solution of 1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 M phosphate buffer, pH 7.4. The samples were dehydrated in a graded ethanol series, followed by butyl-2, 3-epoxypropylether [3], and finally embedded in Epon 812.

Semi-thin sections (0.5 to 1.0 μm) were cut and stained with toluidine blue for 45 seconds. These LM-sections were used for the evaluation of the regeneration and orientation of the myelinated nerve fibers as well as for the fibrous tissue formation. Areas of interest were selected for ultrathin sectioning. The ultrathin sections (50 nm) were stained with uranyl acetate and lead citrate and were examined using a Philips (Eindhoven, Netherlands) EM 201 transmission electron microscope oper-

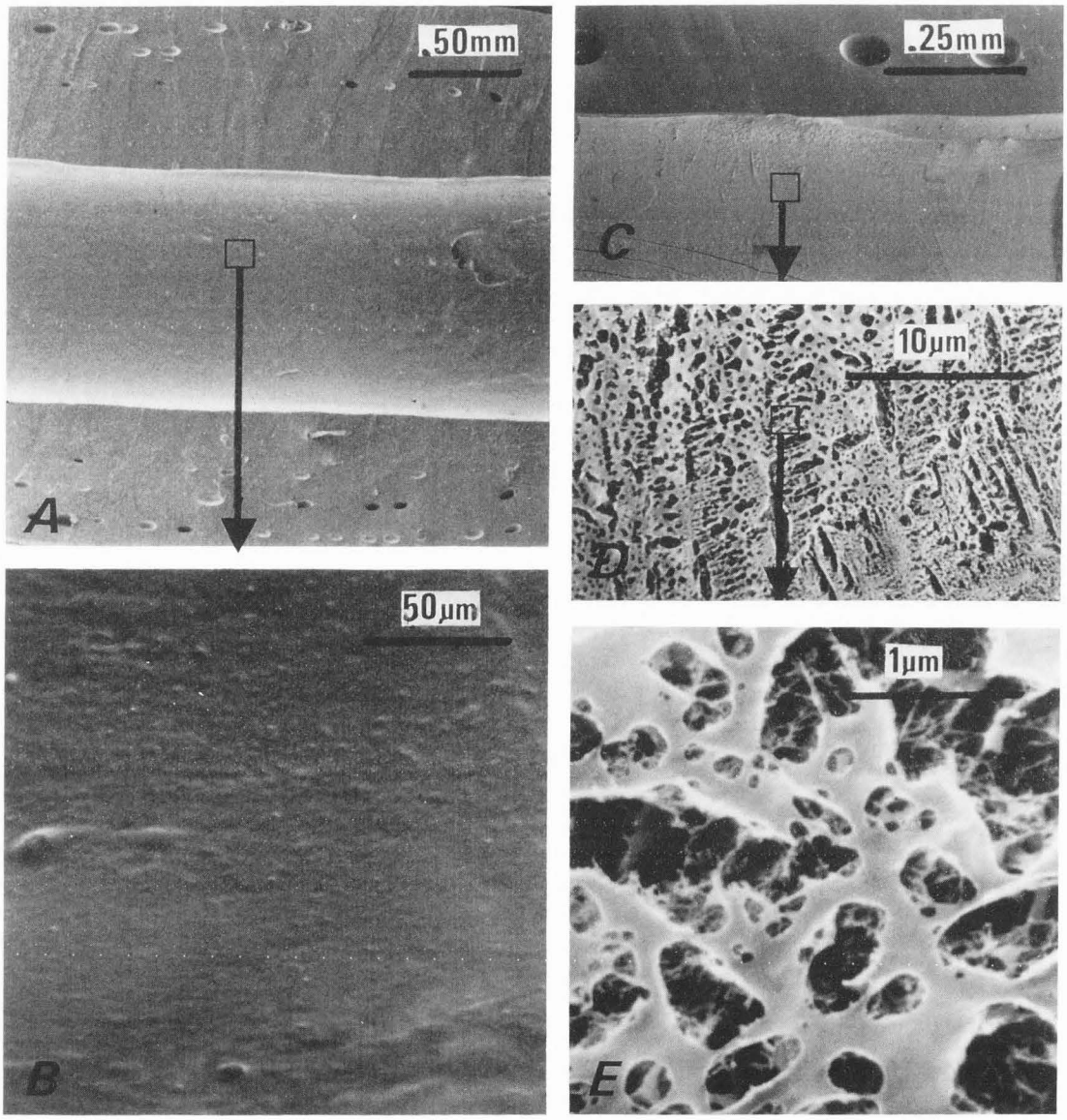

Figure 1. Frozen-hydrated cryo-scanning electron micrographs of a longitudinally transected non-coated poly(DL-lactide-ϵ-caprolactone) nerve guide (A and B) and a fibrin-coated nerve guide (C, D and E). The black boxes in A and C (luminal side of the nerve guides) are shown in detail in B and D, respectively. Note that the coating consists of a dense and randomly orientated fibrin network, whereas the inner surface of the non-coated nerve guide is smooth.

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ated at an accelerating voltage of 60 kV. These samples were evaluated for regeneration and orientation of the non-myelinated nerve fibers, as well as for fibrous tissue/collagen formation.

Results

Macroscopical evaluation

The degradation of both coated and non-coated p(DLLA-ϵ-CL) was identical. Initially the nerve guides were transparent. After two weeks of implantation, the nerve guides were still intact and had a yellow colour. After seven weeks, the nerve guides were still intact, but also swollen and opaque. Macroscopical signs of fragmentation were not observed in this study.

The amount of fibrous tissue surrounding the nerve guides increased in time in both group A and B.

Signs of neuroma formation were not observed either. However, during harvesting of the fibrin-coated nerve guides (group B), seven and eleven weeks after implantation, longitudinally orientated nerve tissue could

be observed on the outside of the nerve guides. This nerve tissue originated from the proximal nerve stump and inserted the (degenerated) nerve, immediately distal to the nerve guide. Nerve tissue was not observed on the outside of the non-coated nerve guides.

Light microscopical (LM) and transmission electron microscopical (TEM) evaluation

Group A: non-coated nerve guides After three weeks of implantation, some myelinated nerve fibers could be observed in the distal nerve stump (Fig. 2b). The proximal nerve stump contained a larger number of myelinated nerve fibers, with a larger average axon diameter (Fig. 2a). In time, both the number and the average axon diameter of the myelinated nerve fibers increased in both the proximal and distal nerve stumps (Figs. 2c, 2d, 2e and 2f). The regenerating nerve fibers were longitudinally orientated, and signs of neuroma formation were not observed. Although the contralateral control nerve contained a smaller number of myelinated nerve fibers with a larger average axon diameter (Fig. 2g), and also a smaller number of non-myelinated nerve fibers, the regenerating nerve had a mature appearance, 10 weeks after reconstruction (Figs. 2e and 2f). The amount of fibrous tissue in the regenerated nerve, ten weeks after reconstruction, was the same when compared with the control nerve.

Group B: fibrin-coated nerve guides After two weeks of implantation, myelinated nerve fibers passed the middle (Fig. 3b) of the nerve guide. The proximal nerve stump contained a larger number of myelinated nerve fibers with a larger average axon diameter (Fig. 3a). Myelinated nerve fibers were not observed in the distal nerve stump (Fig. 3c), but non-myelinated nerve fibers were already present at this time (Figs. 3d and 3e). In time, the number of myelinated nerve fibers increased, but the orientation was not as good as in group A. At first, the number of myelinated nerve fibers inside the nerve guide did not change, but after longer implantation periods it even decreased, whereas the number of myelinated nerve fibers outside the nerve guide increased in time. After four weeks of implantation, some myelinated nerve fibers could be observed in the distal nerve stump. The number of myelinated nerve fibers in the distal nerve stump also increased in time, however, not as fast as in group A.

After two weeks of implantation, an inflammatory response with large numbers of polymorphonuclear cells (PMN's) and macrophages was observed inside the nerve guide (Fig. 4a), whereas only a mild foreign body reaction with macrophages and fibroblasts was observed on the outside (Fig. 4b). In time, the amount of fibrous/scar tissue increased inside the nerve guide.

Figure 2. (*facing page*) Light micrographs showing regenerating nerves, 3, 5 and 10 weeks after reconstruction using a non-coated nerve guide. The regenerated nerves were evaluated 1 mm proximal (Figs. 2A, 2C and 2E) and 1 mm distal (Figs. 2B, 2D and 2F) to the nerve guide. The number and average diameter of the myelinated nerve fibers increased with time in both the proximal and distal nerve stumps. Ten weeks after reconstruction, the regenerated nerves had a mature appearance. The control nerve (Fig. 2G) however, contained a smaller number of myelinated nerve fibers, with a larger average axon diameter. Bars = 32 μm.

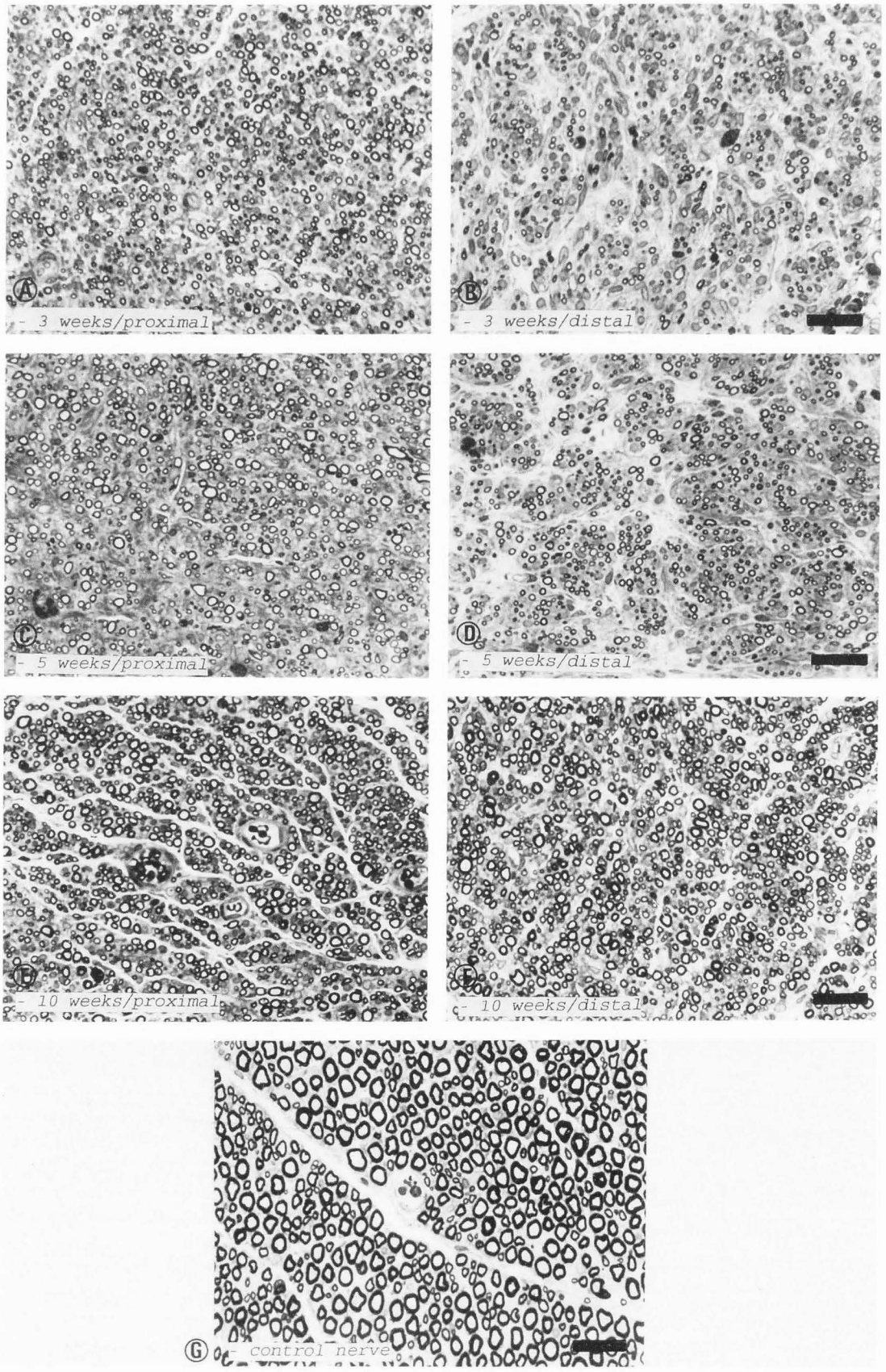
Discussion

Fibrin-matrix formation

This study clearly demonstrates that nerve regeneration in a **fibrin-coated** nerve guide is not faster when compared with a **non-coated** nerve guide, and that nerve regeneration in the **fibrin-coated** nerve guide is even worse after longer implantation periods.

Wound responses are immediately activated when a peripheral nerve is damaged (i.e., post-traumatic response). Blood serum and other extracellular and intracellular fluids immediately fill the lumen of the nerve regeneration chamber [10, 11, 19]. In the case of nerve reconstruction using a nerve guide, a fibrin bridge containing fibroblasts, fibronectin, macrophages, leukocytes and erythrocytes connects the proximal and distal nerve stumps and is formed within one week [2, 6, 10]. This fibrin bridge forms a primary scaffolding for orientating the migration of fibroblasts, Schwann cells and axonal processes across the nerve gap. We may, therefore, conclude that the formation of a fibrin bridge between the severed nerve ends is the first essential event in nerve regeneration, after reconstruction using a nerve guide.

Although the formation of a fibrin bridge seems to be a crucial event in the nerve regeneration after reconstruction using a nerve guide, almost no research has been carried out on this topic. Williams and Varon [19] were one of the few to study the effect of fibrin network formation on nerve regeneration. From that study, it was concluded that a volume of 25 μl and a relatively widely dispersed fibrin matrix functioned best. Nerve guides with a larger volume (75 μl) functioned worst, and nerve guides with smaller volumes (11 μl) and a more dense fibrin network functioned only slightly better. The fibrin-network in every type of nerve guide was randomly orientated, due to the diffusion of fibrin into the lumen of the nerve guide. Therefore, the differences in the speed of the nerve regeneration can only be explained by the differences in the form and density


A - 3 weeks/proximal
B - 3 weeks/distal
C - 5 weeks/proximal
D - 5 weeks/distal
E - 10 weeks/proximal
F - 10 weeks/distal
G - control nerve

of the fibrin network. The fact that nerve regeneration was worst in the 75 μl nerve guides, is probably caused by a too large volume, and not by the density of the fibrin network. The orientation of the regenerating nerve fibers in these nerve guides was worse, probably due to a lower gradient of neurotrophic factors, released by the distal nerve stump.

In our study, both **fibrin-coated** and **non-coated** p(DLLA-ϵ-CL) nerve guides with volumina of 17.7 μl were used for the reconstruction of a 1 cm nerve gap. Three weeks after reconstruction using a **non-coated** nerve guide, myelinated nerve fibers could already be observed in the distal nerve stump! In the **fibrin-coated** nerve guides, the nerve fibers had grown further than the middle of the nerve guide by two weeks. The first myelinated nerve fibers could be observed in the distal nerve stump, four weeks after reconstruction, however, the density of the regenerating nerve fibers was lower when compared with the distal nerve stump in the **non-coated** nerve guide.

Nerve regeneration in a 25 μl silicone nerve guide [19] was less advanced: three weeks after implantation, myelinated nerve fibers had not grown further than the middle of the nerve guide. Even non-myelinated nerve fibers were not observed in the distal nerve stump in the study of Williams and Varon (1985), whereas in our study, non-myelinated nerve fibers could be observed in the distal nerve stumps, two weeks after reconstruction using either the **fibrin-coated** or the **non-coated** nerve guide.

From this study and the study of Williams and Varon (1985), it can be concluded that nerve regeneration through a p(DLLA-ϵ-CL) nerve guide (either coated or not) is faster when compared with a silicon nerve guide. This difference is probably caused by the semipermeability of the biodegradable nerve guide, whereas the silicone nerve guide is non-permeable. Furthermore, it can be concluded that the nerve regeneration in the **non-coated** nerve guide is faster when compared with the **fibrin-coated** nerve guide. This difference is probably caused by a denser fibrin matrix in the **fibrin-coated** nerve guides, and the fact cells enter the core, rather than the periphery of the fibrin matrix [19]. This phenomenon was also observed in the **non-coated** nerve guides in our study. The fibrin in the **fibrin-coated** nerve guides, however, was present at the periphery of the nerve guide. Therefore, a fibrin matrix had still to be formed in the center of the nerve guide.

Fibrin-matrix replacement

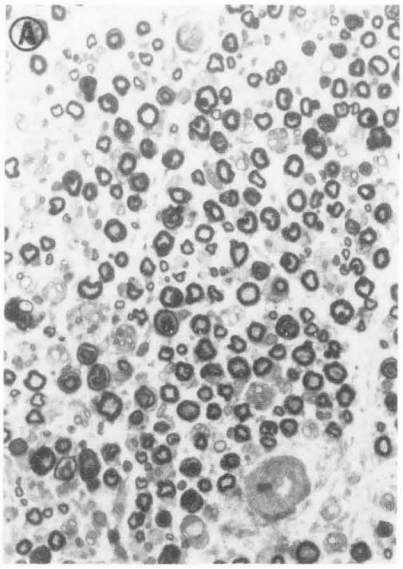
As is the case in every wound response, the fibrin matrix is replaced as soon as this matrix is populated by cells such as fibroblasts and macrophages, and fibrous tissue is formed.

Figure 3. *(facing page)* Light- and transmission electron micrographs showing a regenerating nerve, 2 weeks after reconstruction using a fibrin-coated nerve guide. Myelinated nerve fibers are present in the middle of the nerve guide (Fig. 3B). The number and diameter of the myelinated nerve fibers is larger in the proximal nerve stump (Fig. 3A). Myelinated nerve fibers were not observed in the distal nerve stump (Fig. 3C), but non-myelinated nerve fibers were already present at this time (Figs. 3D and 3E). Figures 3D and 3E are transmission electron micrographs of the regenerating nerve, distal to the nerve guide, showing non-myelinated nerve fibers (N). Figure 3E is a detail from Figure 3D. md: myelin degradation products; N: non-myelinated nerve fiber; m: mitochondria; rER: rough endoplasmic reticulum; bl: basal lamina; S: Schwann cell nucleus. Bars = 40 μm (in Figs. 3A, 3B and 3C), 1.4 μm in 3D and 0.67 μm in 3E).

The fact that the speed and quality of the nerve regeneration in the **fibrin-coated** nerve guides was even worse after longer implantation periods ($\geq$ 3 weeks) could be explained by the fact that the dense fibrin matrix at the periphery inside the nerve guide was replaced after the population of the fibrin matrix by fibroblasts, Schwann cells, vascular sprouts and axonal processes. The replacement of this dense fibrin-coating lead to an inflammatory response with large numbers of PMN's, which was more severe than the foreign body reaction of the p(DLLA-ϵ-CL) of the **non-coated** nerve guides, and resulted in more fibrous tissue formation, which hampered further nerve regeneration through the nerve guide. This lead to a decrease in the amount of nerve tissue inside the nerve guide, whereas the amount of fibrous tissue increased. Due to the scar tissue formation inside the nerve guide, the regenerating nerve fibers had to grow over the outside of the nerve guide until they could grow into the distal nerve stump. The speed of the nerve regeneration in the **fibrin-coated** nerve guide had therefore decreased.

In conclusion, nerve regeneration in **non-coated** nerve guides, constructed of p(DLLA-ϵ-CL), is faster and qualitatively better, when compared with **fibrin-coated** nerve guides. Furthermore, not only fibrin matrix formation, but also fibrin matrix replacement is of importance with regard to speed and quality of peripheral nerve regeneration inside a nerve guide. The more fibrin is present inside the nerve guide, the more fibrin has to be replaced, leading to a more severe inflammatory response with more scar tissue formation, in turn hampering the nerve regeneration.

Since fibroblasts, Schwann cells and axonal proces-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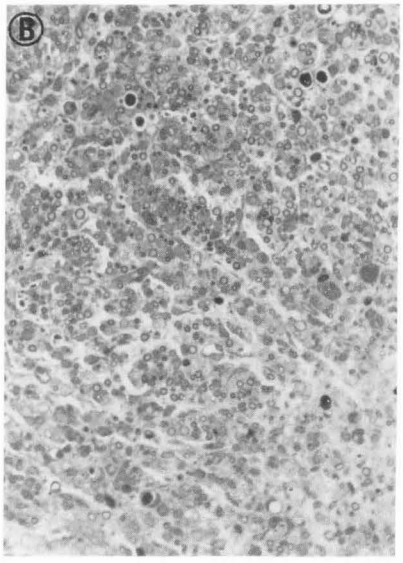
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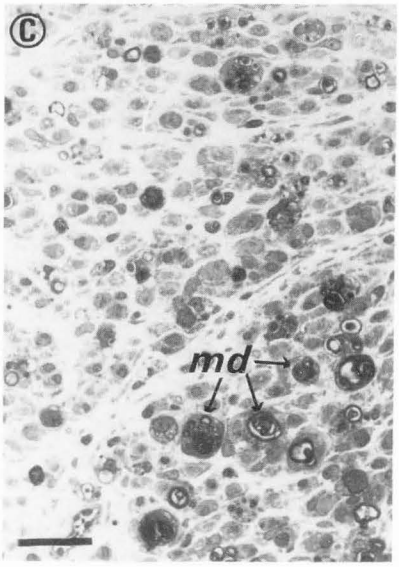
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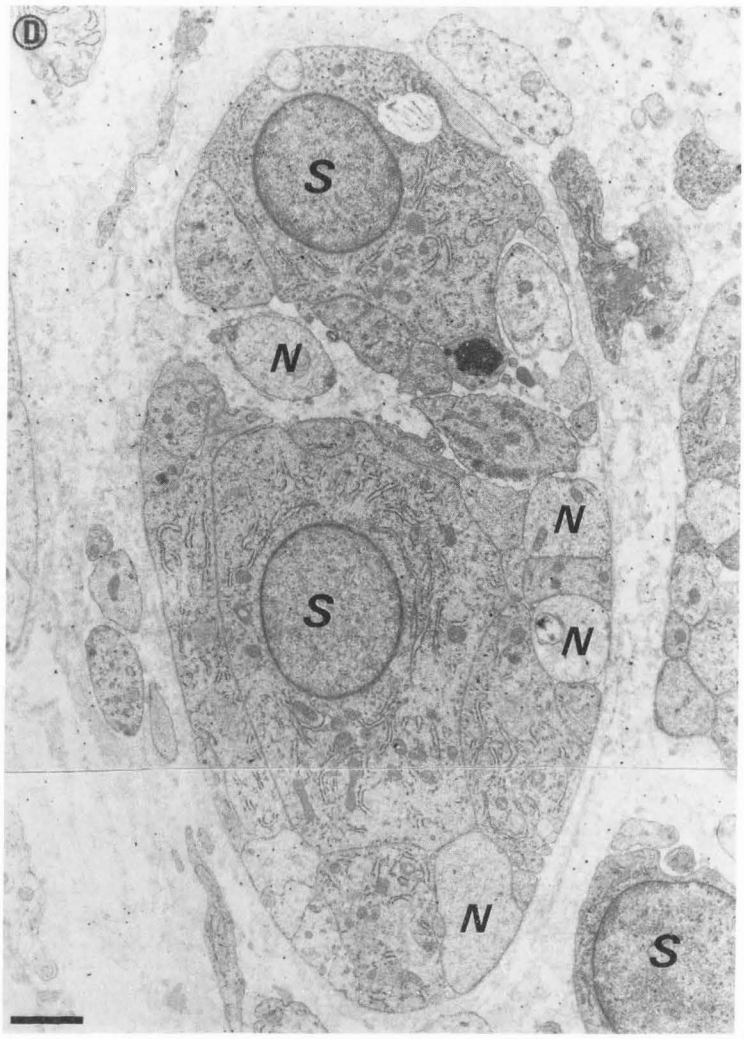
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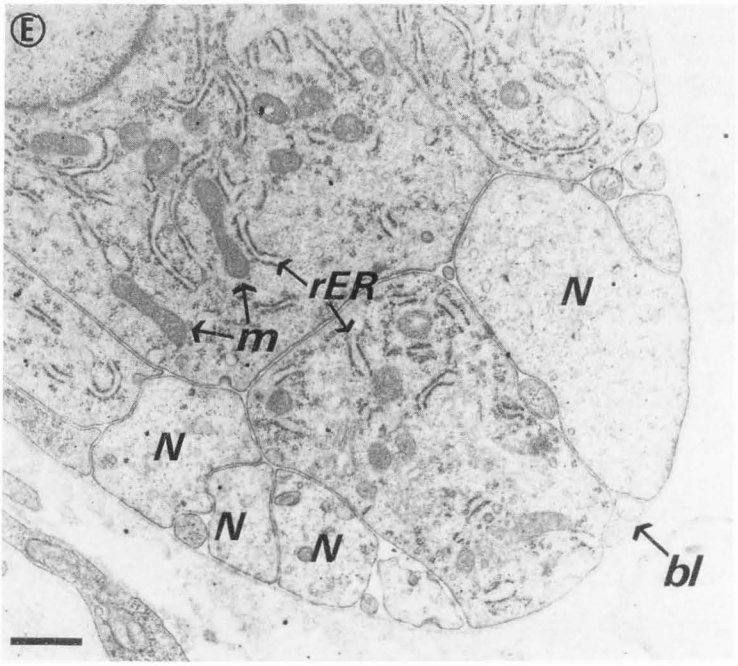
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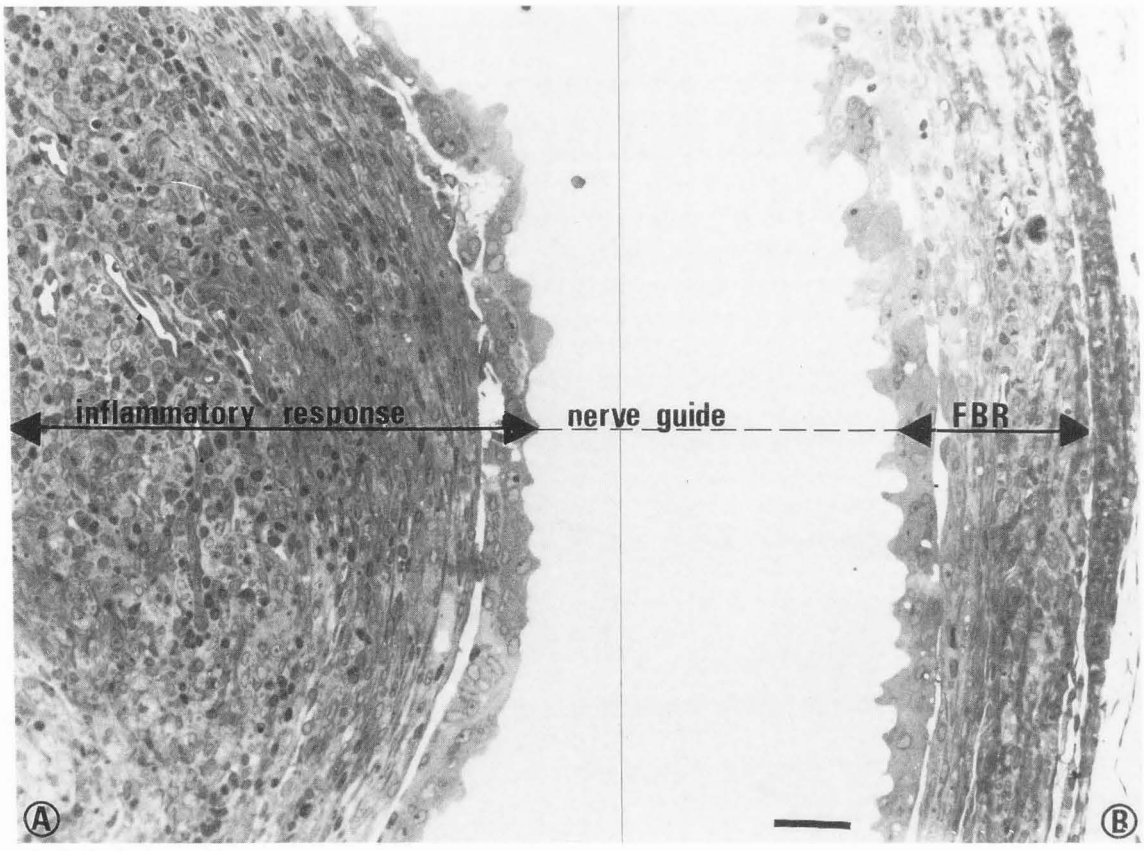

Figure 4. Light micrographs showing the inflammatory response inside the fibrin-coated nerve guide (Fig. 4A), and the foreign body reaction (**FBR**) on the outside of the fibrin-coated nerve guide (Fig. 4B). Note that the **inflammatory response** inside the fibrin-coated nerve guide is more severe than the foreign body reaction (FBR) on the outside. The FBR on the outside is characterized by the formation of a fibrous capsule containing macrophages and fibroblasts, whereas inside the nerve guide an infiltrate of macrophages and polymorphonuclear cells is present. Bar = 40 μm.

ses enter the core rather than the periphery of the fibrin network, in future experiments, a homogeneous, artificial fibrin matrix has to be constructed, instead of a fibrin coating, in order to evaluate the importance of the density of the fibrin matrix formation on the speed and quality of nerve regeneration.

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Discussion with Reviewers

H. Aldskogius: The authors claim that fibrin-coated biodegradable nerve guides may actually provide worse conditions for nerve regeneration compared to non-coated guides as judged by morphological methods. Although their pictures appear to bear out this conclusion, it is a major drawback for the assessment of the significance of their findings that no quantitative data are presented. Such data should be quite easily obtained with regard to number (and size) of myelinated fibers from the light microscopic material already generated for this study. Given this information, a major question left open is, to what extent, the morphological outcome is parallelled by similar differences in restoration of function? Data from previous literature in the field of peripheral nerve injury repair suggest that interpretations from morphological data about functional recovery needs to be done with great caution. For example, in this particular study, the nerve fibers growing outside the nerve guide could eventually contribute quite extensively to target reinnervation, and hence return of functions. These aspects should be discussed by the authors.

Authors: We agree with the reviewer that interpretations from morphological data about functional nerve recovery should be performed with great caution. In this study, we did not perform specific tests to evaluate the restoration of the nerve function. However, one way to evaluate the return of sensory nerve function quickly and easily, is to look for signs of automutilation of the denervated paws. The longer the denervation period, the more likely it is that automutilation will occur. In this study, automutilation was only observed in the group of rats with the fibrin coated nerve guides after periods longer than seven weeks. The difference between the two groups of rats can be explained as follows: due to the inflammatory response inside the fibrin coated nerve guides, scar tissue is formed, hampering the regenerating nerve fibers. These fibers will then have to grow on the outside of the nerve guide, and the denervation period will, therefore, be longer, giving rise to more frequent and more severe automutilation.

At this moment, a study is being undertaken to evaluate functional nerve recovery, after reconstruction of a 1 cm nerve gap, using the same biodegradable nerve guide. To obtain significant data for statistical analysis, walking track analysis and electrostimulation tests are carried out to evaluate the return of motor and sensory nerve function, respectively.

In this study, it was observed that some of the fibers, growing on the outside of the nerve guide, grew into the distal nerve stump. It is likely that these nerve fibers contribute to target reinnervation. However, some nerve fibers grew randomly, forming a neuroma-in-continuity. These nerve fibers will not contribute to target reinnervation.

H. Aldskogius: The authors find that the number of fibers inside the fibrin-coated nerve guides tend to decrease after some time, while fibers outside the nerve guide become more numerous. The mechanisms underlying these events need to be discussed by the authors. In this context, it would also be useful to consider the possibility of differential growth patterns by subpopulations of peripheral nerve axons. Recent studies indicate that guidance cues for sensory and motor axons are different. These fibers also respond to different growth factors. Indeed, also subpopulations of sensory fibers respond to different neurotrophins. These aspects, important as they are to a biological approach to peripheral nerve regeneration, should be at least briefly included in the authors' discussion of their findings.
Authors: The fact that fibrous tissue was formed inside the nerve guide is an explanation for the growth of nerve fibers on the outside of the nerve guide (e.g., a mechanical effect; not necessarily an effect of different neurotrophins working on subpopulations of nerve fibers).

M.S. Shoichet: Can you describe the methods you used to follow the degradation of these nerve guides?
Authors: The degradation of the biomaterial used for the construction of these nerve guides was outlined in detail in two other studies [5, 20]. Briefly, the degradation is characterized by swelling of the biomaterial in the first three months. During this period, the nerve guides loose their tensile strength. After this period, the amount of biomaterial decreases sharply. This decrease is accompanied by a sharp decrease in weight average molecular weight (from 900,000 to 2,700 kg/kmol).

M.S. Shoichet: Can you describe, more fully, how the volume of the nerve guide effects nerve regeneration?
Authors: In nerve guides with a small volume, a relatively dense fibrin matrix is formed, whereas a more widely dispersed fibrin matrix is formed in nerve guides with a larger volume. The density of the fibrin matrix effects the growth rates of the nerve fibers: nerve fibers will grow slower through a dense fibrin network. In this study, an inflammatory response to the randomly oriented (autologous) fibrin coating was observed. Due to scar tissue formation, the growth of regenerating nerve fibers through the coated nerve guide was hampered.

M.S. Shoichet: It is unclear how the fibrin coating in the nerve guide is reducing regeneration. Is the fibrin not oriented parallel to the nerve guide? If so, do you have evidence of this? Or, is the host tissue response to the fibrin inhibiting nerve outgrowth? If so, how can this be overcome to separate out the issues of fibrin-coating influencing nerve regeneration versus the host tissue response influencing nerve regeneration?
Authors: The inner surface of the nerve guide was coated. The fibrin coating was randomly (not longitudinally) oriented as can be observed in Figure 1. The fibrin coating does not inhibit the outgrowth of the nerve fibers, but due to the inflammatory response to the fibrin, the lumen of the nerve guide is occluded by scar tissue, which hampers the nerve regeneration. This severe inflammatory response might be caused by a different three-dimensional texture of the autologous fibrin coating, compared with a physiological fibrin network. In order to evaluate this hypothesis, another technique for fibrin-matrix-formation will have to be developed.

M.S. Shoichet: Could you provide us with quantitative data (i.e., means $\pm$ standard deviation) to support your assertions of the number of myelinated versus non-myelinated fibers in different nerve guides at different times?
Authors: This question has also been raised above by Dr. H. Aldskogius. We agree that morphometric analysis is a good instrument for obtaining data, which can be statistically evaluated. In other studies [5, 21], we performed morphometric analysis to emphasize changes in time or differences between two reconstruction techniques and two kinds of nerve guides. In this study, however, the differences are so clear, that morphometric analysis was not considered to be of any additional support.

Additional Reference

[20] Den Dunnen WFA, Robinson PH, van Wessel R, Pennings AJ, van Leeuwen MBM, Schakenraad JM (1996) Long term evaluation of degradation and foreign body reaction of subcutaneously implanted poly(lactic acid-ϵ-caprolactone). J Biomed Mat Res (in press).

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