Bridging defects in nerve continuity: influence of variations in synthetic fiber composition

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Synthetic filaments introduced into a silicone tube may help to enhance axonal growth over extended defects in nerve continuity [1]. Here we test the influence of number (0, 3, 7 or 15), size (diameter 150 or 250 μm) and material of filaments (polyamide or catgut) enclosed in such tubes (inner diameter 1.98 mm) on axonal growth across a 10 mm defect in rat sciatic nerve. The morphology of the tube content was analyzed four weeks post-surgery. The area of the formed tissue matrix inside the tube showed no difference between the groups. Myelinated axons were observed in the formed tissue matrix inbetween and peripheral to the filaments, however, separated from the filaments by concentric cell layers. The number of myelinated axons was less in the tubes with 15 filaments, most pronounced when catgut filaments were used. In most cases, except in tubes with 15 catgut filaments, fibers had grown into the distal nerve segment (pinch reflex test/light microscopy). We conclude that an intrinsic framework consisting of a limited number of synthetic filaments inside an extrinsic framework (silicone tube) does not disturb nerve regeneration. The formed tissue matrix was neither influenced by the presence or the numbers (if less than or equal to seven filaments), type of filaments nor the size of the filaments indicating the importance of the inserted nerve segments.

1. Introduction

Autologous nerve grafts are used to support axonal growth over extended nerve defects in the extremities as well as in the reconstruction of plexus injuries [2–4]. This technique requires, however, sacrifice of healthy nerves such as the sural nerve which may induce e.g. pain problems [5]. Therefore, there is a need to develop alternatives to autologous nerve grafts. One such alternative to bridge short gaps is to enclose the space between nerve segments in a chamber, e.g. a silicone tube. Inside such a tube a fibrin matrix is spontaneously formed which is invaded by macrophages and which serves as a matrix for ingrowth of non-neuronal cells as well as axons and capillaries [6–9]. Various types of tubes have been used for bridging such defects. Recently, we described a bioartificial nerve graft consisting of a silicone tube in which multiple polyamide filaments constitute an intrinsic framework within the tube [10]. Using such a principle the axonal growth over extended defects in rat sciatic nerves can be supported [1]. It has also been suggested that resorbable filaments are superior to non-resorbable filaments [11, 12] but there is no information concerning the optimal material size or number of filaments which can be used for this purpose. The purpose of this paper was to address this specific question.

2. Material and methods

2.1. Animals and surgical technique

Fifty-eight female Wistar rats, weighing approximately 220 g, were used for the study. The design of the experiment was approved by the local animal ethical committee at Lund University. The rats were anaesthetized by an intraperitoneal injection of pentobarbital ($6\,mg\,ml^{-1}$). The sciatic nerve was exposed at midthigh level and a 2 mm segment was resected. The defect was bridged by a 14 mm long silicone tube (internal diameter 1.98 mm) where the nerve segments were pulled 2 mm into each opening and secured by two 9/0 stitches, thereby leaving a gap of 10 mm between the transected nerve ends. Ten mm long polyamide filaments (Ethilon® Ethicon, Germany, diameter 150 μm, $n = 16$ and 250 μm, $n = 17$) were placed inside this silicone tube. Three (150 μm; $n = 5$ and 250 μm; $n = 7$), seven (150 μm; $n = 5$ and 250 μm; $n = 5$) or 15 (150 μm; $n = 6$ and 250 μm; $n = 5$) filaments were used. In 14 rats resorbable catgut filaments (Ethicon, Germany, diameter 250 μm) were placed inside the silicone tube instead of the polyamide filament and also in these cases three ($n = 5$), seven ($n = 5$) or 15 ($n = 4$) filaments were used. In 11 silicone tubes no filaments were introduced. In all groups the tubes were filled with saline and the wounds were closed by suturing.

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2.2. Evaluation

After 4 weeks the rats were reanaesthetized and the sciatic nerve exposed. The tube was explored and isolated from the surrounding tissue. The distal nerve segment was divided and carefully isolated. A pinch reflex test was performed distal to the tube as previously described [13, 14]. The response expressed as a reflex movement of the back muscles was used to reflect the presence of sensory nerve fibers in the distal segment while no response was taken as an indication of absence of such fibers. The rats were then sacrificed by an overdose of pentobarbital and the specimens were harvested for morphology.

The silicone tube with its content was carefully dissected and the tube was opened by a longitudinal incision. The content of the tube together with a 5 mm nerve segment proximal and distal to the tube were then fixed into 2.5% glutaraldehyde in cacodylate buffer (pH 7.15). Five mm long nerve pieces were cut just distal to the middle of the tube and just distal to the tube. The nerve pieces were postfixed in 1% osmium tetroxide, dehydrated in graded series of ethanol and embedded in Agar 100 resin. Cross-sections, 1 μm thick, were cut and stained with methylene blue and Azure II and sections from the distal nerve segments were coded. All sections were examined by light microscopy. The sections from just distal to the middle of the tube were digitized with Kodak professional DCS 200 Digital Camera and handled with Adobe Photoshop™ in a Macintosh Powerbook PC 8500 computer (Cupertino, CA). The area of the formed tissue matrix around the filaments (excluding the filaments) was measured using the image analysis software (NiH Image 1.59, US). The values are presented as median (interquartile range) and a P-value of < 0.05 was regarded as significant using the Kruskal–Wallis test (Stat View 4.5, Abacus Concept, CA) for measuring the formed tissue matrix in the tube around the filaments.

3. Results

3.1. Macroscopical observations

In all cases where empty silicone tubes were used, a new structure had been formed connecting the proximal and distal nerve segments. In the various filament groups there was also a matrix extending from the proximal to the distal nerve segments and surrounding the filaments which could be clearly seen.

3.2. Pinch reflex test and presence of myelinated fibers in distal segment

The results are summarized in Table I where the exact numbers of positive pinch/total number of nerves are expressed. In the same table the presence of myelinated nerve fibers in the distal segment is also indicated. In most of the cases there was a positive pinch test distal to the tube with a clear response except in experiments where 15 filaments of catgut were placed inside the silicone tube where only one of four nerves elicited a clear positive pinch reflex test and myelinated axons were present.

TABLE I Presence of a positive pinch reflex test and myelinated axons in the distal nerve segment 4 weeks following surgery. The values are positive numbers/total number of nerves.

Filaments	Positive pinch reflex test	Presence of myelinated axons
None	10/11	11/11
Polyamide (ϕ 150 μm)		
3	4/5	5/5
7	4/5	4/5
15	6/6	6/6
Polyamide (ϕ 250 μm)		
3	6/7	6/7
7	5/5	4/5
15	4/5	4/5
Catgut		
3	5/5	3/5
7	5/5	3/5
15	1/4	1/4

3.3. Tissue matrix area

Because of technical reasons it was not possible to calculate the tissue matrix area in tubes where 15 filaments were used. In such sections it was only possible to measure the area in less that 50% of the nerves and therefore sections from nerves with 15 filaments were excluded from the calculation. The area of the tissue matrix is expressed in Fig. 1. There was some variation in the area and therefore median values and interquartile range were used for presentation of the area in Fig. 1 (box plot). There was no statistical difference (Kruskal–Wallis, $P = 0.08$) in nerves where less than or equal to seven filaments or no filaments were used.

3.4. Routine histology

3.4.1. Empty tubes

In all cases there was a well defined nerve structure in the middle of the tube where numerous myelinated nerve fibers were surrounded by a consistent, well-defined, rather thin capsule as a perineurium. The myelinated fibers were organized in minifascicles (Fig. 2a).

3.4.2. Polyamide filaments

3.4.2.1. Diameter 150 μm. In this filament group the findings were similar for three and seven filaments. The

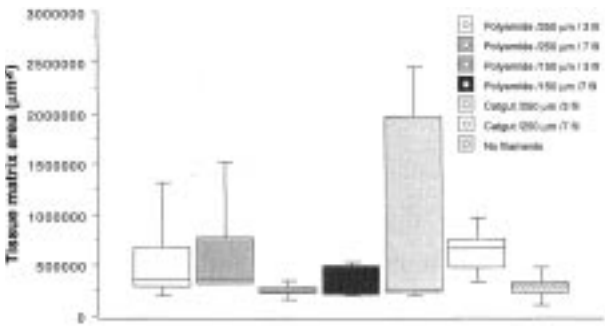
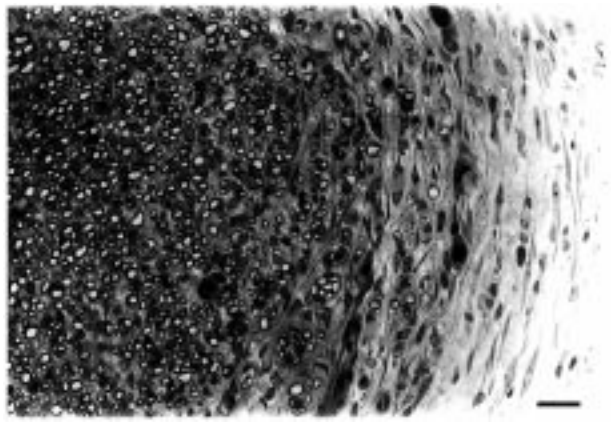
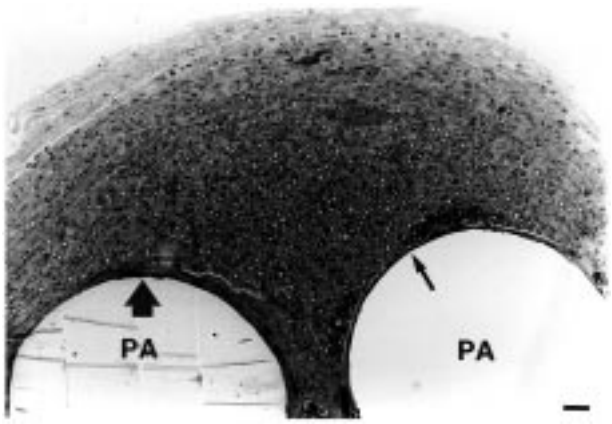


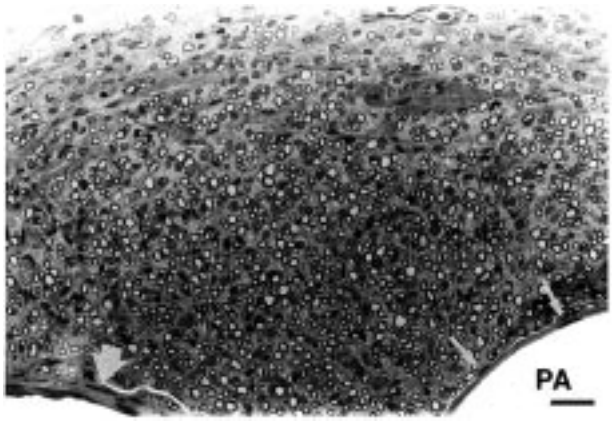
Figure 1 Box plot, displaying median as well as lower and upper quartiles, and whiskers showing minimum and maximum values of the results from the measurements of the tissue matrix area formed in silicone tubes where no, three or seven filaments of different diameters (150 and 250 μm) and material (polyamide and catgut) were used. There was no statistical difference between the various groups ($P = 0.08$).



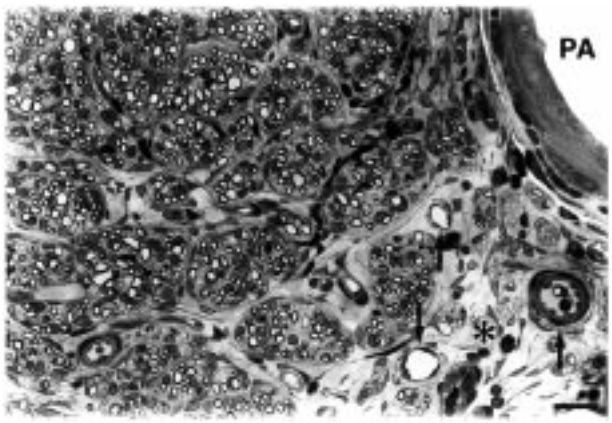
(a)



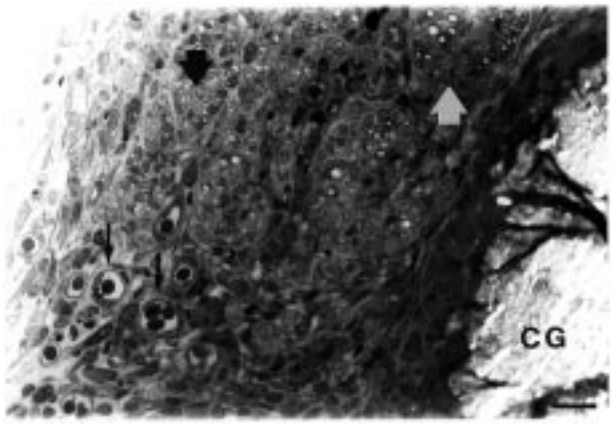
(b)



(c)



(d)



(e)

Figure 2 Cross-sections of tube contents from a segment just distal to the middle of the tube in various situations. (a) Empty tube with no filaments. Note thick capsule and numerous axons in the newly formed nerve structure. (b) Tube with three polyamide filaments (250 μm). Numerous axons growing close to the filament and just separated from the filaments by a single (thin arrow) to several (thick arrow) layers of cells. PA = polyamide filaments (c) Detail of (b). Arrows as in (b). (d) Detail from a tube with three 250 μm polyamide filaments. Note minifascicles and also areas (*) with few axons. The new nerve structure contains blood vessels (arrows). (e) Tube with filaments of catgut (CG). Note extensive reaction around catgut filaments. Myelinated axons are growing in the matrix in minifascicles (thick arrows) interspersed with blood vessels (thin arrows). Bar = 20 μm.

histological sections showed that there was a structure organized around the filaments in all tubes and the whole structure was surrounded by a similar capsule as in the empty tubes. A thin capsule, with one to seven cell layers, was observed around the filaments (Fig. 2b and c). Myelinated axons were organized in minifascicles in between and peripheral to the filaments. Among the minifascicles longitudinally oriented blood vessles were observed (Fig. 2d). However, in tubes where 15 filaments were used more artefacts were seen, but the tissue matrix, which was observed around the filaments, contained the same components as compared to tubes where fewer filaments were used. There were areas between the filaments that only contained connective tissue but no axons.

3.4.2.2. Diameter 250 μm. There was essentially no difference between the histological picture when three and seven filaments were used and the corresponding nerves with filament diameter 150 μm. However, between 15 filaments there were more pronounced areas with connective tissue without myelinated axons. The number of myelinated axons seems also to be generally less in matrixes surrounding 15 filaments.

3.4.3. Catgut filaments

In the group where catgut filaments were placed inside the silicone tube there was also a tissue structure organized around the filaments but the capsule around single filaments was thicker as compared to polyamide filaments. Mononuclear cells were observed in the capsule but also in the matrix (Fig. 2e). The number of myelinated axons was also less than the polyamide group

and in three of four nerves no axons were found in the matrix or in the distal segment.

Discussion

The present study has shown that the principle of creating bioartificial nerve grafts is a valid concept. There was no impairment of nerve regeneration by using filaments in the silicone tube, and the size and number of filaments, if less than or equal to seven, did not influence the regeneration. However, if a large number of filaments (15) were used, especially if the resorbable catgut filaments were introduced, the formation of the tissue matrix and growth into the distal nerve segment were impaired. It is interesting to notice that there was no difference in the formation of tissue matrix in the silicone tube, measured as the area excluding filaments, between the control group and the various experimental groups if less than or equal to seven filaments were used. This indicates that the introduced proximal and distal nerve segment and their size and viability [9, 15] influence the formation of the fibrin matrix which is created between the severed nerve ends [6]. Formation of such a fibrin matrix is important for the regeneration process [16]. Such matrix is invaded by macrophages and one of these produced substances, interleukin-1 (IL-1), can be detected in the matrix [6]. These macrophages have the ability to stimulate the outgrowth of axons [17, 18]. The use of filament materials which induce marked inflammation due to the degradation process of the filament–catgut filament–did not, however, improve the formation of the fibrin matrix or markedly improve the growth of axons through
the silicone tube and into the distal nerve segment at the observed time point (4 weeks). However, it has been shown recently that the use of resorbable filaments such as polyglactin, polydiaxanon and catgut filament, in the long term, stimulate the growth of axons into the distal nerve segments significantly more efficient than non-resorbable polyamide filaments [12].

The reason for using catgut filament was the advantage of that material to induce macrophage invasion [17, 18]. Implantation of such a material follows a specific schedule in the body. Irrespective of the eliciting stimuli an acute inflammatory reaction is induced, initially inducing fluid accumulation around the implantation material, and later invasion of polymorphonuclear granulocytes around the filament. There is a fibrin matrix to which the cells are adhered. After this acute inflammatory reaction the pattern is later characterized by mononuclear cells, proliferation of fibroblasts and blood vessels, and a chronic or granulomatous inflammation occurs [19]. The degradation of catgut filaments is very slow and the reaction around the filaments was clearly noted at 4 weeks, as in the present study. Catgut products are still clearly visible 6 months post surgery and for this reason a polyglactin filament is preferable [11, 12].

The reaction around the polyamide filament was different and there was a very thin capsule around the single filaments consisting of one to several layers of flattened cells. In many cases it was possible to detect myelinated axons very close to the polyamide filaments but the axons did never grow directly on the material. The introduction of filaments did not significantly increase the area of the the formed matrix or improved the outgrowth of axons into the distal nerve segment as compared to silicone tubes with no filaments. This indicates that the filaments are of limited importance when short gaps are bridged by silicone tube but it did not either impair the outgrowth. However, when extended nerve gaps which normally impair the outgrowth of axons (gap ≥ 15 mm) the use of filaments are of significant importance to stimulate the regeneration process [1]. Filaments with small diameter (ϕ 150 μm) seem to be sufficient as a scaffolding and there is no advantage of introducing many filaments (less than or equal to seven) [10].

In conclusion, the regeneration process is not impaired by introduction of filaments into silicone tubes if less than or equal to seven filaments are used. The process is not influenced by the size of the filaments. Resorbable filaments with a very long degradation time, catgut filaments, did not improve formation of the tissue matrix or outgrowth of axons at 4 weeks; however, it may be an advantage to use resorbable filaments with a considerably shorter degradation time (e.g. polyglactin), as recently has been shown [11, 12].

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References

1. G. LUNDBORG, L. B. DAHLIN, M. KANJE, N. TERADA and D. DOHI, *I. Hand Surg.* **22B** (1997) 299.
2. V. R. HENTZ, in ''Operative nerve repair and reconstruction'', vol. 1, edited by R. H. Gelberman (J.B. Lippincott Company, Philadelphia, 1991), pp. 1369.
3. H. MILLESI, G. MEISSL and A. BERGER, *J. Bone Joint Surg.* **54A** (1972) 727.
4. H. MILLESI, *Clin. Plast. Surg.* **11** (1984) 105.
5. L. DAHLIN, K. F. ERIKSSON and G. D. SUNDKVIST, *Diabetic Med.* **14** (1997) 353.
6. L. B. DAHLIN, Q. ZHAO and L. M. BJURSTEN, *Restor. Neurol. Neurosci.* **8** (1995) 199.
7. N. DANIELSEN, *ibid.* **1** (1990) 253.
8. G. LUNDBORG, L. DAHLIN, N. DANIELSEN, H. HANSSON, A. JOHANNESSON, F. M. LONGO and S. VARON, *J. Hand Surg.* **7A** (1982) 580.
9. Q. ZHAO, L. B. DAHLIN, M. KANJE and G. LUNDBORG, *Restor. Neurol. Neurosci.* **5** (1993) 197.
10. G. LUNDBORG and M. KANJE, *Scand. J. Plast. Reconstr. Hand Surg.* **30** (1996) 105.
11. N. TERADA, L. M. BJURSTEN, D. DOHI and G. LUNDBORG, *ibid.* **31** (1997) 1.

12. N. TERADA, L. M. BJURSTEN, M. PAPALOIZOS and G. LUNDBORG, *Rest. Neurol. Neurosci.* **11** (1997) 65.
13. M. KANJE, G. LUNDBORG and A. EDSTRÖM, *Brain Res.* **439** (1988) 116.
14. J. M. KERNS, N. DANIELSEN, B. HOLMQUIST, M. KANJE and G. LUNDBORG, *Exp. Neurol.* **122** (1993) 28.
15. Q. ZHAO, L. B. DAHLIN, M. KANJE and G. LUNDBORG, *Brain Res.* **592** (1992) 106.
16. L. ZENG, A. WORSEG, G ALBRECHT, W. OHLINGER, H. REDL, W. GRISBOLD, K ZATLOUKAL and G. SCHLAG, *Rest. Neurol. Neurosci.* **8** (1995) 107.
17. L. B. DAHLIN, A. MIYAUCHI, P. THOMSEN, N. DANIELSEN and M. KANJE, *ibid.* **9** (1996) 141.
18. A. MIYAUCHI, M. KANJE, N. DANIELSEN and L. B. DAHLIN, *Scand. J. Plast. Reconstr. Surg. Hand Surg.* **31** (1997) 17.
19. A. ROSENGREN, Medical thesis, Lund University (1997).

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