The role of macrophages in bioartificial nerve grafts based on resorbable guiding filament structures

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A 10 mm gap in a rat sciatic nerve was bridged by a bioartificial nerve graft consisting of a silicone tube containing seven longitudinally placed filaments made of non-resorbable material (polyamide [Ethilon®]) or resorbable materials (polydioxanon [PDS®], polyglactin [Vicryl®] or catgut). The purpose was to study the tissue reaction induced by the four different types of materials. At 4 weeks an immunocytochemical technique, using ED1 and ED2 monoclonal antibodies, was used to study the presence and location of macrophages. A large number of macrophages were found accumulating on the surface of catgut and polyglactin, while few were found on the surface of polyamide and polydioxanon filaments. It is concluded that the cell layers on the filament surface mainly consisted of ED1 positive cells and their thickness depends on the filament materials.

1. Introduction

Various types of experimental bioartificial nerve grafts have been made for the treatment of segmental defects of peripheral nerves [1–10]. We have previously presented a new type of bioartificial nerve graft, where multiple synthetic filaments placed inside a silicone tube constitute an effective scaffold for matrix formation and axonal growth [11]. Furthermore we have studied the effects of resorbable synthetic filaments used as guidelines for regenerating axons inside silicone tubes. It was found that resorbable filaments did not interfere with axonal regeneration at the early stage [12]. However, resorbable materials, may induce stronger tissue reactions than non-resorbable materials, and some reports demonstrate that inflammation may improve nerve regeneration [13]. The purpose of the present study was to use immunohistological staining techniques (ED1 and ED2) to study macrophage behaviour inside a bioartificial nerve graft consisting of resorbable or non-resorbable filaments.

2. Materials and methods

2.1 Animals and surgical procedures

Thirty-two female Sprague-Dawley rats weighing around 200 g were used. They were anesthetized by an intraperitoneal injection of 2 ml of a mixture of 0.9% sodiumchloride and sodiumpentobarbiturate (60 mg/ml) in 9/1 proportion. The sciatic nerve on the right side was exposed and resected to give a 10 mm gap. A 14 mm length silicone tube with an inner diameter 1.8 mm (Dow Corning Corporation, Medical Products, Midland, Michigan 48640 USA) was used to bridge the gap between the nerve ends. 2 mm of each nerve end were introduced into the tube and fixed to the wall by two epineural sutures to make a 10 mm gap inside the tube. Seven 10 mm filaments were placed longitudinally inside the silicone tube and the tube was filled with saline.

Four experimental groups, each consisting of eight rats, were set up on four different kinds of filament materials; polyamide group (Ethilon®, Ethicon, Norderstedt, Germany), polydioxanon group (PDS®, Ethicon), polyglactin group (Vicryl®, Ethicon) and catgut group (Ethicon). The filament diameter was the same (250 μm) in each material.

2.2. Evaluation

After 4 weeks the rats were sacrificed using an overdose of sodiumpentobarbital, and the tubes with contents were removed. The nerve structure was removed from the tube, embedded in Tissue Tek® and placed in a freezer at $-70\,^{\circ}C$. Transverse 10 mm thick cryosections at mid-level were made. Immunohistochemistry was performed as previously described [14–17], using monoclonal mouse anti-rat macrophage antibody ED1 or ED2 (Serotec, Oxford, England).The sections were subsequently incubated in biotinconjugated horse anti-mouse lgG (DAKO, Glostrup, Denmark).

The presence and distribution of ED1 and ED2 positive macrophages was investigated by light microscopy in the following four areas: (1) tube interface,

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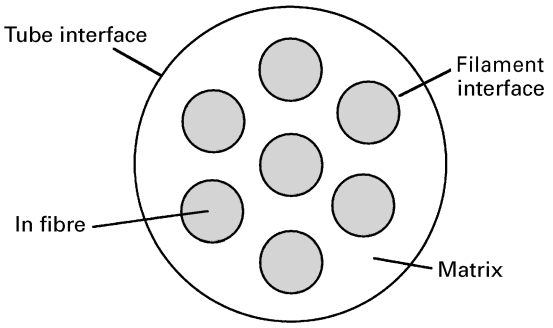

Figure 1 Schematical drawing showing the four areas as defined in this study. See text.

defined as the interface between the regenerating nerve structure and the fluid inside the tube; (2) matrix; (3) filament interface, defined as the interface between the filament and the matrix; and (4) inside filaments (only Vicryl) as shown in Fig. 1. Cell counting was done in the matrix using a grid eyepiece at a magnification of X60. Twenty fields were examined in each group. Each field was 167 by 167 μm wide. Statistical evaluation was done using ANOVA and Shaffe on a Macintosh computer (StatView, Abacus Concepts, California, USA).

3. Results

3.1. Immunocytochemical observation

3.1.1. Polyamide (Ethilon®) group

ED1 and ED2 positive macrophages in moderate numbers were observed in all areas. At the filament interface there was a layer consisting of ED1 positive cells (Fig. 2); only a few ED2 positive cells were observed. In the matrix, ED2 positive cells were more abundant than ED1 positive cells (Fig. 5). At the tube interface, there was no significant difference in the numbers of ED1 and ED2 positive cells.

3.1.2. Polydioxanon (PDS®) group

The ED1 positive cell layer at the filament interface was somewhat thicker than for the polyamide group. ED1 and ED2 positive cells were less abundant in the matrix, compared to the other groups ($p < 0.05$).

3.1.3. Polyglactin (Vicryl®) group

Since each polyglactin bundle consists of multiple small filaments, the histological picture was very different. Many ED1 positive macrophages were present in the bundles, but few ED2 positive cells were observed. There were many ED1 positive polymorphonuclear giant cells, which were negative for ED2, at the bundle interface (Fig. 3). There were moderate numbers of both ED1 and ED2 positive cells at the tube interface.

3.1.4. Catgut group

There was strong staining for ED1 at the filament interface, while no ED2 staining was observed there

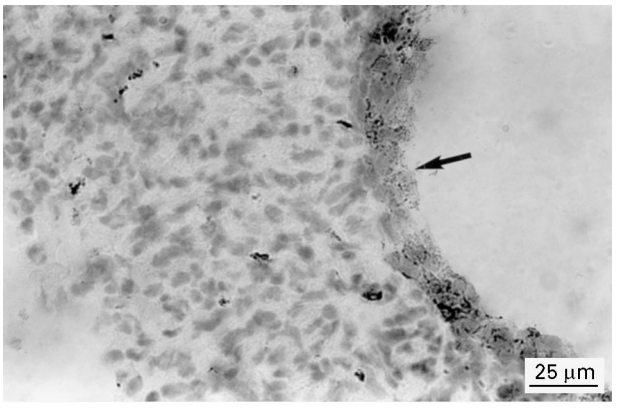

Figure 2 Histological section of specimen with polyamide filaments stained with ED1 antibody. There were ED1 positive cell layers (arrow), which were thinner than around other materials, on the surface of filament.

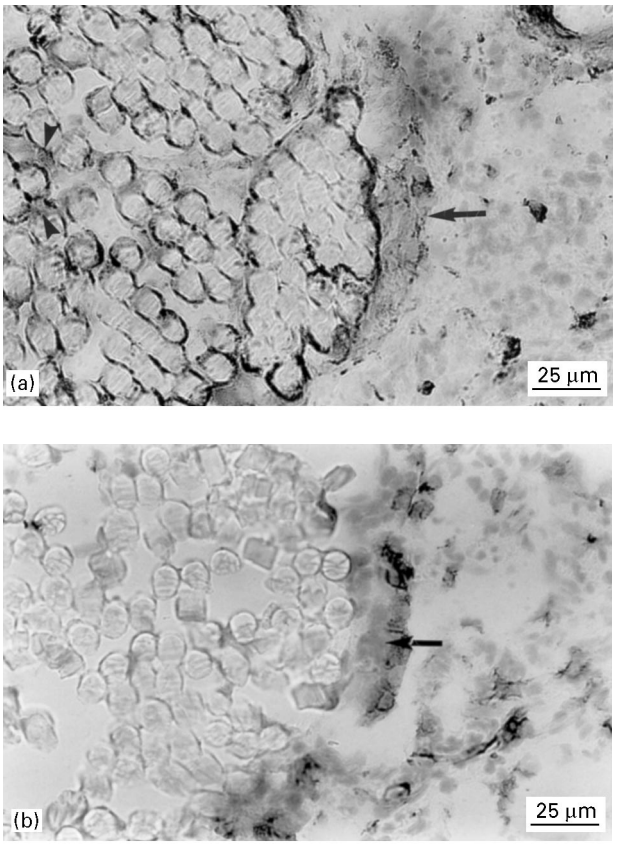

Figure 3 Histological section of polyglactin specimen. (a) ED1 antibody staining. Polymorphonuclear giant cells on the surface of bundles were positive for ED1 (arrow). Most mononuclear cells inside the bundles were positive for ED1 (arrowheads). (b) ED2 antibody staining. Polymorphonuclear giant cells were negative for ED2 (arrow). There were few ED2 positive cells inside the bundles.

(Fig. 4). The ED1 cell layer was the thickest among the four groups. The distribution of ED1 and ED2 cells at the tube interface was moderate, as for the other groups.

3.2. Cell numbers in the matrix

The numbers of ED1 and ED2 positive cells in the matrix is shown in Fig. 5. There was a statistically significant difference between polyglactin and

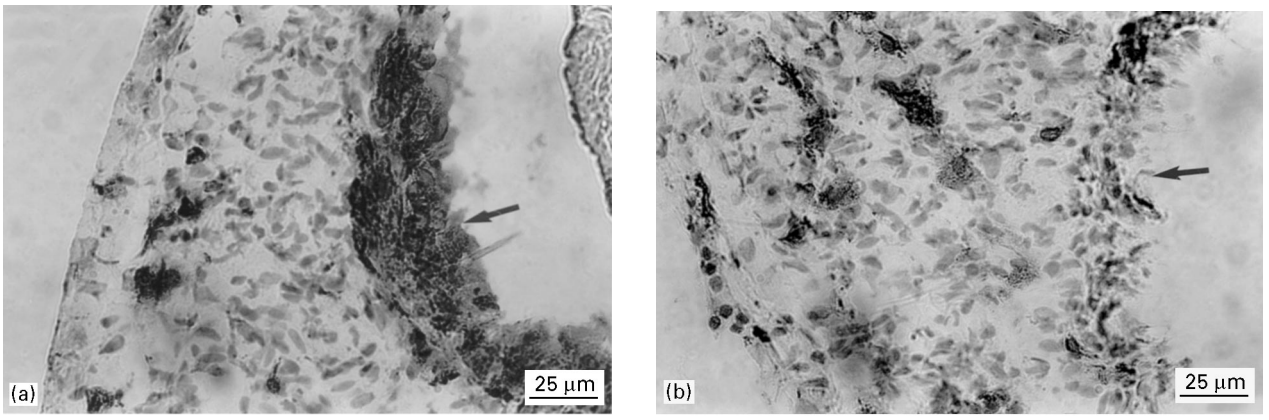

Figure 4 Histological section of specimen with catgut filaments. (a) ED1 antibody staining. There were thick layers of ED1 positive cells on the surface of the filaments (arrow). (b) ED2 antibody staining. The thick cell layers, which were positive for ED1, were negative for ED2 (arrow).

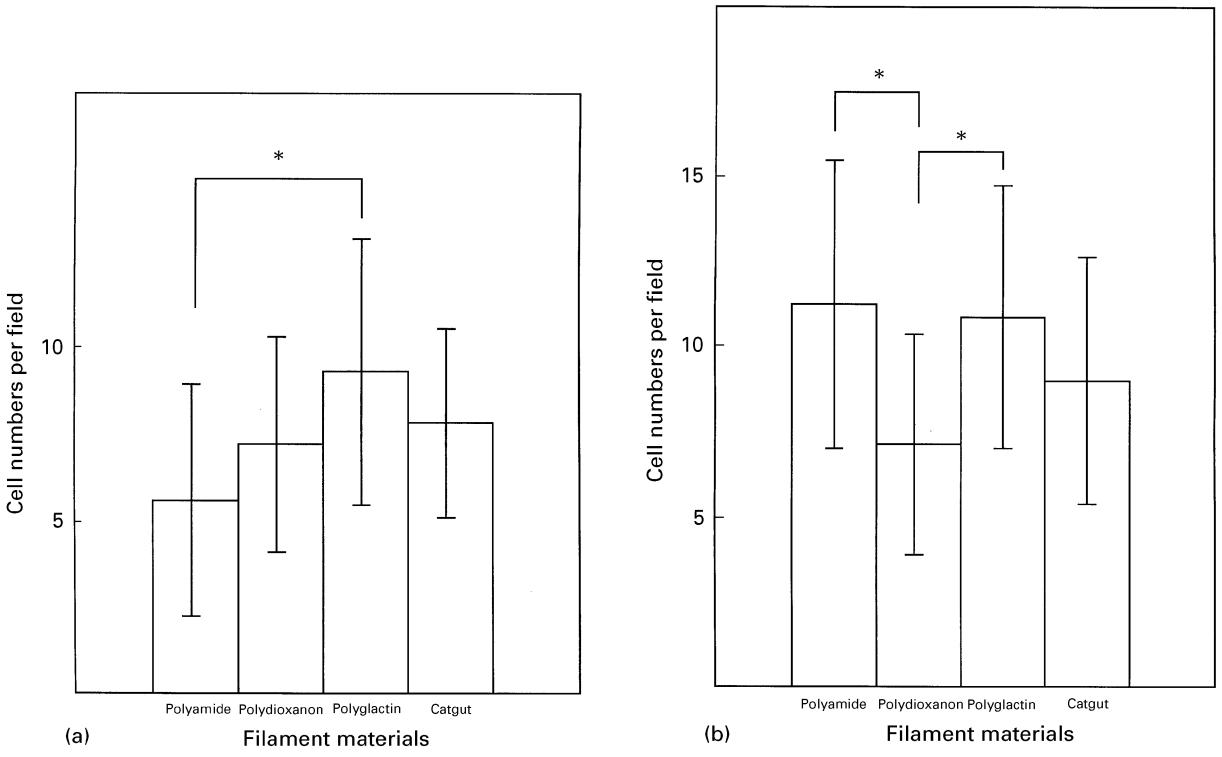

Figure 5 Numbers of cells in the matrix. (a) ED1 positive cell numbers. There was a statistically significant difference between polyglactin and polyamide ($p = 0.0132$, ANOVA and Shaffe). (b) ED2 positive cell numbers. There were statistically significant differences between polyglactin and polydioxanon ($p = 0.0192$, ANOVA and Shaffe) and polyamide and polydioxanon ($p = 0.0027$, ANOVA and Shaffe).

polyamide with regard to ED1 positive cells. With regard to ED2, there was a statistically significant difference between polydioxanon and polyglactin and also between polydioxanon and polyamide.

4. Discussion

In this paper we have further developed our previously described prototype for bioartificial nerve graft based on multiple synthetic filaments providing an intrinsic framework and scaffold for regenerating axons and migrating cells [11]. We have previously described the early results from the use of bioartificial nerve grafts, consisting of silicon tubes containing multiple resorbable filaments, indcating that resorbable materials did not interfere with axonal regeneration [12]. In the present investigation we focus on the occurrence and distribution of macrophages in the regenerating nerve structure as a result of the use of resorbable materials. Dahlin *et al.* [15] reported on the distribution of macrophages and interleukin-Lβ in the fibrin matrix in corresponding tubes containing no filaments. With the use of longitudinally placed synthetic filaments an inflammatory reaction would be expected, especially when resorbable materials are used.

The most interesting finding was the occurrence of an ED1 positive macrophage layer at the filament interface, which showed varying thickness depending on filament materials. Catgut and polyglactin exhibited

a thick ED1 positive cell layer, while polyamide and polydioxanon showed a thinner layer. Furthermore, it was interesting to find that most macrophages inside the polyglactin bundles were positive for ED1, and that polymorphonuclear giant cells on the surface of the polyglactin bundles were positive only for ED1. Usually ED1 is regarded as an indicator of invading macrophages, and ED2 as an indicator of resident tissues macrophages [16–18]. Some reports have demonstrated varying behaviour and roles for ED1 and ED2 macrophages [18–22]. It has also been reported that ED1 positive macrophages accumulate quickly and are active in phagocytosis, while ED2 macrophages are accumulating slowly and play a role in regeneration [21, 22]. However, there is no report concerning tissue reactions at the surface of the resorbable filaments. Our results demonstrate that ED1 positive macrophages act mainly at the filament interface, playing a role in the tissue response to the filament material and presumably to its resorbtion, since the reaction is stronger with rapidly resorbable filaments (catgut and polyglactin).

Concentric cell layers are formed around the filaments as a result of the tissue reactions [12]. However, catgut filaments, which gathered great numbers of ED1 positive cells at the filament interface, did not have thick concentric cell layers as previously described. With the use of polydioxanon filaments, which gathered less ED1 positive cells, thick concentric cell layers were formed. These findings may suggest that ED1 macrophages are not associated with the formation of concentric cell layers in this model.

Axonal regeneration occurs mainly in the matrix [11, 12]. However, our results did not indicate that resorbable filaments induce strong macrophage reactions in the matrix (Fig. 5). Dahlin has suggested that inflammation may act as conditioning lesion [13] and that macrophages may be essential for nerve regeneration [23]. Probably macrophages play some role in the matrix of regenerating nerves. This study shows that it is not enough to identify the presence of macrophages by immunocytochemistry to understand their role for nerve regeneration. Future studies using functional characterization of, for example, cytokine production may resolve these problems.

Acknowledgements

This study was supported by grants form Swedish Medical Research Council (MFR, project 5188), Swedish Council for Work Life Research and Swedish National Board for Technical Development (NUTEK).

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Received 13 May
and accepted 11 July 1996