Peripheral nerve reconstruction with autologous vein, collagen, and silicone rubber tubes
Heyke, G.C.M.
Chapter 3

Vein graft conduits versus conventional suturing in peripheral nerve reconstructions

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Received 16 June 1993; Accepted 16 July 1993
Abstract

Positive results of tubulization in peripheral nerve reconstruction have been established in animals by many investigators. Clinically, tubulization by means of a venous tubulus is accepted as a reliable technique, but histological results are not known and functional analysis is limited. The aim of this investigation was to study the histological effect of venous tubuli in peripheral nerve reconstruction.

In 20 rabbits the saphenous nerves were transected and reconstructed. In ten rabbits (series 1) a venous tubulus was placed around the nerve suture. In another ten rabbits (series 2) a venous tubulus was sutured over a 3-mm nerve gap. Conventional suturing was done in ten contralateral saphenous nerves (series 3, controls). Epineurial stitching was performed. The healing was studied after 3 months and after that histological analysis was performed by means of monoclonal antibody staining.

The results of our experiments show that covering nerve suturing with a venous tubulus did not enhance healing in comparison to the conventional end-to-end suturing, but in contrast evoked extensive fibrous tissue, thereby hampering regeneration of axons.

Microsurgery 14: 584-588 1993
**Introduction**

Many investigators have reported on reconstruction of peripheral nerve, the tubulus method being more successful than conventional suturing or even a nonreconstructed lesion.\(^1\,\)\(^4\) One of the underlying principles for using a tubulus in reconstruction is not only to keep the neurotrophic substances from emerging from the site of the suture\(^5\,\)\(^6\) but also "to barricade the junction of the graft and host against fibroblastic invasion and to prevent constriction of the graft by extraneuronal adhesions,"\(^1\) and to minimize connective tissue proliferation and scar formation. Another positive effect of the application of a tubulus is opposing edema at the nerve suture, retaining longitudinal orientation,\(^3\,\)\(^7\) and hampering the regenerating axons in growing outside the nerve tissue. On the other hand, the effect of a too strong circumferential pressure on the diameter of a nerve could be correlated with its negative effect on conduction and regeneration, as caused by anoxia.\(^7\,\)\(^8\)

In 1941, Weis emphasized the beneficial effect of some traction between the nerve endings, because longitudinal stress enforced longitudinal orientation among the regenerating nerve fibres.\(^9\) However, other investigators found that a decrease of tension at the suture site could reduce connective tissue proliferation and scar formation.\(^10\)

Since the end of the last century, when Glück\(^11\) experimented on decalcified bones as a bridge, many biological materials have been used for tubulization or bridging in nerve reconstruction, like nervous tissue,\(^12\,\)\(^13\) autologous venous segments,\(^14\,\)\(^15\) and skeletal muscle autografts.\(^12\,\)\(^16\)

Venous tubulization as nerve reconstruction is used clinically. However, little is known about histological results, either in the clinical procedure,\(^15\) or in animal models.

In this study we present immunohistochemical data on nerve regeneration after
nerve reconstruction with and without a venous tubulus. The saphenous nerve was used, being an almost totally sensory nerve the transecting of which causes limited harm to the animal.

**Materials and methods**

All animal experiments were carried out following the conditions of the Animal Experimental Law in the Netherlands.

Twenty adult female rabbits (New Zealand) of 3.5 kg body weight were used. Anesthesia was initiated with xylazine and ketamine hydrochloride (Rompun and Ketalar; 10 mg/kg and 50 mg/kg, respectively) administered intramuscularly. Anesthesia was continued by nitrous oxide, oxygen, and Fluothane mixture inhalation. Continuous electrocardiographic (ECG) registration was performed. All surgical procedures were carried out under normal sterile conditions.

After incision of the skin at the medial site of the proximal hindleg and cleavage of the fascia lata over a length of 2 cm, the saphenous magna vein and the saphenous nerve could be explored. The nerve was mobilized over 15 mm.

In all animals the skin at the neck was incised medially, the superficial fascia was cleft, and the external jugular vein was explored and doubly ligated. A segment of 10 mm was taken out and kept in saline at room temperature. Diameter and elasticity of this vein appeared to be sufficient to embrace the saphenous nerve.

Then the saphenous nerve was cut by microsurgical methods. In the first series (n = 10) a venous tubulus was slid over the proximal nerve ending and elevated so that both nerve endings could be sutured by four epineurial stitches (direct method) and the venous tube was placed over the suture. Epineurial vessels always served as a guide for approximation of the nerve endings. In another ten rabbits
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(series 2) the venous tube was slid over the proximal nerve ending and fixed by four epineurial sutures at 0.5 cm proximal to the ending; then the distal nerve ending was inserted into the venous tube and fixed at 0.5 cm distally by four epineurial sutures (indirect method). In this procedure a gap of 3 mm existed due to the retraction of the nerve endings and the elasticity of the venous tissue.

The contralateral saphenous nerve in ten of these animals served as controls; the nerve endings were just sutured by four epineurial stitches (direct method). After nerve reconstruction the fascia and skin of the hind leg and neck were closed. Postoperatively the animals were housed alone and carefully observed. Special events were recorded. In case of a tendency to automutilation, the animal was placed in group quarters and the mutilation mostly stopped. Inflammation was treated by administration of antibiotics.

After 12 weeks the reconstructed nerve was taken out and fixed in Kryofix [50% ethanol, 3% poly-ethylene-glycol (PEG 300)]. Longitudinal sections were taken through the whole nerve sutures. Transverse sections were made 10 mm proximal to the proximal nerve stump and 10 mm distal to the distal nerve ending. Histochemical examination was carried out by indirect NF 90 peroxidase-conjugated monoclonal antibody staining or by indirect NF 90 fluoresceine isothiocyanate (FITC) labeled monoclonal antibody staining and by anti-vimentin Texas Red (TXRD) labeled staining. Visualization was done by light microscopy (LM) or confocal laser scanning microscopy (CLSM, Bio-Rad MRC-600). Data were analyzed by means of an image analysis software program (Fig. 1) and counting was done in standardized areas in the transverse sections, according to the method of Carlson. 

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Results

No motor disturbances were noticed in any animal at the first postoperative day. In two animals with a tubulus (one with the direct and one with the indirect suturing method) a tendency to automutilation appeared, but after change from the solitary cage to a group cage, the behavior became normal. In one rabbit of the direct method an abscess was relieved.

All specimens with a venous conduit showed more connective tissue around the nerve suture, irrespective of direct or indirect procedure, compared to specimens without venous conduits. This finding was confirmed histologically. The connective tissue was situated epi-, peri-, and endoneurially but mostly central in the nerve (Figs. 2, 3; Table 1). After 3 months axons were hardly seen distal to the lesion. Their axonal arrangement was better in the directly sutured tubulus procedure than in the indirectly sutured procedure, but in both tubulus procedures the outgrowing axons seemed to be hampered by the presence of connective tissue.

In the sutured control series (Fig. 4; Table 1) an almost normal number of axons was found at the site of the nerve anastomosis and in the distal part of the nerve in a much more organized pattern, almost without connective tissue excrescence. Interactive counting of the axons per area in the three procedures showed a larger number of outgrowing axons distal to the lesions in series 3 than in series 1 and 2 (Table 1).
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Discussion

The tendency to automutilation in one animal in series 1 and one animal in series 2 probably appeared because of the irritating effect of the venous tubulus on the nerve. This irritation effect was not present when the animals were placed into a group cage.

In rabbits the regeneration rate of sensory nerves is about 3.5 mm/day.\(^\text{18}\)

There was some tension in the directly sutured nerve lesions, caused by the normal retraction in the nerve endings after being cut.

Comparison of directly and indirectly sutured nerve reconstructions with a venous tubulus showed a more orderly axon pattern in the direct procedure. This finding could be due to the small amount of traction present between the directly sutured nerve endings,\(^\text{9}\) and to the absence of traction between the indirectly sutured nerve endings.\(^\text{10}\)

The 3-mm nerve gap present in the indirect procedure resulted in less outgrowth of regenerating axons after 3 months.

Although the advantages of the tubulus method in peripheral nerve reconstruction are acknowledged by many investigators,\(^\text{1-4}\) in our experiments the use of an autologous venous tube gives rise to the formation of obstructive connective tissue and to the decrease of axon regeneration, independent of using either the direct or the indirect suturing technique. After reconstruction without a venous tubulus, however, connective tissue was just present in small quantities. It is possible that the contact between the endothelial cells of the transplanted venous segment and the nervous tissue initiates the development of connective tissue and constriction of the nerve outside, which starts before axons can regenerate.

In conclusion, these results demonstrate that in rabbits the use of autologous venous material as a tube around directly or indirectly sutured peripheral nerves
restricts outgrowth of axons and therefore is a less suitable method of peripheral nerve reconstruction. Because histological results after clinical procedures are rarely known, we advise caution in clinical use of venous tubes in peripheral nerve reconstruction.
References


Table 1 Results of Automatic Counting of the Amount of NF 90 Stained Axons in Fascicles and in a Transverse Section Proximal and Distal to the Lesion.

<table>
<thead>
<tr>
<th></th>
<th>Series 1</th>
<th></th>
<th>Series 2</th>
<th></th>
<th>Series 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
<td>Proximal</td>
<td>Distal</td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>Area of transverse section (t) (µm²)</td>
<td>2,622</td>
<td>1,152</td>
<td>1,659</td>
<td>1,596</td>
<td>2,269</td>
<td>2,143</td>
</tr>
<tr>
<td>Axons(a) as % of area</td>
<td>5.05</td>
<td>1.67</td>
<td>5.61</td>
<td>1.68</td>
<td>5.1</td>
<td>1.8</td>
</tr>
<tr>
<td>a x t/100</td>
<td>132.4</td>
<td>19.2</td>
<td>93.1</td>
<td>26.8</td>
<td>116.0</td>
<td>39.0</td>
</tr>
<tr>
<td>Number of axons/area</td>
<td>31</td>
<td>7</td>
<td>23</td>
<td>12</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>Total size of axons (µm²)</td>
<td>4.3</td>
<td>2.7</td>
<td>4.0</td>
<td>2.2</td>
<td>3.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Number of axons/5,000 µm²</td>
<td>59</td>
<td>30</td>
<td>69</td>
<td>36</td>
<td>67</td>
<td>42</td>
</tr>
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Percentage of outgrowing axons corrected for fascicles per area

Series 1 (direct reconstruction with venous tubulus): 50.84
Series 2 (indirect reconstruction with venous tubulus): 52.17
Series 3 (direct reconstruction without tubulus): 62.98
Figure 1  Cross-section in reconstructed nerve, 10 mm distal to the distal nerve stump. (NF 90 staining, label anti-vimentin Texas Red (TXRD), confocal laser scanning microscopy (CLSM), x 600.) Data registration and analysis of the stained axons by an Image Analyzer software program.
Figure 2  Nerve reconstruction by direct suturing and venous tubulus. Three months postoperatively. Longitudinal section: To the right is distally. Note the diffuse scar tissue and the constriction in the middle of the lesion. (NF 90 staining, x 10.)

Figure 3  Nerve reconstruction by indirect suturing by means of a venous tubulus. Three months postoperatively. Longitudinal section: To the right is distally. Note the large amount of scar tissue and the constriction of the axons in the middle of the lesion. (NF 90 staining, x 10.)
Figure 4  Nerve reconstruction by conventional direct suturing. Three months post operatively. Longitudinal section: To the right is distally. Note the many outgrowing axons in an organized pattern with hardly any scar tissue, without constriction. (NF 90 staining, x 10.)
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