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A new technique of autogenous conduits for bridging short nerve defects. An experimental study in the rabbit

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Summary

Backround. Nerve grafting is the most reliable used procedure to bridge a neural defect, but it is associated with donor site morbidity. In experimental surgery the search for an optimal nerve conduit led to the use of biological and artificial material. Nerve regeneration through epineural conduits for bridging short nerve defect was examined.

Methods. Four groups including 126 New Zealand rabbits were used. There were 3 study groups (A, B and C) and 1 control group (D). A 10-mm long sciatic nerve defect was bridged either with 3 variations of an epineural flap (Groups A, B and C) or with a nerve graft (Group D). Animals from all groups were examined 21, 42 and 91 days postoperatively to evaluate nerve regeneration employing light microscopy and immunocytochemistry. Nerve regeneration was studied in transverse sections at 3, 6 and 9 mm from the proximal stump. Using muscle stimulator the gastrocnemius contractility was examined at 91 days post surgery in all groups.

Findings. Immunohistochemical and functional evaluation showed nerve regeneration resembling the control group, especially in group A, were an advancement epineural flap was used.

Conclusion. An epineurial flap can be used to bridge a nerve defect with success.

Keywords: Defect; conduit; epineurium; nerve; gap.

Introduction

Nerve grafting is the most effective used procedure to repair a neural gap, but it is associated with donor site morbidity [4, 6, 7, 11]. In experimental models the search for an optimal nerve conduit led to the use of autogenous and artificial materials [5, 9, 10, 12]. Clinical implementation of conduits has focused on the use of autogenous tissue (veins, arteries, pseudoseaths, nerve grafts) and artificial conduits (polyglactine, silicon) [6, 7, 10].

Conduit materials does not seem to improve significantly the outcome. The major obstacle in the use of conduits is the limitation in the defect size that can be successfully bridged and is limited in humans to 2.5 cm. The epineurium carries the majority of the nerve vessels, i.e. vasa nervorum. Additional vascular supply comes from the intranervous vascular plexus and from various perforators. There is also another type of reverse vascularization from distal to proximal, involving shorter vessels [4]. In this study we used 3 variations of an epineural flap to bridge a short nerve defect and to study if the epineurium may serve successfully for this reason.

Materials and methods

One hundred and twenty six white New Zealand rabbits, weighing 3.5 kg were used. The animals were allocated to 4 Groups. In the 3 study groups (Groups A, B and C) 36 animals were included, while the rest 18 animals served as control (Group D). In all groups a 10-mm sciatic nerve defect was created and bridged either with 3 variations of an epineural flap (Groups A, B and C) or with a nerve graft (Group D). In all groups the sciatic nerve was exposed under general anaesthesia. The sciatic nerve was exposed under microscope. A 10 mm nerve defect was created using a sharp blade proximal of sciatic nerve bifurcation. An advancement epineural flap harvested from the proximal nerve stump and from the distal nerve stump were employed in Groups A and B, respectively. In Group C a specially designed reversed epineurial flap harvested from the proximal stump was employed. In the control Group D the defect was bridged using the excised portion of the sciatic nerve, which was sutured in its original site.

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Fig. 1. The epineurium harvested from the proximal nerve, forms the epineurial conduit

Following exposure of the sciatic nerve and creation of the defect a 10 mm was designed on the epineurium of the proximal stump in group A and a similar flap on the distal stump in group B. Surgical dissection started in a dorsal longitudinal direction and continued circumferentially to remove the epineurium (Fig. 1). Two millimetre of the epineurium located at the rim of the proximal (Group A) or distal (Group B) nerve stump was preserved to facilitate flap suturing. The excised epineurium was then used to bridge the nerve defect. To prevent collapse of the conduit and to facilitate suturing a 2 mm thick silicon tube was inserted temporarily within the conduit and between the two nerve stumps and removed before final closure. The proximal and distal edge of the nerve was secured on the proximal and distal nerve stumps using four 10–0 Ethilon stitches. The longitudinal flap edges were also approximated using 5–7 stitches. The space within the conduit was filled with a blood clot (Fig. 2), before completing epineurium suturing. In Group C the epineurium in the proximal nerve stump was not completely excised but its distal attachment was preserved. The epineurial flap was reversed pivoting on its distal attachment and sutured on the distal nerve stump with epineurial sutures. In this case the length of the flap was 12 mm.

In Group D the 10 mm defect was repaired using the previously resected nerve segment, which served as an autologous graft using 4 epineurial stitches at each suture line.

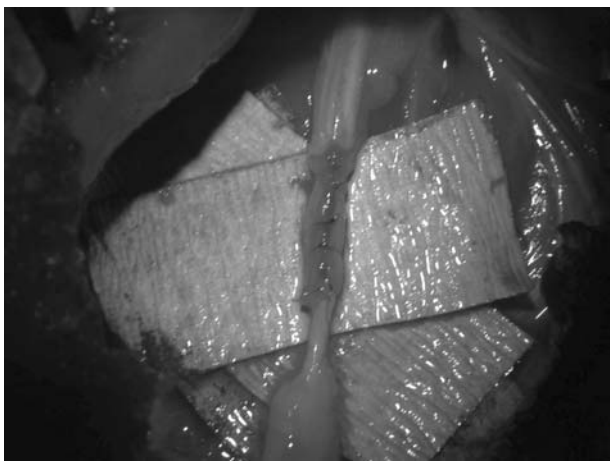

Fig. 2. The nerve defect bridged with the epineurial conduit filled by blood clot (e)

Postoperative evaluation

The nerve regeneration was studied at various intervals using immunochemistry, light microscopy and measurement of the Gastrocnemius contractility. Twelve animals from Groups A, B and C were sacrificed after 3, 6 and 12 weeks, while all control animals were sacrificed after 12 weeks. The conduit area was exposed and the grafted part was excised. Six specimens were used for light microscopy examination and 6 specimens for immunochemistry. After 12 weeks all animals underwent examination of the gastrocnemius contractility in both limbs.

Nerve regeneration was studied in 1 μm transverse sections at 3, 6 and 9 mm from the proximal stump (3 specimens for each group designated S3, S6, S9) and in longitudinal sections stump (3 specimens for each group). The epineurium conduit was resected and immersed in 2.5% glutaraldehyde. After fixation in 1% osmium tetroxide and dehydration in ethanol, the specimens were embedded in Agar 100. The specimens were stained with Toluidine blue and examined by light microscopy. Quantitative morphometry was performed measuring the number of myelinated axons per mm^2 and the mean axon diameter in every section.

In similarity with the light microscopy 6 specimens from each group were examined using immunochemistry. The harvested conduit was rinsed in ice-cold PBS and embedded in Tissue Tek O.C.T. Three micrometre thick transverse and three, 10 mm long longitudinal sections were cut on a cryostat. After fixation in 2.5% paraformaldehyde, the sections were exposed to primary antibodies (DAKO) to identify the components of the newly formed nerve, including 68 KD neurofilament protein, fibrinogen, fibrin and fibronectin. The immunocytochemistry and light microscopy findings at 3 and 6 weeks were only qualitatively analysed.

The isometric contraction force of the Gastrocnemius muscle, which is supplied by the tibial nerve, was measured 13 weeks after the defect bridging in all groups. The animals were anaesthetised and the sciatic nerves and the Gastrocnemius muscles were bilaterally exposed. Electric stimulators were placed proximally and distally to the defect and a recording electrode was placed in the Gastrocnemius muscle 10 mm below the tibial tubercle. A similar procedure was undertaken in the normal limb. Supramaximal electrical stimuli were delivered proximal to the nerve repair site or the respective intact nerve location by a Grass-SD-9 stimulator at a frequency of 100 Hz for 0.6 msec and the gastrocnemius electrode was recording transmitted evoked potentials.

The ratio of the compound muscle action potential between the operated and the normal limb (p-ratio) was recorded [1, 2]. Following contractility measurement the nerve specimens were excised and processed for light microscopy and immunochemistry as described above. The quantitative histomorphometric and electromyographic data were statistically compared using ANOVA and the significance level was set at $p = 0.05$.

Results

Regarding Gastrocnemius contractility the amplitude of the motor response in mV was expresses as the ratio between the operated and the normal side. The amplitude of the gastrocnemius muscle contraction ranged between 5.3 ± 1.2 and 21.8 ± 3.9 mV. The gastrocnemius contractility after 13 weeks compared to the contralateral normal leg was 60.3, 42.1 and 58.7% in groups A, B and C respectively, while in the control group D was 64.1%. The difference between these parameters was statistically not significant ($p = 0.10$).

Histomorphometric results

Employing light microscopy the number of myelinated axons was in group A and B 55 and 43% of the normal, contralateral nerve or 81 and 68% of the control group values. In the control group the respective value was 68% of the normal contralateral sciatic nerve. The difference was between groups A, B and D was statistically highly significant ($p < 0.001$). The mean axonal diameter was in group A and B 59 and 45% of the normal, contralateral nerve or 78 and 62% of the control group values. In the control group this parameter reached 71% of the normal nerve value. The difference was between groups A, B and D was statistically highly significant ($p < 0.001$). On microscopy examination several findings were evident. Three weeks after the operation on microscopy examination of the regenerated nerve presence of myelin sheaths was evident throughout the nerve section with extensive areas of connective tissue between the axons. In the longitudinal sections new myelinated axons could be seen throughout the conduit, which appeared thicker at the proximal third of the conduit. At 6 weeks the myelin sheaths were thicker than before and there was a clear tendency to mini-fasciculation in cross sections (Fig. 3). After 13 weeks the axons constituted a new structure closely resembling the normal nerve. Using immunocytochemistry the epineural conduit was filled with fibrin and fibronectin as part of the healing process, while S100 stain for Schwann cells was positive. Bunger bands (lSchwann cells) appeared in the third week along the conduit. Schwann cell proliferation preceded axonal growth. The proximal $2/3$ of the new structure showed staining of neurofilament proteins. The proximal third was well stained while the second one was slightly stained (progressive axon advancement). At 6 weeks fibrin and fibronectin are present. The conduit is occupied by stained neurofilaments at 13 weeks (Fig. 4).

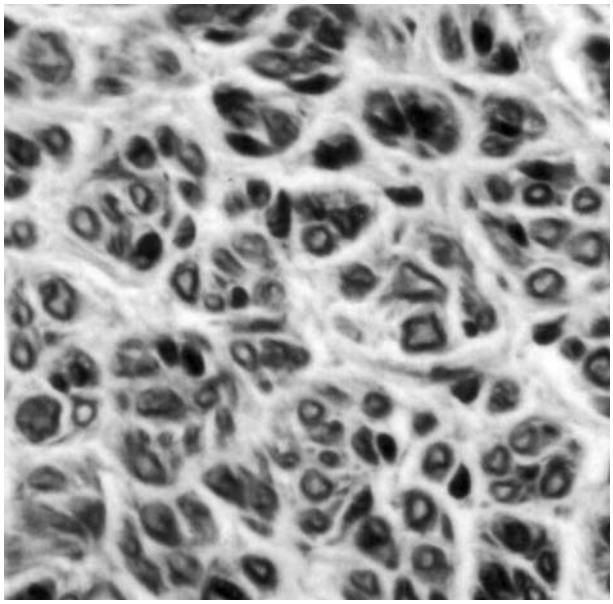

Fig. 3. Myelin sheaths stain with toluidine blue, 3 months postoperative. There is a tendency to mini-fasciculation in cross sections (magnification ×25)

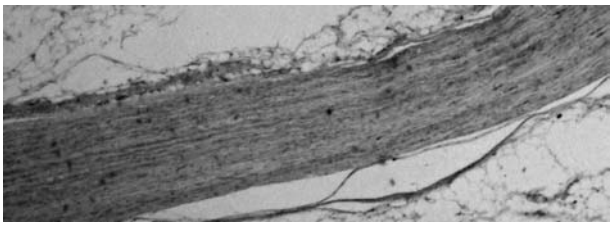

Fig. 4. The conduit is occupied by stained neurofilaments at 3 months (magnification ×25)

Discussion

A short nerve defect was bridged using various epineural flaps. The results of these conduits were comparable with those provided by nerve grafting. The principles of nerve injury have been refined based on an well understanding of nerve biology [4, 6–8]. Harvesting of the epineurium does not hinder nerve function. The epi-

neurium is a connective tissue, which surrounds the fascicles, while carrying the blood supply. From the epineurial plexus vessels arise, running between the nerve fibers. The epineurium defines fascicular groups [4]. To bridge a nerve gap represent a great challenge in surgery [1–7]. When the nerve defect was bridged with a free epineurial flap the resultant nerve regeneration approximated the results of the control group, achieving 93% for muscle contractility and 81% for the microscopy assessed parameters. In the same group the regenerated nerve reached 60.33% of the normal values concerning the contractility force and 55% concerning the parameters of the microscopy evaluation. The regeneration proceeded in a fashion with the progressive axonal maturation. When the epineurium flap was harvested from the distal stump the results were inferior compared to the proximally harvested flap, due to affecting nerve regeneration in significant way. In the latter flap the contractility of the injured side Gastrocnemius reached 42.1% of the normal gastrocnemius contractility. Using a distally attached epineural flap (C) did not improve the results.

Conclusions

The typical nerve grafting provides the best results regarding muscle response and neural regeneration. An epineurial flap may alternatively used to bridge short nerve defects and take advantage of the lesser donor site morbidity. The proximally harvested epineural advancement flap provides comparable results with the nerve graft.

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