

Venous Graft Versus Epineural Flaps in Prevention of Neuroma Formation: An Experimental Study

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ABSTRACT

This article described the effect of two chosen techniques on the prevention of neuroma formation in rats. A total of 36 rats were divided into 3 groups. Right sciatic nerve in all groups was divided. In group I, the proximal nerve stump was left without treatment. In group II, external jugular vein was dissected and divided, then used to cover the proximal nerve stump. In group III the epineurium of the proximal nerve stump was retracted and 5mm of fascicles were excised then epineurium was sutured over the proximal stump. After 4 months rats were examined. The terminal portion of proximal nerve segments in group I demonstrated evidence of neuroma formation. In group II the proximal nerve segments demonstrated no neuroma and no axonal escape in adjacent tissue. Seven rats in group III showed evidence of neuroma formation in their proximal nerve stumps. The remaining 5 rats had no neuroma in their proximal nerve endings. All specimens were examined histologically by both light and transmission electron microscopy. We conclude that there was an effective and easier technique to prevent neuroma formation by insertion of injured nerve ending into lumen of venous graft.

INTRODUCTION

The formation of a neuroma is the physiological consequence of peripheral nerve injury. Regardless of the type of primary care given, some degree of neuroma formation occurs [1]. A significant number of these neuromas are symptomatic, and painful neuroma frequently causes major disabilities [2]. It is considered that undamaged perineurium is an impenetrable barrier for regenerating fascicles. Once this barrier is damaged, regenerating axons escape into the surrounding tissue in a disorganized fashion, accompanied by proliferating fibroblasts, schwann cells and blood vessels. As a result the axons branch irregularly and form whorls, spirals and convolutions, i.e. neuroma [3].

Although as many as 30% of neuromas cause pain, a fully reliable method of preventing or treating painful neuroma has not been found [4]. This reflected in the considerable number of reports on different treatment modalities for neuromas.

Conventional methods of neuroma management include funicular resection with epineural ligation [5], epineural barriers either epineural flaps or epineural graft [6,7,8], epineural stripping or sleeve [9], implantation of nerve end into muscle [10], or bony tunnel [11], Silicon cupping [12], centro-central repair [13], and intralesional injection of steroids, alcohol and phenol [14] and use of laser for prevention of neuroma formation [15].

Though improvement of symptoms of painful neuromas may sometimes obtained by the use of several of these methods, the statistical analysis of the results are variable and sometimes discouraging, and at the present time there is no universally effective cure for painful neuromas. This lead to conclusion that prevention of neuroma formation is an objective that is difficult to achieve [16].

The current animal study was undertaken to investigate and evaluate the influence of epineural flaps and transposition of cut nerve ends into the lumen of vein graft like a glove on the formation of terminal neuroma. We study the utilization of these two procedures and their effectiveness in prevention of formation of neuromas.

MATERIAL AND METHODS

A total of 36 male Sprague dawly rats (300-400gm) were divided into three groups of 12 animals each. They were anesthetized with intraperitoneal ketamine 100mg/kgm with Diazepam 5mg/kg [17]. The right sciatic nerve in each of 36 rats was exposed microsurgically under sterile conditions. Following this, three groups each of 12 rats, were formed according to the techniques performed on the proximal stumps.

Group I: Control group, the proximal stump was left in place without treatment. The end of the nerve was marked using nylon 10/0 stitch.

Group II: External jugular vein was exposed and mobilized. Segment about 1Cm of the vein was dissected and divided. The proximal stump of the nerve was transposed into one end of the external jugular vein. At least 3mm of the nerve was inserted into the vein and held in position with an epineural 10/0 nylon sutures through the venous wall. The other venous end was opened longitudinally in one side, trimmed and folded on nerve stump. The venous wall sutured to epineurium by 10/0 nylon sutures, thus the suture line lies away from the midline at the edge of the nerve stump (Fig. 1).

Group III: Epineurium covering the proximal stump was retracted and a 5mm segment of fascicles was excised. The two distal epineural flaps was closed over the proximal stump using 10/0 nylon sutures Fig. (1). Hemostasis was achieved thereafter and wounds were closed with absorbable stitches.

Evaluation was performed after 4 months. Rats were reanesthetized. The previous operative sites were reexplored. The proximal nerve stumps were exposed and dissected in all rats. The proximal nerve stumps were examined under microscope for evaluation of their size, amount of forward regeneration of axons in millimeters from suture line (Table 1). The end of nerve stump was taken including part of healthy nerve, (6-10mm) were excised.

Table (1): Measurement of amount of nerve regeneration in mm from the original sites at the end of the experiment (4 months).

No. of rats	Amount of nerve regeneration		
	Group I Control	Group II Vein graft	Group III Epineural flaps
1	14	0	10
2	22	0	0
3	18	0	0
4	14	0	22
5	30	0	18
6	28	0	12
7	32	0	0
8	16	0	26
9	24	0	14
10	26	0	28
11	24	0	16
12	20	0	0

Specimens for electron microscope were immediately fixed in 2.5% glutaraldehyde buffered with 0.1M phosphate buffer at pH 7.4 for 2 hours. At 4°C Specimens were dehydrated with ascending grades of ethanol and then were put in propylene oxide for 30 minutes at room temperature impregnated in a mixture of propylene oxide and resin (1:1) for 1 hour then in a mixture of previous reagents at 48°C for 1 hour. The specimens were embedded in Embed-812 resin in BEEM capsules at 60°C for 24 hours [18]. By using Leica ultra cut UCT we obtained semi-thin sections which were stained with toluidine blue for light microscope examination and ultra-thin section were obtained and stained with uranyl acetate and lead citrate and were examined with JEOL JEM 1010 electron microscope.

Statistical analysis:

Data were analysed by Mann Whitney- μ -test to compare axonal escape in all groups. There was highly significant difference between group I and group II, $p < 0.001$. Significant difference was present between group II and group III, $p < 0.05$.

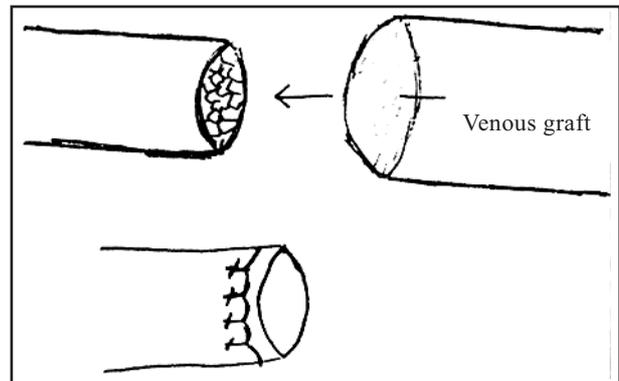


Fig. (1-A): Diagram of venous graft technique.

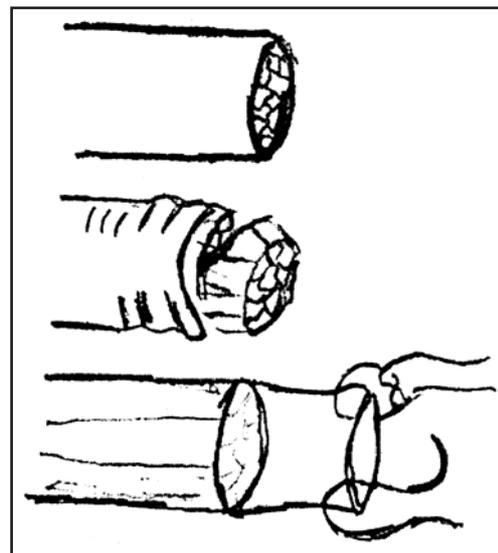


Fig. (1-B): Diagram of epineural flap technique.

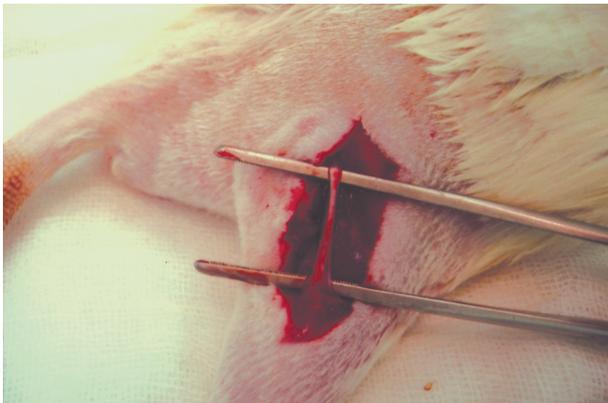


Fig. (2-A): Sciatic nerve after complete dissection.



Fig. (2-B): Classic neuroma. Proximal nerve stump showing dense mass embedded in scarry tissue.

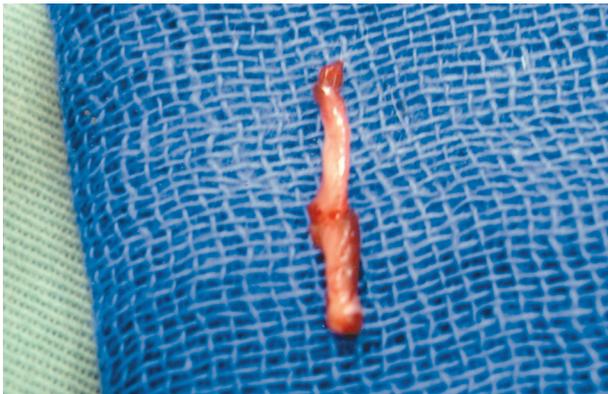


Fig. (2-C): Venous graft cover in group II. No widening of the proximal nerve stump, with no adherence to the surrounding tissues.



Fig. (3): Electron microscope examination of normal proximal nerve stump. Normally arranged axons, with well formed myelin (My). Schwann cells (Sch) are seen enclosing myelinated axons (TEM x8000).

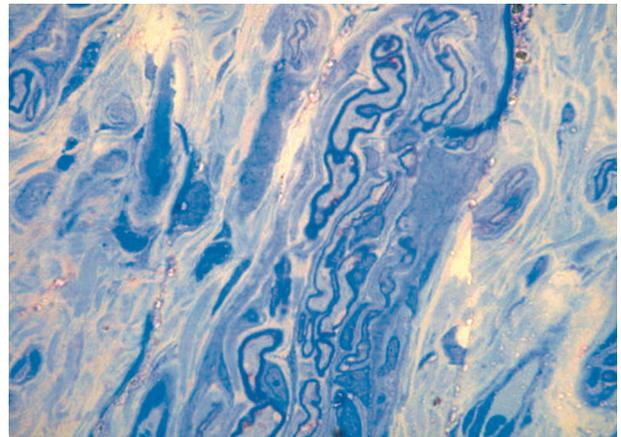


Fig. (4-A): Histologic examination of classic neuroma, demonstrating overgrowth of disorganized nerve fibers, Schwann cells and fibroblasts (Toluidine blue x1000).

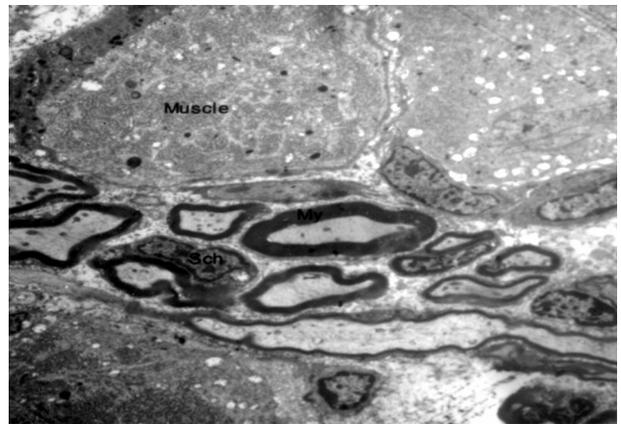


Fig. (4-B): Electron microscope examination (EM) of neuroma. Axons are scattered and disorganized, Schwann cells are irregular and does not surround myelinaed axons (TEM x3000).



Fig. (4-C): Separation of myelin lamellae is seen in cases of neuroma (TEM x15000).

Fig. (5): Venous graft group (II).

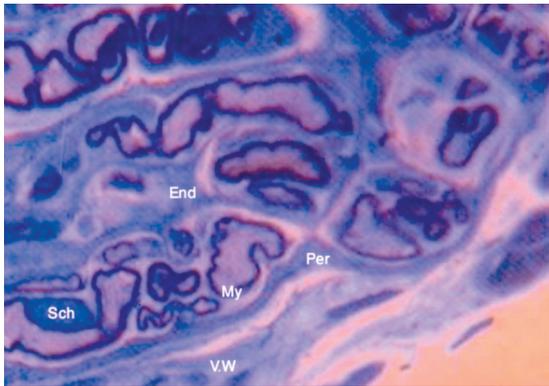


Fig. (5-A): Light microscopy, demonstrating nearly normal nerve trunk. Nerve fibers are normally arranged with dense myelin (My), surrounded with normal prineurium (Per), venous wall (VW) is seen outside nerve trunk (Toludine blue x1000).

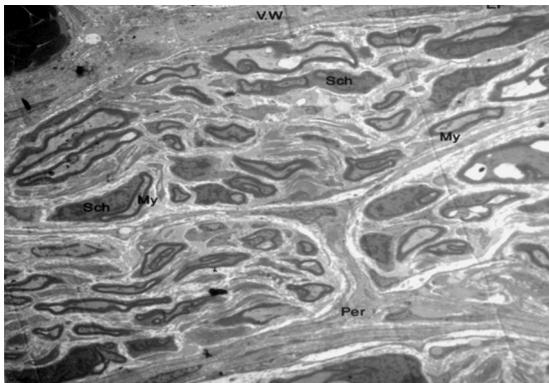


Fig. (5-B): EM examination, there are well formed normally arranged nerve fibers, increased regenerated axons, Schwann cells are plenty (TEM x2000).



Fig. (5-C): EM examination, normal myelinated neve axons with little fibrous tissue (TEM x15000).

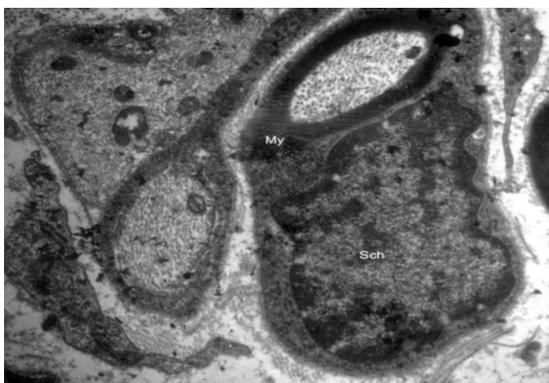


Fig. (5-D): Schwan cells are normal and surrounding myelinated axons (TEM x15000).

Fig. (6): Epineurial flap group III.

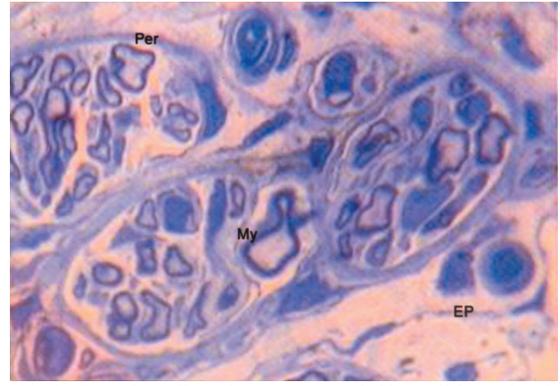


Fig. (6-A): Normal myelinated axons surrounded by well formed perineurium. Epineurium is present outermost (Toludine blue x1000).

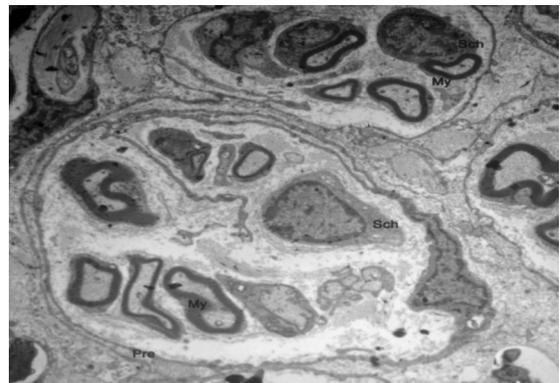


Fig. (6-B): EM examination demonstrating normally arranged, well formed nerve axons, surrounded by normal perineurium with little fibrous tissues in between. Schwann cells are plenty and surrounding myelinated axons (TEM x4000).



Fig. (6-C): Higher magnification show well formed dense myelin, presence of regenerated axons. Perineurium and epineurium are normal (TEM x15000).

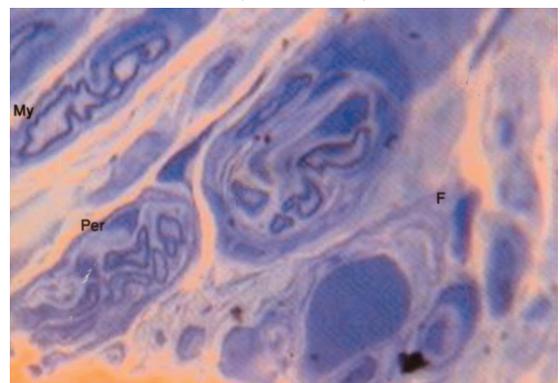


Fig. (6-D): Picture of classic neuroma in the same group. Nerve fibers are scattered. Axonal escape outside the perineurium, with increase in the amount of fibrous tissue (Toludine blue x1000).

RESULTS

The terminal portion of all proximal nerve segments in control group, showed evidence of neuroma formation with distal bulbous swelling embedded in the surrounding soft tissue. Fascicles had caused adhesions and penetration into the surrounding tissue. Axons growth and migration ranged from 8-32mm.

Light microscopy, revealed typical features of amputation neuroma, that demonstrated an overgrowth of disorganized nerve fibers, Schwann's cells, and fibroblasts. High share of connective tissue and low rate of myelinated axons were seen. Regenerating axons extend into adipose tissue or near by skeletal muscle.

Electron microscope examination showed splitting in myelin lamellae and presence of myelin debris with little regenerated myelinated axons. Schwann cells were distorted in shape. There was no any myofibroblasts, but increase in fibroblasts and fibrous tissue formation.

In group II where nerve stumps were implanted into venous lumen. The terminal portion of all proximal sciatic nerve segments demonstrated no evidence of neuroma formation. There were no growths of regenerating fibers into surrounding tissues. No connection or adhesion with other tissue was observed. Non of the stumps showed any axons escaping outside it $p < 0.001$.

Histological analysis of nerve stump ends by light microscopy showed parallel organization of axons. Fascicles were arranged and oriented in the same direction. Intact epineurium, it was in continuity with the venous wall. There was high rate of myelinated axons and low rate of connective and scar tissue compared to control group.

Electron microscope examination revealed that the myelin sheaths were formed of several dark lamellae which appeared very close to each others. Schwann cells were seen enclosing myelinated axons. There were regenerated myelinated axons and little myelin debris were also seen.

In rats whose proximal nerve stump were capped with epineural flaps (group III). Grossly the ends of nerve stump were larger than normal but less than control group. Axons escaped outside the suture line. This happened in 7 rats. Axons sprouting ranged from 6 to 26mm. This may be explained by growth of fascicles between knots through suture lines.

By light microscopy: The specimens had no typical characters of neuromas. There were less scarry and connective tissue. Nerve fibres were organized in areas and disorganized and proliferate behind the epineural layer in other areas. Myelinated axons are apparent, plenty and well formed in many of specimens.

By electron microscopy: Some specimens had a picture of neuroma, especially those with axonal escape outside the suture line. In the other specimens, axons were arranged in regular manner like in a normal nerve. Schwann cells were seen surrounding myelinated axons. Over-proliferated nerve elements were limited behind the intact epineural layer.

DISCUSSION

A neuroma is any neoplasm derived from cells of the nervous system. The formation of a neuroma is the physiological consequence of nerve injury. Regardless of the type of primary care given, some degree of neuroma formation occurs. All methods of treatment attempt to modify the environment in which the neuroma forms [19]. Numerous treatment modalities have been advocated to prevent or treat neuroma formation [4-15]. There is no procedure that is completely and consistently successful in preventing neuroma formation, so any severed nerve will form a neuroma pathologically [8].

Sunderland demonstrated that normal epineurium and perineurium prevent the lateral outgrowth of regenerating axons. He concluded that the proper control of terminal neuroma formation lay in the isolation of neural tissues from surrounding connective tissues by intact epineurium [3].

In this study we compared the effectiveness of two barrier techniques used to prevent neuroma formation. The first one is the use of vein graft to completely cover the cut nerve endings. In the second technique epineural flaps are sutured to cover the exposed fascicles.

Our study revealed considerable difference between treatment and control sides regarding terminal neuroma size and histopathological findings. The neuromas on the control sides were larger in size. Histologically they contained more scar tissues and fewer myelinated axons. In group II in which nerve endings were transposed into vein grafts, no neuromas were found. Histological findings showed significantly more myelinated axons which were arranged in groups and Schwann cells were plenty, with less scar and connective tissues.

The fundamental work by Chiu and colleagues on interpositional vein grafts for nerve defects revealed more rapid and more organized axonal growth along the inner surface of the vein wall [20]. Other studies found that controlled nerve regeneration occurred in a variety of animals using vascular conduits [21]. These studies allowed the supposition of an interaction between regenerating axons and endothelium.

Relocation of a nerve stump into a vein has two effects; the axons emerging from the stump are prevented from growing into scarred environment and the vein lumen is a milieu devoid of neurotrophic factors which play an important role in the development of painful neuroma [22]. This may explain decrease neuroma formation by implantation of nerve endings into muscle. This is due to lack of neurotrophic factors inside the muscle [10,22]. Our results were similar to theirs, and the mechanism of neuroma prevention may be similar to that in the previous study.

Low and associates support our findings. They applied a vein cap to a single cut nerve and noted that the regenerating fibers remains reasonably well organized without the usual adhesions to the vein or surrounding tissues [23]. Other experimental studies were conducted on the prevention of stump neuroma by its transposition into venous lumen. Veins were excluded from blood stream and tied off distally. Koch et al., found considerable differences between control and treatment sides regarding growth and histopathological findings. These results allow the assumptions that there was an interaction between regenerating axons and endothelium [4].

The work reported here was based upon the theory that neuroma formation could be prevented if the cut nerve endings were implanted within lumen of veins. The fascicles fail to grow out of venous graft cap $p < 0.001$. Obviously axonal and Schwann cell regeneration occurred but were limited behind the venous graft barrier.

Vein grafts were preferable to other artificial covers. Lundborg has been successfully using silicon tubes to treat painful neuromas, based on that an open protective tube was the key to successful neuroma prevention. Later on he proved that there was irritation from silicon tubes. He stated that veins may be preferable in a clinical situation, as there has been no irritation associated with their use [24].

Based on these findings, results of the present study and other encouraging clinical results [25,26],

this technique can be used in clinical cases to prevent neuroma formation. Herbert and Filan, in their series of 14 patients treated over 5 years, 13 patients described dramatic pain relief after surgical resection of neuroma and fixation of nerve endings into venous lumen [25].

In group III where the proximal nerve stumps were covered with epineural flaps. There was a considerably high amount of neuroma formation although it was not as much as control group. Capping the nerve end by closure of epineural flaps were done by several authors [6,7,8]. Muehleman and Rahimi used this method, it was appeared that the epineural flaps completely sealed of the proximal stump of rat sciatic nerves. However large number of neuroma was formed [7]. Really epineural flaps sutured in the midline cannot close off the epineurium completely. Small gaps, between sutures of the flaps are large enough for regenerating fascicles to grow through and form neuroma. Axonal escape outside nerve stump was obvious in this group than group II as shown in Table (1), $p < 0.05$. This can explain relatively large number of neuroma in this group when compared to group II.

To overcome axonal escape and relatively large rate of neuroma formation with epineural flaps, several studies were done on use of free epineural grafting. The proximal stump can be covered completely and suture being placed onto lateral walls of the nerve trunk. Thus, fascicles failed to grow out of the epineural barrier. Histologically, axonal and schwann cell regeneration occurred but were limited behind the epineural graft [6,8]. These results may be similar to the result of this article in group II where we used venous graft to cover the proximal nerve stump. Vein grafts are much more better due to its plenty presence and easy dissection without any harm to donor sites.

Our research supports the safe, effective and easier use of venous graft to cover the nerve stump as method of preventing neuroma formation in the rat model. This hopefully provide some scientific basis on which to build a more rational management of neuroma by reducing axonal regeneration. This suggests and encourage clinical trials to be done to achieve best outcome for treatment of painful neuroma.

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