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The effect of omental wrapping on nerve graft regeneration

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SUMMARY. **We have performed 1 cm long nerve grafts in the sciatic nerves of 60 Wistar rats. In 30 cases (n = 30) the grafts were wrapped in omentum. We have studied the nerve regeneration accomplished after 30 and 90 postoperative days from the histological and neurophysiological points of views. Survival of the omentum has been excellent. The histological assessment showed a higher blood vessel population as well as a higher count of axons in the cases where the nerve graft was wrapped in omentum. Areas with neural fibrosis, which are a sign of poor vascularisation, were smaller in the cases with omentum. The amplitude of the contraction in the gastrocnemius muscle was higher in the cases where omentum was used. The endothelial cells of the omentum synthesise fibroblast growth factors (acid and basic) which, due to their angiogenic and neuronotrophic properties, may be the cause of the beneficial effects of omentum on peripheral nerve regeneration.**

Serious traumatic injuries of peripheral nerves usually occur in the context of open injuries associated with tissue loss and fracture. This often causes extensive nerve defects. Treatment for these injuries requires nerve grafts to facilitate reconstruction of the defect and direct the growth and regeneration of axonal sproutings.[1]

One of the most important factors in the biology of nerve grafts is their revascularisation. It is crucial for the survival of the graft and for the degree of axonal regeneration finally achieved.[2]

Omentum has a high angiogenic potential.[3] Numerous surgical techniques have been described using the omentum as a revascularising agent in brain,[4] myocardium[5] and bone.[6,7]

In this study we have evaluated the potential use of omental wrapping of nerve grafts to facilitate nerve regeneration.

Materials and methods

Sixty female Wistar rats weighing between 240 and 300 g were deeply anaesthetised with pentobarbital (Nembutal 35 mg/kg) administered intraperitoneally. In all cases (n = 60), the left-side sciatic nerve was exposed using a muscle-splitting incision and a 6.5 mm segment of the nerve was resected. The resulting gap was repaired with a 10 mm nerve graft taken from the contralateral sciatic nerve, and repaired with 10-0 epineural suture. The operation was performed under a Zeiss operating microscope.

In 30 animals a piece of free omentum from the abdomen of the animal was wrapped completely around the nerve graft, including the proximal and distal segments of the recipient sciatic nerve (Fig. 1). The omentum was secured to the contiguous musculature by 3 8-0 sutures (Fig. 2). All animals were housed in a temperature controlled room, caged separately and given food and water *ad libitum*.

The group of animals with nerve grafts without omentum (n = 30) was assessed at postoperative day 30 (n = 15) and at postoperative day 90 (n = 15). The omental group was similarly evaluated.

Electromyographic recordings

This study was performed on all animals evaluated at the 90th day assessment. The study was carried out with the animal under anaesthesia using pentobarbital. The sciatic nerve was exposed just distal to the sciatic notch and stimulated supramaximally using a TE 42 electromyograph (TECA Corporation, New York, USA). The recording electrode was placed in the gastrocnemius muscle. Distal motor latencies and amplitude were measured.

Histologic and morphometric studies

Histomorphological studies were performed on all animals. The animals were deeply anaesthetised and, through the aorta, they were perfused with 500 ml of heparinised saline solution followed by a fixative containing 2% formaldehyde with a sodium phosphate buffer (pH 7.3). The specimens were carefully dissected with the operating microscope and placed in a fixative solution for 24 h. Transverse 3 μm-thick sections of the medial zone of the nerve graft were stained with Masson's tri-chrome method.

The morphometric evaluation was carried out using a semi-automatic system of image process and analysis IBAS I (Kontron instruments) with a digitising pad and pen linked to an IBM PC AT computer. Using a point-counting grid, the percentage of regenerated myelinated axons, as well as the percentage of blood

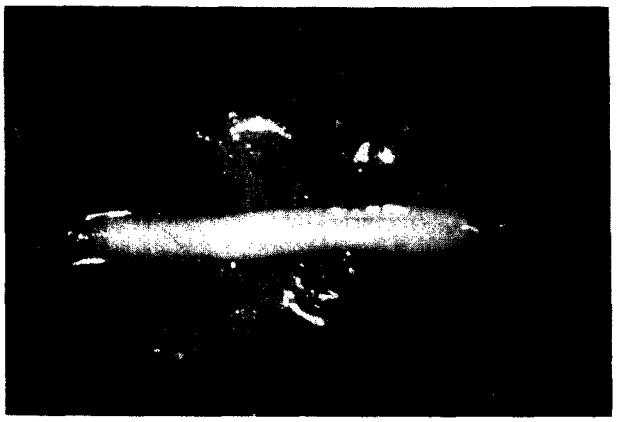

Fig. 1

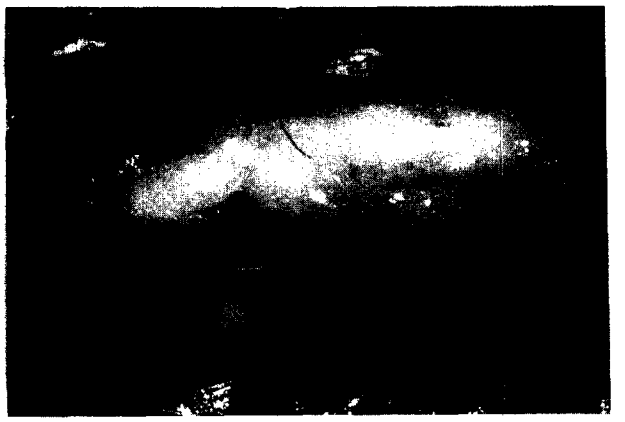

Fig. 2

Figure 1—The omentum placed in the surgical field. **Figure 2**—Final appearance of the omentum completely wrapping both the nerve graft and the proximal and distal segments of the recipient's sciatic nerve. The omentum stays anchored to the adjacent musculature by sutures.

vessels, was estimated carrying out the count in 4 randomised sections magnified by 1000 X. In addition, total nerve graft area and endoneural and epineural fibrosis were assessed.

Statistical assessment

Results were compared using a paired Student's t test, and a non parametric test (Mann-Whitney U) for variables with no normal distribution. A p value of less than 0.05 was considered statistically significant.

Table 1 Neurophysiologic study: significantly higher electromyographic amplitude in the group of animals with nerve graft wrapped in omentum ($p < 0.01$). No significant difference was detected in the distal motor latency between groups ($p > 0.05$).

Group	*N*	*Latency*	*Amplitude*
without omentum	15	mean: 1.93 ms. SD: 0.22	mean: 9.88 mV SD: 2.75
with omentum	15	mean: 1.78 ms. SD: 0.18	mean: 12.96 mV SD: 4.57

Results

Electromyographic study

There was no significant difference between the distal motor latencies for all the animals studied. The amplitude values were significantly greater in the omental wrap group ($p < 0.01$) (Table 1).

Histologic study

Omentum survival was measured when the specimens were taken for histological assessment. In 26 cases (86.5%) omental volume and appearance was judged to be normal. In 4 cases some reduction in the quantity of omental tissue was observed.

In the control nerve graft group, at 30 days only the occasional nerve fibre was identified. Wallerian degeneration was still present. In the grafts wrapped in omentum, the omentum was noted immediately adjacent to the epineurium (Fig. 3). A large number of omental blood vessels at the epineural junction were noted. Omental wrapped grafts demonstrated higher neural area and higher vascular area than the control nerve graft group ($p < 0.001$) (Table 2).

Fig. 3

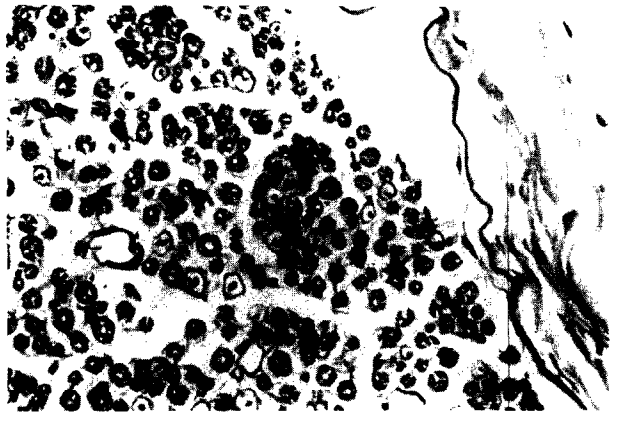

Fig. 4

Figure 3—Masson's trichrome-stained transverse sections X75 of nerve grafts with omentum. Notice the omental adherence to the epineurium and the significant vascularisation. Arrows show omental blood vessels. **Figure 4**—Regenerated myelinated axons in a nerve graft wrapped in omentum (Trichrome stained X400).

Table 2 Percentage of axons and blood vessels in the nerve grafts after 30 days: significantly higher percentage of regenerated myelinated axons and blood vessels in the group of nerve grafts wrapped in omentum ($p < 0.001$).

Group	*N*	*Axons%*	*Vessels%*
without omentum	15	mean: 18.46 SD: 7.96	mean: 5.87 SD: 1.54
with omentum	15	mean: 31.40 SD: 3.93	mean: 11.58 SD: 3.8

Table 3 Percentage of axons and blood vessels in the nerve grafts after 90 days: significantly higher percentage of regenerated myelinated axons and blood vessels in the group of nerve grafts wrapped in omentum ($p < 0.001$).

Group	*N*	*Axons%*	*Vessels%*
without omentum	15	mean: 34.55 SD: 6.02	mean: 6.61 SD: 1.24
with omentum	15	mean: 51.50 SD: 6.63	mean: 10.11 SD: 1.46

Table 4 Epineural fibrosis area and the percentage it represents out of the total area of the nerve graft: the area of epineural fibrosis was significantly smaller in the cases of nerve grafts wrapped in omentum ($p < 0.01$).

Group	*N*	*Epineural fibrosis* mm^2	*% total graft area*
without omentum	15	mean: 0.20 SD: 0.06	mean: 27.4 SD: 9.7
with omentum	15	mean: 0.11 SD: 0.04	mean: 23.1 SD: 4.5

Table 5 Endoneural fibrosis area and the percentage it represents out of the total area of the nerve graft section: the endoneural fibrosis was significantly smaller in the cases of nerve grafts wrapped in omentum ($p < 0.001$).

Group	*N*	*Endoneural fibrosis* mm^2	*% total graft area*
without omentum	15	mean: 0.141 SD: 0.04	mean: 23.1 SD: 7.8
with omentum	15	mean: 0.081 SD: 0.027	mean: 13.6 SD: 3.1

The degree of axonal regeneration was greater at the 90th postoperative day. Well myelinated nerve fibres with an organised distribution were apparent. In the omental wrapped group the neural and vascular areas were greater than in the control nerve graft group ($p < 0.001$) (Table 3, Fig. 4).

Fibrosis tissue (both extraepineurial and central endoneural tissue) was greater in the control group than in the omental wrap group ($p < 0.01$ and $p < 0.001$) (Tables 4–5).

Discussion

Although the systematic use of grafts has resulted in improved results following traumatic injuries of peripheral nerves, these techniques unfortunately continue to give less than optimum results.[89] Many factors influence results, including the type of injury, axonal misdirection and perhaps compromised revascularisation with subsequent fibrosis.[10]

Early revascularisation is critical in nerve graft survival. The angiogenic process has its origin in vessels of the nerve stumps and surrounding tissues that anastomose with vessels in the graft.[2,10] Early revascularisation of the graft is an important factor in determining the efficiency of axonal regeneration.[11–13]

Injection of omental extract into an ischaemic area increases vascular perfusion.[14] Bikfalvi[15] has recently demonstrated that the endothelial cells of the omentum synthesise basic fibroblast growth factor (bFGF). bFGF is a member of a family of protein fibroblast growth factors (FGFs) which stimulate the growth and differentiation of a variety of cells.[16] FGFs induce chemotactic and mitogenic activity in vascular cells and stimulate angiogenesis *in vitro* and *in vivo*.[17] Bikfalvi has demonstrated that omental endothelial cells can develop a mitogenic activity that is inhibited by a polyclonal antibody (antiFGF). His results suggest that omental angiogenic activity could be the consequence of the FGF-like mitogenic activity developed by its endothelial cells.

FGFs also have significant neurotrophic potential.[18] The neurotrophic effects become apparent both in protection from the neuronal damage provoked by the axotomy, and in the stimulation of axonal and neurite growth.[19] In the field of peripheral nerve regeneration, the acidic FGF (aFGF) has been shown to enhance nerve regeneration when added to regenerating nerve stumps.[20] aFGF has also been purified from omentum.[21]

In our work, free omental grafts have shown a high survival rate and have enhanced revascularisation of the nerve graft. The higher vascular perfusion may be responsible for less fibrosis, and support axonal regeneration. These effects may also be related to the angiogenic and neurotrophic action of the FGFs existing in the omentum.

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