REGENERATION OF THE SCIATIC NERVE IN RATS

THE EFFECT OF MUSCLE BASEMENT MEMBRANE

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An orientated substratum has been implicated in the development and regeneration of axons and synapses. We prepared a basement membrane matrix from autogenous striated muscle, used it to repair the sciatic nerve in rats, then investigated the results by histology and electrophysiology.

When treated grafts were coaxially aligned with the nerve fascicles functional recovery appeared within 30 days, with good growth of axons into the distal nerve. Grafts with myotubes at right angles to the nerve fascicles supported nerve regeneration but at a slower rate. Grafts of coaxially aligned but untreated muscle allowed axon penetration only through naturally degenerated muscle fibres, with minimal axon penetration of the distal nerve.

It is concluded that in the rat a treated graft with correctly orientated empty myotubes can facilitate and guide the regeneration of peripheral nerve after injury and so lead to recolonisation of the distal stump with functional recovery.

After injury to peripheral nerve, with a gap between the cut ends, a neuroma is formed at the proximal stump and a glioma at the distal stump (Young 1942). Should these meet in the gap between the cut ends, a potential pathway for nerve regeneration is created (Abercrombie and Johnson 1942; Kline, Hayes and Morse 1964). However, in the absence of surgical treatment, the chances of this are remote and the probability that the conduit will be of sufficient diameter to support functional repair is low (Blackwood and Holmes 1954). To facilitate repair in those cases where direct reapproximation is impossible, nerve grafts are used to bridge the gap (Bunnell 1927; Sanders 1942; Sanders and Young 1942). Such grafts require the sacrifice of a functioning nerve and can only be obtained from an unimportant and small nerve such as the sural nerve in the calf. This restricts the diameter of the donor autograft and the size of its contained fibres, giving problems when a large-diameter nerve needs repair.

Regeneration of fibres into distal nerve after neurotmesis and insertion of a graft is thought to be guided by endoneurial tubes of Schwann cell basement membrane, and by surrounding connective tissue (Holmes and Young 1942; Abercrombie and Johnson 1942; Thomas 1963, 1964, 1966), the Schwann cells having been derived from either the graft, or the proximal or distal stump (Salzer and Bunge 1980). In addition to diffusible growth-producing factors (Lundborg et al. 1982), interest has been directed at possible surface influences upon nerve growth and development (Letourneau 1975; Sanes, Marshall and McMahan 1978). The latter authors have demonstrated a synaptogenic effect of specific areas of the basal lamina of frog muscle when placed in contact with regenerating nerve. It has also been shown that implants of degenerating muscle can be colonised by and can support the growth of peripheral nerve in mice (Keynes, Hopkins and Huang 1984).

Our experiments with rats extend the preliminary study of Keynes et al. in order to investigate whether such growth of regenerating nerve into muscle basement membrane can lead to recolonisation of distal nerve, and eventually to the recovery of function. The extent of axon growth into the graft was studied histologically and electrophysiological observations were used to determine whether the graft and the distal stump had regained the ability to conduct an impulse. Some of our results have been reported in more detail elsewhere (Glasby et al. 1986a, b).

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METHODS

Sprague-Dawley rats of average weight 300 g were anaesthetised with intramuscular Hypnorm (Janssen Pharmaceuticals, UK) (0.5 ml/kg) and intraperitoneal diazepam (2.5 mg/kg). One gluteus maximus muscle was exposed and freed at its iliac attachment. A block was removed

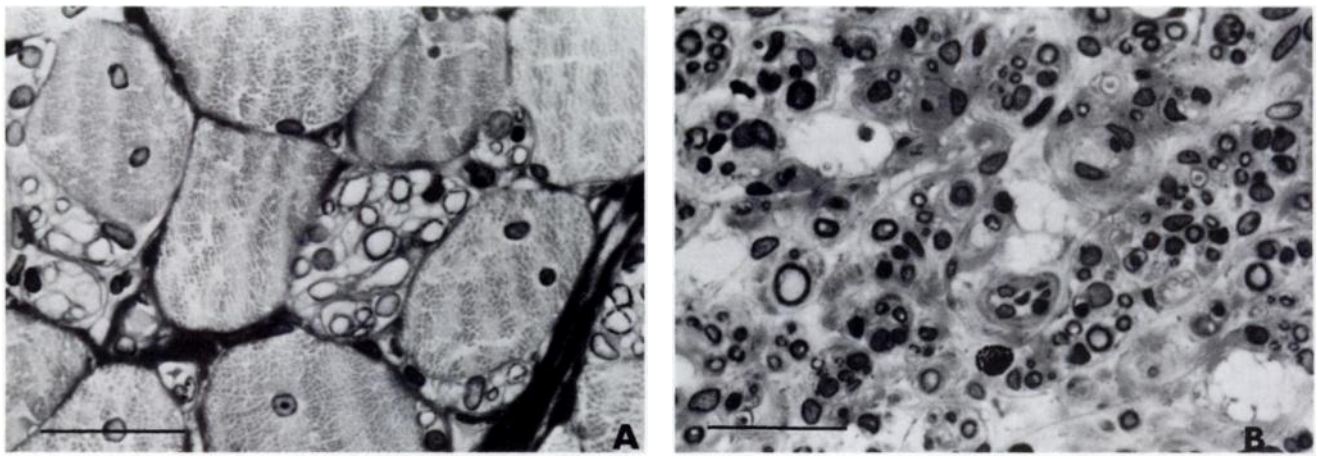

Fig. 1

Transverse sections made 51 days after grafting with untreated muscle. *A*, graft; *B*, distal nerve (scale bars 10 μ).

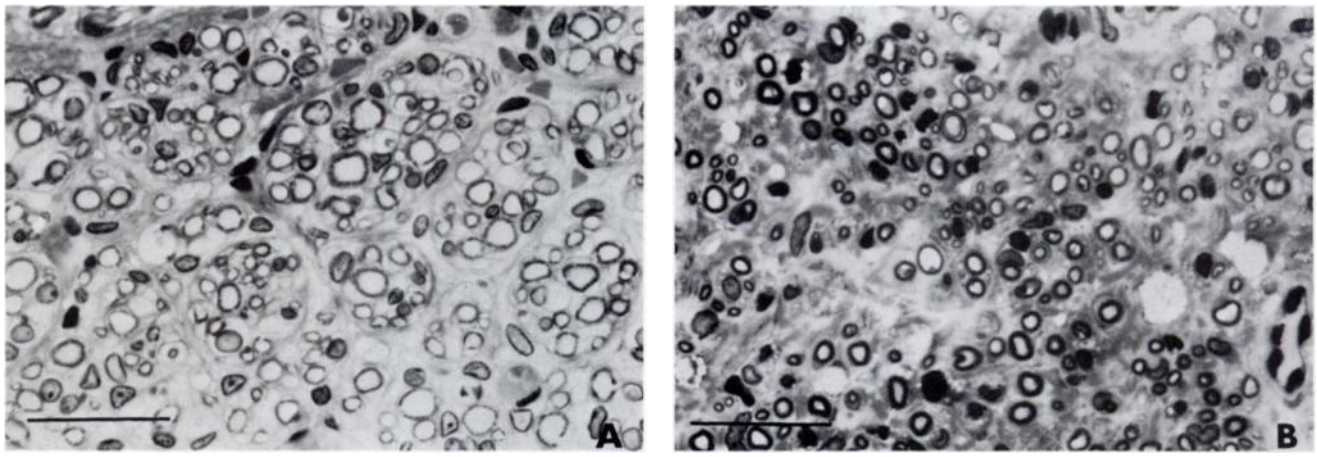

Fig. 2

Transverse sections made 51 days after coaxial grafting with treated muscle. *A*, graft; *B*, distal nerve (scale bars 10 μ).

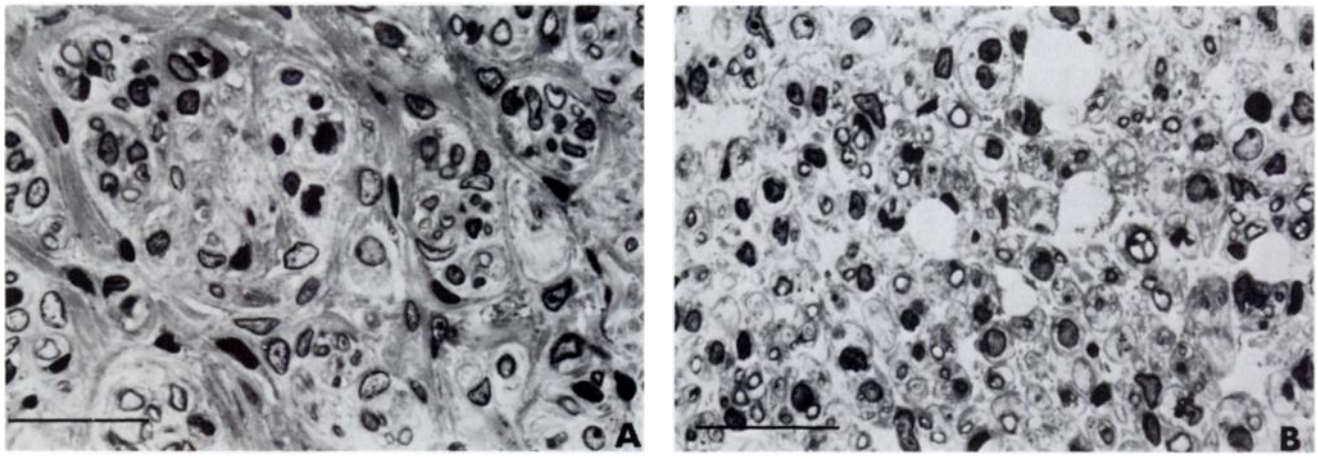

Fig. 3

Transverse sections made 51 days after grafting with treated muscle aligned at 90 to nerve fascicles. *A*, graft; *B*, distal nerve (scale bars 10 μ).

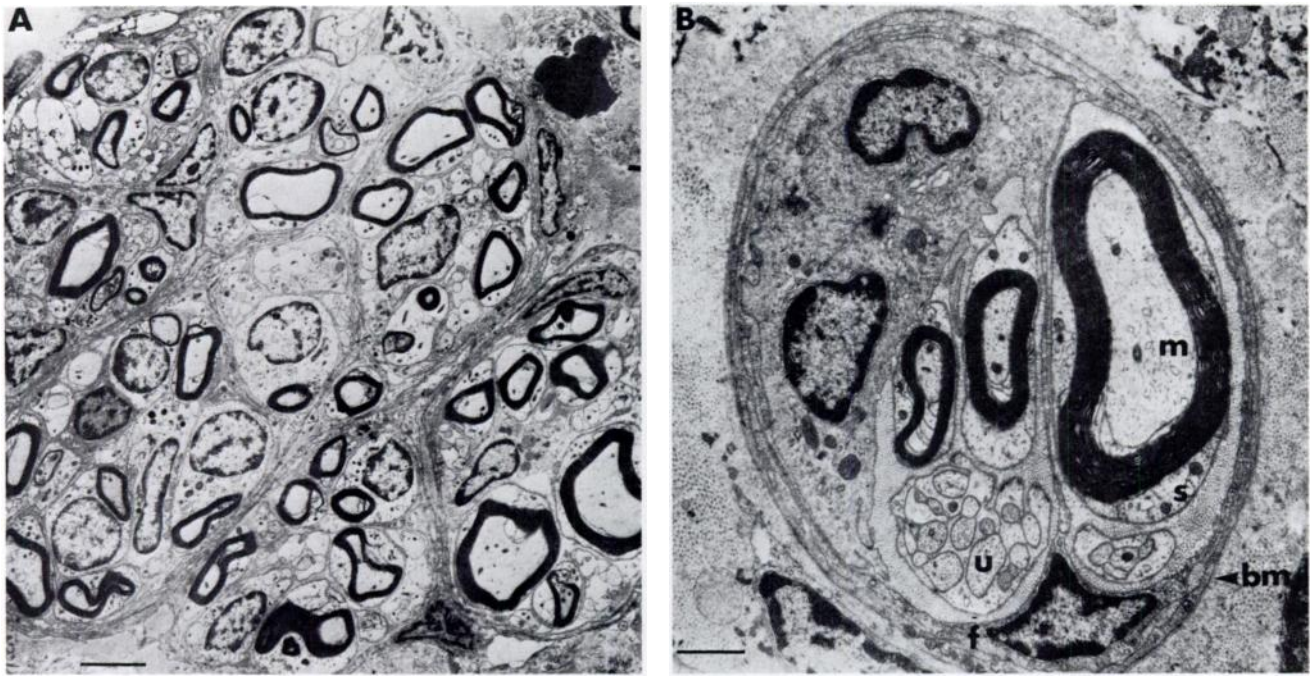

Fig. 4

Electron micrographs of coaxially-aligned treated grafts. *A*, myelinated and non-myelinated fibres can be seen penetrating the graft (scale bar 4.32 μ). *B*, at higher magnification, a single basement membrane tube (*bm*) is seen to contain fibroblasts (*f*), Schwann cells (*s*), myelinated axons (*m*) and unmyelinated axons (*u*). Note that the axons are surrounded by Schwann cell basement membrane tubes orientated concentrically within the muscle basement membrane tube (scale bar 1.0 μ).

from the entire thickness of the muscle, pinned out under tension on Sylgard (Dow Chemical Co.), and immersed in liquid nitrogen to thermal equilibrium. It was then thawed in distilled water to promote osmotic disruption. The ipsilateral sciatic nerve was mobilised with the minimum of disturbance to surrounding tissues and a 10 mm segment of nerve excised from 5 mm distal to the sciatic notch. A 10 mm length of treated muscle, of a diameter similar to that of the sciatic nerve, was prepared and interposed in the nerve gap. The graft was sutured in position with six interrupted 10/0 Prolene (Ethicon, UK) stitches joining the epineurium of the nerve to the periphery of the graft, leaving no exposed axoplasm. Deep tissues were closed with 4/0 catgut and the skin with 5/0 Prolene.

At intervals thereafter the degree of regeneration was assessed. The skin incision was reopened and the nerve mobilised from the sciatic notch to the knee. Stimulating and recording electrodes (Harvard Biosciences, UK) were applied to the nerve on either side of the graft, which was isolated from surrounding tissue by a sheet of dielectric, and used to assess the propagation of extracellular compound action potentials in both directions across the graft. These were displayed on a Model 502 cathode-ray oscilloscope (Tektronix; Oregon, USA). Nerve and graft were then removed, marking proximal and distal ends. They were fixed in cacodylate-buffered 4% gluteraldehyde, post-fixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Araldite. Transverse sections were cut on the ultramicrotome at 1 μ, stained with toluidine blue, and mounted in DPX for light microscopy. Ultra-thin sections cut from the same blocks were stained with lead citrate and uranyl acetate and viewed on an AEI Corinth 275 transmission electron microscope (Associated Electrical Industries, UK). Axon counts of whole diameter axons were made from light microscope slices using a counting graticule.

RESULTS

Our control experiments employed untreated devascularised muscle autografts. In this group, nerve stimulation 51 days after operation, even with high stimulus intensities, did not elicit propagated action potentials across the graft. This correlated, in all five rats of the group, with the histological appearances of sparse axon penetration of the graft and distal nerve with, in the latter, a considerable preponderance of degenerating axons (Fig. 1). Transverse sections of the graft still showed whole muscle fibres, and those few axons present were in groups apparently contained within the residual basement membrane of naturally degenerated muscle fibres (Fig. 1A), since the diameter of such groups of axons approximated that of a muscle fibre.

Interposing treated grafts gave contrasting results. Their treatment with liquid nitrogen and thawing appeared to produce disruption of the muscle architecture with preservation of the basement membrane tubes (see Fig. 5). When the myotubes had been placed

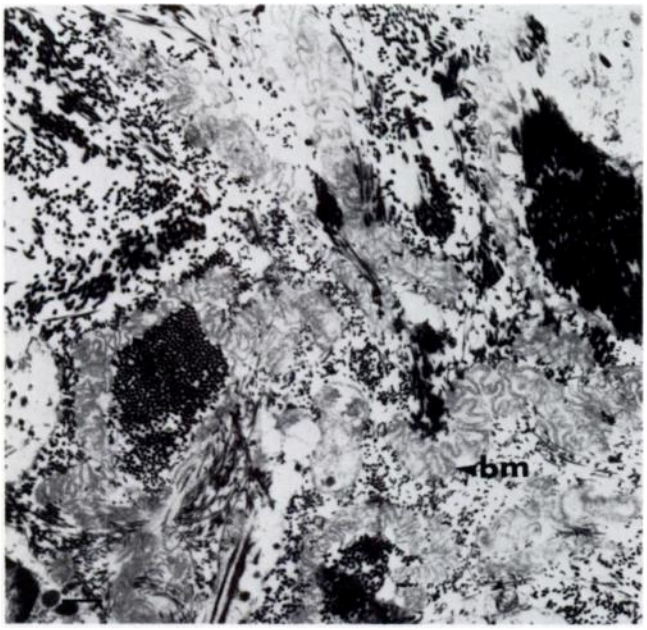

Fig. 5

Electron micrograph of muscle treated by freezing and thawing. Collagen fibres from the perimysium and basement membrane tubes are seen among the cellular debris (scale bar 1.0 μ).

coaxially with the nerve fascicles, electrical conduction was initially lost, but in all five rats in this group conducted action potentials in response to stimulation reappeared between 21 and 30 days. At 30 days, normal electromyograms could be elicited in the ipsilateral flexor hallucis longus. Transverse sections of the grafts at 51 days showed little intact muscle but apparently intact basement membrane tubes. Large numbers of axons were arranged in circular groups within the basement membranes (Figs 2A, 4A and 4B), while axons outside such groups were rare. In transverse sections of distal nerve, there was a high density of healthy axons (Fig. 2B) with few apparently degenerating. These findings suggest that available, appropriately-orientated basement membrane tubes facilitate penetration of the graft and the distal nerve stump by regenerating axons; this result again correlated well with the electrophysiological findings.

In the third group of experiments, the treated graft was placed so that its fibres lay at right angles to the nerve fascicles. No action potentials were propagated across the graft at 51 days in any of the five rats so examined. Transverse sections showed large numbers of myelinated axons within the graft but there were few healthy axons in the distal stump (Fig. 3).

Table I gives the absolute numbers of axons found in the grafts and distal segments of nerve in each group. About twice the number of axons were seen in coaxially-aligned grafts than in aligned fresh muscle grafts or treated muscle aligned at right angles to the nerves. In the distal segments of the nerves, about three times as many axons were seen after growth through a coaxially aligned treated graft as in the other groups after the same period of time.

Table I. Number of myelinated axons after 51 days in rat sciatic nerves grafted with different muscle grafts; there were five nerves in each group

	Number of axons (mean ± s.e. of mean)		
Type of graft	**Proximal nerve**	**Graft**	**Distal stump**
Fresh muscle	5034 ± 126 (all types)	1879 ± 101	642 ± 80
Treated muscle			
Coaxial orientation		4061 ± 482	1886 ± 220
Right-angled orientation		2283 ± 32	637 ± 125

DISCUSSION

Our results suggest that the availability of appropriately-orientated empty basement membrane tubes both facilitates and guides the growth of regenerating nerve in a manner that leads to repopulation of the distal stump with subsequent recovery of function. In both treated and untreated coaxially-aligned grafts, the orientation of the basement membrane tubes was appropriate for growth but only in the degenerating muscle were the inner surfaces of these tubes accessible to the regenerating axons. In the degenerated grafts aligned at right angles, the basement membrane tubes were orientated wrongly and their inner surface could not be gained without penetration by the axon. Over the period of the experiments, up to 51 days, the coaxially-aligned degenerated grafts proved to be the best means of bridging the gap, so that at 51 days, the number of axons in the graft approached that in the proximal nerve. The number of fibres in the distal segment still lagged well behind but was significantly greater than in the other groups.

It is reasonable to suppose that with time there would be continued growth of axons into the distal stump; work in progress in our laboratory has shown that this is the case. Whether the facilitatory effects of treated grafts are due to their physical properties, such as the availability of suitably-orientated tubes for the ingress of Schwann cells or some diffusible growth factor, or to their chemical properties (perhaps some molecule situated on the inside of the basement membrane tube) remains to be investigated. Nevertheless, our results suggest that there may be specific clinical uses for such grafts in situations where direct repair of a peripheral nerve is impossible and where the damaged nerve is too large to be repaired usefully with a conventional nerve graft. Work at progress in our laboratory has shown that treated coaxially-aligned muscle grafts can give successful repairs of primate radial nerves over six-month periods; this will be reported in a later paper.

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