Nerve Regeneration Through Fresh Skeletal Muscle Graft-An Experimental Study in Rabbits

TAN CHONG TIEN A.M., M.D., M.B.B.S., F.R.C.S.*, PONNAMPALAM BALASUBRAMANIAM, M.D, F.R.C.S.,** AND PONNAMPALAM GOPALAKRISHNAKONE, M.D., M.B.B.S., Ph.D.***

ABSTRACT : Two centimetre gaps in rabbit sciatic nerves were coaxially bridged with fresh skeletal muscle grafts and they were re-explored at two weekly intervals for three months. The grafts were examined under the light and electron microscope. Regenerating axons were found to grow through the muscle graft to reach the distal nerve segment by the end of two weeks and form nerve bundles by four weeks. Electrophysiological studies showed conduction of nerve stimulus across the graft by one month. Most of the regenerating axons were found to lie outside the muscle basement membranes. This is in contrast to the findings of others who reported that most of the regenerating axons were found to lie within the empty muscle basement membrane tubes of per-degenerated muscle grafts. Though there were morphological and physiological evidence of nerve regeneration in the experimental group of animals, the clinical examination at the end of three months failed to show clinical evidence of motor or sensory recovery in the affected lower limb. This may be due to fibrosis in the muscle graft.

INTRODUCTION

Injury to peripheral nerve resulting in a large nerve gap has remained a difficult problem. Traditionally autogenous nerve graft is used to bridge the gap. The source of autogenous nerve graft is limited and sacrifice of a donor nerve produces however minor, some functional deficit. Hence, the idea of using an easily available alternative graft material is very attractive.

Several types of graft have been used experimentally to bridge nerve gaps[1,2]. The recent use of skeletal muscle graft to bridge nerve gaps seems to be promising experimentally[3,4] as well as clinically[4]. Norris, Glassby Gattuso and Bowden[5] in 1988 published their results of grafting digital nerve defects with pre-degenerated skeletal muscle grafts in eight patients. Their results compared favourably with those of direct nerve repair or the use of autogenous nerve graft.

Morever,the concept of using basal lamina tubes of skeletal muscle as a graft requires that the muscle graft should be treated first to degenerate its fibres. This can be achieved by incubation of the graft in chemicals or freezing and thawing them in liquid nitrogen. Both methods impose practical difficulties in clinical practice. Since firstly, very few operating theatres have easy access to liquid nitrogen. Secondly, the treatment of the skeletal muscle graft with liquid nitrogen makes it extremely friable. Thirdly, the treatment with a series of chemicals requires many hours of preparation. If untreated fresh skeletal muscle graft could be shown to be as effective as pre-degenerated graft its use as a graft material to bridge nerve gaps, this will be of tremendous advantage. The aim of this study is therfore, to investigate the effectiveness of fresh muscle as a graft conduit for nerve regeneration to bridge nerve gaps.

MATERIALS AND METHODS

New Zealand white rabbits were used for this experiment. In the experimental group, a 2 cm length of left sciatic nerve was resected and the gap was bridged with an equal length of fresh skeletal muscle graft harvested from hamstring muscles. The animals were periodically examined for evidence of sensory and motor recovery of the paralysed foot. The grafts were re-explored at intervals of 1 week, 2 weeks, 1 month, 2 months and 3 months. There were six rebbits in each group of the experimental animals. At the time of re-exploration, the electrophysiological studies of the grafted nerves were done. Besides, the stimulating electrode was placed over the proximal stump and the recording electrode was placed over the distal stump. The proximal nerve stump was stimulated and any impluse that crossed the graft to reach the distal nerve segment was recorded. The graft were then excised together with 2 cm length of the proximal nerve and 2 cm of the distal nerve segment. The proximal and distal nerve segments were excised in continuity with the muscle graft.

* Department of Orthopaedic Surgery, Singapore General Hospital, Singapore

** Department of Orthopaedic Surgery, National University of Singapore, Singapore

*** Department of Anatomy, National University of Singapore, Singapore

Longitudinal and transverse sections of the proximal junction, muscle graft, the distal junction and the distal nerve segment were examined under the light and electron microscope.

There were 18 rabbits in the control group. Two centimetre of the sciatic nerve was excised as in the experimental group but the gap was left free. Microscopic and physiological studies of these nerves were done as in the experimental group of animals.

RESULTS

Control group

In all control animals, there were no regenerating nerve fibres found to grow across the nerve gap even at the end of 3 months and the proximal and distal stumps developed into neuromas. In addition, electrical stimulation of the sciatic nerve of the control group also failed to conduct any impulse across the nerve gap even at the end of 3 months.

Experimental group

By the end of one week (Fig 1), the tremendous proliferation of regenerating axons were seen in the proximal nerve stump and the regenerating nerve fibres were seen to grow into the muscle graft. At the end of two weeks, some of the regenerating axons reached the distal nerve stumps but most of the fibres were still within the muscle grafts (Fig 2). Morever, well defined nerve bundles of the regenerating axons were seen in the distal nerve segment by the end of one month (Fig 3). At the end of 2 and 3 months, the distal nerve segments showed nerve bundles intermingled with remnants of skeletal muscle fibres and fibrous tissue.

The Electron microscopic studies confirmed the presence of large number of regenerating nerve fibres within the muscle graft. However, in contrast to other studies in which pre-treated skeletal muscles were used, most of the regenerating axons in our study were found to be outside the basal membrane tubes of the muscle (Fig 4). Some axons, however,were found to be within the basement membrane. The electrical stimulation of the experimental nerves showed conduction of nerve impulse across the graft by the end of 4 weeks. And the clinical examination of the experimental group of rabbits failed to show sensory or motor recovery in the lower limb.

Figure 1 : One week old nerve-muscle coaxial graft showing proliferation of regenerating axons entering the nerve-muscle junction. Haematoxylin Eosin X100.

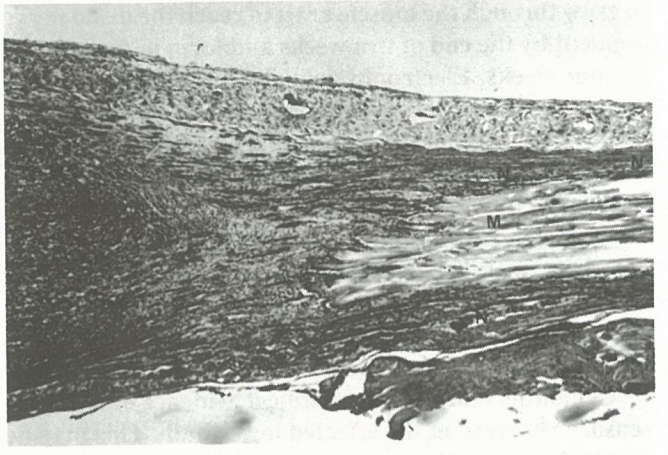
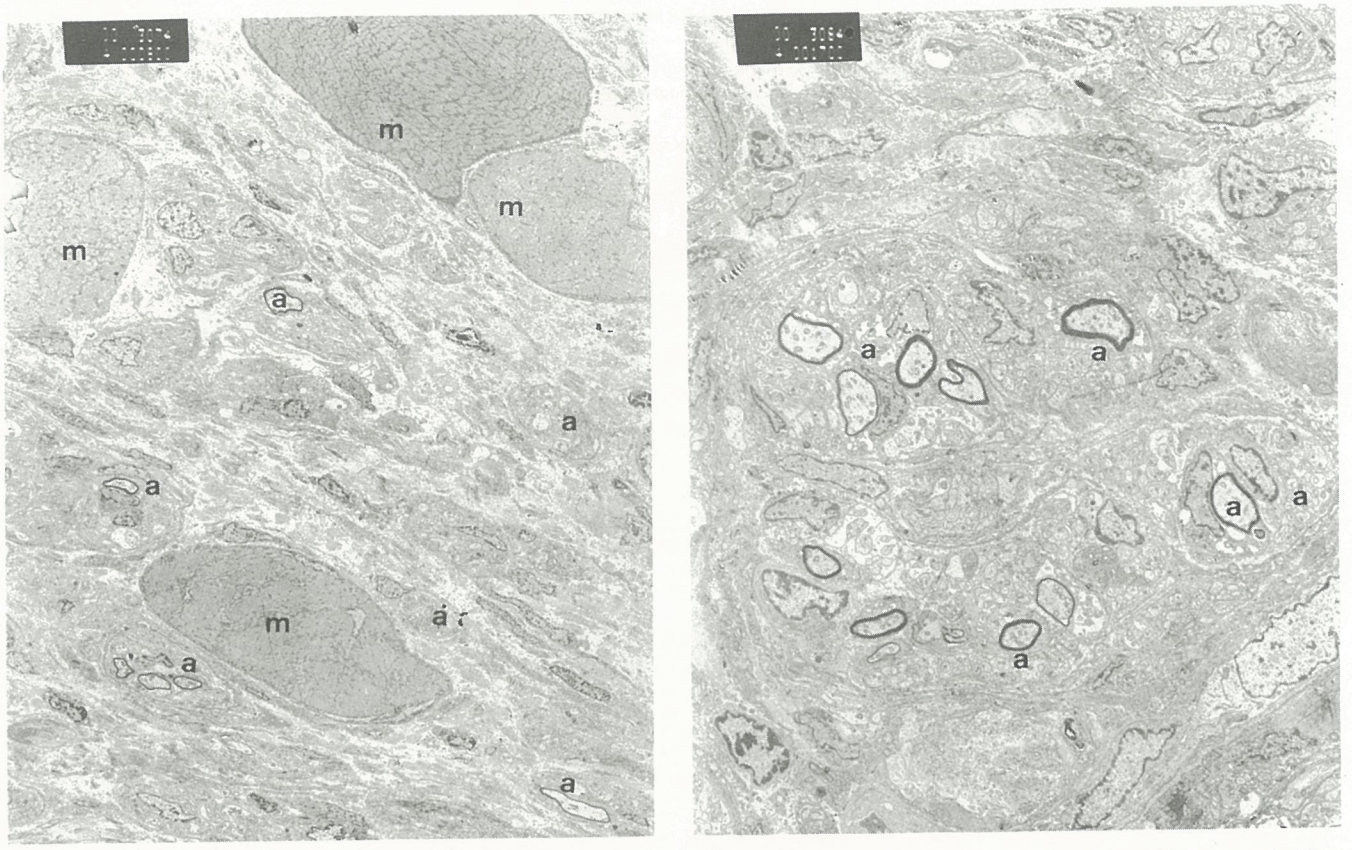


Figure 2 : Two weeks after nerve-muscle coaxial grafting. Regerating axons have reached this graft and formed bundles (N) along the periphery. Remnants of muscle (M) seen with axons within them. Haematoxylin Eosin X100.

Figure 3 : Two months old muscle grafts replaced by nerve bundles. Haematoxylin Eosin X100.

DISCUSSION

Ide et al[6] were the first to suggest the use of skeletal muscle as a possible graft material for bridging nerve gap. In their study on nerve regeneration, they observed that it was the basal lamina tubes of the Schwann cells which were responsible for guiding the regenerating axons through the nerve graft. They suggested that the basal lamina tubes of skeletal muscle may have capability of similar function and confirmed this later in their experimental rat model. Glassby et al[4] study



Figre 4a and 4b : Two electron micrographs are showing the regenerating axons along the surface of muscle cells of the graft at the end of four weeks.
Remark : M = muscle A = axons

of the effect of muscle basement membrane on the regeneration of sciatic nere in rats found that when muscle grafts immersed in liquid nitrogen and then thawed in distilled water to promote osmotic disruption were coaxially aligned with nerve fascicles functional recovery appeared within 30 days with good growth of axons into the distal nerves. They also found that coaxially aligned grafts of untreated muscle allowed axon penetration only through naturally degenerated muscle fibres with minimal axon penetration of the distal nerve. However, our study has shown that coaxially aligned fresh muscle grafts with nerve fascicles allowed regenerating axons to grow through it to reach the distal nerve segment to form nerve bundles and conduct nerve impulses across it. Besides, most of the regenerating nerve fibres in our study were found to lie on the outer surface of the muscle basement membrane. This raises the question of the exact role of the basement membrane of the muscle graft in nerve regeneration. Do the empty muscle basement membrane provide a conduit or just a surface to guide the regenerating axons into the distal segment[6]? We feel that so long as there is a suitable surface it could guide regenerating nerve fibres to the distal segment. It may be the surface of the muscle basement membrane inner or outer that guides the growing axon rather than its shape as an empty tube to serve as a conduit for the regenerating axons to reach the distal nerve segment.

The presence of undisruptive muscle fibres in our study probably caused fibrosis and prevented functional recovery though the regenerating axons formed nerve bundles in the distal segment and conducted electrical impulse across the graft. The predegeneration of the muscle graft by liquid nitrogen or chemicals to empty them of the muscle cytoplasm and preserve their basement membrane probably prevents fibrosis of the regenerating axons along the muscle basement membranes. We feel that as long as there is a suitable surface for the regenerating axons, they will grow along them to reach the distal nerve stump in adequate numbers to form nerve bundles. The fibrosis of the regenerating axons must be minimised or prevented for a functional recovery after nerve injury.

CONCLUSIONS

1. The coaxially aligned fresh muscle graft bridging large nerve gaps in rabbits allow regenerating axons to reach the distal nerve segment to form nerve bundles by the end of one month.

2. The electrical stimulation of the nerve grafted with fresh muscle show conduction of nerve impulse across it at the end of one month.

3. The fibrosis of the regenerating axons prevents clinical recovery of motor and sensory function.

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