

Comparison of Nerve Graft and Artificial Conduits for Bridging Nerve Defects

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Summary

A study of nerve regeneration through a 1cm defect in the peroneal component of the sciatic nerve was performed on sixteen rabbits. Either silicone or polytetrafluoroethylene (PTFE) tubes or nerve graft were used to bridge the defect and the opposite limb was not operated upon. The rabbits that underwent nerve grafting had favourable findings. In the PTFE group, a nerve-like structure was seen at the former gap site and histology confirmed viable axons within the tubes and distal to the repair site. In the silicone tube group, there were no myelinated axons demonstrated. The axonal count for the grafted nerves and the nerves repaired with PTFE tube are on average 80.4% and 38.2% of that of the unoperated nerve, respectively. On average, the percentage anterior compartment muscle weight (expressed as a percentage of the unoperated limb) for the silicone, PTFE and nerve graft groups are 42.3%, 42.1%, and 72.7% respectively.

The results show that although, PTFE conduits can bridge a nerve defect of 1cm, nerve grafting provides a superior and more predictable outcome.

Key Words: Nerve defect, Nerve graft, Artificial conduit, Nerve regeneration

Introduction

Direct suture of the ends of a nerve in a chronic nerve injury is neither always possible nor desirable. Either as a result of nerve stump retraction, actual nervous tissue loss or resection of damaged nerve ends, nerve gaps are produced. The ideal environment for nerve regeneration is a tension free repair. Connective tissue proliferation and scarring between nerve ends are directly related to tension of the suture site. This is detrimental to axonal regrowth.

Extensive mobilisation of the nerve ends or extreme positioning of the extremity in an attempt to perform a tension-free direct repair are now well known to be detrimental to nerve regeneration^{1,2,3,4}. Closure of a significant nerve gap requires it to be bridged. This is a well-accepted principle, however the actual

magnitude which constitutes a significant nerve gap is controversial^{5,6,7}.

At present, traditional interfascicular nerve grafts still remain the best option to bridge nerve defects. Several researchers have tried to find new conduits able to lead the regenerating nerve. Three major reasons fuel the research into developing new conduits: 1) the need for large amounts of grafts 2) avoidance of the time and morbidity of nerve harvesting and 3) the possibility that chemotactic mechanisms acting within conduits may solve the problem of correct topographic orientation of nerve fibers. This process in which the distal stump of the repaired nerve releases factors which guide the regenerating axons into its appropriate neural sleeve is termed neurotropism^{8,9}. The rationale of utilising a conduit is that in addition to acting as a guide for nerve regeneration it is thought that the tube will protect the

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repair from scarring¹⁰ and contain substances that enhance nerve regeneration at the repair site¹¹.

It has been shown in a number of studies in humans and animals that nerves will regenerate across a nerve gap through various biological and prosthetic tubes^{12,13-23}. The aim of the present study is to evaluate the results of silicone and polytetrafluoroethylene conduits in bridging nerve gaps and comparing it with nerve grafts.

Materials and Methods

Experiments were conducted on sixteen rabbits between the age of 7 to 9 weeks. Ethical approval was given from the Subcommittee on the Use and Care of Animals, Faculty of Medicine, University of Malaya. Each rabbit had a 1-cm gap created in the right common peroneal component of the sciatic nerve.

The whole sciatic nerve was not used as it was considered to leave the animal too disabled during the period of nerve recovery and in addition it may automutilate its hind leg due to complete sensory loss of its foot. The tibial component, the larger nerve of the two was not selected, as the animal will have neuropathic ulcer formation on the sole of the feet as the animal is housed in a cage with grill wire flooring.

Either silicone or polytetrafluoroethylene (PTFE) tubes or nerve graft were used to bridge the defect and the other limb was not operated upon. Silicone tubes were obtained from the Faculty of Mechanical Engineering, University of Malaya. PTFE tubes are 18 gauge human intravenous cannulas manufactured by B-Braun@(Meisungen AG). The inner diameter of the tube was about 1-2 times the size of the nerve to avoid compression of the nerve with postoperative oedema and expected nerve growth. One rabbit underwent a sham operation in which the right common peroneal nerve was exposed then closed. Four rabbits each underwent repair by silicone and PTFE tubes respectively. Seven rabbits had nerve grafting. After a period of thirteen to fifteen weeks, the animal was sacrificed and nerve regeneration assessed.

Surgical Procedure

Rabbits were anaesthetised using intravenous sodium pentobarbital. General anaesthesia was supplemented with 0.5% lignocaine injected subcutaneously at the operative site. A single prophylactic dose of ampicillin (50mg) was given intravenously before induction.

Under aseptic conditions, the sciatic nerve was exposed through an incision on the posterior aspect of the right thigh. The common peroneal component was separated from the tibial component of the sciatic nerve using sharp dissection starting distally and progressing proximally with careful attention to the fine epineurial vessels supplying the nerve. The two components can be seen as two distinct nerve bundles with loose intervening perineurium, which then divide roughly 1cm above the level of the knee joint.

The right peroneal component of the sciatic nerve would then have prosthetic tube repair or nerve graft repair. The prosthetic tube was spliced longitudinally, the nerve was secured to the wall of the tube at both ends with sutures 10/0 nylon placed through the epineurium. A 1cm-nerve defect was created within the tube with sharp division with a microscissor. In this manner, the tension within the nerve would be the same as that for the nerve graft repair. The tube was filled with saline and secured closed with sutures 10/0 nylon.

For the nerve graft repair, excising 1cm of the common peroneal nerve created a nerve defect of 1cm. The excised nerve was reversed and used as a nerve graft. The nerve graft was secured without tension with epineurial sutures 10/0 nylon.

All procedures performed on the nerve were aided by the use of a microscope and standard microsurgical instruments. Postoperatively, the limb was not splinted. The rabbits were housed alone and unrestrained in their respective cages.

After a period of between thirteen and fifteen weeks the animal was sacrificed with an overdose of sodium pentobarbital. The extent to which nerve regeneration has occurred was quantified by histological sections of the repaired nerve and the unoperated nerve from the opposite leg taken immediately after the animal was sacrificed. Sections were obtained from the nerve bilaterally just proximal to the fibula head. The anterior compartment muscle of both legs were removed and weighed.

Histological examination

The nerve was fixed in 4% glutaraldehyde for 1 day. Following that the specimen was post fixed in osmium tetroxide. It was gradually dehydrated with increasing concentrations of alcohol then infiltrated with propylene oxide and embedded in resin. A semithin

section of 1 micrometre was taken and the section was stained with toluidine blue. This technique stains the myelin of the axon. Photographs of the histological section under high power magnification were taken and this was assembled to form a photocollage allowing manual axonal counting to be performed.

Results

There was no wound infection, auto-mutilation or ulcer formation in the experimental rabbits. Rabbits that survived the anaesthesia and initial operation remained healthy and there were no late deaths. Two rabbits died during administration of anaesthesia.

Findings at reoperation(sacrifice)

All the seven rabbits that underwent nerve grafting had good results. There was no hair loss in the sensory distribution of the common peroneal nerve and there were no contractures of the ankle. On exposure of the repaired nerve, there was minimal scarring of the graft to the underlying bed. The nerve had no neuroma formation at the repair site and the distal nerve was of satisfactory caliber. On transection of the nerve for histological staining, the foot was seen to flicker with visible dorsiflexion at the ankle.

In contrast, the four rabbits with silicone tube repair had poor results. The ankles had a fixed plantarflexion deformity when the knee was extended indicating contracture of the dorsiflexors. Three of the four rabbits had marked hair loss on the dorsum of the foot. The tube was encompassed within fibrous tissue but relatively free from the underlying muscular bed. On transection of the nerve to remove the tube (and distal nerve specimen) there was no visible dorsiflexion of the foot. The distal nerve stump had remained within the tube lumen and there was anatomical continuity of the proximal and distal stumps by a string of tissue. In addition, in two of the rabbits a small amount of fluid was present within the tube.

In the PTFE tube group of four rabbits, the results were better than that of the silicone tube group. Fixed plantarflexion deformity when the knee was extended (indicating contracture of the dorsiflexors) was not seen in all the rabbits. However, as regards to hair loss on the dorsum of the foot, one had normal hair growth, two had mild hair loss and one had marked hair loss. The amount of hair loss did not correlate with axonal count and muscle weight. On exposure of the repair site there was hardly any scarring at the repair site. In

contrast to the silicone tube group, in all the PTFE tube rabbits, a continuous nerve-like structure was seen at the former gap site and there were no signs of the former lesion (see Figure 1). The tube remained anchored to the nerve and there was some bulging of the nerve proximal to the tube in two of the rabbits.

Muscle weight

Two rabbits that were not operated upon due to immediate anaesthetic death had the anterior compartment muscles of the left and right legs removed for weighing. In the first rabbit the weight was 2.25g for the left limb and 2.30g for the other limb. In the other rabbit the weight was 2.26g and 2.17g respectively. Therefore, the weight of both anterior compartment muscles in an unoperated rabbit was comparable. Expressed as a percentage of the heavier muscle the values for these two rabbits are 97.8% and 96.0% respectively.

The rabbit that underwent the sham operation had very similar muscle bulk and the weight of the lighter muscle was 91.6% that of the contralateral muscle. The individual percentage values for all the rabbits are given in Table I. On average the percentage muscle weight for the silicone, PTFE and nerve graft groups are 42.3%, 42.1%, and 72.7% respectively.

Histology

Histological examination of a normal common peroneal nerve (the unoperated limb i.e. left) revealed a densely packed nerve with axons of varying size and myelin sheath thickness. The axons were numerous, crowded together with sparse intercellular connective tissue or cells. The axons showed characteristic grouping with larger axons travelling together. The architecture of the connective tissue elements was well defined and organised. Blood vessels were not seen in the interior but predominate in the epineurium. (Figure 2A.)

In contrast, the appearance of the grafted nerve was distinctly different. The axons were less densely packed together and more disorganised. The axonal diameter was generally smaller and there was absence of the larger axons. Intervening tissue was more fibrous and cellular with the presence of blood vessels within the nerve interior. These changes are consistent with a reinnervated nerve. (Figure 2B.)

In keeping with the observational findings and the muscle weight, the nerves repaired with the prosthetic

tubes demonstrate inferior results to that of the grafted nerve.

In the silicone tube group of rabbits, there were no myelinated axons in the distal stump and the epineurium is markedly thickened. Sections of the distal stump showed similar features to section taken of the tissue within the tube with cellular infiltration and fibrous tissue within the nerve and the absence of axons.

In contrast, histology of the peroneal nerve distal to the tube in the PTFE group of rabbits demonstrates viable axons. The nerve had axonal size and density comparable to nerve grafting but in general the amount of intercellular tissue was greater than that of the nerve-grafting group. (Figure 2C.). With regards to histological examination, although all the rabbits in the PTFE tube group had viable axons in the distal segment

of the nerve, two rabbits had better axonal density with less non-axonal tissue as compared to the remaining two rabbits. This was reflected in the differing axonal counts. The histological appearance of nerves within the nerve-grafting group was more consistent.

Histological section of tissue within the PTFE tube demonstrated viable axons. Clearly the axons reached the distal stump by growing within the tube rather than on the outside or around the tube.

The axonal counts are given in Table II. The quality of the histological section for Rabbit 6 did not allow for axonal counting. On average the axonal count for the grafted nerves and the nerve repaired with PTFE tube were 80.4% and 38.2% of that of the unoperated nerve. (There were no axons to count in the nerve repaired by silicone tubes).

Table I: Weight of leg muscles (anterior compartment) at time of sacrifice

GROUP	RABBIT	MUSCLE WEIGHT (GRAM)		PERCENTAGE WEIGHT (%)	MEAN PERCENTAGE	STANDARD DEVIATION
		Left leg (L)	Right leg (R)	$\frac{R}{L} \times 100$		
UNOPERATED	Rabbit A	2.26	2.17			
	Rabbit B	2.25	2.30			
SHAM OPERATION	Rabbit 1	5.14	4.71	91.6		
SILICONE TUBE	Rabbit 2	3.70	1.65	44.6	42.3	3.59
	Rabbit 3	3.51	1.30	37.0		
	Rabbit 4	4.32	1.85	42.8		
	Rabbit 5	4.55	2.03	44.6		
NERVE GRAFT	Rabbit 6	3.91	2.63	67.3	72.7	5.94
	Rabbit 7	5.12	3.27	63.9		
	Rabbit 8	5.16	4.15	80.4		
	Rabbit 9	5.24	4.10	78.2		
	Rabbit 10	4.92	3.60	73.2		
	Rabbit 11	5.14	3.61	70.2		
	Rabbit 12	5.04	3.80	75.4		
PTFE TUBE	Rabbit 13	5.80	2.59	44.7	42.1	5.12
	Rabbit 14	6.16	2.12	34.4		
	Rabbit 15	6.36	2.80	44.0		
	Rabbit 16	5.14	2.32	45.1		

Table II: Axon counts of the common peroneal nerve distal to the repair site

GROUP	RABBIT	AXON COUNT		PERCENTAGE COUNT (%)	MEAN	STANDARD DEVIATION
		Left leg (L)	Right leg (R)	$\frac{R}{L} \times 100$		
SHAM OPERATION	Rabbit 1	3576	3300			
NERVE GRAFT	Rabbit 6	2100	Unable (see text)		80.4	10.3
	Rabbit 7	3160	2582	81.7		
	Rabbit 8	2627	2442	93.0		
	Rabbit 9	3389	3057	90.2		
	Rabbit 10	3808	2561	67.3		
	Rabbit 11	3475	2765	79.6		
	Rabbit 12	3729	2629	70.5		
PTFE TUBE	Rabbit 13	4350	698	16.0	38.2	18.8
	Rabbit 14	2463	1258	51.1		
	Rabbit 15	2700	1520	56.3		
	Rabbit 16	2451	720	29.4		

Discussion

Tubes have been used in nerve surgery for decades^{18,20}. The advocates for its use with regard to nerve injury have proposed beneficial modes of action. 1) Cuffing of the nerve to reduce adherence of the nerve to a scarred bed; 2) Wrapping of a nerve repair to prevent axonal loss, obstruct ingrowth of connective tissue into the neurorrhaphy and to capitalise on neurotropism 3) entubulation of nerve stumps to create a conduit for regeneration across a nerve gap.

The use of two different types of tube was to investigate if the intrinsic property of the tube itself would cause a degree of neural degeneration or inhibition of growth. The literature does provide differing results for different types and structure of tubes but the conclusions are mostly anecdotal comparing differing series of experiments with the inherent differences in the choice of nerve and animal species. Some authors^{12,17} prefer bioabsorbable conduits to silicone claiming that the silicone itself may not be conducive for nerve regeneration or that by virtue of its non-permeability causes anoxia within the tube. Lundborg utilises silicone tubes with good results in patients with nerve defects of 5mm^{13,14}. Silicone being non-absorbable is thought to induce less scarring as the process of breakdown of an absorbable conduit involves acute inflammatory cells. However, it is also known that the property of silicone to elicit a fibrous

response is depended upon to create a sheath through which tendon grafts are implanted²⁴. Chronic nerve compression has also been reported in three patients with the use of non-absorbable conduits²⁵.

Muscle weighing was incorporated in to the methodology of this study as a means of quantifying effective axon regeneration and specificity of nerve regeneration. The success of nerve regeneration is ultimately dependent on the reinnervation of the end organ. It is not sufficient for x number of axons to grow through the nerve graft or conduit but the correct axon has to enter its corresponding endoneurial tube in the distal stump for effective nerve regeneration.

Surprisingly, the silicone tubes performed so poorly in this experimental study that at the time of sacrifice there were no axons to count in histological sections taken from the distal nerve end. Similarly, not only did the axons fail to cross the conduit, histological examination of tissue within the silicone conduit suggests that nerve growth arrest had occurred. Muscle weight, the absence of muscular action on transection of the nerve and the observation of hair loss and contractures provided further confirmatory evidence for the failure of the silicone conduits in this study. In the polytetrafluoroethylene (PTFE) group, axonal regeneration across the gap was demonstrated, however relative muscle weight in the PTFE tube group was similar to that of the silicone tubes.

Other authors using nerve conduits report consistently good results in animals with small nerve gaps^{12,14,16,19,23}. It is difficult to postulate the reasons why the prosthetic conduit performed poorly in this study contrasting with the results of published works. This study by design truly matches nerve graft with conduits, as all steps in the surgical technique and the postoperative environment are identical in both groups. Tension in the repair is also identical, as there is no actual nerve defect in the nerve graft group and in the prosthetic tube groups the nerve segment is removed from the tube after the nerve is anchored to the tube on both ends.

The nerve gap length in this study is modest. There is no consensus on the maximum length at which a nerve can pass through a conduit. In the primate model¹⁷ using bioabsorbable tubes, distances of 3cm were successfully crossed. Similarly in humans using vein^{26,27} or bioabsorbable conduits²⁸, satisfactory functional result occurred in bridging a 3cm defect in digital nerves.

The introduction of prosthetic conduits in humans should be viewed with caution. Presently, until there is more conclusive evidence, it is best limited to distal

nerves in the setting of an adjunct to primary repair of a nerve that has sustained a sharp division or at most a small gap. This situation however is amenable to direct repair so more prospective comparative studies are needed. An attractive technical and theoretical advantage of the conduit is that accurate fascicular matching may not be necessary by virtue of neurotropism. As there can be no histological evidence or direct electrical measurement of nerve regeneration, human studies rely on determining motor and sensory recovery. Careful assessment of these criteria over a prolonged period is required to diminish inherent inaccuracies.

At present nerve grafts are still the best option to bridge nerve defects. Whatever the mechanism, conduits have been shown to be able to lead the regenerating nerve and assist in the topographic orientation of nerve fibers^{8,9}. At best compared to nerve grafts the distance that can be bridged by a conduit is small. Equally many have opposed the idea of using nerve conduits. Sir Sydney Sunderland's cautionary comments in 1978 may still hold true; 'The use of nonbiologic material is of no value in the bridging of gaps in nerves and may even introduce new complications in the form of infection, foreign body reaction and more extensive fibrosis'²⁹.

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