Review Article

Indian Journal of Neurotrauma (IJNT)
2008, Vol. 5, No. 1, pp. 15-20

Entubulation techniques in peripheral nerve repair

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Abstract: Peripheral nerve injuries are common, and there is no easily available formula for successful treatment. Although primary neurorrhaphy and nerve autografts are the most effective methods of repair, several newer options are at our disposal today. Though one can help speed up the nerve regeneration process to some extent, success is hindered by additional issues such as number of coaptation sites, supply of donor nerves and the limitations of nerve substitutes. There is now considerable evidence that peripheral nerves have the potential to regenerate if an appropriate microenvironment is provided. A better understanding of the biological processes involved in nerve regeneration process and the realization that nerve grafts serve as a guide for the growing neurons led to the concept of entubulation techniques. For distances of less than 3 cms, either a nerve conduit or an autologous vein graft serves equally well as nerve graft. Seeding the conduits with cultured Schwann cells has pushed the limit of nerve regeneration through a 6 cm gap. In experimental studies with Schwann cell lined bioengineered conduits gaps as large as 8cms can be bridged. Advances in bioengineering has allowed creation of composite neural tubes lined with Schwann cells and neurotropic agents that enhances regeneration of nerve fibers, block the invasion of scar tissue and autodegrade when it is no longer required .The evolution of the concept of entubulation, the early experimentation, the present development and various types of conduits are discussed here.

Keywords: nerve gap, nerve conduits, entubulation

INTRODUCTION

Nerve repair is still a frontier in the art of peripheral nerve surgeries. Peripheral nerve injuries are common and there is no easily available formula for successful treatment. Researches have shown that neurons themselves have the potency to sprout and grow nerve fibers. If an environment that favours recovery of the injured site is set up quickly, growth of the nerve fiber and formation of synapses can be promoted to restore the nerve circuit. Prompt primary neurorrhaphy by end to end suture coaptation is the procedure of choice whenever possible. But, a percentage of nerve fibers are lost at each coaptation site and out of those neurons that cross the repair, as many as 50% of regenerating sensory or motor neurons may never reach the correct end organ[1]. When presented with a nerve defect that precludes end-to-end coaptation surgeon faces a dilemma: should it be repaired by a nerve graft forcing the axons to cross two coaptations or should it be repaired by extensive mobilization of the nerves and end-to-end repair, or should it be repaired by a nerve conduit. Trumble and McCallister recommend limiting stretching of a peripheral nerve to overcome a gap defect to 10% after which the microvascular flow will reduce by 50%[2]. This jeopardizes both mechanical repair and the health of the nerve. Hence nerve autograft still remains the gold standard for bridging nerve gaps. Peripheral nerve repair using nerve autograft has several shortcomings including donor site morbidity, inadequate return of function and aberrant regeneration. When extensive nerve grafts became a necessity allografts and vascularised nerve grafts were introduced. Allografts act as temporary scaffolds across which host axons regenerate but there is need for prolonged immunosuppression using agents like FK 506[3]. For bridging long nerve gaps (more than 20cms) the current recommendation is free vascularised nerve grafts[4,5]. But, the number of dispensable nerves suitable as donor nerves in the human body is limited. This has resulted in development of new techiniques for bridging nerve gaps. Various nerve guidance channels are being developed as alternatives to nerve autografts. This technology, which is commonly called "entubulation repair" has several theoretical advantages over nerve autografting, but the results still are not satisfactory for repairing nerve defects longer than 3 cms. Considering the importance of the cellular component in nerve

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regeneration, the development of artificial grafts, composed of biocompatible nerve guide[6] filled with a neurotropic matrix and seeded with Schwann cells[7] (SCs) has been developed. It is also feasible to manipulate conduit architecture, surface properties, porosity and biodegradability to optimise the regenerative process. This is an interesting option to enhance nerve regeneration and provide an alternative to the classical autologous nerve grafts for bridging longer gaps.

THE EVOLUTION OF ENTUBULATION

The goal of any nerve repair is to direct the regenerating axons successfully into the endoneural tube of the distal end with minimal loss of fibers. With better understanding of the internal topography of the nerve and the biological processes involved in the nerve regeneration process, we now have exciting possibilities that can add a brilliant glow to the ever evolving horizon of peripheral nerve surgeries.

Nerve fibers travel in bundles termed fascicles and they are the smallest unit of nerve fiber that can be manipulated surgically. They do not travel as cables that run parallel throughout the entire length of the nerve. Instead they interconnect resulting in the formation of intraneural plexus. They travel only for a short distance before joining the adjacent fascicle. When an injury results in loss of neural tissue, correct matching of fascicles in proximal and distal stumps becomes practically impossible. So also, there many indeterminate zones with poor localization of fascicle group such as divisions of upper and middle trunks of brachial plexus and the origins of axillary and suprascapular nerves. Hence grouped fascicular repair or epineural repair may not direct the sprouting axons to its native endoneurial tube, rather the daughter neuron is forced to enter a wrong one.

Researches have shown that establishment of a closed environment[8] between the proximal and distal stumps of an injured nerve and given freedom, the young sprouting axons are capable of navigation in a more effective manner to reach their native endoneurial tube. The closed environment between the stumps provides an optimal milieu for axonal growth cones to find their appropriate distal endoneurial pathway. Various autogenous and synthetic conduits have been tried for the entubulation technique with varying results.

To replace the loss of a nerve segment with a tube like structure was first attempted by Gluck[9] utilizing a segment of decalcified bone in 1880. A decade later, Bungner[10] used a segment of cadaveric brachial artery as a tube to bridge a nerve gap of hypoglossal nerve in a dog. Foramitti[11] and Nageotte[12] experimented by using a segment of vessel to repair nerve gap in rodents. The early part of the 20th century saw a resurgence of interest in peripheral nerve injuries due to the multitude of war injuries. Platt[13] in 1920 presented a series of 26 patients who underwent repair of peripheral nerve with nerve gaps using catgut sutures, nerve grafts and vein segments. The grafted region was wrapped around with a tube of tensor fascia lata. The fascial tube was then filled with sterile olive oil. All 26 cases resulted in failure.

The successful application of autogenous sural nerve graft for repair as reported by Sterling Bunnel[14], who revived interest in the entubulation techniques. Weiss in 1943 described the microenvironment within the arterial segments used to bridge nerve gaps[15]. Weiss used the segment of vessel as a coupler rather than a true bridge graft. Parallel to the experiment on entubulation, there was a continuous enhancement of application of autogenous nerve grafts.

The use of non-neural biologic material in bridging nerve gap was sounded like a fantasy in early years but many experimentation and clinical trials proved it to be a reality. Biological conduits made from bone, vein, artery, collagen and muscles were used initially to bridge nerve gaps[16,17]. Veins longer than 3 cms tend to collapse because of their thin walls and absence of pressure from within. To improve regeneration, inside out vein conduits, vein grafts filled with various biodegradable matrix such as muscle (cigarette graft) were also tried. For distances of more than 3cms, nerve conduits or autogenous vein grafts serves equally well as a nerve graft[18].

Cambell and Bassett[19] (1957) conducted a series of studies using Millipore which is a cellulose acetate plastic and that is chemically inert and porous. The porosity allowed free exchange of nutrients while inhibiting in growth of scar tissue. Though nerve regeneration was revealed by histopathological studies 3-4 weeks post-op, the device was found to be calcified and fragmented. Later Chiu[20] et al in 1982 and Rice[21] in 1984 studied the physiological environment provided by autogenous venous nerve conduit (AVNC) and proved that nerve fibers grew across the tube and and restored continuity. Wang later in 1984 observed that the regenerating nerve fiber not only resumed regular normal growth in a proximal to distal orientation, but also passed through the vein graft segment successfully and recaptured the

distal neural motor end plate[22].

The phenomenon of neurotrophism was demonstrated by Lundberg[23] in 1982. The proximal and distal nerve stumps were separated by a mesothelial chamber and it was shown that the fluid that contained within had trophic activity for cultured sensory neurons. It was also shown that distal stump is a source of neurotrophic factors by demonstrating failure of nerve regeneration across a gap when distal stump was absent or was at a distance exceeding10mm from the proximal stump. The regenerating nerve fibres selectively grew down the limb of a "Y" that contained distal nerve stump presumably following a concentration gradient.

Mackinnon[24] and Delton[25] in 1988 compared the efficacy of sural nerve graft and synthetic pseudosynovial tubes in bridging 3 cms ulnar nerve gap and found no statistical difference between the electrophysiological studies of nerve graft and the conduit and it was concluded that entubulation compared favorably with nerve grafting.

The synthetic tubes or conduits for guiding peripheral nerve regeneration are commonly made of materials such as polylactide, polylactide/polyglycolide copolymers, acrylic copolymers, polyvinylidene fluoride, polyglycollic acid[26] mesh, Millipore[27] filter material, silicone, GORE-TEX and preformed mesothelial tubes or various other synthetic polyesters. The shortcomings of using a tube or conduit made of these materials include immune responses, induction of scar tissue, difficulty in application, and development of local elevated concentrations of compounds released after the degradation of a degradable material used in the device. For a tube or conduit made of non-degradable material, a second surgery is often necessary for removal of the tube or conduit.

Biodegradable[28] nerve guides used to bridge nerve defects have been shown to cause relatively less aberrant axonal growth, fibrous scar tissue and neuromas. Masumoto[29] and coworkers in 2000 showed that an artificial nerve conduit made of polyglycollic acid (PGA) collagen tube filled with laminin coated collagen fibers could successfully bridge a nerve gap of 80mm in peroneal nerves of dogs by demonstrating numerous myelinated nerve fibers after a period of 12 months.

Silicone tubes[30] (1-2mm diameter,15 mm length) packed with mixture of Type -1 collagen gel and recombinant Neurocrescin or MDP77 proteins have also been successfully tried. After gelation the tubes are grafted into the nerve defect . After 9-12 weeks nerves bridged the gap to elongate into the distal nerve fascicle. Neurocrescin increased the number of regenerating nerve fibres and MDP77 promoted maturation of the regenerating nerve fibers. Neurolac nerve guide, is a transparent, bioresorbable nerve conduit which can be successfully used for a tensionless nerve repair.

Local delivery of nerve growth factor and local incorporation of hyaluronic acid[31] inside a newly manufactured nerve conduit made from fresh human amniotic membrane[32] has been found to increase axonal regeneration. Human amniotic membrane contains important biochemical factors that play a neurotrophic role in nerve regenerative process and the NGF/HA enhancement promoted axonal regeneration 45% better than the nontreated amniotic tube group. HA, a normal component of intact and regenerating peripheral nerves is a fibroblast derived glycosaminoglycan which is believed to play an important role in wound healing. The early presence of an HA-rich matrix favours the infiltration of migratory cells into injured tissue. HA is involved in the detachment process of the cell cycle that allows cell migration, but also inhibits cell differentiation, thus creating an environment that promotes cell proliferation[33]. The degradation products of HA modulate the inflammatory response and stimulate angiogenesis. Additionally, the prolonged presence of HA may provide the mesenchymal signal for healing by regeneration rather than by scarring and fibrosis.

Magnetically aligned collagen[34] gel rods with preseeded Schwann cells is found to stimulate peripheral nerve regeneration as well as direct the growth cone during neurite elongation in an experimental study. The seeding with Schwann cells accelerates the formation of a bridge giving support to growth cones seeking the distal stump and act as a continuous source of trophic factors Introduction of Schwann cells[35] to the conduit pushed the limit of nerve regeneration through a 6 cms gap in animal studies. Realizing the importance of Schwann cells in the nerve regeneration process, Schwann cells are being cultured to line bioengineered conduits and gaps as large as 8 cms can be bridged successfully[36]. Patients with severed nerves could donate a small portion of the damaged nerve from which Schwann cells could be extracted. While the patients' muscles and tendons are recovering from injury, isolated Schwann cells could be cultured and made to proliferate. These results may

translate into an improved method of entubulation repair of transected peripheral nerves.

Mark I Hobson and Colin J. Green, in an experimental study in rats showed that vascular endothelial growth factor (VEGF)[37], a highly specific endothelial cell mitogen, can enhance vascularisation and, indirectly, axonal regeneration within a silicone nerve regeneration chamber. The study demonstrated that the addition of VEGF significantly increased blood vessel penetration within the chamber from day 5, and by days 10 this correlated with an increase of axonal regeneration and Schwann cell migration. The pattern of increased nerve regeneration due to VEGF administration was maintained up to 180 d, when myelinated axon counts were increased by 78% compared with control. Furthermore the dose-response of blood vessel regeneration to VEGF was clearly reflected in the increase of axonal regrowth and Schwann cell proliferation, indicating the close relationship between regenerating nerves and blood vessels within the chamber.

Madorsky in an animal experiment, studied the effect of entubulation in the regeneration of sensory and motor nerves and showed that sensory neurons regenerated consistently better than motor neurons in the same environment[38]. Therefore, intrinsic differences exist between motor and sensory neuron regeneration in the same nerve.

L Michel La MD in a prospective randomized animal study showed that FK506 (Tacrolimus) applied topically at the time of facial nerve repair using entubulation neurorrhaphy demonstrated superior results in nerve regeneration versus entubulation neurorrhaphy and interposition autograft[39].

ENTUBULATION METHODOLOGY

The proximal and distal nerve stumps of the injured nerves exposed and the neuroma at the proximal end and glioma at the distal end are excised to a level where the nerve appears normal . Both ends are placed into the conduit and fine epineural sutures are placed to fix the nerve inside the conduit. The young regenerating neurons will find their way through the conduit to their native distal endoneureal sheath. Conventional suture coaptations are also wrapped with same to reduce fibroblastic infiltration from the bed between stumps.

Attempts to replace the loss of a cord like nerve segment with a tubular structure was merely a simple attempt to restore mechanical continuity of the nerve trunk. Little consideration was given to the physiological response of injury and the biological sequences of regeneration. Now it has been proved that enclosure of proximal and distal stumps of the injured nerves in a tube leads to accumulation of growth factors, which offers an optimal milieu to facilitate axonal sprouting and regeneration within the conduit. The conduit prevents the loss of axons sprouting from growth cone as well as the Nodes of Ranvier which may be several segments proximal to the stump. The entubulation process prevents the in growth of scar tissue in to the "vital space of regeneration". Regenerating neurons get enough space to navigate to its original Schwann cell tube under the guidance of neurotrophic factors. The presence of conduit also prevents branching, diversion, turning back and termination of growth cones when it comes in contact with scar tissue.

BIOENGINEERING CONDUITS- THE FUTURE

Advances in bioengineering has allowed creation of composite neural tubes lined with schwann cells and neurotropic agents like Nerve Growth Factors(NGF), Glial Cell Derived Growth Factors, Ciliary Growth Factors(CGF) and IL-6 to enhance regeneration of nerve fibres. They block the invasion of scar tissue and autodegrade when it is no longer required. Biological conduits with jetted microspheres that contain nerve growth factors with precise sequencing are the latest developments[40].

One external modality to enhance nerve regeneration is the use of pulsed electromagnetic fields. Although the rate of regeneration is not increased, the number of motor neurons as well as their ability to reach the target organ is significantly improved[41].

CONCLUSION

Although the gold standard for bridging nerve gaps remains nerve autograft, several newer options are there at our disposal. Nerve transfers have revolutionized our approach to nerve gaps from devastating brachial plexus injuries to highly selected sensory nerve injuries. Nerve allografts also may have a promising role in future. The ready availability of biodegradable synthetic grafts to span short nerve gaps would eliminate morbidity associated with short nerve grafts and would capitalize on the potential benefits of neurotropism in directing nerve

regeneration. The future of bioengineered grafts may entail a combination of tissue typing, trophic factors and perhaps the use of embryonic stem cells to create effective nerve conduits that can help in promoting more robust and more precisely directed nerve regeneration.

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