Peripheral nerve regeneration through guidance tubes

Jason S. Belkas*, Molly S. Shoichet† and Rajiv Midha*

*Division of Neurosurgery and Neuroscience Research Program, Sunnybrook & Women’s College Health Sciences Centre, University of Toronto
†Department of Chemical Engineering and Applied Chemistry and Department of Chemistry, Institute of Biomaterials and Biomedical Engineering, University of Toronto

INTRODUCTION
Peripheral nerve injury (PNI) is a serious health problem for society today. It affects 2.8% of trauma patients, many of whom acquire life-long disability1. Approximately 360,000 people in the United States suffer from upper extremity paralytic syndromes yearly, resulting in 8,648,000 and 4,916,000 restricted activity days and bed/disability days, respectively2. Furthermore, 44,000 upper extremity inpatient procedures involved the nervous system in the United States from 1989–19912.

HISTORICAL OVERVIEW
The first attempts at repairing nerve injuries were reported in the 17th century3. By the 19th century, various surgical options and their outcomes for the management of peripheral nerve injury gaps were reported in a review by Huber4. Some of these included stretching or transposing the nerve, considerably mobilizing the proximal and distal stumps with acute joint flexion or bone shortening, utilizing nerve grafts, or bridging the nerve ends with various organic or synthetic materials acting as nerve conduits4. Sanders later classified the management of large peripheral nerve gaps into two general categories: (1) bridge operations (which included all grafting, transposition and tubulization techniques); and (2) manipulative nerve operations (whereby all measures were taken to achieve end-to-end apposition of the nerve stumps)5. In the late 20th century, it was shown that tension across a repair site was adverse to nerve regeneration which led to the preference of nerve grafting over manipulative procedures for repairing any substantial peripheral nerve gap6,7.

Nerve autografts (nerve segments of autogeneic or self origin) were extensively studied in the early nerve grafting experiments. Philipeaux and Vulpian reported transplanting 2 cm segments of lingual nerves into hypoglossal deficits in dogs. Functional recovery was rarely reported in these early studies8, however, experimental validity of the benefits of nerve grafting was established in dogs, rabbits, and guinea pigs9. Clinically, though, results were variable, with only rare favourable cases10.

Positive outcomes with nerve autografting were consistently observed by Seddon who repaired large peripheral nerve deficits in the extremities by using small diameter cutaneous nerve grafts in a “cable” fashion rather than larger caliber grafts which usually were associated with a high incidence of necrosis11. Millesi and colleagues improved upon these clinical results and popularized nerve autografting with the advent of the operating microscope and microsurgical instrumentation and supplies12.

PERIPHERAL NERVE INJURY AND CONVENTIONAL REPAIR
When a peripheral nerve is transected, Wallerian degeneration will occur in all of the axons distal to the injury site13. This begins at the time of injury, and axonal degeneration is evident early whereby the axoplasmic microtubules and neurofilaments disintegrate due to a calcium dependent proteolytic process14. These events of Wallerian degeneration occur because the axon is separated from its trophic (nutritive) source in the nerve cell body (located in the spinal cord, dorsal root ganglia, or autonomic ganglia)15. Within 24 hours, most of the axons along the distal stumps of transected nerves are...
Peripheral nerve regeneration through guidance tubes: Jason S. Belkas et al.

reduced to granular and amorphous debris15. By 48 hours, the myelin sheath has begun to be transformed into short segments which then form into ovoids15. Macrophages migrate specifically to and closely associate with degenerating nerve fibers16. The macrophages mainly are recruited from the circulation, but they are also resident cells which lie just inside or just outside the basal lamina of endoneurial vessels16. These activated cells pass through the basal lamina of degenerating nerve fibres and become phagocytic and foamy in appearance15. Schwann cells proliferate by mitosis on day 3 in response to myelin debris and macrophage-derived cytokines15. These proliferating Schwann cells help degrade the myelin, but they also form longitudinal Schwann cell bands (bands of Bungner) as they divide and remain within the basal lamina lined endoneurial tubes17.

Myelinated and unmyelinated fibers, at some distance proximal to the injury site, will spontaneously sprout new daughter axons18. The sprouts arising from one axon form a “regenerating unit” that is surrounded by a common basal lamina17. The sprouts begin proximally from the nodes of Ranvier at a level where the axons are still intact and these sprouts progress in a distal fashion (across a suture line or graft), growing between the inner surface of the Schwann cell basal lamina and the outer surface of the Schwann cell membrane19. Compartmentation begins after the first few months whereby the regenerating nerve is separated into numerous small nerve bundles, or “mini-fascicles”, leading to the re-establishment of the normal endoneurial environment17. With time, the number of fibers in the distal nerve decreases when some axons reach their targets and mature (due to target-derived growth factors) at the expense of the many sprouts which have not made appropriate connections and are withdrawn20.

If the regenerating units do not reach the endoneurial environment of the distal stump (for instance, if they are blocked by scar tissue), then they will form neuromas that result in a loss of potential nerve function21. The regenerating fibers are often prevented from reaching the distal stump by scar tissue, which can be laid down between the proximal and distal nerve stumps, may lead the regenerating axons to the appropriate end organ. This often requires the resection of a neuroma in continuity and repair of the resulting nerve gap. For repair of gaps longer than 5 mm, the gold standard for bridging the proximal and distal stumps is still the nerve autograft22,23. Although the field of nerve pathophysiology has grown significantly during the last few decades, our understanding of, and advances in, the clinical treatment of peripheral nerve injuries has changed relatively little. To date no tubular or other type of conduit has proved superior to the autologous nerve graft, at least not for reconstruction of the substantial human nerves such as the median or ulnar nerve trunks. Donor nerves utilized commonly are small diameter (2–3 mm) cutaneous nerves harvested from either the arm or leg (e.g. the sural nerve) for repairing large gaps. Nerve grafts contain Schwann cells and basal lamina endoneurial tubes that provide neurotrophic factors24, as well as cell and endoneurial tube surface adhesion molecules25.

Unfortunately, there are disadvantages with nerve autografting. A secondary injury is created to repair the primary one. Morbidity in the donor site can arise in the form of scar and occasional neuroma pain26. Insufficient donor tissue availability presents another obstacle, as the autograft material may be of insufficient length and diameter to optimize the repair27. The microsurgical techniques used to approximate the two stumps of a transected nerve have been optimized23. Surgically, nothing more can be done to enhance the elongation rate and course of regenerating fibers, other than suturing the epineurial or perineurial connective tissue layers together25. Results achieved using a nerve autograft are variable, ranging from extremely poor28 to very good29; including faulty sensory localization and uncoordinated muscle contractions22. The grafted nerve contains thousands of basal lamina endoneurial tubes that are oriented in a linear fashion and can impose nontopographic directionality to a regenerating nerve axon, leading to inappropriate (nonspecific) and incomplete reinnervation of the distal nerve stump and subsequent poor functional recovery30.

Alternatively, a bioengineered graft, sutured in-between the proximal and distal nerve stumps, may provide a more suitable environment for regenerating axons. A major benefit of artificial conduits is that no secondary injury is created to repair the primary one. Use of nerve guidance channels was originally believed to be superior to the conventional endo-to-end suturing repair technique or nerve autografting31. Fewer epineurial sutures are needed in entubulation repair since the nerve stumps are placed into the ends of the tube resulting in less surgical trauma14. Guidance tubes assist in directing axons from the proximal to the distal stump without any interference from the imperfectly aligned degenerating fascicles of the nerve graft or the closely apposed distal stump14. Also, guidance channels are utilized in an attempt to minimize the infiltration of fibrous scar tissue, which can be laid down between the nerve stumps hindering the advance of neurites. Many of the graft properties (e.g. length, diameter, rigidity, permeability, degradability, interior surface, luminal constitution, and much more) can be manipulated to best suit clinical requirements. Furthermore, various soluble factors that are released from the nerve stumps accumulate within these synthetic nerve tubes. Similarly, the conduits themselves can be enhanced with the incorporation of exogenous growth factors into the lumen.

BIOLICAL NERVE GRAFTS

Weiss used non-nerve tissues as alternatives to suture repair of nerve to successfully bridge very short nerve gaps32,33. Since then, conduits from many different biological tissues have been used with varying success. These include the use of arteries33, veins34,35, muscle36-38 and other materials which are extensively reviewed by Doolabh and colleagues22. Other nerve tube conduits have been made from modified biological tissues such as the median or ulnar nerve trunks. Donor nerves utilized commonly are small diameter (2–3 mm) cutaneous nerves harvested from either the arm or leg (e.g. the sural nerve) for repairing large gaps. Nerve grafts contain Schwann cells and basal lamina endoneurial tubes that provide neurotrophic factors24, as well as cell and endoneurial tube surface adhesion molecules.
as laminin\textsuperscript{22} and collagen\textsuperscript{39,40} and have proved successful in specific situations. There are a number of disadvantages with the use of blood vessel, muscle, and other biologic tissues in bridging peripheral nerve defects including tissue reaction, early fibrosis, scar infiltration, and lack of precise control of the conduits' mechanical properties\textsuperscript{22}. These limitations have led to the emergence of conduits made from novel synthetic materials, despite potential problems with biocompatibility.

**REGENERATIVE EVENTS OCCURRING WITHIN A SYNTHETIC CHAMBER**

The aim of the early hollow tube experiments was to offer the regenerating axons optimal conditions where the influence of external non-cellular and humoral factors was minimized, and where only cells and tissue elements normally occurring in a peripheral nerve trunk would influence the regeneration process\textsuperscript{17}.

In 1983, Williams and colleagues took advantage of the fact that silicone tubes were impermeable which facilitated the isolation and characterization of their contents\textsuperscript{41}. They examined the spatial and temporal sequences in which various nerve regeneration events occurred across a 10 mm rat sciatic nerve gap within a silicone chamber\textsuperscript{41}. These events are illustrated in Figure 1. A clear tissue fluid originating from the damaged nerve ends filled the chamber within hours. After 12 h, the 10 mm gap was completely filled with fluid, which demonstrated considerable neurotrophic activities under in vitro conditions\textsuperscript{14}. This fluid containing neurotrophic factors, affecting sensory, motor, and sympathetic neurons, peaked in concentration after 3 to 6 h\textsuperscript{42}. Within the first week, a matrix coalesced (consisting largely of fibrin polymers) that was relatively acellular\textsuperscript{41}. This fibrin matrix provided a scaffold for the immigration and seeding of cells from both nerve stumps during the second week. These cells included Schwann cells, fibroblasts, endothelial cells, and perineurial cells\textsuperscript{41}. The formation of this fibrin matrix is critical for regeneration. If a matrix fails to form, as can happen when a tube is used to repair a long gap, no regeneration will occur\textsuperscript{43}. The thickness and quality of the fibrin matrix can be influenced by the dimensions of the tube\textsuperscript{43}. There is a tendency of the nerve regenerating cable to taper from both the proximal and distal nerve stumps towards the mid-tube area\textsuperscript{17}. The more this cable tapers, the more constraint is placed on the regeneration of axons through it\textsuperscript{14}. The amount of tapering is greater with larger diameter or longer nerve tubes. Axons appear inside the chamber by the second week, and even then only over the first (proximal) 1–3 mm. Some nonmyelinated axons cross the 10 mm gap by the third week. By week 4, myelinated axons can be seen at the chamber midpoint. Schwann cells and fibroblasts advance ahead of the axons in the first few weeks and blood vessels lag behind them. This fact could indicate that the fibrin matrix does not serve as a sufficient substrate for axonal growth and that axonal elongation depends upon the prior presence of Schwann cells that lead the axons. In rats, axonal elongation inside the chamber proceeds at a rate of about 1 mm/day\textsuperscript{41}. This is much slower than the regeneration rate observed in a rat autograft which is approximately 3 mm/day\textsuperscript{24}. In nerve regeneration studies, scar tissue within a nerve conduit is hopefully kept to a minimum as axons can only elongate through this type of tissue at a slow rate of 0.25 mm/day\textsuperscript{42}. It should be noted that these are averaged values. Cajal reported that regenerating axons meander across the apposition of two nerve stumps such that the axons take winding pathways to enter into the distal stump in an asynchronous fashion\textsuperscript{44}.

**SYNTHETIC MATERIALS USED AS NERVE CONDUITS**

As mentioned, synthetic guidance channels are attractive candidates for repairing peripheral nerve defects because their physical and chemical properties (for instance, strength, diameter, porosity, degradation rate) can be precisely manipulated in order to optimize regenerative conditions.

Lundborg and Hansson noted that regeneration through 10 mm long chambers was similar to that within the nerve autografts in rats\textsuperscript{45}. Seckel \textit{et al.} observed successful reconstitution of a nerve trunk of a rat sciatic nerve with negligible inflammation using plasticized
polyester tubes. Regeneration through a collagen-based conduit was as effective as nerve autografting in studies utilizing rodent sciatic and primate median nerves.

Polymers such as poly(lactic acid), polyglycolic acid, and poly(lactic-co-glycolic) acid were early candidates for testing because of their availability, ease of processing, and approval by the FDA. One such degradable polymer studied first as a conduit material over a relatively large nerve gap was polyglactin (Vicryl mesh). Polyglactin was not found to create significant irritation to the regenerating nerve although the regenerative nerve cable morphology differed slightly from that of a normal nerve. Many recent studies investigating the use of biodegradable conduits have shown promise for nerve regeneration applications. The biomaterials used in some of these studies include poly(ester); poly(lactic-co-glycolic) acid, poly(organophosphazene), poly(lactide-co-caprolactone), poly(DL-lactide-co-glycolide), and poly(3-hydroxybutyrate).

Various nerve conduits as described above permit peripheral nerve regeneration. However, they are often not able to facilitate growth over long gaps secondary to collapse, scar infiltration, and early resorption. With regards to biodegradable materials, cytotoxic degradations products have been demonstrated to be released, that may introduce newly recognized problems associated with the resorption process in terms of a substantial macrophage invasion, fibrosis, and disorganized axonal growth.

**BIOMATERIAL CONSIDERATIONS**

Hudson et al. listed several important properties that guidance channels should possess. Conduits should be: easily fabricated with the desired diameter, implanted with relative ease, and easily sterilized. Additionally, they should be flexible, yet able to maintain their structural integrity in vivo. When designing nerve conduits, other factors must also be taken into consideration including tube dimensions, permeability, luminal surface topography, and the conduit's inherent electrical charge. It is preferable if the guidance channels also have the potential to be enhanced by the incorporation of insoluble and soluble proteins, longitudinally aligned fibers, interposed nerve segments, and seeding of neuronal support cells. The dimensions (e.g. length, luminal diameter, tube wall thickness, and cross-sectional area) of the nerve tube must be easily controlled in a reproducible manner. In 1982, Lundborg and colleagues reported that regeneration could occur through a silicone conduit bridging rat sciatic nerve gaps that were at most 10 mm long, provided these tubes were not enhanced with exogenous growth factors. Furthermore, the inner diameter of the nerve conduit must also be taken into account when designing a guidance channel so that the contained nerve does not become constricted. Williams and Varon observed improved regeneration through rat sciatic nerve tubes that had an inner diameter of 1.8 mm (an internal volume capacity of 25 µl) compared to tubes that had inner diameters of 1.2 mm (11 µl) and 3.1 mm (75 µl).

Another group determined that the optimal inner cross-sectional area for non-biodegradable tubes was 2.5–3 times that of the nerve bundle. Wall thickness is another factor that should be considered since decreased neuroma formation was found in Silastic tubes with thinner walls. Rutkowski and Heath noted significantly reduced axonal growth in tubes with wall thicknesses greater than 0.81 mm. While there is a correlation between wall thickness and tube porosity, tube porosity plays a more important role than wall thickness in nerve regeneration through guidance channels.

Permeability of the tube is a key property of biomaterials used in repairing nerve gaps. In general, tubes which are porous and permeable to the surrounding tissue medium exhibit improved nerve regeneration, although the exact mechanism is unclear. Aesbich et al. compared regeneration through semipermeable acrylic copolymer tubes with impermeable silicone elastomer tubes. They noted better regeneration through the semipermeable channels, suggesting that those channels permit the influx of nutrients and growth factors from the external environment. In another study, semipermeable polysulphone tubular membranes with a molecular weight cutoff of 50 kD demonstrated superior regeneration to their 100 kD molecular weight cutoff tubular counterparts. These results suggest that tubes with high porosities having a 100 kD cutoff allow the influx of inhibitory molecules from the external wound-healing environment that would not be included inside lower porosity tubes. Alternatively, others have suggested that growth factors within guidance channels of high porosities diffuse out of the conduits more readily in comparison to lower porosity tubes.

The quality of nerve regeneration can also be influenced by the texture of the inner surface of the conduit used. More robust regeneration was observed through tubes with smooth inner surfaces as opposed to tubes with rough inner surfaces. Likewise, the in vivo foreign body reaction to biomaterials can depend on the topography and the relationship between an implant's surface area to volume. Ratner states that relatively smooth surfaces like those on breast implants are invaginated with macrophages while rougher surfaces such as those on expanded poly(tetrafluoroethylene) vascular prostheses elicit a foreign body type reaction composed of macrophages and giant cells at the surface. Similarly, high surface-to-volume ratio implants (such as fabrics) have higher ratios of macrophages and giant cells at the implantation site than smooth surface implants which have fibrosis as a significant component at the site.

The electrical properties of a biomaterial may also influence nerve regeneration. Piezoelectric biomaterials, such as poly(vinylidene fluoride) (PVDF) and poly(vinylidenefluoride-co-trifluoroethylene), are able to generate transient surface charges under little mechanical strain. Electrically-poled (piezoelectric) PVDF demonstrated improved nerve fiber outgrowth both in vitro and in vivo compared to unpoled (non-piezoelectric) PVDF. Nerve regeneration through
piezoelectrically active poly(vinylidenefluoride-trifluoroethylene) conduits was also enhanced compared to non-poled tubes. In a different study, PC-12 cells cultured on electrically stimulated polypyrrole (an electrically conductive polymer) showed an increase in neurite length compared to non-stimulated ones and tissue culture polystyrene controls.

Extracellular matrix (ECM) proteins, mainly collagen, laminin, and fibronectin, are haptotactic cues that guide growth cones during regeneration. The inclusion of these proteins into tubes can further stimulate axonal elongation. The incorporation of collagen gels within tubes has been shown to improve regeneration relative to saline-filled tubes in several studies. Similar conclusions were also drawn from studies using laminin-filled tubes compared to control tubes. However, the success of the incorporation of these ECM molecules depends on the concentration of these gels since too highly concentrated gels may impede axonal outgrowth. Dilute collagen (1.28 mg/ml) and laminin (4 mg/ml) gels enhanced nerve regeneration significantly better than their more concentrated counterparts (1.92 and 2.56 mg/ml collagen gels and 12 mg/ml laminin gel). Another promising avenue in promoting nerve regeneration is incorporating a laminin-soaked collagen sponge into a guidance channel, which has shown comparable results to tubes enriched with collagen fibers.

Cell adhesion molecules, such as neural cell adhesion molecule (N-CAM), L1, myelin-associated glycoprotein (MAG) and neuron-glial cell adhesion molecule (Ng-CAM) affect cell interactions during the development, maintenance, and regeneration of the nervous system. Specific cell-surface receptors, such as integrins, bind to ECM proteins, such as laminin and fibronectin, in which the amino acid sequences arginine-glycine-aspartic (RGD) acid have been found to be important for binding. Two other notable sequences are tyrosine-isoleucine-glycine-serine-arginine (YIGSR) and isoleucine-lysin-valine-alanine-valine (IKVAV) found in laminin, which have been shown to be active in epithelial and neuron cell attachment and in promoting neurite outgrowth, respectively. Several groups have found that peptide-modified surfaces enhance cell adhesion.

In vitro, YIGSR, IKVAV, and RGD enhanced the interaction of primary neuronal cells with fluoropolymers and directed neuron adhesion and outgrowth.

Peripheral nerve regeneration can be further enhanced by pre-filling nerve tubes with dialyzed plasma, which forms a fibrin gel. This gel resembles the fibrin matrix formed during the early stages of regeneration. As an extension of this reasoning, longitudinally aligned fibers have been incorporated into the lumen of nerve tubes to test their effectiveness. Dubey et al. observed that magnetically aligned type I collagen gel had a directional effect on neurites and Schwann cells from dorsal root ganglia cultured in the gel surface, resulting in increased neurite ingrowth into the gel compared to the control collagen gel. Ceballos et al. demonstrated in vivo that collagen tubes filled with magnetically aligned type I collagen gel significantly improved regeneration over tubes filled with a control collagen gel. They hypothesized that the aligned collagen gel guided the growth cones and Schwann cells by contact-mediated cues. Verdu et al. showed that silicone tubes pre-filled with aligned collagen or laminin-containing gels improved the quality of regeneration in the mouse sciatic nerve. A recent in vitro study showed that magnetically-aligned fibrin gels also guided axons. Another group reported that silicone tubes inserted with longitudinally aligned polyamide, catgut, polydioxanone, normal polyglactin, or quickly-absorbed polyglactin filaments each exhibited a regenerating bridge and some degree of functional recovery across a 15 mm long rat sciatic nerve gap that was not seen with empty silicone tubes after 3 months post-implantation.

It is well known that neurotrophic factors support survival, differentiation, and growth of neurons in the developing nervous system and promote nerve regeneration (reviewed in). Cajal’s revolutionary work has established that axons from a severed peripheral nerve exhibit tropism (the tendency to extend across a gap towards and into the denervated distal stump). It has only recently been demonstrated that the distal nerve indeed provides neurotropic support rather than simply a source of migrating cells. Some of the neurotropic and neurotrophic factors that have shown success in nerve regeneration studies include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), acidic and basic fibroblast growth factors (FGF-1 and FGF-2, respectively), neurotrophin-3 (NT-3), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), ciliary neurotrophic factor (CNTF), interleukin-1 (IL-1) and transforming growth factor beta (TGF-β).

Growth factors have been delivered most commonly with the use of implantable osmotic pumps, or instilled into the site of nerve injury using a variety of carriers including gelfoam, fibrin glue, and genetically engineered cells such as Schwann cells and fibroblasts. The growth factor can also be incorporated into the matrix substance within the guidance conduit. Direct delivery into the local environment, where axons are regenerating, has been shown by Utley et al. to promote better axonal regeneration versus osmotic pump release. Two concerns with delivering factors within the matrix are inadequate bioavailability or bioactivity and the uniform concentration delivered across the device. Cao and Shiochet have encapsulated neurotrophic factors in biodegradable microspheres that slowly release their contents as they degrade, which improve bioavailability and bioactivity.

Another innovative approach to improve nerve regeneration across long gaps is interposing multiple nerve segments between multiple silicone conduits. In studies conducted on rats, these types of grafts enhanced regeneration, but were inferior to a single long nerve graft. A clinical study by Tang in 1995 involved the interposition of multiple nerve segments to bridge...
Figure 2: Representative photomicrographs of 1 µm toluidine-blue stained cross-sections of 16 week PHEMA-MMA tubes at mid-graft level from [12]. A: Low power photomicrograph of a tube with a contained nerve regenerating cable (RC). The tube wall was biphasic; having an inner spongy (IS) layer and an outer gel-like (OG) layer. A fibrous capsule (FC) formed around the artificial tube. Magnification 40 x. B: Higher power photomicrograph of the regenerating cable that was reasonably abundant with unmyelinated fibres (arrows) and adequately myelinated fibres. Schwann cells (*) and a blood vessel (arrowhead) were also present in the regenerating cable. Magnification 1000 x.
2.0–4.5 cm gaps. Good motor and sensory recovery was observed at follow-up. It is believed these nerve segments help keep the conduits open over the lengthy gaps and are a source of neurotrophic factors, ECM proteins and Schwann cells.

Other components that have been incorporated into the lumen of tubes to promote nerve regeneration include testosterone, gangliosides, catalase, adrenocorticotropin, glial-derived protease inhibitor, forskolin, pyronin, matrigel and hyaluronic acid.

Many laboratories have combined a few of the above approaches in order to optimize nerve regeneration in animal models. Poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) (PHEMA-MMA) hydrogel tubes have been utilized to bridge 10 mm long rat sciatic nerve injury gaps. These tubes had an inner diameter of 1.3 mm, a wall thickness of 0.25 mm, and were permeable to small molecules up to 10 kDa in size. When filled with 10 μg/ml of FGF-1 dispersed in a 1.28 mg/ml collagen-1 gel matrix, these tubes demonstrated comparable regeneration to nerve autografts at eight weeks post-implantation. In a longer-term study, robust regenerating nerve cables were maintained within the PHEMA-MMA tubes at 16 weeks (Figure 2).

NERVE CONDUITS USED IN CLINICAL TRIALS

Some of the experimental studies described above have led to clinical trials using nerve conduits to improve peripheral nerve regeneration. The ulnar nerve and the median nerve were successfully reconstructed using silicone conduits in three young adult male patients with gap lengths that ranged from 3 to 5 mm. However, these impermeable, non-biodegradable tubes elicited an inflammatory and fibrotic reaction and produced chronic nerve compression, requiring their removal after regeneration had occurred through them.

Expanded polytetrafluoroethylene has been used in the clinical setting with some success in repairing median and ulnar nerve gaps up to 4 cm in length. Using biodegradable polyglycolic acid (PGA) conduits, excellent sensory recovery was seen in 13 of the 16 patients for the repair of digital nerve gap lengths averaging 1.7 cm and in three of four patients with a 2.4 cm average gap length in median nerves. In a randomized prospective study, PGA tubes have also been proven to be successful in the clinical repair of digital nerves with defects up to 3 cm. Partly based on these results, PGA tubes (Neurotube, Neureogen LLC, Bel Air, MD) were recently approved by the FDA for the repair of peripheral nerve injuries. Collagen nerve tubes (NeuraGen, Integra Neurosciences, Plainsboro, NJ) have also attained this status because of their success in non-human primates, as well as Phase I–II clinical safety studies. However, many of these clinical studies are limited primarily to short defects of the small-caliber digital nerve. A recent comprehensive review of the literature pertaining to the clinical use of nerve conduits is provided by Meek and Coert.

CONCLUSION

Tissue engineering is a dynamic and innovative field that allows and indeed fosters collaboration amongst scientists, physicians, and the industry in order to make significant and meaningful advances in clinical care. There is much promise in using tissue engineering approaches to improve the surgical treatment of nerve injuries. Many different projects are being undertaken with the aim of maximizing neurological recovery after peripheral nerve injury. It is reasonable to presume that the most effective guidance channel for nerve repair in the future would be one that has been designed with at least some combination of ideas and principles detailed in this review.

REFERENCES

3. Ferrara G. Nuova Selva di Citurgia Divisa tre Parti Venice; S Combi, 1608
5. Sanders FK. The repair of large gaps in the peripheral nerves. Brain 1942; 65: 281–337
7. Miyamoto Y. Experimental study of results of nerve suture under tension vs. nerve grafting. Plast Reconstr Surg 1979; 64: 540–549
8. Philippine JM, Vulpius A. Note sur des essais de greffe d'un tronc de nerf lingual entre les deux bouts du nert hypoglosse, après excision d'un segment de ce dernier nert. Arch de Physiol Norm et Path (Par) 1870; 8: 618–620
Peripheral nerve regeneration through guidance tubes: Jason S. Belkas et al.


30 De Medinaceli L, Rawlings RR. Is it possible to predict the outcome of peripheral nerve injuries? A probability model based on prospects for regenerating neurites. Biosystems 1987; 20: 243–258


33 Weiss P. The technology of nerve regeneration: a review. J Neurosurg 1944; 1: 400–450


35 Tang JB. Vein conduits with interposition of nerve tissue for peripheral nerve defects. J Reconstr Microsurg 1995; 11: 21–26


38 Hall S. Axonal regeneration through acellular muscle grafts. J Anat 1997; 190: 57–71


42 Danielsen N, Varon S. Characterization of neurotrophic activity in the silicone-chamber model for nerve regeneration. J Reconstr Microsurg 1995; 11: 231–235


60 Mackinnon SE, Dellon AL. An alternative to the classical nerve stump for peripheral nerve repair. J Neurosurg 1988; 70B: 179–187


Peripheral nerve regeneration through guidance tubes: Jason S. Belkas et al.
Peripheral nerve regeneration through guidance tubes: Jason S. Belkas et al.

120 Tang JB. Vein conduits with interposition of nerve tissue for peripheral nerve defects. J Reconstr Microsurg 1995; 11: 21–26
125 Keynes RJ. The effects of pyronin on sprouting and regeneration of mouse motor nerves. Brain Res 1982; 253: 13–18
132 Merle M, Dellon AL, Campbell JN, Chang PS. Complications from silicon-polymer intubulation of nerves. Microsurgery 1989; 10: 130–133
Listed below are queries relating to your manuscript. Please answer the queries and return with the corrected proof.

<table>
<thead>
<tr>
<th>Query no</th>
<th>Section</th>
<th>Para</th>
<th>Query</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Abstract</td>
<td></td>
<td>Please supply</td>
</tr>
<tr>
<td>2</td>
<td>Keywords</td>
<td></td>
<td>Please supply – up to six words</td>
</tr>
<tr>
<td>3</td>
<td>References</td>
<td>129</td>
<td>Has this paper been accepted for publication? If so please give details.</td>
</tr>
</tbody>
</table>