Peripheral nerve regeneration through guidance tubes

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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 disability[1]. 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, respectively[2]. Furthermore, 44,000 upper extremity inpatient procedures involved the nervous system in the United States from 1989–1991[2].

HISTORICAL OVERVIEW

The first attempts at repairing nerve injuries were reported in the 17th century[3]. By the 19th century, various surgical options and their outcomes for the management of peripheral nerve injury gaps were reported in a review by Huber[4]. 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 conduits[4]. 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 gap[6,7].

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 studies[8], however, experimental validity of the benefits of nerve grafting was established in dogs, rabbits, and guinea pigs[4,9]. Clinically, though, results were variable, with only rare favourable cases[10].

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 necrosis[11]. Millesi and colleagues improved upon these clinical results and popularized nerve autografting with the advent of the operating microscope and microsurgical instrumentation and supplies[12].

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 site[13]. 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 process[14]. 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

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reduced to granular and amorphous debris[15]. By 48 hours, the myelin sheath has begun to be transformed into short segments which then form into ovoids[15]. Macrophages migrate specifically to and closely associate with degenerating nerve fibers[16]. 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 vessels[16]. These activated cells pass through the basal lamina of degenerating nerve fibres and become phagocytotic and foamy in appearance[15]. Schwann cells proliferate by mitosis on day 3 in response to myelin debris and macrophage-derived cytokines[15]. 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 tubes[17].

Myelinated and unmyelinated fibers, at some distance proximal to the injury site, will spontaneously sprout new daughter axons[18]. The sprouts arising from one axon form a "regenerating unit" that is surrounded by a common basal lamina[17]. 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 membrane[19]. 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 environment[17]. 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 withdrawn[20].

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 results in a loss of potential nerve function[21]. The goal of nerve repair is to direct the regenerating nerve fibers into the proper distal endoneurial tubes that will 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 autograft[6,22,23]. 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[6]. Nerve grafts contain Schwann cells and basal lamina endoneurial tubes that provide neurotrophic factors[24], as well as cell and endoneurial tube surface adhesion molecules[25].

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 pain[26]. Insufficient donor tissue availability presents another obstacle, as the autograft material may be of insufficient length and diameter to optimize the repair[27]. The microsurgical techniques used to approximate the two stumps of a transected nerve have been optimized[23]. 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 together[23]. Results achieved using a nerve autograft are variable, ranging from extremely poor[28] to very good[29]; including faulty sensory localization and uncoordinated muscle contractions[22]. 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 recovery[30].

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 end-to-end suturing repair technique or nerve autografting[31]. Fewer epineurial sutures are needed in entubulation repair since the nerve stumps are placed into the ends of the tube resulting in less surgical trauma[14]. 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 stump[14]. 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.

BIOLOGICAL NERVE GRAFTS

Weiss used non-nerve tissues as alternatives to suture repair of nerve to successfully bridge very short nerve gaps[32,33]. Since then, conduits from many different biological tissues have been used with varying success. These include the use of arteries[33], veins[34,35], muscle[36-38], and other materials which are extensively reviewed by Doolabh and colleagues[22]. Other nerve tube conduits have been made from modified biological tissues such

as laminin[22] and collagen[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[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[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[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[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[14]. This fluid containing neurotrophic factors, affecting sensory, motor, and sympathetic neurons, peaked in concentration after 3 to 6 h[42]. Within the first week, a matrix coalesced (consisting largely of fibrin polymers) that was relatively acellular[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[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[43]. The thickness and quality of the fibrin matrix can be influenced by the dimensions of the tube[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[17]. The more this cable tapers, the more constraint is placed on the regeneration of axons through it[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

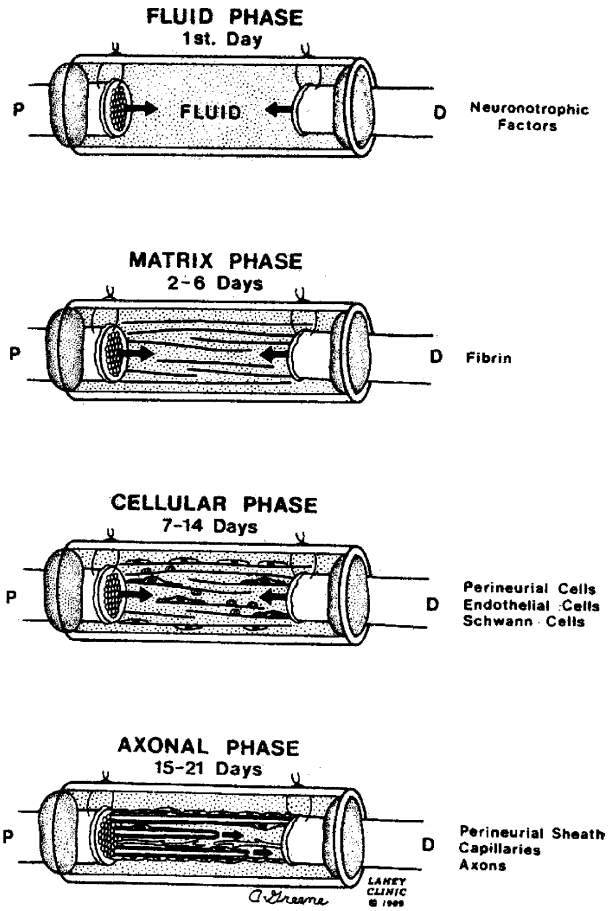

Figure 1: Events occurring within a silicone tube bridging the proximal (P) and distal (D) stumps in a 10 mm rat sciatic nerve gap as reported by Williams *et al.*[41]. See text for details. (Reprinted by permission of John Wiley & Sons, Inc.[14])

chamber proceeds at a rate of about 1 mm/day[41]. This is much slower than the regeneration rate observed in a rat autograft which is approximately 3 mm/day[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[44]. 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[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[45]. Seckel *et al.* observed successful reconstitution of a nerve trunk of a rat sciatic nerve with negligible inflammation using plasticized

polyester tubes[46]. Regeneration through a collagen-based conduit was as effective as nerve autografting in studies utilizing rodent sciatic and primate median nerves[39].

Polyesters such as polylactic 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[47]. One such degradable polymer studied first as a conduit material over a relatively large nerve gap was polyglactin (Vicryl mesh)[48]. 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[48]. 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(phosphoester)s[49], poly(lactic-co-glycolic) acid[50], poly(organophosphazene)s[51], poly(L-lactide-co-μ-caprolactone)[52], poly(DL-lactide-co-glycolide)[53] and poly(3-hydroxybutarate)[54].

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[22]. With regards to biodegradable materials, cytotoxic degradation 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[22,23].

BIOMATERIAL CONSIDERATIONS

Hudson *et al*. listed several important properties that guidance channels should possess[47]. 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[47]. 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[55]. 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)[56]. Another group determined that the optimal inner cross-sectional area for non-biodegradable tubes was 2.5–3 times that of the nerve bundle[57]. Wall thickness is another factor that should be considered since decreased neuroma formation was found in Silastic tubes with thinner walls[57]. Rutkowski and Heath noted significantly reduced axonal growth in tubes with wall thicknesses greater than 0.81 mm[58]. 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[58].

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[23,59–63] although the exact mechanism is unclear[22,64]. Aesbischer *et al*. compared regeneration through semipermeable acrylic copolymer tubes with impermeable silicone elastomer tubes[61]. 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 polysulfone tubular membranes with a molecular weight cutoff of 50 kD demonstrated superior regeneration to their 100 kD molecular weight cutoff tubular counterparts[65]. 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[65]. 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[58].

The quality of nerve regeneration can also be influenced by the texture of the inner surface of the conduit used[47,66]. More robust regeneration was observed through tubes with smooth inner surfaces as opposed to tubes with rough inner surfaces[66]. 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[67]. 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[67]. 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[67].

The electrical properties of a biomaterial may also influence nerve regeneration[47]. Piezoelectric biomaterials, such as poly(vinylidene fluoride) (PVDF) and poly(vinylidenefluoride-co-trifluoroethylene), are able to generate transient surface charges under little mechanical strain[68]. Electrically-poled (piezoelectric) PVDF demonstrated improved nerve fiber outgrowth both *in vitro*[68] and *in vivo*[69] compared to unpoled (non-piezoelectric) PVDF. Nerve regeneration through

piezoelectrically active poly(vinylidenefluoride-trifluoroethylene) conduits was also enhanced compared to non-poled tubes[70]. 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[71].

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[72–74]. Similar conclusions were also drawn from studies using laminin-filled tubes compared to control tubes[75,76]. 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[77,78]. Dilute collagen[77,78] (1.28 mg/ml) and laminin[77] (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 enhanced with collagen fibers[79].

Cell adhesion molecules, such as neural cell adhesion molecule (N-CAM), L1, myelin-associated glycoprotein (MAG) and neuron-glia cell adhesion molecule (Ng-CAM) affect cell interactions during the development, maintenance, and regeneration of the nervous system[22]. Specific cell-surface receptors, such as integrins[80], bind to ECM proteins, such as laminin and fibronectin[81], in which the amino acid sequences arginine-glycine-aspartic acid (RGD) have been found to be important for binding[82,83]. Two other notable sequences are tyrosine-isoleucine-glycine-serine-arginine (YIGSR) and isoleucine-lysine-valine-alanine-valine (IKVAV) found in laminin, which have been shown to be active in epithelial and neuron cell attachment[84] and in promoting neurite outgrowth[85], respectively. Several groups have found that peptide-modified surfaces enhance cell adhesion[86–89]. *In vitro*, YIGSR, IKVAV and RGD enhanced the interaction of primary neuronal cells with fluoropolymers[90,91] and directed neuron adhesion and outgrowth[92].

Peripheral nerve regeneration can also be further enhanced by pre-filling nerve tubes with dialyzed plasma, which forms a fibrin gel[93]. 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[94]. 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[95]. They hypothesized that the aligned collagen gels guided the growth cones and Schwann cells by contact-mediated cues[94]. 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[96]. A recent *in vitro* study showed that magnetically-aligned fibrin gels also guided axons[97]. 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[98].

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[99–101]). 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)[44]. It has only recently been demonstrated that the distal nerve indeed provides neurotropic support[102,103] rather than simply a source of migrating cells[38]. 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)[47,104].

Growth factors have been delivered most commonly with the use of implantable osmotic pumps[105], or instilled into the site of nerve injury using a variety of carriers including gelfoam[106,107], fibrin glue[108,109], and genetically engineered cells such as Schwann cells[58,110–114] and fibroblasts[115]. The growth factor can also be incorporated into the matrix substance within the guidance conduit[14]. 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[116]. Two concerns with delivering factors within the matrix are inadequate bioavailability or bioactivity and the uniform concentration delivered across the device. Cao and Shoichet have encapsulated neurotrophic factors in biodegradable microspheres that slowly release their contents as they degrade, which improve bioavailability and bioactivity[117].

Another innovative approach to improve nerve regeneration across long gaps is interposing multiple nerve segments between multiple silicone conduits[118]. In studies conducted on rats, these types of grafts enhanced regeneration[118,119], but were inferior to a single long nerve graft[118]. A clinical study by Tang in 1995 involved the interposition of multiple nerve segments to bridge

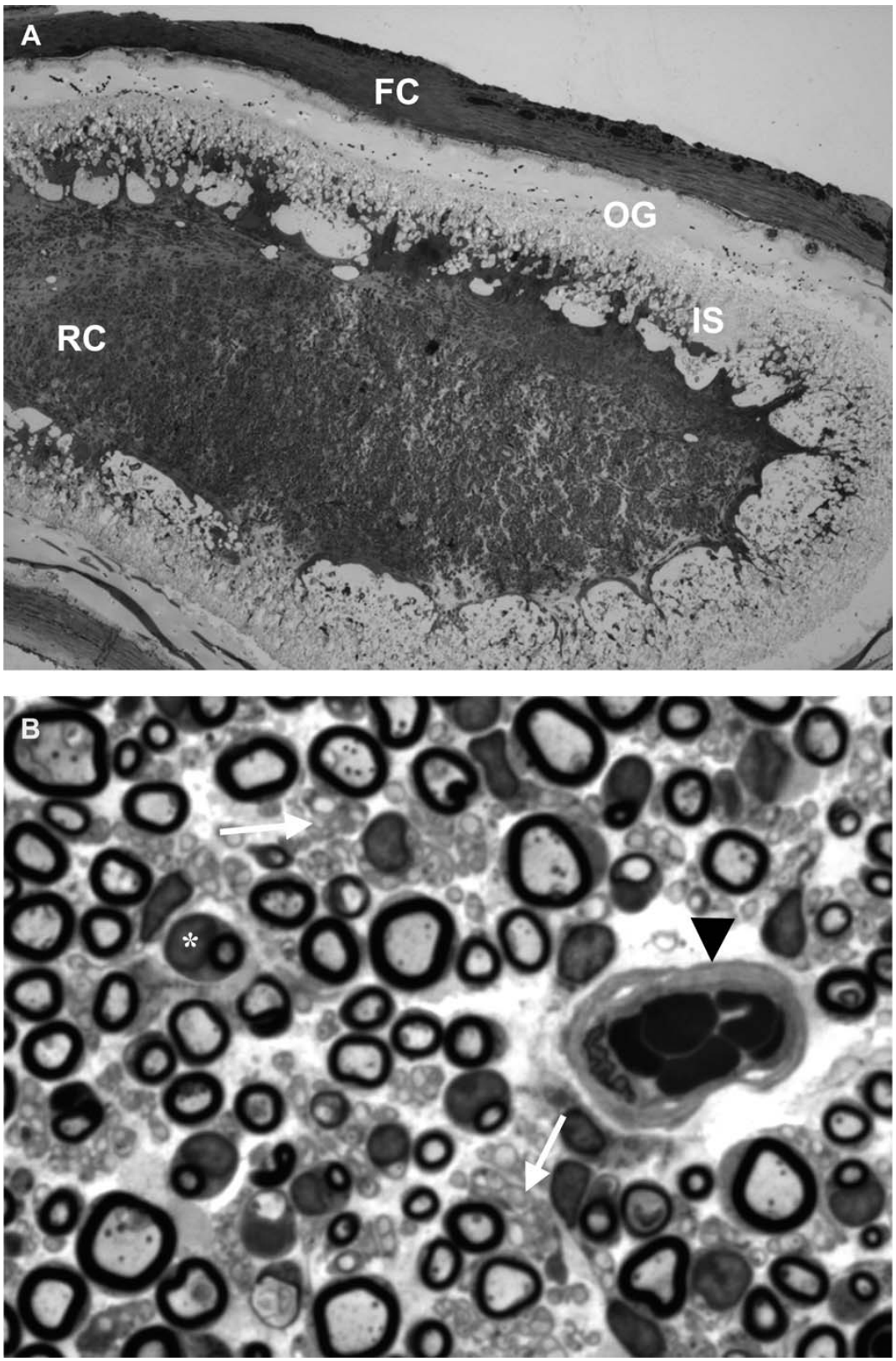

Figure 2: Representative photomicrographs of 1 μm toluidine-blue stained cross-sections of 16 week PHEMA-MMA tubes at mid-graft level from[129]. **A:** Low power photomicrograph of a tube with a contained nerve regenerating cable (RC). The tube wall was biphasic; having an inner spongy (IS) layer and an outer gel-like (OG) layer. A fibrous capsule (FC) formed around the artificial tube. Magnification 40 ×. **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 ×

2.0–4.5 cm gaps[120]. 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[22].

Other components that have been incorporated into the lumen of tubes to promote nerve regeneration include testosterone, gangliosides, catalase[121], adrenocorticotropin[122], glial-derived protease inhibitor[123], forskolin[124], pyronin[125], matrigel[126] and hyaluronic acid[127].

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[78], a wall thickness of 0.25 mm[78], and were permeable to small molecules up to 10 kD in size[128]. 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[74]. In a longer-term study, robust regenerating nerve cables were maintained within the PHEMA-MMA tubes at 16 weeks (*Figure 2*)[129].

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[130] and the median nerve[131] 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[132], 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[133]. 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[28,134]. 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[135]. Partly based on these results, PGA tubes (Neurotube, Neuroregen 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[40,136] 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[137].

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

1 Noble J, Munro CA, Prasad VSSV, Midha R. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. *J Trauma* 1998; **45**: 116–122
2 Kelsey J, Praemer A, Nelson L, Felberg A, Rice D. Upper extremity disorders: frequency, impact, and cost. London: Churchill-Livingstone, 1997: pp. 26, 42
3 Ferrara G. Nuova Selva di Cirurgia Divisia tre Parti Venice. S Combi, 1608
4 Huber GC. A study of the operative treatment for loss of nerve substance in peripheral nerves. *J Morphol* 1895; **11**: 629–735
5 Sanders FK. The repair of large gaps in the peripheral nerves. *Brain* 1942; **65**: 281–337
6 Millesi H. Nerve grafting. *Clin Plast Surg* 1984; **11**: 105–113
7 Miyamoto Y. Experimental study of results of nerve suture under tension vs. nerve grafting. *Plast Reconstr Surg* 1979; **64**: 540–549
8 Philipeaux JM, Vulpian A. Note sur des essays de greffe d'un troncon du nerf lingual entre les deux bouts du nerf hypoglosse, après excision d'un segment de ce denier nerf. *Arch de Physiol Norm et Path (Par)* 1870; **8**: 618–620
9 Huber C. Repair of peripheral nerve injuries. *Surg Gyn Obstet* 1920; **30**: 464–471
10 Delageniere H. A contribution to the study of the surgical repair of peripheral nerves. *Surg Gyn Obstet* 1924; **39**: 543–553
11 Seddon HJ. The use of autogenous grafts for the repair of large gaps in peripheral nerves. *Br J Surg* 1947; **35**: 151–167
12 Millesi H, Meissl G, Berger A. The interfascicular nerve-grafting of the median and ulnar nerves. *J Bone Joint Surg* 1972; **54A**: 727–750
13 Waller AV. Septieme memoire sur le systeme nerveux. *Cr Hebd Acad Sci (Paris)* 1852; **35**: 301
14 Seckel BR. Enhancement of peripheral nerve regeneration. *Muscle Nerve* 1990; **13**: 785–800
15 Chaudhry V, Glass JD, Griffin JW. Wallerian degeneration in peripheral nerve disease. *Neurol Clin* 1992; **10**: 613–627
16 Stoll G, *et al*. Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. *J Neurocytol* 1989; **18**: 671–683
17 Lundborg G. *Nerve Injury and Repair*. London: Churchill-Livingstone, 1988: pp. 149–195
18 Morris J, Hudson A, Wedell G. A study of degeneration and regeneration in the divided rat sciatic nerve based on electron microscopy. *Z Zellforch Mikrosk Anat* 1972; **124**: 76
19 Ide C, Tohyama K, Yokota R, Nitatori T, Onodera S. Schwann cell basal lamina and nerve regeneration. *Brain Res* 1983; **288**: 61–75
20 Brushart TM, Tarlov EC, Mesulam MM. Specificity of muscle innervation after epineurial and individual fascicular suture of the rat sciatic nerve. *J Hand Surg* 1983; **8**: 248–253
21 Mackinnon SE, Dellon AL, Hudson AR, Hunter DA. Alteration of neuroma formation by manipulation of the microenvironment. *Plast Reconstr Surg* 1985; **76**: 345
22 Doolabh VB, Hertl MC, Mackinnon SE. The role of conduits in nerve repair: a review. *Rev Neurosci* 1996; **7**: 47–84
23 Dahlin LB, Lundborg G. Use of tubes in peripheral nerve repair. *Neurosurg Clin N Am* 2001; **12**: 341–352

24 Hudson AR, Morris J, Weddell G, Drury A. Peripheral nerve autografts. *J Surg Res* 1972; **12**: 267–274
25 Martini R. Expression and functional roles of neural cell surface molecules and extracellular matrix components during development and regeneration of peripheral nerves. *J Neurocytol* 1994; **23**: 1–28
26 Ortiguela ME, Wood MB, Cahill DR. Anatomy of the sural nerve complex. *J Hand Surg* 1987; **12A**: 1119–1123
27 Mackinnon SE, Hudson AR. Clinical appreciation of peripheral nerve transplantation. *Plast Reconstr Surg* 1992; **90**: 695–699
28 Mackinnon SE, Dellon AL. *Surgery of the Peripheral Nerve: Nerve Repair and Nerve Grafting*. New York: Thieme, 1988: pp. 89–129
29 Millesi H. Reappraisal of nerve repair. *Surg Clin N Am* 1981; **61**: 321–340
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
31 Seckel BR, Ryan SE, Gagne RG, Chiu TH, Watkins E Jr Target-specific nerve regeneration through a nerve guide in the rat. *Plast Reconstr Surg* 1986; **78**: 793–800
32 Weiss P. Scientific apparatus and laboratory methods. Reunion of stumps of small nerves by tabulation instead of sutures. *Science* 1941; **93**: 67–68
33 Weiss P. The technology of nerve regeneration: a review. *J Neurosurg* 1944; **1**: 400–450
34 Walton RL, Brown RE, Matory WE Borah GL, Dolph JL. Autogenous vein graft repair of digital nerve defects in the finger: a retrospective clinical study. *Plast Reconstr Surg* 1989; **84**: 944–952
35 Tang JB. Vein conduits with interposition of nerve tissue for peripheral nerve defects. *J Reconstruc Microsurg* 1995; **11**: 21–26
36 Norris RW, Glasby MA, Gattuso JM, Bowden REM. Peripheral nerve repair in humans using muscle autografts. A new technique. *J Bone Joint Surg (Br)* 1988; **70B**: 530–533
37 Glasby MA, Gschmeissner SE, Huang CLH, de Souza BA. Degenerated muscle grafts used for peripheral nerve repair in primates. *J Hand Surg* 1986; **11B**: 347–351
38 Hall S. Axonal regeneration through acellular muscle grafts. *J Anat* 1997; **190**: 57–71
39 Archibald SJ, Krarup C, Shefner J, Li S-T, Madison RD. A collagen-based nerve guide conduit for peripheral nerve regeneration in rodents and nonhuman primates. *J Comp Neurol* 1991; **306**: 685–696
40 Archibald SJ, Shefner J, Krarup C, Madison RD. Monkey median nerve repaired by nerve graft or collagen nerve guide tube. *J Neurosci* 1995; **15**: 4109–4123
41 Williams LR, Longo FM, Powell HC, Lundborg G, Varon S. Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: parameters for a bioassay. :*J Comp Neurol* 1983; **218**: 460–470
42 Danielsen N, Varon S. Characterization of neurotrophic activity in the silicone-chamber model for nerve regeneration. *J Reconstr Microsurg* 1995; **11**: 231–235
43 Zhao Q, Dahlin LB, Kanje M, et al. Repair of the transacted rat sciatic nerve: matrix formation within silicon tubes. *Restorative Neurol Neurosci* 1993; **5**: 197–204
44 Ramon y Cajal S. *Degeneration and Regeneration of the Nervous System*, vol. 1. London: Oxford University Press, 1928
45 Lundborg G, Hansson HA. Nerve regeneration through preformed pseudosynovial tubes. A preliminary report of a new experimental model for studying the regeneration and reorganization capacity of peripheral nerve tissue. *J Hand Surg (Am)* 1980; **5**: 35–38
46 Seckel BR, Chiu TH, Nyilas E, Sidman RL. Nerve regeneration through synthetic biodegradable nerve guides: regulation by the target organ. *Plast Reconstr Surg* 1984; **74**: 173–181
47 Hudson TW, Evans GR, Schmidt CE. Engineering strategies for peripheral nerve repair. *Clin Plastic Surg* 1999; **26**: 617–628
48 Molander H, Olsson Y, Engkvist O, et al. Regeneration of peripheral nerve through a polyglactin tube. *Muscle Nerve* 1982; **5**: 54–57
49 Wang S, Wan AC, Xu X, et al. A new nerve guide conduit material composed of a biodegradable poly(phosphoester). *Biomaterials* 2001; **22**: 1157–1169
50 Bryan DJ, et al. Enhanced peripheral nerve regeneration elicited by cell-mediated events delivered via a bioresorbable PLGA guide. *J Reconstr Microsurg* 2003; **19**: 125–134
51 Aldini N, Fini M, Rocca M, Giavaresi G, Giardino R. Guided regeneration with resorbable conduits in experimental peripheral nerve injuries. *Int Orthop* 2000; **24**: 121–125
52 Valero-Cabre A, Tsironis K, Skouras E, et al. Superior muscle reinnervation after autologous nerve graft or poly-L-lactide-epsilon-caprolactone (PLC) tube implantation in comparison to silicone tube repair. *J Neurosci Res* 2001; **63**: 214–223
53 Sundback C, Hadlock T, Cheney M, Vacanti J. Manufacture of porous polymer nerve conduits by a novel low-pressure injection molding process. *Biomaterials* 2003; **24**: 819–830
54 Young RC, Wiberg M, Terenghi G. Poly-3-hydroxybutyrate (PHB): a resorbable conduit for long-gap repair in peripheral nerves. *Br J Plast Surg* 2002; **55**: 235–240
55 Lundborg G, Dahlin LB, Danielsen N, *et al*. Nerve regeneration in silicone chambers: influence of gap length and of distal stump components. *Exp Neurol* 1982; **76**: 361–375
56 Williams LR, Varon S. Modification of fibrin matrix formation in situ enhances nerve regeneration in silicone chambers. *J Comp Neurol* 1985; **231**: 209–220
57 Ducker TB, Hayes GJ. Experimental improvements in the use of Silastic cuff for peripheral nerve repair. *J Neurosurg* 1968; **28**: 582–587
58 Rutkowski GE, Heath CA. Development of a bioartificial nerve graft. II. Nerve regeneration in vitro. *Biotechnol Prog* 2002; **18**: 373–379
59 Uzman BG, Villegas GM. Mouse sciatic nerve regeneration through semipermeable tubes: a quantitative model. *J Neurosci Res* 1983; **9**: 325–338
60 Mackinnon SE, Dellon AL, Hudson AR, Hunter DA. Chronic nerve compression – an experimental model in the rat. *Ann Plast Surg* 1984; **13**: 112–120
61 Aebischer P, et al. Blind-ended semipermeable guidance channels support peripheral nerve regeneration in the absence of a distal nerve stump. *Brain Res*. 1988; **454**: 179–187
62 Dellon AL, Mackinnon SE. An alternative to the classical nerve graft for the management of the short nerve gap. *Plast Reconstr Surg* 1988; **82**: 849–856
63 Rodriguez FJ, Gomez N, Perego G, Navarro X. Highly permeable polylactide-caprolactone nerve guides enhance peripheral nerve regeneration through long gaps. *Biomaterials* 1999; **20**: 1489–500
64 Hadlock T, Elisseeff J, Langer R, Vacanti J, Cheney M. A tissue-engineered conduit for peripheral nerve repair. *Arch Otolaryngol Head Neck Surg* 1998; **124**: 1081–1086
65 Aebischer P, Guenard V, Brace S. Peripheral nerve regeneration through blind-ended semipermeable guidance channels: effect of the molecular weight cutoff. *J Neurosci* 1989; **9**: 3590–3595
66 Aebischer P, Guenard V, Valentini RF. The morphology of regenerating peripheral nerves is modulated by the surface microgeometry of polymeric guidance channels. *Brain Res* 1990; **531**: 211–218
67 Ratner BD, Hoffman AS, Shoen FJ, Lemons JE. *Biomaterials Science: an Introduction to Materials in Medicine*. London: Academic Press, 1996
68 Valentini RF, Vargo TG, Gardella JA Jr, Aebischer P. Electrically charged polymeric substrates enhance nerve fibre outgrowth in vitro. *Biomaterials* 1992; **13**: 183–190
69 Aebischer P, Valentini RF, Dario P, Domenici C, Galletti PM. Piezoelectric guidance channels enhance regeneration in the mouse sciatic nerve after axotomy. *Brain Res* 1987; **436**: 165–168
70 Fine EG, *et al*. Improved nerve regeneration through piezoelectric vinylidenefluoride-trifluoroethylene copolymer guidance channels. *Biomaterials* 1991; **12**: 775–780
71 Schmidt CE, Shastri VR, Vacanti JP, Langer R. Stimulation of neurite outgrowth using an electrically conducting polymer. P*roc Natl Acad Sci USA* 1997; **94**: 8948–8953
72 Satou T, Nishida S, Hiruma S, et al. A morphological study on the effects of collagen gel matrix on regeneration of severed rat sciatic nerve in silicone tubes. *Acta Pathol Jpn* 1986; **36**: 199–208
73 Rosen JM, *et al*. Artificial nerve graft using collagen as an extracellular matrix for nerve repair compared with sutured autograft in a rat model. *Ann Plast Surg* 1990; **25**: 375–387

74 Midha R, Munro CA, Dalton PD, Tator CH, Shoichet MS. Growth factor enhancement of peripheral nerve regeneration through a novel synthetic hydrogel tube. *J Neurosurg* 2003; **99**: 555–565

75 Madison R, da Silva CF, Dikkes P, Chiu TH, Sidman RL. Increased rate of peripheral nerve regeneration using bioresorbable nerve guides and a laminin-containing gel. *Exp Neurol* 1985; **88**: 767–772

76 Madison RD, da Silva C, Dikkes P, Sidman RL, Chiu TH. Peripheral nerve regeneration with entubulation repair: comparison of biodegradable nerve guides versus polyethylene tubes and the effects of a laminin-containing gel. *Exp Neurol* 1987; **95**: 378–390

77 Labrador RO, Buti M, Navarro X. Influence of collagen and laminin gels concentration on nerve regeneration after resection and tube repair. *Exp Neurol* 1998; **149**: 243–252

78 Midha R, Shoichet MS, Dalton PD, Cao X, Munro CA, Noble J, Wong MK. Tissue engineered alternatives to nerve transplantation for repair of peripheral nervous system injuries. *Transplant Proc* 2001; **33**: 612–615

79 Toba T, *et al*. Regeneration of canine peroneal nerve with the use of a polyglycolic acid-collagen tube filled with laminin-soaked collagen sponge: a comparative study of collagen sponge and collagen fibers as filling materials for nerve conduits. *J Biomed Mater Res* 2001; **58**: 622–630

80 Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987; **48**: 549–554

81 Ruoslahti E. Fibronectin and its receptors. *Annu Rev Biochem* 1988; **57**: 375–413

82 Pierschbacher MD. Ruoslahti E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. *Nature* 1984; **309**: 30–33

83 Grant DS, *et al*. Two different laminin domains mediate the differentiation of human endothelial cells into capillary-like structures in vitro. *Cell* 1989; **58**: 933–943

84 Graf J, *et al*. A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67,000 laminin receptor. *Biochemistry* 1987; **26**: 6896–6900

85 Jucker M, Kleinman HK, Ingram DK. Fetal rat septal cells adhere to and extend processes on basement membrane, laminin, and a synthetic peptide from the laminin A chain sequence. *J Neurosci Res* 1991; **28**: 507–517

86 Brandley BK., Schnaar RL. Covalent attachment of an Arg-Gly-Asp sequence peptide to derivatizable polyacrylamide surfaces: support of fibroblast adhesion and long-term growth. *Anal Biochem* 1988; **172**: 270–278

87 Hubbell JA, Massia SP, Drumheller PD. Surface-grafted cell-binding peptides in tissue engineering of the vascular graft. *Ann NY Acad Sci* 1992; **665**, 253–258

88 Ranieri JP, *et al*. Neuronal cell attachment to fluorinated ethylene propylene films with covalently immobilized laminin oligopeptides YIGSR and IKVAV. II. *J Biomed Mater Res* 1995; **29**: 779–785

89 Bellamkonda R, Ranieri JP, Aebischer P. Laminin oligopeptide derivatized agarose gels allow three-dimensional neurite extension in vitro. *J Neurosci Res* 1995; **41**: 501–509

90 Tong YW, Shoichet MS. Peptide surface modification of poly(tetrafluoroethylene-co-hexafluoropropylene) enhances its interaction with central nervous system neurons. *J Biomed Mater Res* 1998; **42**: 85–95

91 Tong YW, Shoichet MS. Novel peptide modification of poly(tetrafluoroethylene-co-hexafluoropropylene) via surface amine-functionalization enhances the interaction with central nervous system neurons. *J Biomater Sci* 1998; **9**: 713–729

92 Saneinejad S, Shoichet MS. Patterned poly(chlorotrifluoroethylene) guides primary nerve cell adhesion and neurite outgrowth. *J Biomed Mater Res* 2000; **50**: 465–474

93 Williams LR, Danielsen N, Muller H, Varon S. Exogenous matrix precursors promote functional nerve regeneration across a 15-mm gap within a silicone chamber in the rat. *J Comp Neurol* 1987; **264**: 284–290

94 Dubey N, Letourneau PC, Tranquillo RT. Guided neurite elongation and schwann cell invasion into magnetically aligned collagen in simulated peripheral nerve regeneration. *Exp Neurol* 1999; **158**: 338–350

95 Ceballos D, et al. Magnetically aligned collagen gel filling a collagen nerve guide improves peripheral nerve regeneration. *Exp Neurol* 1999; **158**: 290–300

96 Verdu E, Labrador RO, Rodriguez FJ, et al. Alignment of collagen and laminin-containing gels improve nerve regeneration within silicone tubes. *Restor Neurol Neurosci* 2002; **20**: 169–179

97 Dubey N, Letourneau PC, Tranquillo RT. Neuronal contact guidance in magnetically aligned fibrin gels: effect of variation in gel mechano-structural properties. *Biomaterials* 2001; **22**: 1065–1075

98 Arai T, Lundborg G, Dahlin LB. Bioartificial nerve graft for bridging extended nerve defects in rat sciatic nerve based on resorbable guiding filaments. *Scand J Plast Reconstr Surg Hand Surg* 2000; **34**: 101–108.

99 Richardson PM. Neurotrophic factors in regeneration. *Curr Opin Neurobiol* 1991; **1**: 401–406.

100 Logan A, Oliver JJ, Berry M. Growth factors in CNS repair and regeneration. *Prog Growth Factor Res* 1994; **5**: 379–405

101 Ebadi M, *et al*. Neurotrophins and their receptors in nerve injury and repair. *Neurochem Int* 1997; **30**: 347–374

102 Politis MJ, Ederle K, Spencer PS. Tropism in nerve regeneration in vivo. Attraction of regenerating axons by diffusible factors derived from cells in distal nerve stumps of transected peripheral nerves. *Brain Res* 1982; **253**; 1–12.

103 Zheng M, Kuffler DP. Guidance of regenerating motor axons in vivo by gradients of diffusible peripheral nerve-derived factors. *J Neurobiol* 2000; **42**, 212–219

104 Raivich G, Kreutzberg GW. Peripheral nerve regeneration: role of growth factors and their receptors. *Int J Dev Neurosci* 1993; **11**: 311–324

105 Baffour R, *et al*. Synergistic effect of basic fibroblast growth factor and methylprednisolone on neurologic function after experimental spinal cord injury. *J Neurosurg* 1995; **83**: 105–110

106 Thompson JA *et al*. Site-directed neovessel formation in vivo. *Science* 1988; **241**: 1349–1352

107 Bregman BS, McAtee M, Dai HD, Kuhn PL. Neurotrophic factors increase axonal growth after spinal cord injury and transplantation in the adult rat. *Exp Neurol* 1997; **148**: 475–494

108 Cheng H, Cao Y, Olson L. Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function. *Science* 1996; **273**: 510–513

109 Guest JD, *et al*. Influence of IN-1 antibody and acidic FGF-Fibrin glue on the response of injured corticospinal tract axons to human Schwann cell grafts. *J Neurosci Res* 1997; **50**: 888–905

110 Xu XM, Guenard V, Kleitman N, et al. A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. *Exp Neurol* 1995; **134**, 261–272

111 Guest JD, Rao A, Olson L, Bunge MB, Bunge R.P. The ability of human Schwann cell grafts to promote regeneration in the transected nude rat spinal cord. *Exp Neurol* 1997; **148**: 502–522

112 Hadlock T, Sundback C, Hunter D, Cheney M, Vacanti JP. A polymer foam conduit seeded with Schwann cells promotes guided peripheral nerve regeneration. *Tissue Eng* 2000; **6**: 119–127

113 Cheng B, Chen Z. Fabricating autologous tissue to engineer artificial nerve. *Microsurgery* 2002; **22**: 133–137

114 Evans GR, Brandt K, Katz S, *et al*. Bioactive poly(L-lactic acid) conduits seeded with Schwann cells for peripheral nerve regeneration. *Biomaterials* 2002; **23**: 841–848

115 Nakahara Y, Gage FH, Tuszynski MH. Grafts of fibroblasts genetically modified to secrete NGF, BDNF, NT-3, or basic FGF elicit differential responses in the adult spinal cord. *Cell Transplantation* 1996; **5**: 191–204

116 Utley DS, Lewin SL, Cheng ET, *et al*. Brain-derived neurotrophic factor and collagen tubulization enhance functional recovery after peripheral nerve transection and repair. *Arch Otolaryngol Head Neck Surg* 1996; **122**: 407–413

117 Cao X, Shoichet MS. Delivering neuroactive molecules from biodegradable microspheres for application in central nervous system disorders. *Biomaterials* 1999; **20**, 329–339

118 Maeda T, Mackinnon SE, Best TJ, Evans PJ, Hunter DA, Midha RT. Regeneration across 'stepping-stone' nerve grafts. *Brain Res* 1993; **618**: 196–202

119 Scherman P, Lundborg G, Kanje M, Dahlin LB. Neural regeneration along longitudinal polyglactin sutures across short and extended defects in the rat sciatic nerve. *J Neurosurg* 2001; **95**: 316–323

120 Tang JB. Vein conduits with interposition of nerve tissue for peripheral nerve defects. *J Reconstr Microsurg* 1995; **11**: 21–26
121 Müller H, Williams LR, Varon S. Nerve regeneration chamber: evaluation of exogenous agents applied by multiple injections. *Brain Res* 1987; **413**: 320–326
122 Strand FL, Kung TT. ACTH accelerates recovery of neuromuscular function following crushing of peripheral nerve. *Peptides* 1980; **1**: 135–138
123 Guenther J, Nick H, Monard D. A glia-derived neurite-promoting factor with protease inhibitory activity. *EMBO J* 1985; **4**: 1963–1966
124 Kilmer SL, Carlsen RC. Forskolin activation of adenylate cyclase in vivo stimulates nerve regeneration. *Nature* 1984; **307**: 455–457
125 Keynes RJ. The effects of pyronin on sprouting and regeneration of mouse motor nerves. *Brain Res* 1982; **253**: 13–18
126 Guenard V, *et al*. Syngeneic Schwann cells derived from adult nerves seeded in semipermeable guidance channels enhance peripheral nerve regeneration. *J Neurosci* 1992; **12**: 3310–3320
127 Wang KK, Nemeth IR, Seckel BR, *et al*. Hyaluronic acid enhances peripheral nerve regeneration in vivo. *Microsurgery* 1998; **18**: 270–275
128 Luo Y, Dalton PD, Shoichet MS. Investigating the properties of novel poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) hydrogel hollow fiber membranes. *Chem Mater* 2001; **13**: 4087–4093
129 Belkas JS, *et al*. Peripheral nerve regeneration through a synthetic hydrogel nerve tube. 2003; in preparation
130 Lundborg G, Dahlin LB, Danielsen N. Ulnar nerve repair by the silicone chamber technique. Case report. *Scand J Plast Reconstr Surg Hand Surg* 1991; **25**: 79–82
131 Lundborg G, Rosen B, Abrahamson SO, Dahlin L, Danielsen N. Tubular repair of the median nerve in the human forearm. Preliminary findings. *J Hand Surg [Br]* 1994; **19**: 273–276
132 Merle M, Dellon AL, Campbell JN, Chang PS. Complications from silicon-polymer intubulation of nerves. *Microsurgery* 1989; **10**: 130–133
133 Stanec S, Stanec Z. Reconstruction of upper-extremity peripheral nerve injuries with ePTFE conduits. *J Reconstr Microsurg* 1998; **14**: 227–232
134 Mackinnon SE, Dellon AL. Clinical nerve reconstruction with a bioabsorbable polyglycolic acid tube. *Plast Reconstr Surg* 1990; **85**: 419–424
135 Weber RA, Breidenbach WC, Brown RE, et al. A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. *Plast Reconstr Surg* 2000; **106**: 1036–1045
136 Krarup C, Archibald SJ, Madison RD. Factors that influence peripheral nerve regeneration: an electrophysiological study of the monkey median nerve. *Ann. Neurol* 2002; **51**: 69–81
137 Meek MF, Coert JH. Clinical use of nerve conduits in peripheral-nerve repair: review of the literature. *J Reconstr Microsurg* 2002; **18**: 97–109

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