POLYMERIC BIOMATERIALS FOR NERVE REGENERATION

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I. Introduction

Each year, over 10,000 Americans sustain spinal cord injuries (Teng *et al*., 2002). In addition, numerous surgeries are performed every year to try to repair peripheral nerve damage (Miller *et al*., 2001a). Central nervous system repair is impeded partly by myelin-associated inhibitors and if the axons can traverse the injury site, there is a possibility of regrowth in the unscarred areas and of functional recovery (Teng *et al*., 2002). Grafting is a common approach to facilitate peripheral nerve regeneration to provide guidance to the regenerating axons. Grafting methods include autografts and allografts (Ide *et al*., 1983; Keeley *et al*., 1993; Wang *et al*., 1992). However, a major drawback of autografts is that they partially deinnervate the donor site to repair the injury site. Problems with allografts include tissue rejection and lack of donor tissue. These problems could eventually

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be minimized by tissue engineered nerve grafts based on polymers for transplantation and alternative methods to engineer an artificial environment to mimic the physical and chemical stimulus that promotes nerve regeneration.

Polymers are being extensively investigated to help facilitate nerve regeneration (Bellamkonda and Aebischer, 1994). Entubulization methods involving polymers where a conduit is used to connect the nerve endings has great potential as a repair method for peripheral nerve regeneration. The conduit allows for neurotropic and neurotrophic communication between the nerve stumps and also provides physical guidance for the regenerating axons similar to the grafts (Fig. 1) (Heath and Rutkowski, 1998). The closely fitting tubes facilitate axonal regeneration by inducing rapid development of a highly organized capsule that isolates the repair site and guides and aligns endoneurial components (Stensaas *et al.*, 1989). Entubulization minimizes unregulated axonal growth at the site of injury by providing a distinct environment, and allows for trophic factors emitted from the distal stump to reach the proximal segment, which enhances physiological conditions for nerve regeneration. The spatial cues also induce a change in tissue architecture, with the cabling of cells within the microconduit (Pearson *et al.*, 2003). The conduits can also be environmentally enhanced with chemical stimulants like laminin and nerve growth factor (NGF), biological cues such as from Schwann cells and astrocytes, the satellite cells of the peripheral and central nervous systems, and lastly, physical guidance cues.

Another area where polymers are making a significant difference in nerve regeneration is the use of polymers to encapsulate and release trophic factors, or encapsulate cells that release nerve growth factor or other agents that enhance the regeneration process. This chapter details the advances in the use of polymeric biomaterials that have been explored for nerve regeneration in the peripheral and central nervous systems.

II. Polymers for Regeneration in the Peripheral Nervous System

Current polymeric entubulization repair methods for peripheral nerve regeneration use various nondegradable and biodegradable materials. The most common nondegradable material investigated has been silicone rubber. Medical grade silicone rubber, polydimethylsiloxane, maintains its shape and can be filled with neurotrophic factors or extracellular

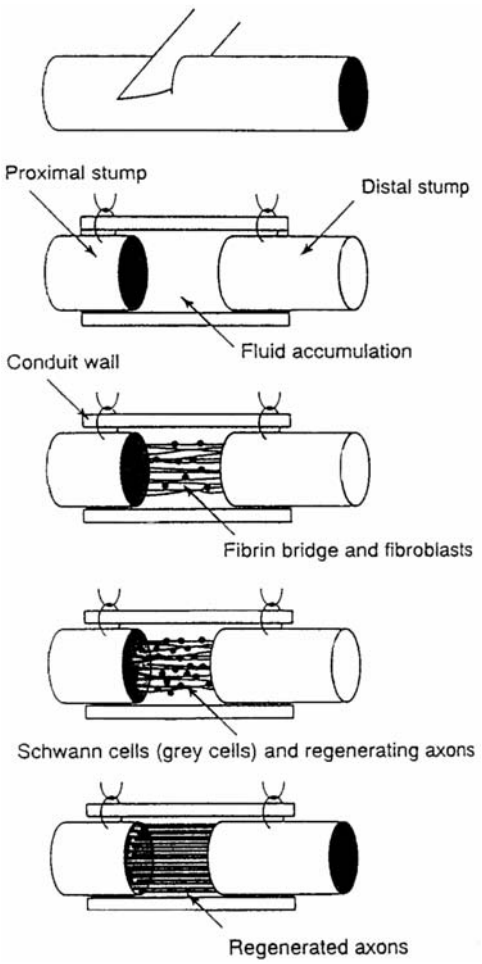

FIG. 1. Silicone-chamber model showing the progression of events during peripheral-nerve regeneration. After bridging the proximal and distal nerve stumps, the silicone tube becomes filled with serum and other extracellular fluids. A fibrin bridge containing a variety of cell types connects the two stumps. Schwann cells and axons processes migrate from the proximal end to the distal stump along the bridge. The axons continue to regenerate through the distal stump to their final contacts. [Reproduced with permission from Heath and Rutkowski (1998).]

matrix (ECM) components. The silicone tubes provide an impermeable conduit for endoneurial fluid that creates an environment favoring the regeneration of axons and Schwann cells. Unfortunately, the nerve guide remains in the body and can cause a chronic immune response.

A second surgery is required after regeneration to prevent secondary nerve injury due to compression (den Dunnen *et al.*, 1996a).

Biodegradable materials such as collagen, poly(glycolic acid) and poly(lactic acid) have been used to make conduits for entubulization repairs. These polymers completely degrade in the body eliminating the need for a second surgery. One problem with some biodegradable polymers is that they tend to swell and can deform causing compression to the regenerating nerve (den Dunnen *et al.*, 1996b; Henry *et al.*, 1985).

A. Nondegradable Polymers for Entubulization

Silicone has been one of the most commonly investigated nondegradable polymer for nerve entubulization, especially for peripheral nerve regeneration in animal models (Wang-Bennett and Coker, 1990). Some of the earliest studies have shown that silicone tubes sutured to the proximal and distal ends of a severed nerve can promote directed growth (Seckel *et al.*, 1986) and regeneration to bridge a 6–10 mm gap in the nerve (Luo and Lu, 1996). However, in human studies with a small 3–4 mm gap between nerve endings, the silicone tubes did not exhibit a significant improvement in regeneration compared to conventional microsurgical repair of the nerve trunk (Lundborg *et al.*, 1997).

1. Chemical Cues

The fluid filling these cylindrical chambers was found to contain trophic factors (Lundborg *et al.*, 1982a,b,c), but local application of the accumulated fluid to silicone chambers at the time of implantation did not have any significant effect on nerve elongation rates (Sebille and Becker, 1988). One of the components of the fluid, fibrin, was injected into silicone chambers prior to implantation and was found to enhance regeneration in the short-term, but no difference was observed after five weeks (Chen, 1992). However, filling the silicone chambers with a resorbable fibrin sponge matrix was found to enable axons to bridge a 10-mm gap in a sciatic nerve transection within 14 days under appropriate substrate conditions (Dubovy and Bednarova, 1996). Similarly, filling the silicone compartment with a collagen gel prior to implantation led to more rapid and directed outgrowth of sprouting axons towards the distal side, but led to fewer fibroblasts and Schwann cells (Satou *et al.*, 1986). An extracellular gel containing collagen, laminin, and fibronectin was filled into silicone, tubes to enhance regeneration and both qualitative and quantitative histology of the regenerated nerves revealed a more mature ultrastructural organization with larger cross-sectional area and higher number of

myelinated axons in the combination gel group than the controls (Chen *et al.*, 2000). Similar results were observed with polyethylene tubes filled with pure collagen or collagen–NGF mixtures (Da-Silva and Langone, 1989). Relative adhesiveness of various chemically modified substrates was however not a good predictor of the rate of axon growth or the degree of axon fasciculation (Lemmon *et al.*, 1992). However, a conduit made of a perm-selective material such as an acrylic copolymer that allows solute transport was found to promote much better peripheral nerve regeneration compared to an impermeable silicone conduit (Aebischer *et al.*, 1988). Gore-Tex has also been explored as a conduit material with limited success (Young *et al.*, 1984).

Various chemical cues have been used in conjunction with the silicone chambers to accelerate regeneration (Liu, 1996). Using a nitrocellulose strip soaked in fibroblast growth factor (FGF) to partition the silicone chamber into two compartments was found to lead to faster migration of all cellular elements including perineurial-like cells, vasculature, and Schwann cells and revealed that two separate nerve structures had formed, one on either side of the partition (Danielsen *et al.*, 1988a,b). Nerve growth factor added to silicone chambers has also been shown to promote regeneration in the short-term of facial and sciatic nerves (Chen *et al.*, 1989; Rich *et al.*, 1989). However, over 10 weeks, ultrastructural analysis demonstrated no difference in the distribution of axonal diameters or myelin thickness between the regenerated groups, and there was essentially complete regeneration of both the NGF and control regenerative groups demonstrating that NGF provides an early but limited neurotrophic effect on nerve regeneration (Hollowell *et al.*, 1990; Spector *et al.*, 1993). This could be due to the short half-life of NGF *in vivo*. Use of silicone chambers filled with thyroid hormones have also been found to significantly increase the number, the mean diameter of myelinated axons, and the thickness of myelin sheaths compared to control samples, even after eight weeks of peripheral nerve regeneration (Voinesco *et al.*, 1998).

2. *Electrical Cues*

Electrical stimuli have also been investigated to promote neurite outgrowth. Neurons cultured on electrically charged piezoelectric polymers such as poly(vinylidene fluoride) exhibited significantly greater levels of process outgrowth and neurite lengths than the control samples (Valentini *et al.*, 1992). An electrically conductive polymer, oxidized polypyrrole, was found to significantly enhance neurite outgrowth of PC12 cells when they were subjected to an electrical stimulus (Schmidt *et al.*, 1997; Shastri *et al.*, 1997).

3. *Directed Growth*

Several methods have been developed to control neuronal outgrowth on substrates (Buettner, 1996; Clark, 1996; Tessier-Lavigne, 1994). Oriented growth of the axons was promoted by filling silicone tubes with longitudinal fibers made of nonabsorbable polyamide or absorbable materials such as polyglactin and catgut. No axons were found growing in direct contact with the filaments and the filaments did not seem to disturb axonal growth across the tube (Terada *et al.*, 1997). Silicone tubes prefilled with oriented collagen and laminin gels produced by gravitational or magnetic forces were found to increase the success and the quality of regeneration in long nerve gaps. The laminin gel was found to perform better than the collagen gel in promoting sciatic nerve regeneration (Verdu *et al.*, 2002). Silicone tubes were also filled with collagen or poly(lactide) fibers to enhance regeneration (Itoh *et al.*, 2001). To avoid problems associated with removing a nondegradable material after nerve regeneration, silicone tubes were used to create pseudo nerves, which contain longitudinal Schwann cell columns without axons and surrounded by perineurium-like tissue (Zhao *et al.*, 1992). These pseudo-nerves were applied as a graft to repair nerve defects in rats and were found to induce nerve repair to a similar extent as a real graft (Zhao *et al.*, 1997). Alternative approaches involve the use of degradable materials as conduits for nerve regeneration, as discussed in the following section.

B. Degradable Polymers for Entubulization

In order to avoid common problems associated with nondegradable polymeric conduits such as chronic immune response and a second surgery to prevent nerve injury due to compression, degradable polymers have been investigated extensively for entubulization applications. The degradable polymers can be either synthetic or biological. The most commonly used synthetic degradable polymers for entubulization have been poly(L-lactide), poly(glycolide), and their copolymers (Schugens *et al.*, 1996; Widmer *et al.*, 1998) and the most commonly used biological polymer has been collagen (Tong *et al.*, 1994). The biological polymers such as collagen have also been used extensively in conjunction with the synthetic biodegradable as well as nondegradable polymers.

When using synthetic polymers, the use of copolymers enables tailoring of the degradation rates to periods varying from a few days to a few months. The degradation of the polymers eliminates the need for a second surgery after the regeneration is complete. The degradation products are usually biocompatible and have been found to cause no

adverse reactions: however, in some cases where synthetic polymers are used, there is a local drop in pH inside the conduit due to acidic degradation products. But studies with poly(lactic acid) devices affixed to divided nerves have shown that even though there are significant amounts of degradation products formed, there was no adverse reaction to the biodegradable substance or its metabolites (de Medinaceli *et al.*, 1995). The natural polymers suffer from disadvantages associated with increase immunogenicities compared to synthetic polymers (Navarro *et al.*, 1996), and variability based on source.

Polyglactin mesh tubes were used to bridge defects 7–9 mm in length and were found to reduce formation of neuromas and growth of scar tissue from surrounding structures (Molander *et al.*, 1982). Copolymers of lactic and glycolic acid were used to bridge peripheral nerve transections (Nyilas *et al.*, 1983) and the local environment was manipulated further by the addition of the proteins collagen, fibrinogen, and anti-Thy-1 antibody to the nerve guide lumens at the time of operation (Madison *et al.*, 1984). The results for poly(L-lactide) (PLLA) were significantly improved over those for 75:25 poly(DL-lactic-*co*-glycolic acid) (PLGA) and suggest that PLLA porous conduits may serve as a better scaffold for peripheral nerve regeneration (Evans *et al.*, 1999). However, longer evaluation of polymer degradation is warranted (Evans *et al.*, 2000). Laminin-containing gels filled in the bioresorbable conduits enabled improved initial regeneration compared to empty conduits (Madison *et al.*, 1985) at two weeks, but was found to be inhibitory at six weeks (Madison *et al.*, 1987). For PLGA nerve guides, studies revealed a reduction in the total axon count and the number of myelinated axons in the presence of exogenously added Schwann cells compared to saline controls. In contrast, the addition of glial growth factor (GGF) alone enhanced the total number of axons and significantly increased the number of blood vessels. Although combining GGF with Schwann cells negated this effect, this combination resulted in the highest myelination index and the fastest conduction velocities recorded, demonstrating a potential role for GGF in nerve regeneration (Bryan *et al.*, 2000). These results can be explained by a simple reaction–diffusion model that was developed to describe the mass transport of nutrients and nerve growth factor within a bioartificial nerve graft (Rutkowski and Heath, 2002b). The results suggest that at higher porosities, more growth factors diffuse out of the conduit, while at low porosities there is competition for nutrients. Increasing the Schwann cell seeding density enhances growth but also leads to an increase in the number of axons along the length of the conduit. This is indicative of branching of the axons, which requires additional resources to maintain and can lead to painful neuroma formation (Rutkowski and Heath, 2002a).

Biodegradable conduits made of poly(L-lactide-*co*-epsilon-caprolactone) (PLC) polymers have been evaluated long-term and found to enable sciatic nerve regeneration with very mild foreign body reaction for two years (den Dunnen *et al*., 1993; Nicoli Aldini *et al*., 1996; Perego *et al*., 1994). The diameter of the nerve guide was seen to have a significant role as nerve guides with smaller lumens showed nerve compression due to a pronounced swelling of the degrading tube and increased foreign body reaction (den Dunnen *et al*., 1995; Meek *et al*., 1997, 1999). To minimize diffusion limitations, a nerve guide comprised of an inner microporous layer of PLC (pore size 0.5–1 μm) and an outer microporous layer (pore size 30–70 μm) of polyurethane/poly(L-lactide) mixture was investigated (Hoppen *et al*., 1990). These conduits were filled with denatured muscle tissue in order to enable slightly faster regeneration than the empty conduits, and also enable regeneration across longer nerve gaps of 15-mm (Meek *et al*., 1996, 2001). Syngeneic, isogeneic, and autologous Schwann cells were suspended in Matrigel and seeded in permeable PLC guides. Transplants of autologous Schwann cells resulted in slightly lower levels of reinnervation than autografts, but higher recovery and number of regenerated fibers reaching the distal nerve than transplants of isologous and syngeneic Schwann cells, although most of the differences were not statistically significant (Rodriguez *et al*., 2000). The main problem with PLC nerve conduits is their propensity to swell, especially in the first three months, and that can have a negative influence on the regenerating nerve. Therefore, PLC nerve guides are most suited for clinical situations involving short nerve gaps in small nerves (den Dunnen *et al*., 2000).

Poly-3-hydroxybutyrate (PHB), a polymer obtained from bacterial sources, has also shown promise in peripheral nerve regeneration as a conduit material and demonstrated good regeneration in comparison with nerve grafts (Hazari *et al*., 1999b; Young *et al*., 2002). Transected radial nerves were also wrapped in PHB sheets to promote regeneration (Hazari *et al*., 1999a). Polymers made of noncrystallizable blocks of poly(glycolide-*co*-(epsilon-caprolactone))-diol and crystallizable blocks of poly((R)-3-hydroxybutyric acid-*co*-(R)-3-hydroxyvaleric acid)-diol were used to fabricate nerve conduits since they have elastomeric properties and their degradation rates can be modulated. These conduits were seen to promote sciatic nerve regeneration in the majority of the cases (Borkenhagen *et al*., 1998). Polyphosphazenes have also demonstrated success similar to those seen with PLLA and PLC conduits in peripheral nerve regeneration (Aldini *et al*., 1999; Nicoli Aldini *et al*., 2000). Poly(phospho esters) have been found to promote peripheral nerve regeneration and have advantages over some other biodegradable polymers due to their lack of swelling and no crystallization after implantation.

However, the mechanical strength of the polymers tested was lacking since tube fragmentation and even breakage was observed less than five days after implantation (Wang *et al*., 2001).

Enhancement of regeneration was observed following subcutaneous priming of bioresorbable PLGA guides *in vivo*. Four weeks after nerve reconstruction, regeneration of the peripheral nerve through the cell-infiltrated guides displayed a significant increase in the total axon number and myelination status recorded in primed over unprimed guides, demonstrating the importance of cell-mediated events in the regeneration process. Factors capable of eliciting Schwann-cell migration were identified, providing a rationale for selection and use of exogenous factors for the enhancement of peripheral-nerve regeneration (Bryan *et al*., 2003).

The ECM protein laminin was covalently coupled to agarose hydrogels and was found to significantly enhance neurite extension from three-dimensionally cultured embryonic chick dorsal root ganglia and PC12 cells (Yu *et al*., 1999). However, fibronectin was shown to induce greater Schwann cell proliferation than laminin, making it a potentially important component of nerve guidance channels (Chafik *et al*., 2003). Sciatic nerve regeneration was facilitated by laminin–fibronectin double coated biodegradable collagen grafts in rats (Tong *et al*., 1994).

1. Localized and Directed Cell Placement and Growth

Several methods have been developed to control positioning and directed growth of cells on biodegradable polymeric substrates. Axonal guidance has also been achieved using diffusible repellants and attractants (Cao and Shoichet, 2001; Tessier-Lavigne, 1994). In order to provide better control of interactions between scaffolds and cells, poly(lactic acid-*co*-amino acid) graft copolymers with poly(lysine), poly(aspartic acid), and poly(alanine) side chains of varying lengths were synthesized (Harkach *et al*., 1996). A tubular nerve guidance conduit possessing the macroarchitecture of a polyfascicular peripheral nerve was created. Methods of generating micron-scale patterns of any biotinylated ligand on the surface of a biodegradable polylactide-poly(ethylene glycol) block copolymers were developed. These were used to obtain spatial control over nerve cells (Patel *et al*., 1998; Shakesheff *et al*., 1999).

Control of axonal growth was also achieved by forming two-dimensional adherent patterns of collagen. The axons grew along the collagen-adsorbed pathways and completed the honeycomb-like patterning, as designed (Matsuda *et al*., 1992). Following implantation of any absorbable device there occurs a proliferation of fibrous tissue, which along

with material from the degrading implant forms a composite membranous structure called a neomembrane. Neomembranes have been proposed as potential candidates that can be used for guiding tissue regeneration, including nerve regeneration (Ashammakhi, 1996). Tubular nerve guidance conduits possessing the macroarchitecture of a polyfascicular peripheral nerve were created using a dip coating method and preseeded with Schwann cells to enable more robust and more precisely directed nerve regeneration (Hadlock *et al.*, 1998). A biohybrid nerve guide containing fibers or microfilaments of poly(lactic acid) (Ngo *et al.*, 2003) coated with Schwann cells (Steuer *et al.*, 1999) or laminin (Rangappa *et al.*, 2000) were fabricated and shown to greatly improve nerve regeneration.

A magnetically aligned collagen gel filling a collagen nerve guide was found to enhance peripheral-nerve regeneration (Ceballos *et al.*, 1999; Dubey *et al.*, 1996). Collagen conduits, filled with collagen fibers or sheets to provide guided nerve regeneration, were effective in providing a scaffold for regenerating nerve tissue (Itoh *et al.*, 2001). Two types of pore structures in PLLA foams—oriented or interconnected pores, were produced depending on the mechanism of phase separation. Microscopic observations of the cells seeded onto the polymer foams showed that the interconnected pore networks were more favorable to cell attachment than the anisotropic ones. In the oriented pores, abundant cell migration was observed at the outer surface of the polymer implant, but not within the macrotubes (Maquet *et al.*, 2000).

To address this issue of directional growth, micropatterned biodegradable polymer films were fabricated and inserted on the inside of poly(D,L-lactide) (PDLA) conduits. The micropatterned surfaces were preseeded with Schwann cells in order to provide guidance to axons at the cellular level. Over 95% alignment of the axons and Schwann cells was observed on the micropatterned surfaces with laminin selectively attached to the microgrooves (Miller *et al.*, 2002). Acceleration of neurite extension from rat dorsal root ganglia was also observed within micron-scale tubes in the range of 200 to 635 microns. Within these hydrogel-filled conduits, neurites were observed to extend more rapidly than when cultured within the hydrogel alone and a change in tissue architecture was observed with the cabling of cells within the microconduit (Pearson *et al.*, 2003). Various fabrication techniques have been used for these biodegradable polymer conduits as described in the following section.

2. Fabrication Methods

A common method of fabricating porous conduits is to use a combined solvent casting (Luciano *et al.*, 2000; Widmer *et al.*, 1998) and

extrusion technique involving salt mixed in with the polymer. The salt is then leached out leaving a conduit with an open ended pore structure. Using this method, PLGA and PLLA conduits were fabricated and the modulus and failure strength of PLLA conduits were approximately 10 times higher than those of PLGA conduits (Widmer *et al.*, 1998).

Tubes of poly-L-lactic acid or polylactic-*co*-glycolic acid copolymer were formed using a dip-molding technique and were created containing 1, 2, 4, or 5 sublumina, or "fascicular analogs" (Hadlock *et al.*, 1998). Liquid–liquid phase separation of solutions of amorphous PDLA and semicrystalline PLLA in solvent mixtures was used to produce a porous scaffold for cell transplantation. Freeze–drying of phase-separated polylactide solutions was found to produce flexible and tough foams with an isotropic morphology. Interconnected pores of 1–10 microns in diameter resulted from the spinodal decomposition of the polylactide solutions with formation of co-continuous phases (Schugens *et al.*, 1996). A thermally induced polymer–solvent phase separation was used to create PLLA foams with two types of pore structures—oriented or interconnected pores, depending on the mechanism of phase separation, which in turn depends on the thermodynamics of the polymer–solvent pair (Maquet *et al.*, 2000).

Poly(phosphoester) conduits were fabricated by immersing mandrels coated with a solution of the polymer in chloroform into nonsolvent immersion baths, followed by freeze or vacuum–drying (Wan *et al.*, 2001) (Fig. 2). To control micropositioning of neural cells and guidance of extending axons in a given region, a novel surface photoprocessing method was developed. Ultraviolet irradiation with the use of a photomask placed on a substrate hydrophilically modified the irradiated regions. Collagen was adsorbed only on the nonirradiated hydrophobic portions to guide axonal growth (Matsuda *et al.*, 1992). Microcontact printing techniques have also been developed to attach protein selectively to certain regions of the substrate (James and Davis, 2000). A novel transfer patterning method was developed to fabricate biodegradable films with microgrooves with laminin selectively adsorbed to the grooves. A combination of photolithography, reactive ion etching, solvent casting, and surface-tension-based techniques were used to fabricate these films to provide guidance to axons at the cellular level (Miller *et al.*, 2001a,b, 2002) (Fig. 3).

C. Comparative Studies

In order to compare the relative performance of various types of conduit materials, several studies have been conducted as described in this section.

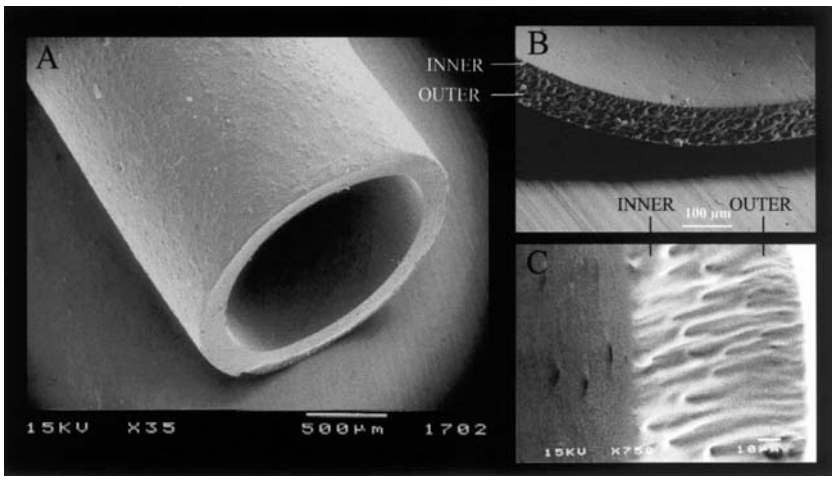

FIG. 2. (A) Nerve guide conduit from the poly(phosphoester), poly(bis(hydroxyethyl) terepthalate-ethyl ortho-phosphate/terephthaloyl chloride) (M_w: 14,900, solution concentration: 34% (w/w), immersion bath: water, drying method: freeze–drying); (B) cross-section of conduit showing distinct coating layers; (C) cross-section of conduit with finger-like cavities. [Reproduced with permission from Wan *et al.* (2001).]

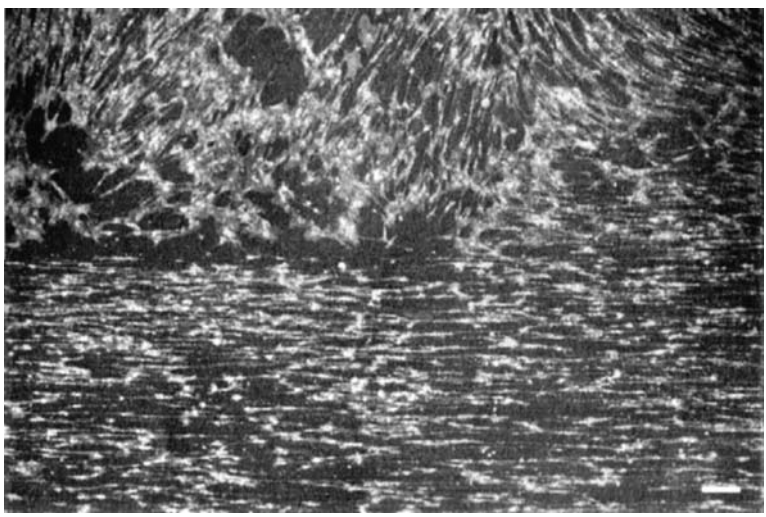

FIG. 3. Half smooth (top) and half 10/20/3 µm patterned laminin coated (200 µg/mL) PDLA solvent-cast substrate with DiI-labeled neurons and Schwann cells showing nonaligned and aligned cells, respectively. Bar = 50 µm. [Reproduced with permission from Miller *et al.* (2001a).]

Silicone tubes prefilled with NGF were compared to autologous nerve grafts in their ability to bridge an 8-mm nerve gap in rabbit facial nerves. The number of regenerating myelinated axons in the autologous nerve grafts at five weeks was significantly greater than the number of myelinated axons in the silicone tubes. But in the nerve grafts, majority of the axons were found in the extrafascicular connective tissue and did not find their way into the distal nerve stump. Thus, functional recovery of autologous nerve graft repairs may not be superior to that of entubulization repairs (Spector *et al.*, 1995).

Comparative studies of biodegradable conduits and silicone conduits have been conducted with various polymers. Peripheral nerve repair using degradable poly(organo)phosphazene tubular conduits was found to be enhanced compared to the regenerated nerve fiber bundle obtained with the silicone conduits (Langone *et al.*, 1995). Recordings of compound muscle action potential (CMAP) after sciatic nerve regeneration was found to be similar with autografts and with PLC tubes but lower with silicone tubes. The number of neurons with multiple projections was also lower in autografts and PLC tubes compared to silicone tubes, thereby indicating that the PLC tubes and superior to silicone tubes for peripheral nerve regeneration (Navarro *et al.*, 1996; Valero-Cabre *et al.*, 2001). However, a study designed to compare regeneration of rat peroneal nerve across a 0.5-cm gap repaired with a sutured autograft versus an artificial nerve graft of PGA filled with collagen showed using electrophysiological analyses that the axonal regeneration was statistically inferior to that in the autograft (Rosen *et al.*, 1989).

Schwann cells incorporated in a collagen matrix and injected into PLLA conduits were found to demonstrate comparable SFI values compared to isograft controls, but showed a statistically lower number of axons for both the high and low density Schwann cells groups and the collagen samples compared to the isograft controls (Evans *et al.*, 2002). These results can be explained by a simple reaction diffusion model (Rutkowski and Heath, 2002b) described earlier.

A comparative study conducted with two different types of conduits, one biological, obtained with homologous glutaraldehyde preserved vein segments and the other synthetic bioabsorbable, made with PLC, were evaluated as guides for nerve repair. Nerve regeneration was effective with both conduits, but the count of myelinated axons showed a significant difference between the synthetic and biological tubes. Synthetic conduits were found to be better than those obtained with preserved vein segments for peripheral nerve reconstruction (Giardino *et al.*, 1995).

Poly(L-lactide-*co*-epsilon-caprolactone) (PLC) nerve guides were found to yield faster and higher levels of nerve innervation compared to conduits

made of collagen, silicone, or teflon. Resorbable tubes promoted regeneration in a higher proportion of mice than durable tubes. There was only minimal inflammatory reaction within the remnants of collagen tubes, but not in the other materials (Navarro *et al.*, 1996). PLC nerve guides were found to be superior to autologous nerve grafts in enabling faster as well as qualitatively better nerve regeneration to bridge a 1-cm gap (den Dunnen *et al.*, 1996a,b). In another study, the highest number of regenerated myelinated fibers at mid tube and distal nerve were found in highly permeable PLC guides. Impermeable PLC guides allowed slightly worse levels of regeneration, while low-permeable PLC guides promoted neuroma and limited distal regeneration. The lowest number of regenerated fibers were found in permanent polysulfone tubes (Rodriguez *et al.*, 1999). Therefore, resorbable permeable polymeric nerve guides with chemical cues seem to show the best potential for peripheral nerve regeneration.

III. Polymers for Regeneration in the Central Nervous Systems

In addition to the use of polymeric conduits for peripheral nerve regeneration, polymers, specifically hydrogels, have been used extensively to help rectify central nervous system disorders and promote regeneration. Oriented growth has been achieved not just in the peripheral nervous system, but also in the central nervous system using silicone tubes. Electrically mediated guidance of axonal growth has also been achieved using silicone tubes containing a cathodal electrode (Fig. 4). A robust regeneration of spinal cord axons into the tube was observed in more than half the cases with the imposed electric field, while there were rarely any axons found in the control guidance channels (Borgens, 1999).

Nitrocellulose implants treated with biological materials were introduced into neonatal rat spinal cords before the arrival of corticospinal tract (CST) axons. Implants with living cells from spinal cord primary cultures and acellular implants coated with laminin supported the adhesion and growth of CST axons. This suggests that laminin or some other adhesive factor produced by immature neuroglial cells may be normally involved in CST axon growth and guidance (Schreyer and Jones, 1987). Nerve growth factor (NGF)-treated nitrocellulose implants were used to promote growth across a complete transection lesion of a rat spinal cord and were found to be significantly better than the nontreated implants at promoting regrowth (Houle and Ziegler, 1994).

Solid fetal spinal cord tissue seeded into semipermeable mini guidance channels and implanted into a lesion site in the rat adult spinal cord

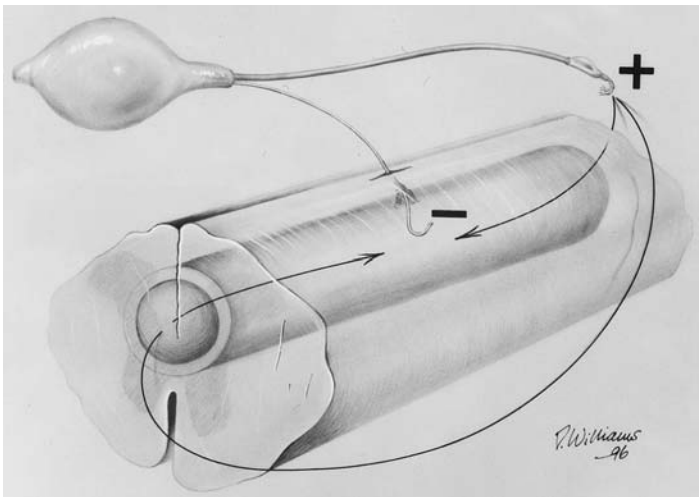

FIG. 4. Artist's drawing of the stimulator, silicone rubber tube or guidance channel, and the electrical circuit within the spinal cord. The tube was implanted into the dorsal spinal cord. The uninsulated tip of the cathodal electrode (negative) was sealed within the center of the tube, while the anodal electrode (positive) remained outside the vertebral column, sutured to paravertebral musculature. The body of the stimulator was surgically placed within the fat pad at the base of the guinea-pig's neck. To complete a circuit, current must flow initially into each end of the hollow tube as diagrammed. For diagrammatic purposes, the drawing is not made to scale. [Reproduced with permission from Borgens (1999).]

was found to serve as a permissive bridge for longitudinally directed axonal growth (Bamber *et al.*, 1999). Instead of autografts, a dual scaffold structure made of biodegradable polymers and seeded with neural stem cells was developed to address the issues of spinal cord injury. Unique biodegradable polymer scaffolds were fabricated where the general design of the scaffold was derived from the structure of the spinal cord with an outer section that mimics the white matter with long axial pores to provide axonal guidance and an inner section seeded with neural stem cells for cell replacement and mimic the general character of the gray matter (Lavik *et al.*, 2001). The seeded scaffold led to improved functional recovery as compared with the lesion control or cells alone following spinal cord injury. Implantation of the scaffold-neural stem cells unit into an adult rat hemisection model of spinal cord injury promoted long-term improvement in function (persistent for one year in some animals) relative to a lesion-control group (Teng *et al.*, 2002) (Fig. 5).

Poly(alpha-hydroxy acids) with seeded Schwann cells or Schwann cell grafts were also found to be effective candidates for spinal cord

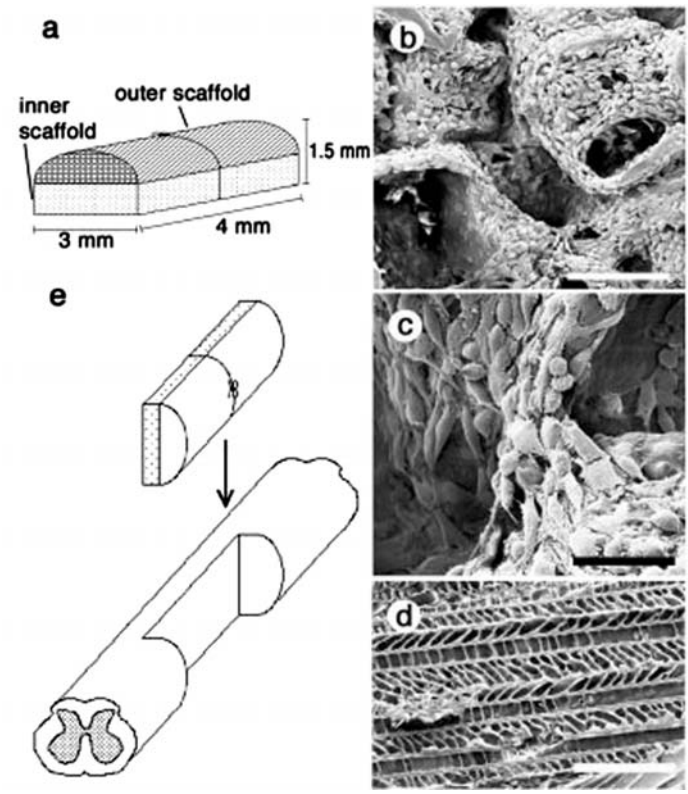

FIG. 5. (*a*) Schematic of the scaffold design showing the inner and outer scaffolds. (*b* and *c*) Inner scaffolds seeded with NSCs. (Scale bars: 200 μm and 50 μm, respectively.) The outer section of the scaffold was created by means of a solid–liquid phase separation technique that produced long, axially oriented pores for axonal guidance as well as radial pores to allow fluid transport and inhibit the ingrowth of scar tissue (*d*; scale bar, 100 μm). (*e*) Schematic of surgical insertion of the implant into the spinal cord. [Reproduced with permission from Teng *et al.* (2002).]

regeneration (Gautier *et al.*, 1998; Oudega *et al.*, 2001). PDLLA mixed with poly(ethylene oxide)-block-poly(D,L-lactide) (PELA) copolymers were made into foams by freeze drying and the ability of these foams to be integrated and to promote tissue repair and axonal regeneration in transected rat spinal cords was investigated. The polymer construct was able to bridge the cord stumps by forming a permissive support for cellular migration, angiogenesis, and axonal regrowth (Maquet *et al.*, 2001). Unsintered hydroxyapatite/poly-L-lactide (u-HA/PLLA) composite films were found to have good biocompatibility, osteoconductivity, and a

fast primary degradation rate, with potential application to spinal surgery (Matsumoto *et al.*, 2002).

Synthetic hydrogels have been used to serve as artificial matrices for neural tissue reconstruction, for the delivery of cells and for the promotion of axonal regeneration required for successful neurotransplantation. Cultured neurons were found to attach to hydrogel substrates prepared from poly(2-hydroxyethylmethacrylate) (PHEMA) but grow few nerve fibers unless fibronectin, collagen, or nerve growth factor was incorporated into the hydrogel. This provides a mechanism to provide controlled growth on hydrogel surfaces (Carbonetto *et al.*, 1982). Ionic poly(glyceryl methacrylate) p(GMA)–collagen hydrogels containing polar chemical groups, either basic amino groups or acidic carboxyl groups, were evaluated after long-term implantation in the cerebral cortex. This approach was found to be a new avenue to modulate the brain scar formation (Woerly *et al.*, 1992). Hydrogels have been created with bioactive characteristics for neural cell adhesion and growth (Woerly, 1993). Arg–Gly–Asp peptides (RGD) were synthesized and chemically coupled to the bulk of poly(*N*-(2-hydroxypropyl) methacrylamide) (PHPMA) based polymer hydrogels. These RGD-grafted polymers implanted into the striata of rat brains promoted and supported the growth and spread of glial tissue onto and into the hydrogels (Woerly *et al.*, 1995). Cultured Schwann cells, neonatal astrocytes or cells dissociated from embryonic cerebral hemispheres were also dispersed within PHPMA hydrogel matrices and found to promote cellular ingrowth *in vivo* (Woerly *et al.*, 1996). These polymer hydrogel matrices were found to have neuroinductive and neuroconductive properties and the potential to repair tissue defects in the central nervous system by promoting the formation of a tissue matrix and axonal growth by replacing the lost of tissue (Woerly *et al.*, 1998, 1999, 2001a,b). Biocompatible porous PHMPA hydrogels (NeuroGels) were used to provide a permissive environment across a 3-mm gap in cat spinal cord to promote regeneration. Results indicated that functional deficit, as assessed by treadmill training, and morphological changes following double transection of the spinal cord can be modified by the implantation of NeuroGel. Most of the regenerating axons were found to be myelinated and were found to have grown at least 12-mm into the dorsal cord tissue 15 months after surgery (Woerly *et al.*, 2001a,c).

The mechanical properties of the substrate were found to have a significant influence on neurite behavior. Neurons grown on softer substrates formed more than three times as many branches as those grown on stiffer gels (Flanagan *et al.*, 2002). PHEMA hydrogels, coated with collagen and

infiltrated *in vitro* with cultured Schwann cells, were implanted into lesioned optic tracts to act as prosthetic bridges to promote axonal regeneration (Lesny *et al*., 2002; Plant *et al*., 1995, 1998). Collagen impregnated PHEMA sponges were found to provide a safe supportive material for regenerating spinal cord axons (Giannetti *et al*., 2001). Hydrogel tubes of poly(2-hydroxyethyl methacrylate-*co*-methyl methacrylate) (p(HEMA-*co*-MMA)) were investigated as potential nerve guidance channels in the central nervous system since their mechanical properties were similar to those of the spinal cord, where they were implanted (Dalton *et al*., 2002). Hydrogel tubes of (p(HEMA-*co*-MMA)) were made by liquid–liquid centrifugal casting to yield tubes that were soft and flexible, consisting of a gel-like outer layer, and an interconnected macroporous, inner layer, and with mechanical properties similar to that of spinal cord (Dalton *et al*., 2002). Photochemical methods were developed to bind NGF to microporous PHEMA hydrogels to build stable concentration gradients and control cell growth (Kapur and Shoichet, 2003).

Poly-3-hydroxybutyrate (PHB) fibers coated with alginate gels and fibronectin were implanted into lesion cavities after cervical spinal cord injury in rats. Implantation of the PHB graft reduced cell loss by 50%, a rescuing effect similar to that obtained after treatment with brain-derived neurotrophic factor or neurotrophin-3 (NT-3). In the absence of PHB support, implants of only alginate hydrogel or fibronectin, or their combination had no effect on neuronal survival. After addition of neonatal Schwann cells to the PHB graft, regenerating axons were seen to enter the graft from both ends and to extend along its entire length (Mosahebi *et al*., 2003; Novikov *et al*., 2002).

Even though early success has been demonstrated with the use of polymers for peripheral and central nerve regeneration, it can be enhanced further by providing site-specific release of growth factors and other chemical cues to the regenerating axons, as described in the next section.

IV. Polymers for Controlled Release

Growth factor administration may also be a useful treatment for neurodegenerative diseases, such as Alzheimer's disease or Parkinson's disease, which are characterized by the degeneration of neuronal cell populations. It was found that the NGF promoted nerve regeneration within conduits at an early stage, but the effect did not last after one month. This was attributed to the rapid decline in NGF concentrations in the conduit due to degradation in aqueous media and leakage from the

conduit (Hollowell *et al.*, 1990). This limitation can be overcome by providing controlled release of NGF. Controlled-release polymer delivery systems may be an important technology in enabling the prevention of neuronal degeneration, or even the stimulation of neuronal regeneration, by providing a sustained release of growth factors to promote the long-term survival of endogenous or transplanted cells (Haller and Saltzman, 1998).

Basic fibroblast growth factor (b-FGF), for instance, has been shown to enhance the *in vitro* survival and neurite extension of various types of neurons including dorsal root ganglia. One of the earliest studies involved controlled release of b-FGF and Alpha 1-glycoprotein (alpha 1-GP) from synthetic nerve guidance channels. After an initial burst, linear release was obtained from the conduits for a period of at least two weeks and four weeks postimplantation. The dip molding technique was used to fabricate tubes containing b-FGF, bovine serum albumin (BSA), alpha 1-GP, etc., for controlled release. Only the tubes releasing b-FGF or b-FGF and alpha 1-GP displayed regenerated cables bridging both nerve stumps, which contained nerve fascicles with myelinated and unmyelinated axons (Aebischer *et al.*, 1989).

Polymeric implants providing controlled release of nerve growth factor (NGF) for one month were developed and found to improve neurite extension in cultured PC12 cells (Powell *et al.*, 1990). Neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) and neurotrophin-3 (NT-3) were released from synthetic guidance channels for facial nerve regeneration. Nerve cables regenerated in the presence of GDNF showed a large number of myelinated axons while no regenerated axons were observed in the absence of growth factors, demonstrating that GDNF, as previously described for the sciatic nerve, a mixed sensory and motor nerve, is also very efficient in promoting regeneration of the facial nerve, an essentially pure motor nerve (Barras *et al.*, 2002). Inosine, a purine analog that promotes axonal extension, was loaded into PLGA conduits for controlled release during sciatic nerve regeneration. Inosine loaded PLGA foams were fashioned into cylindrical nerve guidance channels using a novel low pressure injection molding technique. After 10 weeks, a higher percentage cross sectional area composed of neural tissue was found in the inosine-loaded conduits compared with controls (Hadlock *et al.*, 1999). Biodegradable polymer foams for controlled release and to provide a permissive environment for spinal cord regeneration were formed by freeze–drying (Maquet *et al.*, 2001).

To provide for prolonged, site-specific delivery of NGF to the tissue in a convenient manner without affecting the properties of the conduit, biodegradable polymer microspheres of poly(L-lactide)*co*-glycolide

containing NGF were fabricated. Biologically active NGF was released from the microspheres, as assayed by neurite outgrowth in a dorsal root ganglion tissue culture system (Camarata *et al.*, 1992; Pean *et al.*, 1998, 1999) NGF co-encapsulated in PLGA microspheres along with ovalbumin was found to be bioactive for over 90 days (Cao and Schoichet, 1999). NGF release from biodegradable poly(phosphoester) microspheres produced using a double emulsion technique exhibited a lower burst effect but similar protein entrapment levels and efficiencies when compared with those made of PLGA (Xu *et al.*, 2002). These NGF-loaded poly(phosphoester) microspheres were successfully implanted to bridge a 10-mm gap in a rat sciatic nerve model (Xu *et al.*, 2003).

Biodegradable polymeric microspheres for controlled delivery of growth factors are commonly produced using the water-in-oil-in-water emulsion method. The use of sodium chloride in the dispersing phase of the double emulsion markedly reduced the burst effect from PLGA microspheres loaded with NGF by making the microparticle morphology more compact. Unfortunately, it was found to induce pronounced NGF denaturation (Pean *et al.*, 1998). Co-encapsulation of PEG 400 improved the stability of NGF and allowed a continuous release from PLGA microspheres produced using a double emulsion method because the PEG reduced contact of NGF with the organic phase (Pean *et al.*, 1999).

A pharmacotectonics concept was illustrated by researchers, in which drug-delivery systems were arranged spatially in tissues to shape concentration fields for potent agents. NGF-releasing implants placed within 1–2 mm of the treatment site enhanced the biological function of cellular targets, whereas identical implants placed ~3 mm from the target site of treatment produced no beneficial effect (Mahoney and Saltzman, 1999). Because of some limitations with controlled delivery systems, alternatives such as encapsulation of cells that secrete these factors are discussed in the next section.

V. Cell Encapsulation

A significant use of polymers has been to encapsulate cells that secrete growth and neurotrophic factors and implant them as a treatment for neurodegenerative disorders and to promote nerve regeneration. For instance, neural transplantation as an experimental therapy for Parkinsonian patients has been shown to be effective in several clinical trials. However, grafting combined with a treatment of neurotrophic factors improves the survival and growth of grafted embryonic dopaminergic neurons.

Continuous trophic support may be needed requiring long-term delivery of neurotrophic factors to the brain (Sautter *et al.*, 1998). A number of proteins have specific neuroprotective activities *in vitro*; however, the local delivery of these factors into the central nervous system over the long term at therapeutic levels has been difficult to achieve. Direct administration at the target site is a logical alternative, particularly in the central nervous system, but the limits of direct administration have not been defined clearly. For instance NGF must be delivered within several millimeters of the target to be effective in treating Alzheimer's disease (Mahoney and Saltzman, 1999). Cells engineered to express the neuroprotective proteins, encapsulated in immunoisolation polymeric devices and implanted at the site of lesions have the potential to alter the progression of neurodegenerative disorders. The polymers used for encapsulation should allow transport of nutrients and oxygen to the cells, but also afford immunoprotection. Long-term cell viability *in vivo* in these constructs due to diffusional limitations has been the major drawback of this approach.

An expression vector containing the human nerve growth factor gene (hNGF) was transfected into a baby hamster fibroblast cell line (BHK). Using an immunoisolatory polymeric device, encapsulated BHK-control cells and those secreting hNGF (BHK–hNGF) were transplanted unilaterally into rat lateral ventricles. Human nerve growth factor gene (hNGF) was found to be released by the encapsulated BHK-hNGF cells for over a year (Winn *et al.*, 1996) suggesting that implantation of polymer-encapsulated hNGF-releasing cells can be used to protect neurons from excitotoxin damage (Emerich *et al.*, 1994a,b). In the lesioned rat brain, chronic delivery of human nerve growth factor by the encapsulated BHK cells provided nearly complete protection of axotomized medial septal cholinergic neurons for up to six months *in vivo* and long-term encapsulated cell survival was confirmed by histologic analysis (Winn *et al.*, 1994). Encapsulated hNGF-secreting BHK cells were also found to promote the functional recovery of hemi-Parkinsonian rats (Date *et al.*, 1996a,b). Instead of fibroblasts, a Schwannoma cell line derived from a transgenic mouse, was transfected with a human NGF cDNA and encapsulated in a polymer capsule. The hNGF transgene was expressed for at least three weeks after implantation and the cells did not overgrow the capsule (Schinstine *et al.*, 1995). Transplantation of polymer-encapsulated cells genetically engineered to release nerve growth factor was found to allow a normal functional development of the visual cortex in dark-reared rats (Pizzorusso *et al.*, 1997).

Ciliary neurotrophic factor (CNTF) decreases naturally occurring and axotomy-induced cell death and has been evaluated as a treatment for neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS)

and Huntington's disease (Emerich *et al*., 1997). Effective administration of this protein to motoneurons has been hampered by the exceedingly short half-life of CNTF, and the inability to deliver effective concentration into the central nervous system after systemic administration *in vivo*. BHK cells stably transfected with a plasmid construct containing the gene for human or mouse CNTF were encapsulated in polymer fibers and found to continuously release CNTF and slow down motoneuron degeneration following axotomy (Tan *et al*., 1996). Implantation of polymer-encapsulated cells genetically engineered to continuously secrete glial cell line-derived neurotrophic factor to the adult rat striatum was found to improve dopaminergic graft survival and function (Sautter *et al*., 1998). Therefore cell encapsulation is a potentially important method in nerve regeneration, and can be used alone or in conjunction with other methods such as entubulization.

VI. Conclusions

Polymeric materials have great potential in facilitating nerve regeneration. The use of polymeric conduits in facilitating peripheral nerve regeneration has been demonstrated successfully and polymers have shown great promise in addressing spinal cord injuries as well. This regeneration process with various polymers, both degradable as well as nondegradable, has been enhanced further by promoting directed growth and by the addition of chemical cues such as laminin, nerve growth factors and other agents incorporated in the conduits to be released in a controlled fashion. Polymers also play an important role in encapsulating cells that release factors to promote nerve regeneration.

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