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SPINAL CORD REPAIR STRATEGIES: SCHWANN CELLS, NEUROTROPHIC FACTORS, AND BIODEGRADABLE POLYMERS

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Injury to the adult mammalian spinal cord leads to permanent loss of controlled neurological function. Endogenous repair mechanisms fail to reestablish functional synoptic connections. Moreover, neurological outcome usually gets worse in time, due to neurodestructive processes inherent to the adult spinal cord. Surgical repair strategies need to focus on replacing damaged/ lost nervous tissue, promoting axonal regeneration and reestablishing functional synaptic contacts. This review will discuss the current understanding of the potential beneficial role of Schwann cells, neurotrophic factors, biodegradable polymers or combinations thereof in spinal cord injury. Replacement, of injured spinal tissue with a Schwann cell graft promotes axonal regeneration and myelination. Neurotrophic factors initiate and/or enhance events that are crucial for functional recovery, such as cell survival and axonal regeneration. Biodegradable polymers can be used as a scaffold for cell implantation and/or as a drug delivery vehicle. The complex nature of spinal cord injury demands a combinatorial restorative approach. For the future, the challenge will be to combine individual growth-promoting properties such that neurological recovery in spinal cord injured humans can be achieved. ***Biomed Rev 1999; 10: 75-88.***

INTRODUCTION

Damage to the spinal cord initiates a cascade of neural and neuronal events, which results in permanent damage and loss of neurological function. Within minutes to hours after an injury, neutrophils, polymorphonucleargranulocytes, andmacrophages invade the damaged area (9) and local resident microglial cells become activated (70). These inflammatory events, together with ischemia (39,125) and Ca^{2+} influx into cells (39), are thought to contribute to secondary injury, which causes progressive loss of spinal tissue (13,50,141). In human spinal cordinjury, the loss of gray matter is often localized to one or a few segments, but its extension across the diameter of the cord leads to significant loss of white matter (131). This results in the interruption of descending and ascending fiber tracts and further functional impairment (25).

Initially following injury some neurons switch into a restorative mode, as evidenced by a transient upregulation of regeneration-associated genes (127,128). Nevertheless, spontaneous axonal growth does not occur mainly due to the presence of reactive astrocytes (44) and neurite growth-inhibitory molecules (37,44, 100) at the site of injury. This failed regeneration leads to axonal dieback, neuronal atrophy, and possibly death (74).

Acute treatment strategies for petients with spinal cord injury focus on preventing injury-induced spinal tissue loss. Currently, in the United States, a high dose of the corticosteroid and antioxidantmethylprednisoloneis administered within 8 hours after injury (17,18). This reportedly reduces spinal tissue loss,

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but only modestly improves motor and sensory function (16,18). Due to the lack of efficacy of current acute treatments, permanent neurological damage is inevitable and surgical strategies have to be developed aimed at replacing damaged spinal tissue and promoting axonal regeneration.

SPINAL CORD IMPLANTATION STRATEGIES

Central nervous system (CNS) neurons are intrinsically able to regenerate their axon following damage. However, injury-induced tissue loss, gliosis and expression of axonal growth-inhibitory factors create an environment hostile to axonal growth within the adult spinal cord. Implantation of an axonal growth-permissive environment has long been recognized as an effective approach to replace damaged spinal tissue and promote axonal regeneration. Implants that have shown to promote axonal regeneration in nonprimate mammals include fetal tissue (4,20,109,110,126), peripheral nerve grafts (2,34,35,93-95,112), cultured and genetically modified fibroblasts (75, 85,123,129,134), olfactory ensheathing cells (73,104-106) and Schwann cells (SC) (24,26,32,55,96,137-139). In this review, the use of SC in spinal cord repair strategies will be dealt with especially, but other implants will also be mentioned.

One implant may be preferred over the others depending on the type and size of the injury. A contusion cavity may be filled with a suspension of fetal tissue (49,60,109), fibroblasts (85), olfactory ensheathing glia, or SC (82,99,136), which can be achieved without much further damage to spared spinal tissue. A partial or complete transection of the spinal cord may require solid implants of fetal tissue, peripheral nerve or preformed grafts of fibroblasts or SCs that bridge the lesion gap. Although some modestrecovery following implantation of only an axonal growth-promoting graft has been reported, it is generally accepted that a combination of interventions is necessary to achieve substantial recovery of neurological function.

SCHWANN CELL IMPLANTATION IN THE INJURED SPINAL CORD

Early this century Santiago Ramon y Cajal (107) implanted peripheral nerve grafts into the spinal cord and observed central axon growth. Although this growth appeared to be abortive, Cajal and others recognized the powerful growth-promoting properties of the major cellular component of peripheral nerves, the SC. Richard Bunge was one of the first to acknowledge the possibility to isolate and culture these cells from a patient's nerve for autologous implantation in injured spinal cord. Presently, large numbers of SC can be generated from adult rat peripheral nerve (90) and from human nerve (29,71,114). SC secrete neurotrophic factors, express cell adhesion molecules, and generate several extracellular matrix molecules, which all can positively influence axonal growth. The efficacy of SC in promoting axonal regeneration in the injured spinal cord has been studied extensively (22-24,26,54). Adult rat sciatic nerve SC, mixed in Matrigel, a basal lamina matrix, and contained in a semipermeablepolyacrylonitrile/polyvinylchoride(PAN/PVC) polymer tube (Fig. 1 a), implanted in the completely transected adult rat thoracic spinal cord promote axonal growth (137,139). Within a few weeks, the spinal cord stumps are bridged by a tissue cable (Fig. lb,c) that contains SC (Fig. le,f), ensheathed unmyelinated andmyelinatedaxons (Fig. Id,e,g), blood vessels (Fig.Id), fibroblasts, meningeal cells and usually a few macrophages (32,96,137-139). An estimated 25% of the total number of fibers is myelinated by SC. Retrograde tracing experiments revealed that the maj ority of the axons derive from propriospinal neurons located as far rostrally as the third cervical spinal cord level and as far caudally as the fourth sacral level (139). The responding axons grew across the graft but did not enter the opposite cord stump. Also, supraspinal fibers were not found in these grafts. Important for future human application is that implantation of isolated human SC in the nude (T-cell deficient) rat elicits an axonal growth response (55).

Following implantation of aSC graft, usually loss of nervous tissue at the graft-host cord interfaces occurs and this may restrict an optimal regenerative response. A combination of a SC implant and methylprednisolone treatment shortly after the injury/implantation limits the loss of tissue at the interface, enhances the number of axons, and promotes supraspinal growth into the SC graft (32). The observed preservation of nervous tissue and enhanced axonal growth may be related to the methylprednisolone-induced decrease in the number of microglia/macrophages near the injury site (98).

As alluded to above, other grafts such as fetal tissue, peripheral nerve, genetically modified fibroblasts or olfactory ensheathing glia have been used successfully in injured spinal cord. The main advantage of SC over these grafts is that SC also myelinate at least a portion of the regenerating fibers. Following injury, oligodendrocytes near the lesion site die (1,43), which results in demyelination, even of initially undamaged axons. SCs could replace these oligodendrocytes and remyelinate new axon sprouts as well as demyelinated intact axons. Implantation of a SC graft alone in the injured adult rat spinal cord usually does not result in growth of axons from the graft into the spinal tissue beyond (24,139). Clearly, additional interventions need to be combined with SC implantation to achieve reentry of regenerating fibers into the spinal cord (see below). Nevertheless, the studies mentioned above have established the SC as an important candidate for future surgical repair strategies aimed at functional recovery in humans with spinal cord injury.

MEUROTROPHIC FACTORS IN THE INJURED SPINAL CORD

During CNS development neurons depend for their survival and function on neurotrophic factors, which are produced by neurons and glial cells in their vicinity or innervation territory (72). The neurotrophin family includes nerve growth factor (NGF), brain-

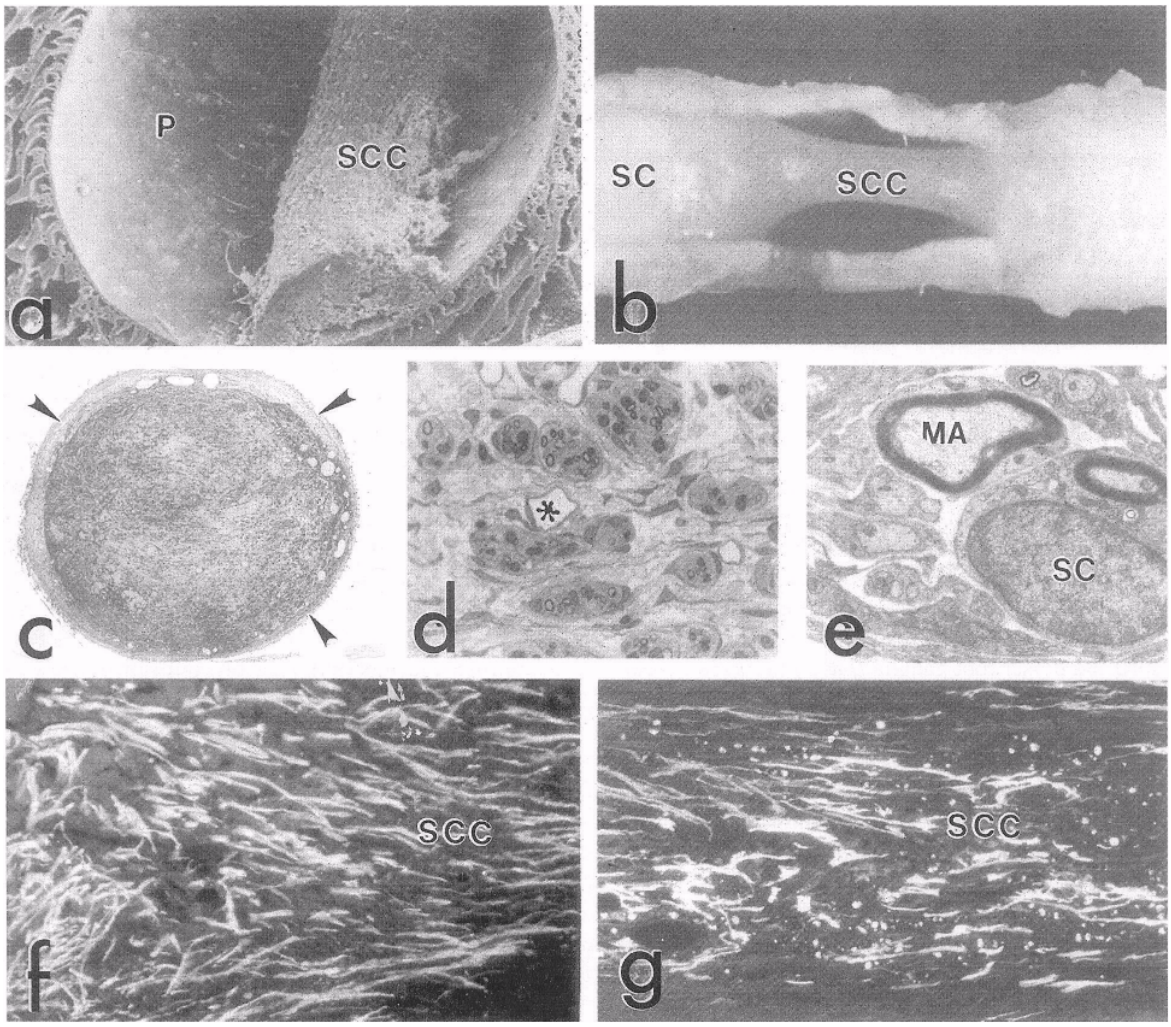

Figure 1. Schwann cell implantation in the completely transected adult rat thoracic spinal cord. Schwann cells mixed with Matrigel and seeded (a) into a nondegradable, semipermeable polyacrylonitrile/polyvinylchoride tube (P) form a compact Schwann cell cable (SCC). Four weeks after implantation (b), the SCC has fused well with the spinal cord (SC) bridging the opposite cord stumps. At this time, the cable is surrounded by a few layers offibroblasts (c) and contains fascicles ofmyelinated axons (d) as well as blood vessels (asterisks in d). Panal (e) demonstrates myelinated (MA) and unmyelinated axons present in the SCC at 4 weeks after implantation. Immunocytochemistry for SI 00 and neurofilaments revealed that (f) the Schwann cells and (g) ingrowing axons are longitudinally arranged within the cable.

derived neurotrophic factor (BDNF), neurotrophin-3, -4/5 and-6 (NT-3, NT-4, NT-6) (7,15), which are all homodimers of two approximately 120 amino acid proteins (~ 13 kD; 62). Neurotrophic factors are structurally and functionally homologous and 50-60% identical in amino acid sequence. They bind to and activate high-affinity transmembrane tyrosine kinase (Trk) receptors that interact with several identified intracellular signaling pathways (7,15,31,58,122). NGF specifically activates TrkA, BDNF and NT-4 activates TrkB and NT-3 activates TrkC, and in some cell types TrkB. All the neurotrophic factors can bind to the low-affinity p75 neurotrophin receptor ($p75^{NTR}$), amember of the tumor necrosis factor receptor superfamily(15,31,58), which is involved in increasing or decreasing Trk phosphorylation in the presence or absence, respectively, of the ligand. Through TrkA-independent activation of the ceramide pathway (30,42), $p75^{NTR}$ also plays a role in neuronal apoptosis (46, 103).

Following injury to the adult rat CNS, the synthesis of several neurotrophic factors is up regulated (38,89). This response may be mediated by cytokines, such as interleukin-1 (IL-1), which is released by microgliaand immune cells (38). The injury-induced increase in the level of neurotrophic factors is not sufficient or does not occur at the right time to effectively prevent degeneration and promote regeneration in the CNS.

Neurotrophic factor-promoted axonal growth into an intraspinal graft

As described above only a relatively small percentage of the available injured axons usually regenerate into spinal cord grafts. It has been demonstrated repeatedly that increasing the levels of neurotrophic factors in the graft environment enhances the axonal growth response. A 2-week infusion of BDNF and NT-3 directly into a SC graft placed in the completely transected rat spinal cord increases the number of responding fibers twofold and recruits fibers from neurons in 10 different brainstem nuclei (138). Most of the responding supraspinal fibers are vestibulospinal fibers. It is not clear whether both neurotrophins are necessary to elicit this growth response. SC genetically modified to produce and release NGF and placed in a hemisection were shown to promote axonal growth of neurons located in the spinal cord and dorsal root ganglia (DRG) (130,134). Also, implantation of a 5mm long trail of BDNF-secreting SC into the distal stump of the transected adult rat spinal cord promotes regeneration of axons from neurons of brainstem nuclei across the transection site into the implant (88). The growth response appears specific for the secreted neurotrophic factor since many of the responding axons were found to derive from neurons known to express TrkB.

It is clear that an increased level of neurotrophic factors enhances the axonal growth response into intraspinal SC implants. It is important to note that not every combination of growth factors will effectively promoting axonal regeneration. For instance, the combination of a SC graft with insulin-like growth factor and platelet-derived growth factor did not result in enhanced axonal growth in the injured adult rat spinal cord (96). In this particular study, relatively more myelinated axons (with thicker myelin sheaths) but fewer unmyelinated axons were present in the graft and more nervous tissue loss was observed in the graft-host cord interface. These findings and other (32) suggest that the magnitude of the regenerative response is related to preservation of the interface.

Neurotrophic factors have also been demonstrated to effectively increase the growth response in combination with other intraspinal grafts than SC grafts. An enhanced regenerative response was found in grafts containing fibroblasts genetically modified toproduceNGF(12,67,129),BDNF(67,75),orNT-3(53). Fibroblasts secreting BDNF or NT-3 were found to induce oligodendrocyte proliferation and myelination of regenerating axons when placed in a contused adult rat spinal cord (85).

Neurotrophic factor-promoted axonal growth from a graft into spinal tissue

To effectively repair spinal cord injury, axons need to regenerate from the implant into the spinal cord and establish functional synapses with neurons that are involved in the lost neurological function. Spontaneous growth of fibers from a graft into the spinal cord has been shown sporadically and only over short distances. More substantial reentry of fibers into the spinal cord can be achieved by increasing the level of neurotrophic factors a distance away from the graft (Fig. 2; 94,95). The rationale for these experiments is based on the *in vitro* observation that NGF cannot induce axonal outgrowth into an environment with lower NGF levels (28). In adult rats with a 1-week old conditioning lesion of the sciatic nerve and apredegenerated peripheral nerve bridge (Fig. 2a,c) in the thoracic dorsal funiculus (93), a 2-week infusion of NGF into the spinal cord 3-5 mm rostral from the rostral end of the nerve graft (Fig. 2b,d) promotes outgrowth of approximately 50% of the sensory fibers into the dorsal funiculus white matter (Fig. 2c,d,e; 94). The number of axons decreased further away from the graft and 10-20% reached the infusion site (Fig. 2e) but not farther, probably because of the chemoattractant effects of NGF (56,57,72). A largely similar response was seen after infusion of BDNF, NT-3 or a combination of all three neurotrophic factors (95). Possibly, infusion of neurotrophins over a time period longer than 2 weeks would further enhance axonal growth into the spinal cord. Recently, neurotrophic factor infusion was shown to promote growth of descending fibers from a SC implant into the distal spinal cord (140). In this study, the factors were infused over a 4-week period and the reentering axons could be seen beyond the site of infusion.

Normally, in the adult rat, sensory projections of dorsal root ganglia (DRG) neurons regenerate very well in aperipheral nerve or dorsal root environment after a crush injury (102) but do not cross the dorsal root-spinal cord interface, even after a conditioning lesion of the peripheral nerve (94). Infusion of NGF in the lumbar cord 3-4 mm away from the entrance zone of the crushed L4 dorsal root (L3,5, and 6 roots were resected) results in growth of approximately 20% of the sensory axons from the root into the spinal cord towards the infusion site (94). Clearly, limited growth of axons from a graft into spinal tissue results at least partially from insufficient levels of neurotrophic factors in the spinal cord and/or from their chemoattractant properti es that would limit growth away from the neurotrophic factor source, i.e., the graft environment. However, in the studies alluded to above, the improved regeneration into the spinal cord involves only a small number of axons and the elongation of the long projecting axons is limited to relatively short distances when considering the total length of the spinal cord. To accomplish topographical and functionally appropriate connections, which usually requires growth over longer distances, more than merely implantation of a graft and increasing the level of neurotrophic factors may be necessary.

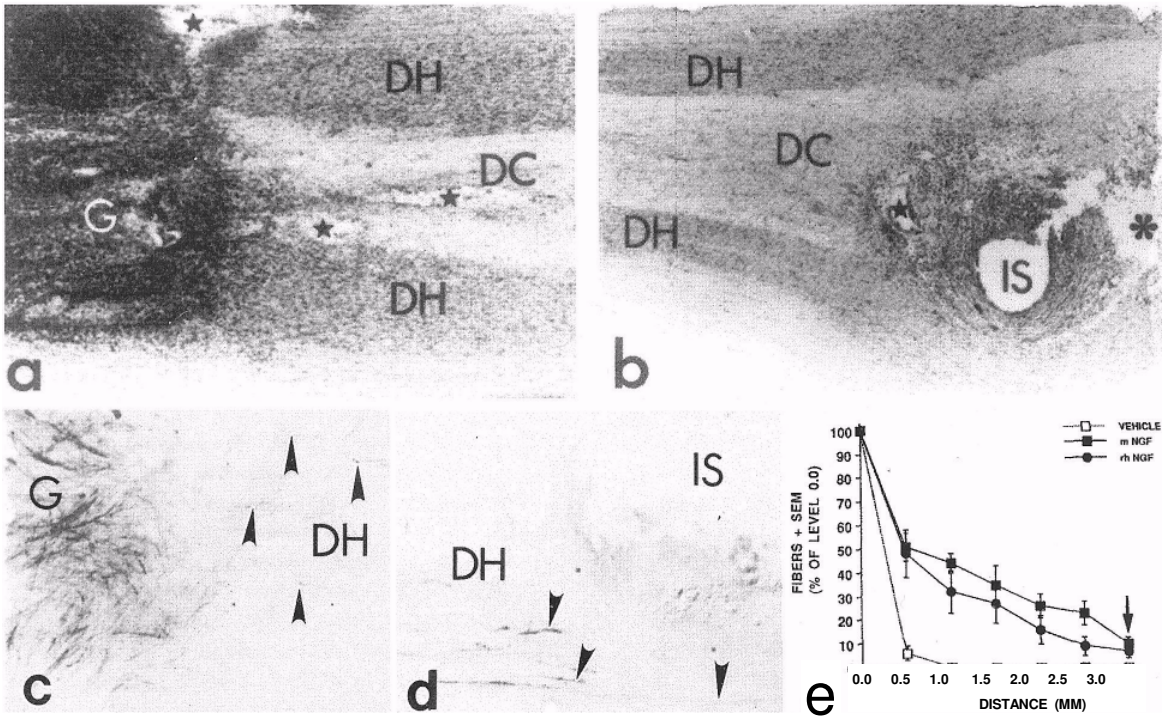

Figure 2. *Neurotrophins promote axonal growth from a graft into the spinal cord. A peripheral nerve graft (G) implanted into the spinal cord dorsal columns (DC) fuses well with spinal cord tissue (a). A few small cavities are observed near the graft-host cord interface (stars in a) in the DC or dorsal horn (DH). Similarly, placement of a metal infusion canulla into the dorsal spinal cord (b) results in relatively minor damage (star in b) of the spinal tissue around the infusion site (IS). The asterisk (b) is placed in an area that was damaged during histological preparation of the tissue. A 2-week infusion of mouse NGF (m NGF) or recombinant human NGF (rh NGF) 3-4 mm way from the rostral nerve graft-host cord interface (c) promotes outgrowth of sensory fibers from the graft into the spinal cord dorsal columns (DC). These fibers grow as far as the infusion site (IS, d) but not further (e). In the graph (e), the 0.0 level represents the rostral end of the peripheral nerve graft The arrow indicates the 3mm level where the infusion canulla was placed.*

Other strategies to improve growth into the spinal cord have also been successful. Combining a SC graft with methylprednisolone treatment shortly after injury/implantation resulted in reentry of axons into the distal cord, albeit over a relatively short distance (24). Substantial growth of fibers into the spinal cord was also achieved by combining a SC graft with olfactory ensheathing glial cells implanted in the graft-host cord interfaces (105). Serotonergic fiber growth into the distal cord was observed after stabilizing the transected spinal cord using compressive wiring of the dorsal vertebral processes (33). Also, this stabilizing technique combined with implantation of 18 intercostal nerve grafts resulted in growth of several long descending fiber tracts, including the corticospinal tract, through the nerve grafts into the distal spinal tissue (34). The nerve grafts routed spinal cord fiber tracts from nonpermissive white matter to permissive gray matter and were embedded in fibrin glue containing acidic fibroblast growth factor. These findings are exciting as well as surprising and need to be confirmed in the future. In addition, elucidation of the mechanisms responsible for these findings would make it possible to effectively promote other fiber tracts involved in locomotion.

Recently, implantation of olfactory ensheathing glia in between spinal cord stumps following a complete transection (106) or into an electrolytic lesion in the dorsal funiculus (73) was also shown to promote axonal growth across the injury site and into 'spinal tissue beyond. In these studies the olfactory ensheathing cells were accompanying the reentering axons, suggesting that as long as regenerating fibers are not or minimally exposed to interface tissue they will grow across this growth barrier. Clearly, the cellular and molecular characteristics

of the graft-host cord interfaces are of crucial importance to axonal reentry into the spinal cord. Strategies such as the chemical neutralization or destruction of these growth inhibitors (19,117-119,142) may prove to be successful forreentry of fibers and, consequently enhancing the chances for functional recovery.

MECHANISMS OF THE NEUROTROPHIC FACTOR-ENHANCED AXONAL REGENERATION

Neurotrophic factor-promoted axonal growth into an intraspinal graft may be due to the chemoattractant effects of the factors, which would result in growth towards the higher concentrations, i.e., the implant environment. Alternatively, the presence of neurotrophic factors in the graft and especially near the graft-host interface may allow for the internalization of the factors by transected axons. Subsequent retrograde transport to their cell body (41,92) can then activate second message systems involved in regeneration. For example, it has been shown that BDNF infused near the red nucleus induces the expression of regeneration-associated genes in axotomized rubrospinal neurons (128). The switch from synthesizing function-related to regeneration-related proteins elicits axonal growth (14,51). At this time it is unclear whether neurotrophic factors can also activate intracellular signaling at the distal end of growing axons (63). It is important to expanding our knowledge about where neurotrophic factors act since this will govern where treatments would be best applied.

An alternative mechanism by which high levels of neurotrophic factors in the graft environment can enhance axonal regeneration is by preventing or limiting injury-induced axonal and spinal degeneration at the graft-host cord interfaces. This would increase the number of available injured axons to grow into the graft. Normally, following spinal cord injury, axons disintegrate and form swellings or terminal clubs on the proximal axonal stumps (48,65). Release of hydrolytic enzymes from these clubs is thought to significantly contribute to the secondary spinal cord damage. Neurotrophic factors can prevent the formation of terminal clubs in the transected ascending sensory tracts (116). Also, neurotrophic factors can prevent axonal dieback or degeneration (133) and neuronal atrophy (21). This may be relevant to humans with a contusion injury, where immediately after the damage a large number of axons may still be intact. Early administration of neurotrophic factors may prevent degeneration of the undamaged axons and thus maintain functional axonal connections, i.e., limit neurological dysfunction.

The mechanisms of neurotrophic factor-promoted axonal growth from a graft into the spinal cord are unclear. Possibly, the chemoattractant effects of neurotrophic factors may elicit growth of fibers from the graft into the otherwise growth-inhibitory adult spinal cord tissue. This would imply that neurotrophic factors always have to be presented in front of growing axons to induce regeneration into the appropriate target tissues. However, an implant of BDNF-secreting fibroblasts in the injured rubrospinal tract promotes axonal growth into the spinal cord distal to the implant, i.e., away from the highest concentration the neurotrophic factor (75). An other mechanism could be neurotrophic factors modify local cells (astrocytes, oligodendrocytes, microglia and white blood cells) such that they release regeneration-promoting substances, which would make the spinal tissue more permissive for axonal growth. Alternatively, neurotrophic factors may affect the expression of neurite growth-inhibitory molecules at the graft-host cord interfaces.

The likely role of neurotrophins in future repair strategies for spinal cord injury brings about two other important issues; where and how to deliver the neurotrophic factors to achieve an optimal growth response. Different methods of delivery are currently being explored. Important considerations for treatments are the location and extent of the injured region since, ideally, the treatment should only affect this region. Also, as with any pharmacological intervention, it would be important to be able to control the dose and the time-course of administration, including the termination of the neurotrophic factor treatment.

Injections or infusions of neurotrophic factors directly into the injured spinal cord region would result in local effects and also permit regulation of the dose and timing of the treatment. Also, injections of viral vectors with genes for new and continuous synthesis of neurotrophic factors by spinal cord cells or of molecules that mimic the binding sites of neurotrophic factors to their receptors (62) could result in locally increased levels of neurotrophic factors. Increased levels of neurotrophic factors can also be achieved by implanting slow-release polymers j that contain neurotrophic factors (59). However, a disadvantage of these site-specific delivery techniques is that it will most likely induce additional damage to the already injured spinal cord.

An alternative delivery technique for neurotrophins for which the spinal cord is readily accessible is intrathecal administration. In most injury cases, neurotrophins may be able to enter the cord from the cerebrospinal fluid because for at least a short period of time the injury site has a reduced blood-brain barrier (BBB). At later time points, carrier molecules such as antibodies to | transferrin receptor could be used to shuttle neurotrophic \ factors across the intact/restored BBB (52,68). Another potential j delivery approach is based on the fact that almost all neurotrophic j factors are produced in the CNS. Thus, agents may be developed ′ that can upregulate the synthesis of these endogenous sources : (115). However, these delivery techniques result in a systemic administration of neurotrophic factors and, as with all pharmacological agents, may result in side effects because many neurotrophic factors also play physiological roles in nonnervous tissues. Side effects were observed in the recent clinical trials with CNTFfor amyotrophic lateral sclerosis (3).

POLYMERS IN SPINAL CORD REPAIR

Natural and synthetic polymers have been used extensively in spinal cord repair strategies as amatrix for cellular implants and/ or a scaffold for regenerating axons. Fibrin containing acidic fibroblast growth factor and combined with peripheral nerve grafts and stabilization of the lesion site was found to promote growth of long descending fiber tracts across and beyond the implanted area (34). Implants of solid (81) or fluid collagen (64) also promote axonal growth in the injured adult rat spinal cord. Other polymers that have been used in spinal cord repair strategies include carbon filaments (66), nitrocellulose membranes (61), polyacrylonitrile-poly vinylalcohol tubes (83), polyacrylonitrile-poly vinylchloride tubes (32,55,96,137,138), and polymethacrylate porous hydrogels (135).

Polymer structures in spinal cord injury paradigms need to fulfill several requirements. The device should be *(i)* porous to allow migration of cells and/or ingrowth of axons as well as exchange of nutrients, *(ii)* supporting or even promoting axonal regeneration, *(Hi)* three-dimensional with preferably longitudinally oriented pores, *(iv)* biodegradable, and (v) biocom-palible, i.e., not induce an inflammatory or immune response above that normally seen in spinal cord injury. The incorporation of axonal growth-promoting factors within the polymer would enhance the axonal growth response. Moreover, with a biodegradable polymer the release of these, growth-promoting factors would be temporary for as long as the degradation takes place.

Polymers that potentially fulfill all the requirements listed above are those derived from lactic and glycolic acids, the aliphatic poly(ohydroxyacids). The main advantage of this family of polymers is that their degradation and mechanical properties can be fully accustomed. These properties depend on the ratio of the L-lactic (L-LA), D-lactic (D-LA) and glycolic (GA) acid repeating units, their distribution along the macromolecular backbone and their molecular weight distribution (132). Poly (a-hydroxyacids) can be manufactured into porous structures with controlled porosity, pore size and orientation (78, 79). Moreover, following implantation, these polymers do not provoke immunologic reactions (86) and are completely resorbed (6). PLA/GA were used as temporary prostheses and scaffolds for cell implantation in a wide variety of structures and shapes (78,79). Besides in injured peripheral nerves in animals (40,108) and humans (77), poly(oc-hydroxyacids) have also been used for the repair of cartilage and liver (36), skin (11), and urothelial tissues (5).

In the CNS, poly(oc-hydroxyacids) implants have been used mainly as controlled delivery systems for bioactive (macro)molecules (10,69,87). Rods and microspheres made of po!y(D,L-lactic acid) were implanted in the rat brain for the delivery of neurotrophic factors (69). They were found to provoke a typical astrocytic and inflammatory response in the brain (69,87). At present, a clinical trial is underway with microspheres loaded with 5-fluorouracil injected prior to radiotherapy of patients with glioblastoma (10).

ALIPHATIC POLY(oc-HYDROXYACIDS) IN SPINAL CORD REPAIR STRATEGIES

So far, aliphatic poly(oc-hydroxyacids) have sporadically been used in the injured spinal cord. In an attempt to design biodegradable scaffolds for the implantation of SC in the injured adult spinal cord, the biocampatibility of poly(oc-hydroxyacids) with SC and adult spinal tissue was investigated (47). *In vitro,* it was demonstrated that PLA/GA and its breakdown products had no adverse effect on the morphology, survival and proliferation of cultured rat SC (Fig. 3a-c). In fact, the SCs had formed extensions that were in close contact with the PLA/GA (Fig. 3c). *In vivo,* a2-mm long PLA/GA disc implanted in between the stumps of a transected adult rat thoracic spinal cord was shown to integrate well into spinal tissue at two weeks after implantation (47). At all time-points post-implantation, the astrocytic (Fig. 3d *versus* 3e) and inflammatory response (Fig. 3f *versus* 3g) near the lesion site was largely similar in both experimental and controls animals, from which a 2-mm long spinal segment was removed only. Neuro filament-positive fibers were found growing into the PLA/GA disc or the remains thereof. Moreover, GAP-43, a protein indicative of regenerating axons, was found in some of these fibers in the remains of the PLA/GA discs (47). The results suggest that poly(a-hydroxyacids) and their breakdown products are not hostile to SC nor to regenerating axons. In another study, PLA needles with 100 lim wide longitudinal grooves and embedded in fibrin glue were implanted in a similar complete adult spinal cord transection model (80). Cell migration, angiogenesis and axonal growth was observed within this implant. The longitudinal orientation of the needles and their macropores appeared to benefit blood vessel and axonal growth (80).

In most of the experiments alluded to above, in which SC were implanted into the injured spinal cord, the SC were enclosed in a nondegradable PAN/PVC tube. A tubular scaffold for cell implantation may improve axonal regeneration by limiting the formation of scar tissue and by allowing the accumulation of neurite growth-promoting factors (23). These properties are thought to be especially beneficial for axonal growth in the early phase of the regeneration response (45). However, in later stages of the growth response the presence of a tubular scaffold may actually restrict axonal growth or the maintenance of fibers that have grown into the implant due to constriction of the spinal cord, toxicity, or foreign body reaction, which may develop over the long term. The use of a biodegradable tubular scaffold for SC implantation into the injured spinal cord may circumvent these detrimental effects. Preliminary results have revealed that axons do grow into an implant of SC contained in a tubular PLA scaffold implanted into the completely transected adult rat thoracic spinal cord (97). However, the number of axons within

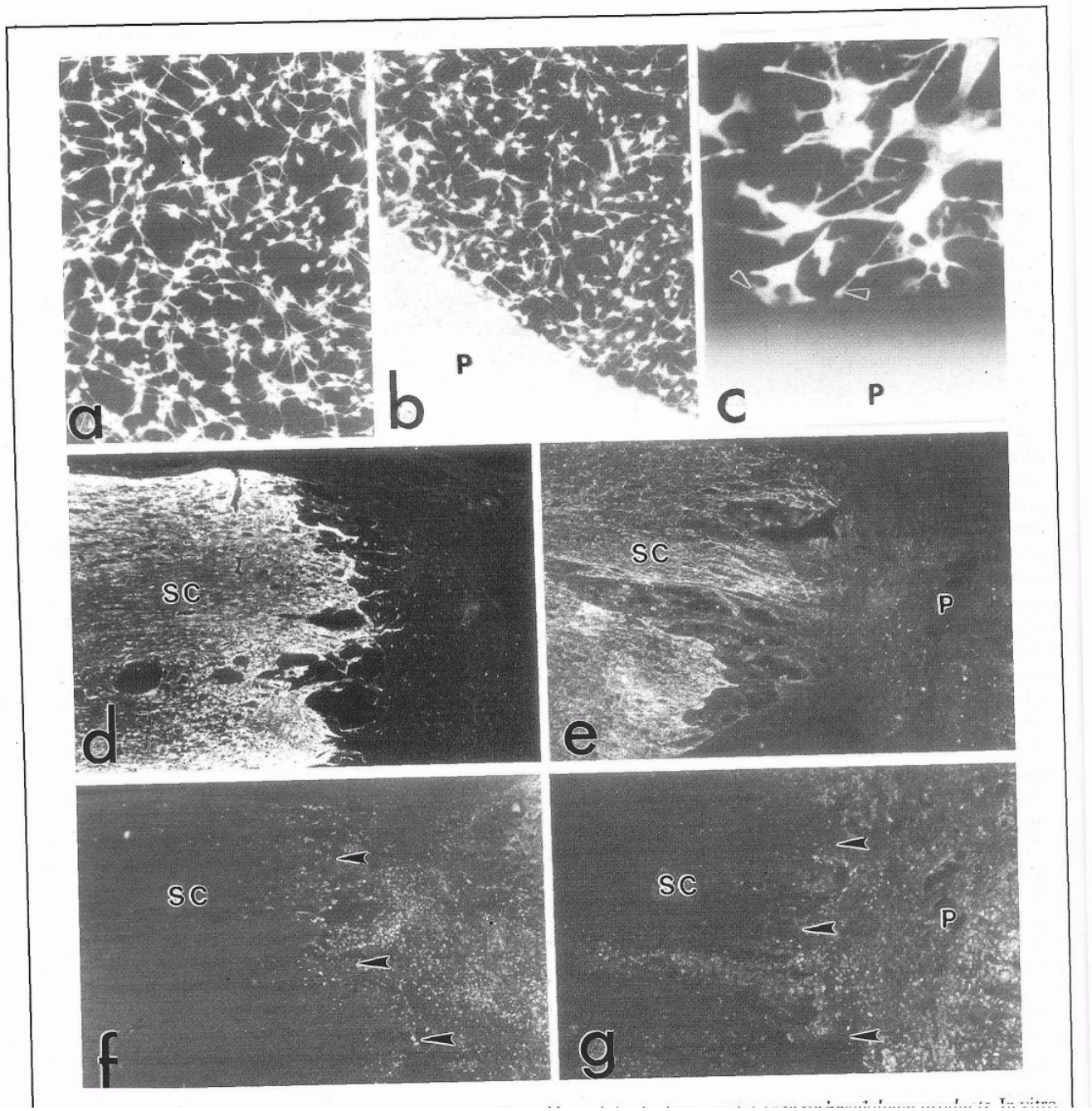

Figure 3. Schwann cells and spinal cord tissue are not affected by poly (a-hydroxyacids) or their breakdown products. In vitro

Schwann cells proliferate at a similar rate without (a) or with (b) poly(a-hydroxy acids) p) present in the cuUuredtshThe Schwann cells even form extensions (c) that grow towards the polymer. In vivo, *implantation of a 2 mm-long poly(a-h^Zacid cylinder (P in panel e andg) does not result in an astrocytic (d* versus *e) or inflammatory (/*versus *g) response beyond ^a,leer after a transection and removal of 2 mm of spinal cord (SC) only (d andf). Panel d ande show horizontal cr^ostat sections stained with antibodies against glial fibrillary acidic protein, a marker for astrocytes Panel fond g show hor^'^ostat sections stainedwith antibodies against ED-1, a marker for microgliaSmacrophages. The arrow heads pomt at the rostral end of the transected spinal cord.*

the tissue cable decreased due to damage by parts of the degrading scaffold. Possibly, the nonuniformly breakdown of these tubes may have resulted in the formation of large pieces of the scaffold that caused damage to the tissue cable inside. Future experiments need to focus on the development and use of biodegradable scaffolds that degrade uniformly. Also, the incorporation of growth promoting factors into these tubular SC scaffolds or the implantation of SC containing multichannel scaffolds with or without these factors may substantially improve the axonal regeneration response.

CONCLUSION

The consequences of a spinal cord injury are devastating and the complexity demands a multifactorial repair strategy. SC have been shown repeatedly to possess properties beneficial for spinal cord repair strategies. Neurotrophic factors have been shown to play a crucial role in enhancing the regenerative response and to achieve lengthy regeneration across and beyond an intraspinal implant. Neurotrophic factors are also likely to play a role in preventing degeneration of axons after spinal cord injury. A clinically relevant question is whether chronically injured neurons remain responsive to SC and/or neuro trophic factors. Neutralization of axonal growth inhibitors in injured spinal tissue can result in lengthy regeneration of a limited number of axons (27,118). Neurotrophic factors combined with such an approach may further enhance the regenerative response (117). Another promising area in spinal cord repair strategies, which need further in depth study, is the pharmacological prevention of additional loss of tissue and/or spared axonal connections. The development of such acute treatments may, in some but not all spinal cord injuries, simplify the complex task of promoting long-distance regeneration and functionally appropriate reinnervation.

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