JOURNAL OF NEUROTRAUMA
Volume 23, Number 3/4, 2006

Pp. 496–507

Bioengineered Strategies for Spinal Cord Repair

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ABSTRACT

This article reviews bioengineered strategies for spinal cord repair using tissue engineered scaffolds and drug delivery systems. The pathophysiology of spinal cord injury (SCI) is multifactorial and multiphasic, and therefore, it is likely that effective treatments will require combinations of strategies such as neuroprotection to counteract secondary injury, provision of scaffolds to replace lost tissue, and methods to enhance axonal regrowth, synaptic plasticity, and inhibition of astrocytosis. Biomaterials have major advantages for spinal cord repair because of their structural and chemical versatility. To date, various degradable or non-degradable biomaterial polymers have been tested as guidance channels or delivery systems for cellular and non-cellular neuroprotective or neuroregenerative agents in experimental SCI. There is promise that bioengineering technology utilizing cellular treatment strategies, including Schwann cells, olfactory ensheathing glia, or neural stem cells, can promote repair of the injured spinal cord. This review is divided into three parts: (1) degradable and non-degradable biomaterials; (2) device design; and (3) combination strategies with scaffolds. We will show that bioengineering combinations of cellular and non-cellular strategies have enhanced the potential for experimental SCI repair, although further pre-clinical work is required before this technology can be translated to humans.

Key words: bioengineering; biomaterials; drug delivery systems; neural stem cells; scaffolds; Schwann cells; spinal cord injury

INTRODUCTION

BIOMATERIALS HAVE BEEN WIDELY USED in medical applications, including tissue engineered scaffolds and drug delivery vehicles for spinal cord injury (SCI). To repair the damaged central or peripheral nervous system (CNS/PNS), biomaterial polymers have been employed as guidance channels or as delivery vehicles for cells, and neuroprotective or neuroregenerative factors. Because the pathophysiology of SCI is complicated, various combination strategies, including the provision of scaffolds to replace lost tissue, neuroprotection from second injury, enhancement of axonal regrowth and synaptic plasticity, and inhibition of astrocytosis, are required for SCI repair. There is promise that bioengineering technology combined with cellular treatment strategies such as Schwann cells or neural stem cells grafts can promote repair of the injured spinal cord. Here, we review bioengineering strategies for traumatic spinal cord repair and in doing so reflect on some similar strategies that have been pursued in peripheral nerve repair. For spinal cord regeneration, it is important that CNS axons regenerate in order for lost functions to be regained. This review is divided into three parts: (1) degradable and non-degradable biomaterials,

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(2) device design, and (3) combination strategies with scaffolds.

BIOMATERIALS

Degradable Biomaterials

Collagen. Collagen is one of the major proteins present in mammals, accounting for 25–30% of the total protein in the human body. Among the 19 varieties of collagen in humans, type I collagen has been most commonly used as a primary component of scaffolds to promote axonal regeneration after SCI in rodents. Bridging the transected spinal cord with collagen gels induced vascularization in the bridging collagen and led to the ingrowth of a small number of myelinated axons from the host spinal cord (de la Torre, 1982; Marchand et al., 1993). In addition, combination therapy of collagen gels with several neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3), stimulated axonal regeneration, including axons from the corticospinal tract, after dorsal spinal cord transection, and promoted partial functional recovery (Houweling et al., 1998a,b). It is of interest that implantation of collagen rolls enclosing cultured Schwann cells into the cavity of the injured rat spinal cord contributed to axonal regeneration, although only a few axons originating from the host spinal cord were found in the implanted acellular collagen rolls (Paino and Bunge, 1991). Remarkably, a recent report showed that implantation of plain collagen filaments significantly enhanced functional recovery after spinal cord transection; however, the death rate of the animals up to 12 weeks after implantation of collagen filaments was more than 40%, which suggests the possibility of cytotoxicity associated with the grafted collagen filaments (Yoshii et al., 2003a,b; Yoshii et al., 2004). Thus, the promise of collagen for spinal cord repair is somewhat disputed and may depend on the form of collagen used (i.e., tubes vs. filaments) or with what it is combined. In PNS injury, implantation of silicon tubes filled with collagen gels to bridge transected sciatic nerve stumps failed to improve functional recovery (Terris et al., 1999). In contrast, implantation of collagen filaments resulted in 30-mm-long axonal regeneration accompanied by myelination after sciatic nerve transection in the rat (Yoshii et al., 2003a,b). The collagen gel, unless used at very dilute concentrations, may present a physical barrier to regenerating axons (Midha et al., 2003). Collagen gels have been used as "drug delivery agents" in order to provide localized delivery of therapeutic agents after SCI. Our group has had some success with subarachnoid-injected collagen gels to deliver growth factors to enhance proliferation of endogenous stem/progenitor cells in the spinal cord (Jimenez Hamann et al., 2005).

Fibronectin. Fibronectin (FN) is an extracellular matrix glycoprotein involved in many cellular processes, including tissue repair, embryogenesis, blood clotting, and cell migration/adhesion (Yamada and Olden, 1978). FN exists mainly as an insoluble glycoprotein dimer that serves as a linker in the extracellular matrix, and as a soluble disulphide-linked dimer found in plasma. FN mats from human plasma have been used as a scaffold for repair of injured peripheral nerves or the spinal cord in experimental animals (Ejim et al., 1993). For PNS injury, implantation of FN mats impregnated with NT-3, but not naive FN mats, after rat sciatic nerve transection showed a beneficial effect on neuromuscular interaction and induced a significant number of myelinated axons into the scaffolds at 8 months (Sterne et al., 1997). In contrast, in SCI, FN mats containing neurotrophic factors such as NT-3, BDNF, or nerve growth factor (NGF) did not stimulate axonal outgrowth from the graft into the host spinal cord (King et al., 2004), although plain FN acted as a guidance cue for some axonal regrowth from the host spinal cord (Phillips et al., 2004). In addition, the implantation of anti–transforming growth factor $\beta 2$ (TGF$\beta 2$)–incubated FN mats failed to enhance axonal regeneration after partial spinal cord transection (King et al., 2004). Thus, fibronectin has not demonstrated significant promise in SCI repair strategies, despite being an important extracellular matrix protein.

Alginate/agarose. Alginate is a linear polysaccharide produced by brown algae, and is composed of $(1 \rightarrow 4)$ linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues. While not strictly biodegradable, calcium-crosslinked alginate dissolves with calcium chelation, and because of its biocompatibility, hydrophilicity, and low toxicity, alginate has been used as a scaffold to fill the cavity in the damaged spinal cord (Freier et al., 2005a,b; Suzuki et al., 2002). Freeze-dried alginate sponge or gel acted as a guide for the amputated axons and reduced astrocytosis between the grafted alginate and the host spinal cord after spinal cord transection in young rats (Kataoka et al., 2004; Suzuki et al., 2002). Alginate sponge was also beneficial as a three-dimensional scaffold for transplantation of hippocampus-derived neurosphere cells into the injured spinal cord in rats (Wu et al., 2001). In addition, BDNF-producing fibroblasts encapsulated within alginate survived in the injured spinal cord without immune suppression and contributed to some functional recovery (Tobias et al., 2005). However, one of the major problems with alginate is the mitogenic and cytotoxic impurities in commercially available algi-

nates (Zimmermann et al., 2001). Thus, ultra purification of commercial alginates is required prior to cell transplantation.

Agarose is a linear polysaccharide extracted from sea weed and a thermal gelling hydrogel. Recently, it was reported that freeze-dried agarose scaffolds containing nerve growth factor (NGF) had a favorable effect on axonal regeneration after CNS injury (Stokols and Tuszynski, 2004). While the origin of those axons was not described, agarose has also demonstrated promise *in vitro,* where it has been used to guide cell adhesion and neurite outgrowth (Luo and Shoichet, 2004).

Hyaluronic acid. Hyaluronic acid (HA) is a naturally occurring linear polysaccharide with repeating disaccharide units composed of glucuronic acid and *N*-acetylglucosamine, and has an important role in many tissue repair processes (Wang et al., 1998). Several *in vivo* and *in vitro* experiments show that hyaluronan-based conduits are completely biodegradable, non-cytotoxic, and biocompatible (Avitabile et al., 2001). For repair of PNS injury, topical application of HA gel enhanced axonal regrowth and reduced neural adhesion in rat sciatic nerve injury (Ozgenel, 2003). For repair after SCI, transplantation of cultured embryonic spinal cord tissue embedded in HA into the gap after spinal cord transection resulted in slight functional recovery (Rochkind et al., 2002). Recently, a HA-poly-D-lysine copolymer hydrogel was introduced as a scaffold to repair brain defects in rats (Tian et al., 2005) and thus this biomaterial may warrant further investigation for SCI repair.

Chitosan. Chitin, a co-polymer of *N*-acetyl-glucosamine and *N*-glucosamine units, is the second most abundant polysaccharide in nature next to cellulose, and is the principal component of exoskeletons of crustaceans and insects, and cell walls of some bacteria and fungi. Chitosan is a naturally occurring substance that is chemically similar to cellulose, a plant fiber, and is prepared from chitin by *N*-deacetylation (Khor and Lim, 2003). Because of its properties of non-toxicity, biocompatibility, and biodegradation, chitosan channels have been used as an artificial bridge for repair in rodent sciatic nerve injury (Itoh et al., 2003) and dogs (Rosales-Cortes et al., 2003). In addition, transplantation of cultured Schwann cells on chitosan tubular scaffolds was beneficial in peripheral nerve injury (Yuan et al., 2004). There have been no reports of chitosan scaffolds for CNS repair, a strategy we are pursuing in our laboratory (Freier et al., 2005b).

Poly-β-hydroxybutirate. Biopolyesters are high-molecular-weight biodegradable and biocompatible polymers synthesized by microorganisms in nature. Among a wide variety of biopolyesters, a poly-β-hydroxybutirate (PHB) scaffold has been used as an artificial conduit for peripheral nerve repair in rodents and cats (Hazari et al., 1999; Mohanna et al., 2003). Furthermore, transplantation of *lac*Z-labeled Schwann cells in a conduit composed of PHB into the gap between the stumps of the transected rat sciatic nerve enhanced axonal regeneration (Mosahebi et al., 2001). To treat SCI, implantation of PHB fibers containing alginate hydrogel, fibronectin, and neonatal Schwann cells protected the neurons in the red nucleus from secondary neuronal atrophy and enhanced axonal regeneration into the implant after rat cervical spinal cord hemisection (Novikov et al., 2002).

Poly(glycolic acid)/poly(latcitic acid). Poly(glycolic acid)/poly(lactic acid) (PGA/PLA), a member of the α-hydroxy acid class of compounds, is composed of synthetic biodegradable aliphatic polyesters. PLA and copolymers have been widely used as a temporary extracellular matrix in tissue engineering. An example is artificial skin grafts for surgical repair of skin defects in plastic and reconstructive surgery (Freier et al., 2005a). Also, PGA tubes have been successfully used as guidance channels for axonal reconnection in human PNS injury (Mackinnon and Dellon, 1990). Modified PGA tubes filled with laminin-coated collagen material guided peripheral nerve elongation across a wide nerve gap without tube collapse (Matsumoto et al., 2000). Also, poly(L-lactic acid) (PLLA) tubes have been used to reconnect transected peripheral nerves (Evans et al., 1999). With respect to SCI, implantation of tubular scaffolds made of high-molecular-weight PLLA mixed with 10% PLLA oligomers seeded with cultured Schwann cells promoted abundant axonal growth into the tubes accompanied by extensive vascularization, and there was minimal tube collapse after 4 months. In contrast, poly(D,L-lactic acid) tubes completely collapsed by 2 months after grafting in the spinal cord (Oudega et al., 2001). In addition, grafts of poly(D,L-lactic acid) scaffolds impregnated with BDNF enhanced axonal regeneration in a rat spinal cord transection model (Patist et al., 2004). One of the disadvantages associated with PLLA is its rapid degradation based on the hydrolysis of the polyester bond with the rate dependent on polymer crystallinity, molecular weight, shape, wall morphology, among others (von Recum et al., 1995). To control the degradation rate of polymers, various synthetic polymers, including poly(D,L-lactic acid-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), or a blend of PCL and PLGA, have been examined (Cao and Shoichet, 1999; Day et al., 2004; Wu and Ding, 2004). Remarkably, implantation of PLGA scaffolds seeded with murine neural stem cells significantly improved functional recovery after rat spinal cord hemisection (Teng et al., 2002). However, it seems to be difficult

to control the degradation rate precisely. For example, the grafted PLGA cylinders completely dissolved within 3 weeks of rat spinal cord transection (Gautier et al., 1998).

Polycarbonate. Three-dimensional tubular scaffolds made of polycarbonate have been used for CNS repair (Montgomery and Robson, 1993; Montgomery et al., 1996). Rolled polycarbonate membranes coated with poly-L-lysine and filled with cultured Schwann cells or embryonic astrocytes were implanted into the rat brain, and the tubes filled with Schwann cells contained more regenerating axons after 6 months (Montgomery and Robson, 1993). Interestingly, the implantation of tubes made of plain polycarbonate supported a degree of axonal growth after rat spinal cord hemisection similar to that of polycarbonate tubes coated with poly-L-lysine in combination with cultured Schwann cells (Montgomery et al., 1996). Polycarbonates, like the polyesters, are hydrophobic and generally not adhesive to cells; however, unlike polyesters, polycarbonates degrade to non-acidic degradation products that are more bioacceptable.

Polyethylene glycol. Polyethylene glycol (PEG) is a hydrophilic polymer that has low protein and cell adhesion properties. PEG has demonstrated neuroprotective properties, perhaps due to its ability to seal damaged cell membranes and suppress oxidative stress following CNS injury (Luo and Shi, 2004). *In vivo* experiments indicated that the application of an aqueous solution of PEG after SCI reduced the volume of the cavity in the damaged spinal cord and produced some functional recovery in guinea pigs and dogs (Borgens et al., 2002; Laverty et al., 2004).

Non-Degradable Biomaterials

Poly(2-hydroxyethyl methacrylate) or poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) (PHEMA/PHEMA-MMA). One of the highly desirable properties of non-degradable conduits for repairing SCI is structural stability. Non-degradable materials must be biocompatible and non-toxic. Our group has focused on PHEMA-MMA or PHEMA, important constituents in soft contact lenses and soft tissue substitutes (Corkhill et al., 1989), as excellent non-degradable biomaterials based on the mechanical properties of porous tubes that have been designed to be similar to that of the spinal cord (Dalton et al., 2002; Flynn et al., 2003). With peripheral nerve repair, PHEMA-MMA hydrogel channels filled with FGF-1 led to extensive axonal regeneration into the channels after sciatic nerve transection (Midha et al., 2003). Furthermore, significant axonal regeneration within coil-reinforced PHEMA-MMA porous tubes was observed 16 weeks after tube implantation in a 10-mm-gap between the stumps of the transected sciatic nerve in rats. The PHEMA-MMA tubes demonstrated excellent mechanical integrity and patency (open-ness), and consistently demonstrated autograft-equivalent regeneration when filled with FGF-1 and fibrin (Katayama et al., 2005). PHEMA sponge implantation into the cavity of the injured spinal cord enhanced some axonal regrowth after contusion SCI in rats (Giannetti et al., 2001). Remarkably, the implantation of empty PHEMA-MMA channels between the stumps of the completely transected spinal cord in rats resulted in a thick tissue bridge containing many axons (Tsai et al., 2004). However, many of the channels partially collapsed by 8 weeks after implantation, similar to what had been observed in preliminary studies in the PNS (Belkas et al., 2005a,b). To eliminate the problem of collapse we implanted the coil-reinforced PHEMA-MMA/PHEMA channels (Katayama et al., 2005), and introduced a new method of spinal fixation with surgical wire. In order to enhance axonal regrowth, different combinations of neurotrophins (neurotrophin-3 or FGF-1) and matrixes (fibrin, collagen or methylcellulose) were applied inside the channels. Matrix and neurotrophin inclusion within the PHEMA-MMA hydrogel guidance channels improved specific supraspinal and local axonal regeneration after complete spinal cord transection (Tsai et al., 2005).

Poly[N-(2-hydroxypropyl)methacrylamide] (PHPMA). PHPMAs are linear and water-swellable crosslinked polymers, and are being developed for drug delivery or gene therapy applications (Parker et al., 2005; Prokopova-Kubinova et al., 2001). Synthetic hydrogel PHPMA has been reported as potentially useful for spinal cord repair as well as PHEMA (Woerly et al., 1998). As a delayed treatment relevant for clinical application in chronic SCI, PHPMA hydrogel grafted into the cavity of the rat injured spinal cord 3 months after severe SCI significantly reduced the volume of the cavity and induced axonal growth into the grafts accompanied by proliferation of blood vessels and functional recovery (Woerly et al., 2001). PHPMA hydrogel was used to bridge the stumps of the transected spinal cord and induced myelination of axons by migrating Schwann cells from the host spinal cord, resulting in reduced astrocytosis around the reconstructed site in experimental animals (Woerly et al., 2004). In addition, the combination strategy of BDNF and ciliary neurotrophic factor (CNTF) producing fibroblasts and PHPMA hydrogel grafts promoted significant axonal regrowth after brain injury in rats (Loh et al., 2001). It is interesting to note that PHPMA hydrogel implantation into the rat cortex induced a more massive growth of connective tissue than that of PHEMA (Lesny et al., 2002).

Poly(acrylonitrile-co-vinylchloride) (PAN/PVC). PAN/PVC, a copolymer of polyacrylonitrile and polyvinylchloride, is structurally stable and non-toxic *in vivo* (Shoichet and Rein, 1996). As shown in an *in vitro* experiment (Flanagan et al., 2002), an inner wall composed of a scaffold of polyacrylamide must be covered with Matrigel™ to facilitate adhesion of neural cells. With PNS injury, PAN or PVC alone, or PAN/PVC guidance channels enhanced peripheral nerve regeneration in rodents (Aebischer et al., 1990). In contrast, PAN/PVC conduits alone without any additional treatment were unable to stimulate axonal growth in CNS injury (Bunge, 2002; Xu et al., 1997). For example, PAN/PVC channel implantation containing simple Matrigel™ did not produce axonal regeneration from the host spinal cord after spinal cord transection in the rat (Xu et al., 1997). Due to their high biocompatibility, PAN/PVC scaffolds have frequently been combined with cultured rat/human-Schwann cells or olfactory ensheathing glia (OEG) in SCI and shown some promise (Bamber et al., 2001; Chen et al., 1996; Fouad et al., 2005; Iannotti et al., 2003; Jones et al., 2001; Ramon-Cueto et al., 1998; Xu et al., 1997, 1999). Also, PAN/PVC channels filled with Schwann cells have been combined with neurotrophic factors, such as BDNF, NT-3 or GDNF and showed enhanced regeneration of myelinated axons in rat spinal cord hemisection (Iannotti et al., 2003). Overall PAN/PVC is a promising biomaterial; however, it requires organic solvents for its synthesis into tubular constructs, which complicates its clinical applicability, unlike PHEMA or PHEMA-MMA tubes, which are synthesized in water.

Carbon filaments. Carbon has been used in many different forms and structures for various biomedical applications. Carbon filaments have been used frequently in orthopedic surgery because of their strength and biocompatibility (Schepsis and Greenleaf, 1990). The implantation of carbon filaments promoted axonal regrowth after spinal cord transection in rats, although functional recovery was not achieved (Khan et al., 1991). Also, carbon filaments implanted into the cavity created after contusion SCI provided scaffolding for considerable regrowth of axons in rats (Chauhan et al., 1999). Furthermore, the combination of cultured fetal spinal cord tissue and carbon filaments aided the repair of the injured adult rat spinal cord (Liu et al., 1995). Recently, it was shown *in vitro* that carbon nanofibers minimized astrocyte interactions (McKenzie et al., 2004). Moreover, carbon nanotubes coated with the bioactive molecule 4-hydroxynonenal enhanced embryonic rat-brain neuron elaboration of neuronal growth, which exhibited extensive branching (Mattson et al., 2000).

Silicone. Silicone is the generic name for a family of silicone-carbon-based polymers. Depending on the length and complexity of the polymer, silicone may exist as a liquid, gel, foam, resin, or rubbery material (Muzaffar and Rohrich, 2002). Silicone has been frequently used in cosmetic medicine for more than 40 years (Humble and Mest, 2004), although there are several reports of complications associated with silicone (Busch, 1994; Muzaffar and Rohrich, 2002). Silicone tubular scaffolds have been examined in repair of peripheral nerve injury in experimental animals since the early 1980s (Lundborg et al., 1982; Valero-Cabre et al., 2004). However, one disadvantage associated with silicone tubes is that long-term implantation for bridging transected peripheral nerves causes chronic nerve compression of regenerating axons (Merle et al., 1989). For SCI repair with silicone scaffolds, silicone rubber tube implantation combined with constant electric current stimulator improved axonal regrowth in spinal cord hemisection in guinea pigs, although there were only a few regenerated axons within empty silicone tubes after implantation (Borgens, 1999). It should be noted that silicone implantation created massive astrocytosis in the intact rat brain (Turner et al., 1999). Overall, silicone tubes, while biocompatible, have not been the material of choice for further repair after injury.

DEVICE DESIGN

Gels

Gels are viscous, crosslinked liquids and are advantageous for filling in a small area, such as the cavity remaining after contusion SCI or the gap between the stumps of the transected cord. Obliteration of a cavity with a gel may prevent astrocytosis from expanding and provide regenerating axons with a scaffold. Gels may also be beneficial as vehicles to uniformly disperse living cells or drugs into the resulting cavity. However, some disadvantages associated with gel scaffolds include the inability to guide regenerating axons and insufficient persistence time due to degradation and weak structure.

Sponges

Sponges are porous three-dimensional structures that provide the implant for a relatively wider tissue defect after CNS injury, such as a brain tissue defect after severe cervical infarction. A biodegradable sponge device may provide a wide and rigid scaffold for transplantation of neural stem/ progenitor cells. Similar to gels, sponges have the disadvantage of mechanical weakness.

Tubes

Tubes are porous hollow fiber membranes that provide the pathway for axonal regrowth across the lesion site for repair in both the PNS and CNS where bridging is necessary. Implanted tubes for repair of SCI must remain patent (i.e., open) long enough for axonal regeneration, whether degradable or non-degradable.

Whether the design strategy calls for a gel, sponge or tube, it will require a combination strategy including cells or growth promoting cues, including the haptotactic and chemotactic signaling molecules.

COMBINATION STRATEGIES INVOLVING BIOENGINEERED BIOMATERIALS

Combination strategies have gained momentum for spinal cord repair, as introduced here and in more detail in other reviews in this issue.

Schwann Cells

It has been widely accepted that Schwann cells have great potential to enhance axonal regrowth both in the PNS (Frostick et al., 1998) and CNS (Blakemore, 1977). Schwann cells produce many neurotrophic factors, synthesize extracellular matrix, and express a variety of cell adhesion molecules to repair the damaged spinal cord (Bixby et al., 1988; Frostick et al., 1998). Furthermore, tubular guidance channels can easily allow transplantation of a high concentration of cultured Schwann cells into the injured spinal cord. The combination strategy of guidance channel implantation and cultured rat or human Schwann cells has been found to be a minimally effective treatment for repair of experimental SCI, particularly by Bunge's group (Bamber et al., 2001; Bunge, 2002; Fouad et al., 2005; Iannotti et al., 2003; Xu et al., 1995, 1997). They also reported that infusion of neurotrophic factors, such as BDNF or NT-3, or administration of methylprednisolone in combination with implantation of PAN/PVC tubular scaffolds seeded with cultured Schwann cells stimulated axonal regeneration from brain stem motor nuclei after SCI (Bunge, 2002; Jones et al., 2001; Xu et al., 1995, 1999). Notably, a recent report showed that implantation of a non-degradable tubular scaffold made of polyethersulfone ultrafiltration membrane seeded with bone-marrow stromal cell–derived Schwann cells promoted central axon regrowth and achieved significant functional recovery after rat spinal cord transection (Kamada et al., 2005). While Schwann cells have shown great promise, it is important to measure their survivability after implantation (and over time) in order to gain greater perspective on their importance and impact on regenerative strategies. This is true for other cell strategies as well.

Olfactory Ensheathing Glial Cells

Olfactory ensheathing glial cells (OEGs) are non-myelinating glial cells that provide ensheathment for unmyelinated olfactory axons within both CNS and PNS portions of the olfactory pathway. Although OEGs do not synthesize myelin when in contact with small diameter sympathetic axons, they can be induced to express a myelinating phenotype if the diameter of these axons is extended by increasing the size of the target tissue (Boyd et al., 2005). OEG transplantation has been shown to be a valuable treatment to promote axonal regeneration and functional improvement after SCI (Ramon-Cueto and Santos-Benito, 2001). Interestingly, delayed transplantation of OEGs into the contused rat spinal cord also enhanced axonal regrowth/sparing from brain stem motor nuclei (Plant et al., 2003). With regard to the combination strategy of scaffolds and OEGs, Schwann cell-filled PAN/PVC guidance channel implantation combined with OEG transplantation into both stumps of the transected spinal cord promoted long distance regeneration of descending supraspinal and ascending propriospinal axons within both stumps after rat spinal cord transection (Ramon-Cueto et al., 1998). According to one recent report, the combination therapy of implantation of Schwann cell-seeded channels, OEG grafts into the spinal cord stumps and continuous delivery of chondroitinase abc (to dissolve the glial scar) significantly improved functional recovery after rat spinal cord transection (Fouad et al., 2005).

Neural Stem/Progenitor Cells

Synthetic three-dimensional biodegradable scaffolds seeded with neural stem/progenitor cells (NSCs) have been one of the most interesting strategies in the field of biomaterials (Kulbatski et al., 2005). A scaffold seeded with NSCs for repairing CNS lesions can provide a platform for the cells enabling repair of large neural defects. Also, the scaffold may induce NSCs to differentiate. For example, collagen gel was an effective extracellular matrix for NSCs (Lin et al., 2004). Alginate sponge contributed to the survival and differentiation of rat hippocampus-derived neurosphere cells after transplantation into the injured rat spinal cord as described above (Wu et al., 2001). Furthermore, artificial scaffolds made of synthetic biodegradable polymers such as PGA, PLA, and their copolymers have shown potential in combination with NSCs transplantation (Lavik et al., 2002). For example, the implantation of a PLGA scaffold seeded with mouse NSCs achieved functional recovery in rat SCI

(Teng et al., 2002). Human embryonic stem cells can differentiate on three-dimensional PLGA scaffolds (Levenberg et al., 2003). In addition, the implantation of PGA-based scaffolds seeded with mouse NSCs into a massive tissue defect after hypoxic brain injury significantly reduced parenchymal loss (Park et al., 2002). Recently, it was revealed that fibrous PLLA scaffolds also have potential as NSC carriers *in vitro* (Yang et al., 2005). Interestingly, an artificial nanofiber scaffold selectively induced rapid differentiation of mouse NSCs into neurons and not astrocytes (Silva et al., 2004). While NSCs hold great promise, a constant risk with stem cells is their ability for proliferation and multi-potentiality which may lead to proliferation of undesirable cell and tissue masses. The idea of using the biomaterial to promote differentiation is exciting.

Neurotrophic Factors

Neurotrophic factors (NFs) are proteins with multiple functions that enhance neuronal survival, proliferation, migration and differentiation, axon growth and synaptic plasticity. They not only contribute to neuronal survival and differentiation during development (Black, 1999; Nagtegaal et al., 1998), but also promote repair and recovery after CNS injury in the adult (Blesch et al., 2002; Jones et al., 2001). In fact, administration of NFs, such as NT-3, NT-4/5, or BDNF, into the lesion limits neuronal damage and promotes regenerative activity after SCI (Blesch et al., 2004; Coumans et al., 2001; Houweling et al., 1998a,b; Koda et al., 2004; Namiki et al., 2000). The combination of grafting cultured Schwann cells or autologous peripheral nerves with FGF-1, NT-3, or BDNF accomplished remarkable anatomical connectivity of severed axons and functional recovery after rat spinal cord transection (Blits et al., 2003; Lee et al., 2004; Xu et al., 1995). Tubular PAN/PVC conduits in combination with NFs (specifically NT-3, BDNF or GDNF) and cultured Schwann cells seeded on the inner lumen of the tubes provided promising results in a SCI rat model (Bamber et al., 2001; Bunge, 2002; Iannotti et al., 2003; Xu et al., 1995), whereas similar strategies that used FGF2 instead of the other neurotrophins did not enhance axonal regrowth, but did contribute to neuronal survival in the spinal cord adjacent to the site of rat spinal cord transection (Meijs et al., 2004).

CONCLUSION

Tissue engineering has enhanced the potential for SCI repair, resulting in partial functional recovery in experimental SCI, although more pre-clinical work is required before this technology can be translated to humans. To date, synthetic guidance channels by themselves have been insufficient to achieve significant anatomical or functional recovery in SCI. Combination therapy—utilizing biomaterial scaffolds to deliver growth factors and Schwann cells, OEG grafts, or stem cells—is the preferred strategy. However, there remain significant challenges with both the biomaterials and the stem cells. Non-degradable materials are complicated by not being degradable resulting in either their permanent implantation or a second surgery requiring their removal. Biostable materials may collapse over time, leading to neuromas. Biodegradable materials obviate the need for a second surgery, yet are complicated by the degradation products produced, which must neither be cytotoxic nor cause an inflammatory reaction, and the rate of degradation which must follow that of regeneration. Cell therapy provides great promise as the cells provide many of the factors required for a regenerative environment; however, maintaining cell viability after implantation remains a significant challenge. Moreover, engineered cells have the additional challenge of maintaining cell function and stem cells have the added challenge of differentiating to the desired cell type. Alternate delivery strategies of neuroprotective or neuroregenerative molecules face the challenge of delivery across the blood–spinal cord barrier and having sufficient efficacy for recovery to be observed by the current behavioral models. Despite these many challenges, there is significant hope that the combination strategies of biomaterials, cells and therapeutic molecules will promote functional repair to the injured spinal cord.

ACKNOWLEDGMENTS

We are grateful to the Natural Sciences and Engineering Council of Canada and the Ontario Neurotrauma Foundation for funding.

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