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Current tissue engineering and novel therapeutic approaches to axonal regeneration following spinal cord injury using polymer scaffolds[☆]

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Abstract

This review highlights current tissue engineering and novel therapeutic approaches to axonal regeneration following spinal cord injury. The concept of developing 3-dimensional polymer scaffolds for placement into a spinal cord transection model has recently been more extensively explored as a solution for restoring neurologic function after injury. Given the patient morbidity associated with respiratory compromise, the discrete tracts in the spinal cord conveying innervation for breathing represent an important and achievable therapeutic target. The aim is to derive new neuronal tissue from the surrounding, healthy cord that will be guided by the polymer implant through the injured area to make functional reconnections. A variety of naturally derived and synthetic biomaterial polymers have been developed for placement in the injured spinal cord. Axonal growth is supported by inherent properties of the selected polymer, the architecture of the scaffold, permissive microstructures such as pores, grooves or polymer fibres, and surface modifications to provide improved adherence and growth directionality. Structural support of axonal regeneration is combined with integrated polymeric and cellular delivery systems for therapeutic drugs and for neurotrophic molecules to regionalize growth of specific nerve populations.

Keywords

Spinal cord injury; Axonal regeneration; Polymer scaffold; Tissue engineering; Neurotrophins

1. The rationale for tissue engineering approaches in the context of respiratory compromise

Current interventions offer little hope of functional recovery for patients after a spinal cord injury (SCI). In the United States, the incidence of SCI is 32 injuries per million population, approximately 11,000 new injuries per year, affecting a young group of people of median age 26, that is predominately male (82%). Road traffic accidents, acts of violence, falls, and sports injuries account for the majority of injuries. The average inpatient stay is 9 months, during and

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following which the patient's life, in virtually all aspects, is profoundly changed (Lali et al., 2001). 45.7% of the 253,000 persons living in the United States with the residual of spinal cord injury have permanent and complete paraplegia or tetraplegia, irreversible loss of neurologic function below the level of the injury (National Spinal Cord Injury Association, 2009).

The majority of patients die from respiratory complications. Injury at any level of the spinal cord will impair respiratory function, through the destruction of motor nuclei and descending motor tracts innervating diaphragmatic, thoracic, intercostal and abdominal accessory muscles. Equally impaired are ascending sensory signals for muscle control via stretch reflexes, for cough, vomit and secretion clearance and from peripheral respiratory chemoreceptors. These axons project through the spinal cord to and from a neural network in the brainstem comprising three interconnected centers, the pontine group, and the medullary dorsal and ventral respiratory group. The pontine group (parabrachial/Kölliker-Fuse complex) controls respiratory timing, receives input from lung stretch receptors, and links respiration to behavioural cues; the dorsal group receives afferents from respiratory chemo and mechanoreceptors, and coordinates respiratory-cardiac reflexes; the ventral group (Bötzinger complex) projects inspiratory neurons, expiratory motor neurons rostrally, and includes a pre-complex generating the respiratory rhythm. Axons descend in the spinal cord in the anterolateral white matter to phrenic, intercostal and abdominal motor neurons, laterally in the high cervical cord near the spinothalamic tract for autonomic function and with the corticospinal tracts for voluntary respiratory control (Nogues and Benarroch, 2008).

Accordingly, respiratory failure with spinal cord injury occurs as a consequence of alternations in tidal volume, ventilation and its pattern, diminished responses to hypercapnia, reduced lung and chest wall compliance, and progressive respiratory muscle fatigue due to compensatory breathing rates. Hypoxia from respiratory compromise can further the neurologic injury. Common secondary pathology includes (aspiration) pneumonia, atelectasis and the complications of mechanical ventilation (Lane et al., 2008). Injury to the cord can also induce paralytic ileus worsening aspiration. More severe respiratory compromise occurs with higher levels of injury with risk to phrenic motor nuclei located in cervical spinal cord segments C3-C5 (occasionally as low as C7) (Zimmer et al., 2007).

Pathological (Quencer and Bunge, 1996) and imaging studies (Bodley, 2002) demonstrate tissue destruction with cysts and gliosis in the area of injury, along with atrophy in adjacent segments of cord. Strategies aimed at preventing immediate and delayed secondary damage need to be administered within minutes or hours of injury. Even if ideal protective agents were available, many patients would not be in circumstances where this would be available or successful. The area of cysts and glial scarring does not contain cells or tissue that contribute to regeneration and is consequently both a gap and a barrier to regeneration. There are, therefore, only two ways to re-establish neurologic function below the block: bypassing the area or rebuilding functional tissue within the cyst/scar. A functional bypass might be established by nerve autograft connections from areas above the lesion to distal effectors (cord or muscle) (Tadie et al., 2002). The second approach is to replace the cyst/scar with functional tissue, promoting the development of neural tissue bridges to carry regenerating axons from above to roots or muscles below the lesion (Friedman et al., 2002). For future use in patients, replacement of a segment of cord would be suitable for those with massive damage to the cord with no evidence of residual functional tissue in the area. Unfortunately, this accounts for a significant number of patients.

Animal models of spinal cord injury include complete transection (thoracic), hemisection (dorsal or unilateral), and contusion injuries (forceps and computer-controlled weight impact). These models approximate common human pathology, open cord laceration (1/4 of injuries) and closed compression/contusion injuries (3/4). Biomaterial polymers may be delivered as

gels, suitable for contusion and small tears, as devices designed to fill larger defects (sponges) or to bridge large gaps and traverse the glial scar (tubes and multichannel scaffolds (Hiroshi et al., 2006). While deep tears or transections are rare in human injury, complete or partial transections in animal models are useful as proof of concept, and for the controlled study of axonal regeneration (Talac et al., 2004). Animal models of respiratory dysfunction focus on high cervical injury producing diaphragm hemiplegia, but no studies to date have employed polymer-based tissue engineering strategies specifically in this context. Given the severity of patient morbidity and the rates of mortality associated with respiratory compromise, neurologic repair is an important therapeutic goal. A relatively short distance, from the medulla to phrenic C3-C5 or within the phrenic segments for example, needs be bridged by new neuronal tissue. Equally, respiratory innervation associates with discrete tracts, corticospinal and spinothalamic, and repair may often be unilateral given a lateral injury and diaphragmic hemiplegia. Such tracts represent ideal targets for polymer scaffold implantation given their limited scope and clinical importance.

2. Important bioengineering considerations

The classification of biomaterials for the spinal cord is based on whether the materials are naturally derived or synthetic, whether they are hydrogels, whether or not they are biodegradable, as well as other sub-classifications based on specific modification or functional adaptation (surface charged, drug-delivery etc.). Regardless of the source or application, the material must have properties which are biocompatible specifically within the spinal cord environment (Kohane and Langer, 2008). These properties in turn influence the regenerative capacity of engineered structural support for neurite outgrowth at a macro (i.e. fascicular) and micro (axonal) level.

2.1. Scaffold biocompatibility within the spinal cord environment

In relation to blood, cerebral spinal fluid is low in cellular nutrients. Scaffold permeability to various molecular sizes becomes crucial for access to oxygen and nutrients and removal of metabolic wastes. The degree to which a material swells within the aqueous environment of the spinal cord must be known if the scaffold is to maintain an appropriate alignment and not compress regenerating nerves. Degradation kinetics may be accelerated by the ingrowth of axons and by the deposition of extracellular matrix by support cells of the CNS or by the therapeutic cell line seeded within the scaffold. Stiffness, permeability, swelling, strength and degradation are of course specific to the particular polymer employed, are all readily modified through changes in polymer concentration or constituent ratios. de Ruiter et al. (2008) exemplify the type of *in vitro* characterization and methodology required to develop an implant of any polymer type. In this study, the authors present a series of methods to characterize multichannel nerve tubes for properties of bending, deformation, swelling, and degradation and introduce a new method to test the permeability of multichannel nerve tubes from the rate of diffusion of different-sized fluorescent dextran molecules. Equally, the implantation methodology must be developed. While the material is placed within rigid spinal column, the spine may require further fixation (Fig. 1) (Rooney et al., 2008). Scaffolds in unfixed spines have a greater tendency to produce scoliosis and become displaced (Fig. 2). The material should be of sufficient softness not to physically damage the cord as the animal moves. The degradation products of the polymer, and any residual agents used in its fabrication, cannot be locally or systemically cytotoxic or elicit an immune response which will further gliosis, and be destructive both to the scaffold complex and any regenerating axons (Liu and Cao, 2007). Cells detect mechanical characteristics of the environment through adhesion complexes and the actin cytoskeleton, and the stiffness of the substrate may be of critical importance (Discher et al., 2005). Finally the tensile strength of the material – its ability to hold a suture for example – will contribute to its clinical use.

2.2. Material fabrication and porosity

Scaffold biomaterials for spinal cord placement are fabricated by dissolving the monomer in an aqueous or organic solvent to produce a liquid state that is polymerized into macromers by a chemical, thermal or photo-crosslinking reaction. Additional reagents may be added to enhance the crosslinking reaction. Synthetic polymers often employ the use of chemical initiators and accelerators to fine-tune polymerization rates. The majority of spinal cord scaffolds is made by injection molding. To create pores in the structure, porogens are incorporated into the polymer mix. Commonly used porogens include sodium chloride crystals, ice crystals, gas bubbles introduced by peroxides or air-foaming, and gelatin composite materials. In each case, the polymer forms around the porogen, which itself is removed from the final structure leaving only the space it occupied (Fig. 3). The size of the pore therefore can be controlled by adjusting the variables that control crystal size and the direction of crystal growth (Madaghiele et al., 2008), or by adjusting the water content of a hydrogel.

A continuous porous structure closely mimicking the intrinsic mechanical characteristics of the original tissue may provide a better environment for regeneration (Discher et al., 2005; Deguchi et al., 2006). Material porosity is essential for cell attachments, allows for greater distances that may be bridged, and improves functional recovery following transection (Jeng and Coggeshall, 1985; Vleggeert-Lankamp et al., 2007; Reynolds et al., 2008). Porosity also allows for tissue vascularization of the avascular scaffold implant, influences cell migration and phenotype, and will improve implant stability at the cord-scaffold interface through interlocking between implant and cord tissue (Dadsetan et al., 2008).

2.3. Macroengineering

Nerve tracts in the human spinal cord have diameters of 100–1000 μm . It is the role of scaffold macroengineering to design conduits whose architecture optimizes axonal growth potential through the alignment of fascicular groups (Fig. 4). Channel sizes depicted in the templated agarose scaffold are on the order of 200 μm (Fig. 4A), and 450 μm in the PLGA scaffold (Fig. 4C). In the first study of its kind, Wong et al. (2008) directly compared porous poly (ϵ -caprolactone) synthetic polymer scaffolds cast in five different architectures, cylinder, tube, multichannel, and open-path design with and without a central core (Fig. 4B). The findings demonstrated not only that open path designs were improvements over the other three designs in terms of regenerative capacity, but also that the other more closed designs adversely affected the surrounding cord, doubling the defect length. In a multichannel model, the channel size is of importance. Spinal cord scaffolds with multiple longitudinally aligned channels of 450 and 660 μm were constructed from PLGA using injection molding. When seeded with Schwann cells, this scaffold design supported robust axonal growth (Moore et al., 2006), although also demonstrated the formation of a rim of fibrous tissue surrounding the core of regenerated neurons. Further work evaluating the relationship between scaffold channel diameter and the number of axons regenerating showed a larger area of fibrous tissue and a reduced axon number in the larger channel size (Krych et al., 2009).

2.4. Microengineering

A large myelinated axon in the CNS has a diameter of 15–20 μm . In order to create functional reconnections, the scaffold must also regionally align separated neuron groups at an axonal and growth cone level. Scaffold microengineering refers to designing features that are in the order of a few microns in at least one dimension (Khademhosseini and Langer, 2007). In the context of the spinal cord, this includes micro-structures that will provide precise directionality to growth and improve axonal adherence at the level of the advancing growth cone. It is conceptually useful to consider the re-alignment of nerve fascicles as 3-dimensional bundles of axons which themselves prefer to grow or elongate along the planes of 2 dimensions (Bellamkonda, 2006). Microgrooves can be placed in the polymer surface by laser etching,

affecting contact guidance and alignment of neurites. *In vitro* work has demonstrated optimal sizes of 2 μm minimum groove depth (Clark et al., 1991), with more narrow ridges (5 μm versus 10 μm) improving the number neurites aligned as well as the number of focal contact adhesions in a given cell (Goldner et al., 2006). Further improvements in neurite outgrowth are seen with coating the grooved surface with collagen or laminin peptides, Fig. 5(A and B) (Yao et al., 2009). Axonal growth cones preferentially advance up gradients of laminin (Adams et al., 2005). For the spinal cord, gradients have been made in natural and synthetic polymers with laminin (Dodla and Bellamkonda, 2006).

Axons also show a preference to grow along the length of micro and nanofibres of polymers such as collagen and synthetic polymers, Fig. 5(C and D). Polymer fibres are made by monomer self-assembly, producing a randomized fibre orientation of larger caliber fibres, or by electrospinning for parallel alignments of nanometer scale fibres (50 nm–30 μm). This technique uses electric charge to draw and elongate threads of liquid polymer (collagen, PLGA, PCL) from a syringe pump source. The solvent evaporates from the airborne filament which is laid down upon an electrically grounded plate or rod, or onto a spindle apparatus (Yang et al., 2005). Collagen filament bridges with fibres of 20 μm show promise as 2-dimensional surfaces to guide axons, improving axonal density and motor function, but appear also to have significant cytotoxicity with high animal mortality in a rabbit model (Yoshii et al., 2004; Yoshii et al., 2009). A goal of scaffold microengineering is to pack larger channels of scaffolds with these fibrous substrates to approximate normal axonal densities (Schnell et al., 2007).

3. Biomaterials for the spinal cord

3.1. Natural polymers

The spinal cord lacks a support matrix equivalent to the endoneurium and perineurium in peripheral nerve, one that can act as conduits to approximate disconnected axonal groups. Furthermore the axonal density of the spinal cord far surpasses that of peripheral nerve with far less extracellular matrix support. The rationale for polymer implants is to replace a damaged area of the cord with just such a structural matrix. Natural polymers are biological fibrillar protein, polysaccharide, or glycosylaminoglycan (GAG) carbohydrates which form hydrogels. These polymers already have an intrinsic function such as extracellular matrix or structural support, and a degradation profile by *in vivo* enzymes that is in keeping with their natural role. Hydrogels are mesh networks of insoluble polymer fibres, through which water can freely flow to osmotically swell or shrink the overall structure. Hydrogel polymers are attractive materials for use in the spinal cord. They are macroporous, soft materials readily allowing cell adhesion and migration, while nutrients and wastes are easily exchanged. They can be easily shaped to fit the defect, their elasticity and degradation may be adjusted by component density. Table 1 details the main natural polymers and highlights examples of their application within the spinal cord. Two spinal cord extracellular matrix (ECM) components are used, collagen and hyaluronic acid (HA); two polymers are derived from marine plants, agarose and alginate, and chitosan is derived from insect or crustacean shells.

3.1.1. In vivo extracellular matrix polymers—Being the predominant extracellular matrix protein, type I collagen has intrinsic properties including molecular sites for cell adhesion and migration, inherent signaling transduction for proliferation and differentiation, and mechanical properties similar to soft tissue. Antigenicity is low, provided the origin species is the same as the host. Solutions of collagen are polymerized by adjusting pH, or with the addition of ionic salts. Whereas early use focused on its application as a 3-dimensional matrix growth, it was soon realized that collagen itself has a limited capacity to support axon growth (Marchand et al., 1993), and that its use required further functionalization. An important trend for the use of collagen is in combined strategies, particularly as a growth or elution matrix

within or on the surface of other polymer types (Tsai et al., 2006) or as a composite material (Cheng et al., 2003). Axonal extension onto collagen can be improved through covalent modification, or the incorporation of cell-adhesion molecules such as laminin, to provide directional guidance as a gradient along collagen fibres (Yao et al., 2009). Collagen also has the advantage of being thermoresponsive, gelling at physiologic temperatures, making it attractive for use as an injectable polymer delivery system. This property enables the incorporation of neurotrophic factors, drugs or cells at the time of gelation without thermal damage to the factor or cell line. Incorporation of neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF) (Houweling et al., 1998) improved axonal counts and animal function, including a specific regional improvement of corticospinal tract density with the use of NT-3. Degradation of the collagen *in situ* allows for sustained release of the growth factor, as well as an improved surface for cell attachments.

The glycosaminoglycan HA was thought to be a good material candidate given its role as extracellular matrix in the brain, but its success in supporting axonal growth is modest. As a scaffold material, HA has been used with benefit as a matrix for cultured embryonic spinal cord tissue placed into transected cord (Rochkind et al., 2002). HA has however been developed into an extremely useful co-polymer gel with methylcellulose for intrathecal drug delivery (Gupta et al., 2006). Whereas methylcellulose gels at increasing temperature, unmodified HA quickly disperses *in vivo*. The combination of acetate-modified HA with methylcellulose (HAMC) has the distinctive property of already gelling at room and physiologic temperature prior to its delivery, but become liquid when subjected to the mechanical shear forces involved with syringe and needle injection (Katz and Burdick, 2009). Collagen embedded epidermal growth factor (EGF) (Shoichet et al., 2007), and erythropoietin (Kang et al., 2009), have been safely delivered with sustained release *in situ* from HAMC. The latter agent enhanced neuroprotection with reduced cavitation size and increased neuron numbers following clip compression of the spinal cord.

3.1.2. Polymers from marine life—Agarose is used in many of the same ways as collagen for spinal cord repair. It is a linear polysaccharide derived from seaweed and crosslinked by temperature gradients through hydrogen bonding. Agarose is thermoresponsive, but at temperatures lower than 37 °C. It has been used as an injectable system when it can be rapidly cooled *in situ* using liquid nitrogen vapour (Jain et al., 2006). Such a system is now being developed for direct topical delivery of dexamethasone onto the injury site, from drug eluting nanoparticles suspended within an agarose implant (Chvatal et al., 2008). Early inflammatory infiltrates and lesion size were reduced by day 7. Like collagen, agarose itself is relatively impenetrable by axons, but serves as an excellent axonal growth substrate, particularly when functionalized with laminin gradients (Dodla and Bellamkonda, 2006). Tuszynski and co-worker have used a freeze drying method to form agarose scaffolds containing linear guidance pores with a mean diameter of 120 µm (Stokols and Tuszynski, 2004, 2006). This process involves the formation of ice crystals whose size and direction of growth can be controlled by the temperature gradient (Tabesh et al., 2009). Pore size in the scaffold can also controlled by the freezing rate and pH, with the faster rate creating smaller sizes (Sachlos and Czernuska, 2003). Integrating BDNF into the scaffold material (Fig. 6) and in separate experiments, BDNF-secreting mesenchymal stem cells scaffold channels, significantly improved the scaffold's capacity to promote regeneration (Stokols and Tuszynski, 2006). BDNF within lipid microtubules has also been incorporated into agarose scaffolds, enhancing axonal growth for the length of the scaffold but not into the distal cord (Jain et al., 2006).

Alginate is obtained from algae and the polymer solution is crosslinked by calcium into a sponge-like structure. Such a structure supported axonal extension in the spinal cord and limited gliosis (Kataoka et al., 2001). Hippocampal neurospheres and BDNF-secreting fibroblasts have been seeded onto alginate and placed into the transected cord (Nomura et al., 2006). Agarose

and alginate requires ultrapurification prior to use, given that commercial preparations often contain mitogens and cytotoxic byproducts.

Chitosan is a GAG carbohydrate polymer derived by chemical deacetylation of chitin, the major structural polysaccharide found in crustacean, shellfish and insect shells. Cell adhesion to the structure is determined by the extent of its positive charge, itself a function of the degree of alkaline deacetylation (Nisbet et al., 2008). Further improvements in cell attachment are seen with the addition of poly-L-lysine to the polymer mix, and a thermoresponsive polymer can be made with the addition of glycerol phosphate salts (Crompton et al., 2007). Chitosan scaffolds support axonal growth in the spinal cord (Freier et al., 2005), and the polymer may be used to encapsulate therapeutic cell lines (Yuan et al., 2004), Fig. 7(lower). Recent work by Shoicet and co-workers demonstrates chitosan's use as extramedullary and intramedullary conduits capable of supporting neural stem cell differentiation in the transected cord (Nomura et al., 2008; Zahir et al., 2008).

3.1.3. Polymers derived from the blood—Plasma derived polymers, fibronectin and fibrin, are being used as spinal cord scaffolds. Fibronectin mats are formed with linearly aligned fibres which can orient axonal growth (King et al., 2003) and sequester growth factor within its pores for gradual release (Phillips et al., 2004). Fibrin scaffolds are formed from monomers following fibrinogen cleavage by thrombin and crosslinked with Factor XIIIa (Willerth and Sakiyama-Elbert, 2007). It is a natural matrix for wound repair having inherent cell-binding sites. Heparin has been crosslinked to fibrin scaffolds using a bidomain Factor XIIIa-heparin linker peptide for use as an affinity-based delivery system for growth factors, including NT-3 (Taylor and Sakiyama-Elbert, 2006) and for factors to differentiate embryonic stem cells seeded within the matrix. A recent study implanting fibrin polymer scaffolds into a dorsal hemisection model demonstrated delayed astrogliosis and improved neuron fibre extension (Johnson et al., 2009), Fig. 7(upper panels).

3.2. Synthetic polymers

Whereas the use of natural polymers in the spinal cord takes advantage of their inherent properties, their natural role to some extent being the basis of their function or their modification, using synthetic polymers offers wider scope to design and control the characteristics of the material. The synthetic polymers used thus far in the spinal cord are either biodegradable materials based around polyesters of lactic and glycolic acid (PLA and PGA), are biodegradable hydrogels based on polyethylene glycol (PEG), or are non-biodegradable hydrogels based on methacrylate. Early spinal cord scaffolds were made from the same materials as were in common clinical use for surgical repair of peripheral nerve and skin grafting. Rapid advances in hydrogel chemistry have produced materials more suited to the spinal cord in their mechanical properties. The trend now is towards using these highly aqueous, soft polymers given the similarity of their properties to spinal cord tissue, and the versatility with which their chemistry and architecture can be adjusted. Functionalized synthetic polymers have included gradients and surface charge modification for cell adhesion, neurotrophic gradients, and have opened the field to using scaffolds themselves as drug delivery and gene delivery vehicles to novel extent. The technical ability to rapidly photocrosslink synthetic hydrogels has also enabled a remarkable degree of sophistication in macro and micro-architecture through the use of photolithography. Please see Table 2 for a listing of synthetic polymers and examples of their use within the spinal cord.

3.2.1. Poly α -hydroxy acid polymers—At the time of our previous review (Friedman et al., 2002), much of the work in scaffold design focused on the use of biodegradable synthetic polymers, particularly the poly (α -hydroxy acids) PLA, PGA and their co-polymer PLGA. These polymers are polyester links of lactic and glycolic acid which are hydrolyzed *in vivo* to

release lactide and glycolide, dissolving the material. The pH around the grafted site accordingly will become more acidic. These compounds were a good initial choice for spinal cord placement, having a long track record of FDA approved clinical use as an absorbable suture material, and as grafting material for skin and for peripheral nerve repair (Mackinnon and Dellon, 1990; den Dunnen et al., 1993). The transition to CNS applications was based on the idea that such scaffolds could provide a corresponding PNS-like endoneurial and perineurial guidance structure to regenerating spinal cord axons, improved with the addition of myelinating Schwann cells within the scaffold (Hadlock et al., 2000).

PLA has been used to make scaffolds. Having shown the resorbability and biocompatibility of PLA with Schwann cells and the spinal cord (Gautier et al., 1998), the University of Miami group placed single channel Schwann cell loaded scaffolds into the transected rat spinal cord. The PLA materials used were structurally unstable, fragmenting and collapsing, but proved to support the extension of axons and vascular growth into the graft (Oudega et al., 2001). More recently, macroporous PLA foam scaffolds made with longitudinally aligned pores were fabricated using a freeze drying technique. BDNF was dissolved into the scaffold matrix but did not improve an overall low yield in axon numbers through the graft (Patist et al., 2004). This study was extended to incorporate Schwann cells into the foam that had been genetically engineered to secrete a bi-functional neurotrophin (D15A) with BDNF and NT-3 activity (Hurtado et al., 2006). Axonal regeneration was modest at 6 weeks, and few Schwann cells survived the first week of scaffold placement. There has been recent interest in the use of PLA nanofibres as a cell substrate (Wang et al., 2009).

The degradation rate of PLA can be somewhat controlled in the co-polymer PLGA by altering the ratios of the PLA and PGA in composite. The *in vitro* characteristics (bending, swelling, deformation, degradation and permeability) of varying PLA:PGA ratios have been extensively determined for PLGA spinal cord scaffolds implants (de Ruiter et al., 2008). Multichannel PLGA scaffolds have been demonstrated to support robust axonal regeneration when seeded with Schwann cells without functional improvements (Moore et al., 2006). As detailed in the section below, PLGA degradation kinetics are the basis for drug delivery via microspheres embedded in polymer scaffolds.

3.2.2. Synthetic hydrogels—Polyethylene glycol is a biodegradable synthetic polymer of ethylene oxide units. Its role is somewhat unique in that it is an ‘exclusionary’ compound, immunoprotecting the areas to which it is applied by keeping out cell infiltrates, and equally is used as a delivery system for cells, neurotrophins and genetic constructs. It has been formulated into gels for topical application onto the injured spinal cord (Borgens et al., 2002), combined with PLA for neurotrophin delivery of a photo-inducible gel, or as a formulation of ‘pegylated’ BDNF as an intrathecal infusion (Soderquist et al., 2008). Intravenous solutions with magnesium sulfate are being investigated as a first line immunomodulatory therapy in acute spinal cord injury (Kwon et al., 2009).

Poly(2-hydroxyethyl methacrylate) (pHEMA) compounds were initially used as non-biodegradable materials for soft contact lenses. Professor Shoicet’s group in Toronto has extensively developed pHEMA and pHEMA-co-methyl methacrylate (pHEMA-MMA) for use as spinal cord scaffolds. Early applications included pHEMA sponges (Giannetti et al., 2001), evolving to guidance channels (Tsai et al., 2004), with a variety of surface modifications to improve cell adherence and axonal extension (Moore et al., 2006; Tsai et al., 2006) including neurotrophic gradients and adhesion gradients within a chitosan composite (Yu et al., 2007). Microfluidic techniques are employed to create functional concentration gradients in scaffolds, again providing cues for directionality of axonal growth. The technique involves casting a scaffold using 2 or more inlet ports coupled with rapid polymerization. An important advantage of this polymer class is the modification of surface charge with the addition of quaternary amine

groups or of a second methacrylate subtype. Many cell types adhere better to a positively charged surface. Lesny et al. (2006) evaluated the in growth of neural tissue in a dorsal hemisection injury bridged with four pHEMA composites, demonstrating an improvement in axonal regeneration into the core of the scaffold and a reduction in astrocyte infiltration in the positively charged scaffold.

Very sophisticated architecture is possible with these compounds. The scaffolds have been reinforced with coils, as well as being made as with neurotrophin PLGA microspheres. Multilayered macroporous pHEMA composites, including innermost channel layers that elute neurotrophic factors, have been made by liquid-liquid centrifugal casting. Thin layers of the polymer liquid are forced against the side of the mold by the centrifugal force and polymerized. The polymers can also be crosslinked with light with the addition of a photoinitiator. Using photolithography, in which discrete sections of the polymer mix are exposed to light while others are masked, open channel size and pore size were recently controlled with remarkable precision (Bryant et al., 2007).

4. Biomaterials as therapeutic cell systems in the spinal cord

As one of the field's principle contributors, Bellamkonda advocates that scaffold strategies for nerve regeneration should combine four main components, a permissive growth substrate (hydrogel or micro/nanofibre), a neurostimulatory extracellular matrix (protein or peptide), the provision of neurotrophic factors, and glial or support cells (Schwann cells, neural stem cells). Cell therapies can be delivered to the spinal cord by direct injection into the cord substance, intrathecal infusion, or by polymeric microspheres or scaffolds. It has been shown that a variety of cells support axonal regeneration within polymer scaffold models in the cord. Cells can be loaded onto polymer scaffolds when suspended in a support matrix such as fibrin or Matrigel™. If the scaffold is a macroporous hydrogel, the cells migrate and become resident within that porous structure, and if the scaffold is designed to be multichannel, select channels can be seeded with different cell types allowing for regional topography (Friedman et al., 2002).

4.1. Schwann cells

Early work in the spinal cord was again derived from peripheral nerve repair strategies. Schwann cells myelinate peripheral nerve, and will naturally migrate from peripheral-central nerve junctions such as the dorsal root ganglia during times of cord injury to support repair (Oudega et al., 2005). Here they play a structural role, lay down extracellular matrix proteins like laminin, and provide paracrine trophic support through secretion of nerve growth factor (NGF), NT-3, BDNF, ciliary neurotrophic factor (CNTF) and basic fibroblast growth factor (bFGF) (Willerth and Sakiyama-Elbert, 2008). Xu et al. (1995) placed Schwann cells expanded in culture from harvested rat sciatic nerve into a poly(acrylonitrile-co-vinylchloride) (PAN/PVC) nerve conduit and into a complete cord transection model. The study demonstrated axonal extension and myelination of about a quarter of the regenerated sensory nerves as seen with electron microscopy, and that the extent of the repair could be enhanced by administering methylprednisolone in the acute phase of the injury (Chen et al., 1996). The Miami group also utilized peripheral nerve grafts placed directly into the transected spinal cord serving as natural conduits for axonal extension and as a source of Schwann cells (Oudega and Hagg, 1996, 1999). This work was extended to cell-seeding polymer scaffolds, demonstrating evoked nerve conduction potentials across the graft only in the Schwann cell group (Pinzon et al., 2001). In all applications, it is important to understand whether cells survive in the grafted material (natural or synthetic). We have demonstrated cell survival for up to 6 weeks after transplantation through a multichannel scaffold, and that in this model, Schwann cells have a

higher capacity than stem cell neurospheres to enhance axonal regeneration in the transected cord, Fig. 8 (Olson et al., 2009).

4.2. Olfactory ensheathing cells (OECs)

Olfactory ensheathing glia are cells within the peripheral and central component of the olfactory system that contribute to the regenerative capacity of olfactory neurons. The average lifespan of olfactory receptor neurons is 4 weeks. These neurons are bipolar cells projecting to the nasal epithelium and to the olfactory bulb. New receptor neurons are derived from a stem cell layer at the base of the nasal epithelium, from where axons are projected through the cribriform plates into olfactory bulbs. The role of OEC is to enfold and guide growing axons to the bulb in bundles of unmyelinated axons, and once at the bulb to interact with resident astrocytes and fibroblasts to finalize the connections (Franssen et al., 2007). While extensive work (over 40 *in vivo* studies) has been done with cell injections into the cord, few studies have utilized polymer scaffolds. In one of the first, and most successful studies Bunge's group used Schwann-cell seeded PAN/PVC conduits in a thoracic transection model, now in combination with stereotactic injection of OECs at four midline depths. The OECs induced axonal growth in through the SC-containing channel and for distances of 2.5 cm, the longest distance observed thus far for OECs (Ramon-Cueto et al., 1998). Followup studies showed functional recovery without the use of scaffolds (Ramon-Cueto et al., 2000). Lu and Ashwell (2002) used collagen matrix soaked with OECs to bridge a dorsal transection and with similar efficacy to pieces of olfactory lamina propria and OEC injections. Chuah et al. (2004) encapsulated OECs into polyvinylidene fluoride particles for injection following dorsal transection which increased the numbers of collateral branches from the intact ventral cord. A strategy which used Schwann cells embedded in a Matrigel™ bridge, injections of OECs into the distal and proximal cord stumps following transection, and alternate day delivery of Chondroitinase ABC via an intrathecal catheter, resulted in select fibre regeneration and some recovery of function (Fouad et al., 2005).

4.3. Neural stem cells

Neural stem cells (NSCs) are a pluripotent, self-renewing population of precursor cells that give rise to astrocytes, oligodendrocytes, and neurons in the CNS. Their role in regenerative medicine is therefore to remyelinate axons growing from the injured cord, to themselves become neuronal links within the injury (Lowry and Temple, 2007), and to elaborate neurotrophic factors to stimulate regrowth (Lu et al., 2003). In animal models, they are derived from fetal brain homogenates, from which they are cultured as spherical aggregates (neurospheres), which can be in turn be subcultured for *in vitro* differentiation or *in vivo* transplantation. Stem cells for use in humans pose several problems. Their nature as multipotent cells risks unrestrained proliferation to desired or to unwanted cell lineages. They must be derived from fetal tissue in order to preserve the full range of pluripotency, or be obtained in the adult from brain or spinal cord biopsy; the latter as a source may provide cells whose lineage is more restricted to a neuronal phenotype. Cross-species implantation will not avoid immune surveillance. When injected into the cord following contusion injury (Cao et al., 2001) or a hemi-section model (Chow et al., 2000), neurospheres tend to adopt an astrocyte morphology and the neurite connections which do form tend to create pain circuits (Hofstetter et al., 2005). That indeed no study has shown large scale neuronal differentiation of engrafted stem cells suggests the injured cord, regardless of the injury model, is not a permissive environment (Enzmann et al., 2006). There have been numerous efforts to control cell lineage with growth factors, location and timing of cell harvest, and genetic transduction prior to implantation.

Many researchers have turned to polymer scaffold substrates as a biomechanism to differentiate the cells into neurons either *in vitro* prior to implantation, *in vivo* in conjunction with a scaffold implant, or to encapsulate the cells with polymer for immune protection (Zhong and

Bellamkonda, 2008). Spinal cord scaffolds then may well play a central role should neural stem cells become useful for human clinical therapy. Recent *in vitro* work has set out to define which polymer compounds and structures are permissive or inhibitory to stem cell viability and differentiation. The most promising substrates include the use of 3-dimensional fibrin scaffolds, seen *in vitro* to support and differentiate mouse embryonic stem cells when cell culture and scaffold fabrication conditions were optimized (Willerth et al., 2006). Further work evaluated the effects of growth factors, their doses and combinations on stem cell differentiation to neurons and oligodendrocytes (Willerth et al., 2007). PLGA has also been shown to support NSC viability and neurite outgrowth to a greater extent than poly- ϵ -caprolactone (PCL) and PLA (Bhang et al., 2007). *In vivo* work done by the Langer lab has used PLGA scaffolds seeded with NSCs in a hemisection model: this scaffold had complex architecture, with an inner layer seeded with NSCs approximating the gray matter, and an outer layer whose pores were oriented longitudinally for axon guidance and radially for permeability. Scaffolds were left in the animal for up to a year with persistent functional improvement, including hindlimb weight bearing and improved coordination seen between 2 and 3 months post implantation (Teng et al., 2002). Our group has also seeded neurospheres into multichannel PLGA scaffolds in a direct comparative study with Schwann cells, Fig. 9, demonstrating the capacity of PLGA to support neurosphere differentiation (Olson et al., 2009).

Micro and nanostructures influence stem cell growth and differentiation *in vitro*. A micropatterned polystyrene surface provided growth differentiation and direction to hippocampal stem cells with laminin (Recknor et al., 2006) and with growth factor cues without adhesion molecules (Oh et al., 2008). Fibrous nanostructures differentiate NSCs and may even control cell lineage through the structure itself. Silva et al. showed self-assembled nanofibres could rapidly differentiate NSCs to neurons and inhibit the development of astrocytes (Silva et al., 2004). Similarly, electrospun nanofibres of PCL polymer differentiated cells and provided a stimulus and directionality to neuron growth (Xie et al., 2009).

5. Biomaterials as drug and cell based delivery systems in the spinal cord

The therapeutic potential of spinal cord scaffolds is enhanced by the development of integrated polymer drug delivery systems and cell lines that are genetically modified to secrete neurotrophic factors. Polymeric delivery from scaffolds is achieved by means of the materials inherent properties: its porosity and permeability for the sequestration and diffusion of a drug its degradation kinetics for release of the entrapped drug, its chemical affinity to a drug by means of a linker moiety, or by being a non-biodegradable material for the preservation of a gradient (Willerth and Sakiyama-Elbert, 2007). We have seen in the discussion of individual polymer types a number of ways each polymer type can be used to deliver neurotrophins or drugs. The drug or factor can be released from the material itself, from integrated micro- or nano-spheres or tubules of a different material, or by means of a scaffold's capacity to support a genetically modified cell line *in vivo*.

5.1. Micro- and nano-spheres and nanoshells

Micro- and nano-spheres refer to particulate synthetic polymer of the order of microns or nanometers in diameter. By far the most frequently used polymer is PLGA, as its degradation rate and thus drug release is readily controlled by the proportion of PLA to PGA. For example, 85:15 PLA to PGA will degrade significantly more slowly than a 50:50 ratio. PLGA microspheres are typically 2–45 μm in size. Alginate-chitosan microspheres are also used. Micro and nanospheres are produced by microemulsion techniques, whereby an aqueous solution is emulsified in an organic phase polymer solution to create spherical droplets which are then extracted into another external aqueous phase (Benoit et al., 2000). The size of the

droplet can be controlled by the emulsion agitation rate, the aqueous and organic phases used, and the addition of surfactants to modify the surface tension between the phases (Khademhosseini and Langer, 2007). The drug is usually stabilized with protein (bovine serum albumen) or with zinc, added to the aqueous phase and becomes encapsulated upon polymerization of the droplets. Other techniques include aerosol freeze drying. The rate of drug delivery will depend on the initial concentration in the sphere and the size of the sphere for a polymer of given degradation kinetics. The particles are injected as a suspension, or can be suspended in a scaffold of another material, in which case the pore size of the material must enable diffusion or cell access within. Alternatively, as shown in chitosan scaffolds (Fig. 10), the spheres can be localized directly to the channel wall by centrifugation (spin-coating) techniques (Kim et al., 2008). Nanoshells may be useful in the delivery of hydrophobic drugs from scaffolds. These spherical particles combine the benefits of PEG liposomes with polymeric shell, and are fabricated from PLGA polymer, lecithin phospholipid and a PEG core using modified emulsion techniques (Chan et al., 2009).

Microspheres in scaffolds allow the opportunity for sustained local delivery of therapeutic molecules within the blood–brain barrier. Neurotrophins embedded in PLGA microspheres have been used in a variety of polymer scaffolds with NGF (Mahoney et al., 2006) being the most extensively characterized. Cyclic AMP has been shown in many models to induce or enhance axonal growth (Murray and Shewan, 2008). Similarly the work of Bunge and co-workers has demonstrated that neurotrophins enhance axonal growth in regenerating spinal cord (Blits et al., 2003) and that this may be further enhanced by c-AMP (Pearse et al., 2004). Chondroitinase ABC (ch-ABC) an enzyme which degrades a variety of chondroitin and heparin sulfate proteoglycans (Plant et al., 1998) as well as hyaluronan, disrupts the glial scar matrix and facilitates axonal repair. Treatment with ch-ABC significantly enhanced axon regeneration *in vitro* (Plant et al., 1995) and following brain (Nash et al., 2002) or peripheral nerve lesions (Richardson et al., 1980) and, most importantly, SCI in rats (Stichel et al., 1999). Furthermore, functional recovery in rats after SCI has been observed (Bradbury et al., 2002). We have demonstrated sustained release of dibutyryl cAMP for over 3 weeks when incorporated into microspheres. Finally, while the efficacy of systemic delivery of methylprednisolone in the acute setting for SCI remains an area of contention, polymeric delivery of the steroid is more beneficial in animal models than systemic steroid in reducing the lesion volume when delivered from a scaffold (Kim and Martin, 2006) or from an injectible nanoparticle colloid (Kim et al., 2009).

5.2. Neurotrophin delivery and the promise of regional control

A common fabrication technique is to incorporate a neurotrophic factor into the polymer mix prior to polymerization, whereby the entire scaffold elutes the drug in an isotropic fashion. Natural materials are particularly well suited to this approach, given their polymerization rarely involves processes that will denature or destroy the factor. This approach was among the earliest used in the spinal cord, whereby a collagen matrix with NT-3 was placed into a transection model (Houweling et al., 1998). The collagen construct specifically attracted corticospinal sensory tracts, promoting partial recovery, and in doing so exemplified important principles of polymer scaffold design. The chemotrophic effect of neurotrophins locally delivered from polymers enhanced the regenerative capacity of the scaffold, and did so in a manner that can be regionally controlled on the basis of the neurotrophin's target axonal population.

A second delivery approach is to use synthetic multichannel channel scaffolds whose conduits are filled with eluting polymer. Iannotti et al. (2003) filled a minichannel PAN/PVC with Matrigel™ containing recombinant glial cell derived neurotrophic factor (GDNF), or GDNF Matrigel™ with additional Schwann cells. GDNF attracted axons primarily of propriospinal

phenotype, and the effect of Schwann cells was synergistic. The polymer matrix in the channel itself can also significantly impact the axonal phenotype regenerated. When filled with either Matrigel™, collagen, fibrin, or methylcellulose, fibrin matrix within pHEMA-MMA channels demonstrated preferential regeneration of descending reticular neurons, while methylcellulose promoted growth of vestibular and red nucleus neurons (Tsai et al., 2006).

Thirdly, genetically modified cells that over-express neurotrophic factors have been placed in the injured cord. Initial work, and the vast majority of work to date, involves the injection-engraftment of cell suspensions with or without combinations of ‘cell-bridges’ of cell suspension matrices. Early on, fibroblasts and Schwann cells were transfected with retrovirus to express NT-3 (Grill et al., 1997), NGF (Grill et al., 1997) and BDNF (Menei et al., 1998) respectively. In these 3 studies, NT-3 cells supported corticospinal tract growth in a bilateral dorsal column transection model. NGF secreting cells engrafted by the same group in the same model supported dorsolateral primary sensory axons and cerulospinal axons, but did not support the growth of corticospinal or motor axons. Suspensions of BDNF-Schwann cells implanted into a complete transection whose ends were re-apposed, enhanced the growth primarily of reticular and raphe neurons.

In the context of scaffold delivery, lentivirus was used to transfect Schwann cells to secrete a bifunctional neurotrophic protein (D15A) with NT-3 and BDNF functionality, but cell survival was poor in a PLA scaffold. BDNF-secreting MSCs in templated agarose scaffolds survived to significantly improve regeneration over that supported by the scaffold alone (Stokols et al., 2006). Different cell types and thus different neurotrophic delivery systems can be placed within the same scaffold framework to align corresponding axon groups (Fig. 9A). From the initial studies cited above, a great deal of work has clarified the sensitivity of select axons to specific neurotrophic support (see Lu and Tuszynski (2008) for a recent review). NGF is involved in the growth of nociceptive cells, BDNF those that modify the activity of motor neurons, NT-3 the corticospinal tracts and dorsal sensory axons, NT4/5 proprioceptive and motor modifying inputs, and GDNF the proprioceptive, dorsal sensory, and nociceptive neurons. Despite these approaches to augment neurotrophic support and phenotypic selection, there remains no convincing evidence that axonal populations are extending beyond the cellular or polymer bridges to form lasting functional connections. Furthermore, there is evidence that the inherent regenerative capacity of the axonal subtypes may be different. As recently suggested, within the spinal cord, propriospinal may exhibit a greater regenerative capacity than rubrospinal and reticulospinal, each of which is superior to corticospinal (Blesch and Tuszynski, 2009).

6. Conclusions and future directions

This review has highlighted some of the novel tissue engineering approaches to spinal cord repair using implantable polymers in thoracic transection models. Restoration of respiratory function will be a critical application for biomaterial approaches given the degree of mortality and morbidity associated with respiratory compromise after spinal cord injury. Important bioengineering considerations, fabrication techniques for macroengineering and microengineering, along with some prominent applications and modifications of the main materials in use for scaffolds, have been presented as an overview of the field. Regarding polymer materials, these continue to evolve rapidly in the sophistication of their chemistry and design. New applications with the polymer Polypyrrole (Ppy) represent an exciting avenue for nanofibre and drug delivery systems. Used in the past as coating for neuroelectrodes, Ppy is electrically conductive, and voltage stimulus can induce axonal growth and its direction, along with the release of integrated neurotrophic factors and dexamethasone from the polymer surface.

The integration of Schwann cells, olfactory ensheathing cells and neural stem cells into the polymer structures have improved their regenerative capacity, as has the use of scaffolds as delivery devices for therapeutic agents, particularly neurotrophic factors. Designing scaffolds and seeded cell lines to release neurotrophins for specific axonal locations and sensitive phenotypes may regionalize repair. The use of human embryonic stem cell implantation in polymer scaffolds will be directed by recent FDA approval for Phase 1 clinical trials, and the outcomes of renewed studies in animals. The absence of their use has influenced the development of alternate stem cell types for scaffolds including mesenchymal stem cells and induced pluripotent stem cells (iPSCs) as potential sources both of new neurons as well as drug delivery cells. Further advances in polymers as genetic delivery tools, particularly for siRNA, non-viral and viral gene delivery with or without targeted genomic integration, is an exciting therapeutic prospect. It is hoped that combination strategies will maximize our ability to regenerate spinal cord tissue through the glial scar and recreate connections. The caveat of course is that the investigation of many possible combinations of material, geometry, functionalization strategies, and integrated drug or cell based therapies will need to be properly controlled and systematic. The ability to control these variables with increasing precision, through rapid technological advances such as were highlighted here, enables scaffold models of spinal cord repair to be highly informative about individualized facets of the repair process.

References

- Adams DN, Kao EY, Hypolite CL, Distefano MD, Hu WS, Letourneau PC. Growth cones turn and migrate up an immobilized gradient of the laminin IKVAV peptide. *J Neurobiol* 2005;62:134–147. [PubMed: 15452851]
- Balgude AP, Yu X, Szymanski A, Bellamkonda RV. Agarose gel stiffness determines rate of DRG neurite extension in 3D cultures. *Biomaterials* 2001;22:1077–1084. [PubMed: 11352088]
- Bamber NI, Li H, Lu X, Oudega M, Aebischer P, Xu XM. Neurotrophins BDNF and NT-3 promote axonal re-entry into the distal host spinal cord through Schwann cell-seeded mini-channels. *Eur J Neurosci* 2001;13:257–268. [PubMed: 11168530]
- Bellamkonda R. Peripheral nerve regeneration: an opinion on channels, scaffolds and anisotropy. *Biomaterials* 2006;27:3515–3518. [PubMed: 16533522]
- Bellamkonda R, Ranieri JP, Bouche N, Aebischer P. Hydrogel-based three-dimensional matrix for neural cells. *J Biomed Mater Res* 1995;29:663–671. [PubMed: 7622552]
- Benoit JP, Faisant N, Venier-Julienne MC, Menei P. Development of micro-spheres for neurological disorders: from basics to clinical applications. *J Control Release* 2000;65:285–296. [PubMed: 10699288]
- Bhang SH, Lim JS, Choi CY, Kwon YK, Kim BS. The behavior of neural stem cells on biodegradable synthetic polymers. *J Biomater Sci Polym Ed* 2007;18:223–239. [PubMed: 17323855]
- Blesch A, Tuszynski MH. Spinal cord injury: plasticity, regeneration and the challenge of translational drug development. *Trends Neurosci* 2009;32:41–47. [PubMed: 18977039]
- Blits B, Oudega M, Boer GJ, Bartlett Bunge M, Verhaagen J. Adeno-associated viral vector-mediated neurotrophin gene transfer in the injured adult rat spinal cord improves hind-limb function. *Neuroscience* 2003;118:271–281. [PubMed: 12676157]
- Bodley R. Imaging in chronic spinal cord injury—indications and benefits. *Eur J Radiol* 2002;42:135–153. [PubMed: 11976011]
- Borgens RB, Shi R, Bohnert D. Behavioural recovery from spinal cord injury following application of polyethylene glycol. *J Exp Neurol* 2002;205:1–12.
- Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN, Fawcett JW, McMahon SB. Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature* 2002;416:636–640. [PubMed: 11948352]
- Bryant SJ, Cuy JL, Hauch KD, Ratner BD. Photo-patterning of porous hydrogels for tissue engineering. *Biomaterials* 2007;28:2978–2986. [PubMed: 17397918]

- Cao QL, Zhang YP, Howard RM, Walters WM, Tsoulfas P, Whittemore SR. Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to a glial lineage. *Exp Neurol* 2001;167:48–58. [PubMed: 11161592]
- Cao X, Shoichet MS. Defining the concentration gradient of nerve growth factor for guided neurite outgrowth. *Neuroscience* 2001;103:831–840. [PubMed: 11274797]
- Carone TW, Hasenwinkel JM. Mechanical and morphological characterization of homogeneous and bilayered poly(2-hydroxyethyl methacrylate) scaffolds for use in CNS nerve regeneration. *J Biomed Mater Res B App l Biomater* 2006;78:274–282.
- Chan JM, Zhang L, Yuet KP, Liao G, Rhee JW, Langer R, Farokhzad OC. PLGA-lecithin-PEG core-shell nanoparticles for controlled drug delivery. *Biomaterials* 2009;30:1627–1634. [PubMed: 19111339]
- Chen A, Xu XM, Kleitman N, Bunge MB. Methylprednisolone administration improves axonal regeneration into Schwann cell grafts in transected adult rat thoracic spinal cord. *Exp Neurol* 1996;138:261–276. [PubMed: 8620925]
- Cheng M, Deng J, Yang F, Gong Y, Zhao N, Zhang X. Study on physical properties and nerve cell affinity of composite films from chitosan and gelatin solutions. *Biomaterials* 2003;24:2871–2880. [PubMed: 12742725]
- Chow SY, Moul J, Tobias CA, Himes BT, Liu Y, Obrocka M, Hodge L, Tessler A, Fischer I. Characterization and intraspinal grafting of EGF/bFGF-dependent neurospheres derived from embryonic rat spinal cord. *Brain Res* 2000;874:86–106.
- Chuah MI, Choi-Lundberg D, Weston S, Vincent AJ, Chung RS, Vickers JC, West AK. Olfactory ensheathing cells promote collateral axonal branching in the injured adult rat spinal cord. *Exp Neurol* 2004;185:15–25. [PubMed: 14697315]
- Chvatal SA, Kim YT, Bratt-Leal AM, Lee H, Bellamkonda RV. Spatial distribution and acute anti-inflammatory effects of Methylprednisolone after sustained local delivery to the contused spinal cord. *Biomaterials* 2008;29:1967–1975. [PubMed: 18255138]
- Clark P, Connolly P, Curtis A, Dow J, Wilkinson C. Cell guidance by ultrafine topography *in vitro*. *J Cell Sci* 1991;99:73–77. [PubMed: 1757503]
- Crompton KE, Goud JD, Bellamkonda RV, Gengenbach TR, Finkelstein DI, Horne MK, Forsythe JS. Polylysine-functionalised thermoresponsive chitosan hydrogel for neural tissue engineering. *Biomaterials* 2007;28:441–449. [PubMed: 16978692]
- Dadsetan M, Hefferan TE, Szatkowski JP, Mishra PK, Macura SI, Lu L, Yaszemski MJ. Effect of hydrogel porosity on marrow stromal cell phenotypic expression. *Biomaterials* 2008;29:2193–2202. [PubMed: 18262642]
- De Laporte L, Lei Yan A, Shea LD. Local gene delivery from ECM-coated poly(lactide-co-glycolide) multiple channel bridges after spinal cord injury. *Bio-materials* 2009;30:2361–2368.
- de Ruiter GC, Onyeneho IA, Liang ET, Moore MJ, Knight AM, Malessy MJ, Spinner RJ, Lu L, Currier BL, Yaszemski MJ, Windebank AJ. Methods for *in vitro* characterization of multichannel nerve tubes. *J Biomed Mater Res A* 2008;84:643–651. [PubMed: 17635012]
- Deguchi K, Tsuru K, Hayashi T, Takaishi M, Nagahara M, Nagotani S, Sehara Y, Jin G, Zhang H, Hayakawa S, Shoji M, Miyazaki M, Osaka A, Huh NH, Abe K. Implantation of a new porous gelatin-siloxane hybrid into a brain lesion as a potential scaffold for tissue regeneration. *J Cereb Blood Flow Metab* 2006;26:1263–1273. [PubMed: 16407853]
- den Dunnen WFA, Van Der Lei B, Schakenraad JM, Blaauw EH, Stokroos I, Pennings AJ, Robinson PH. Long-term evaluation of nerve regeneration in a biodegradable nerve guide. *Microsurgery* 1993;14:508–515. [PubMed: 8271930]
- Dewitt DG, Kaszuba SN, Thompson DM, Stegemann JP. Collagen I-Matrigel scaffolds for enhanced Schwann cell survival and control of 3D cell morphology. *Tissue Eng A*. 2009 (Epub February 20, 2009).
- Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science* 2005;310:1139–1143. [PubMed: 16293750]
- Ditor DS, John SM, Roy J, Marx JC, Kittmer C, Weaver LC. Effects of polyethylene glycol and magnesium sulfate administration on clinically relevant neurological outcomes after spinal cord injury in the rat. *J Neurosci Res* 2007;85:1458–1467. [PubMed: 17410603]

- Dodla MC, Bellamkonda RV. Anisotropic scaffolds facilitate enhanced neurite extension *in vitro*. *J Biomed Mater Res A* 2006;78A:213–221. [PubMed: 16892507]
- Duerstock BS, Borgens RB. Three-dimensional morphometry of spinal cord injury following polyethylene glycol treatment. *J Exp Biol* 2002;205:13–24. [PubMed: 11818408]
- Enzmann GU, Benton RL, Talbott JF, Cao Q, Whittemore SR. Functional considerations of stem cell transplantation therapy for spinal cord repair. *J Neurotrauma* 2006;23:479–495. [PubMed: 16629631]
- Flynn L, Dalton PD, Shoichet MS. Fiber templating of poly(2-hydroxyethyl methacrylate) for neural tissue engineering. *Biomaterials* 2003;24:4265–4272. [PubMed: 12853258]
- Fouad K, Schnell L, Bunge MB, Schwab ME, Liebscher T, Pearse DD. Combining Schwann cell bridges and olfactory-ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord. *J Neurosci* 2005;25:1169–1178. [PubMed: 15689553]
- Franssen EH, de Bree FM, Verhaagen J. Olfactory ensheathing glia: their contribution to primary olfactory nervous system regeneration and their regenerative potential following transplantation into the injured spinal cord. *Brain Res Rev* 2007;56:236–258. [PubMed: 17884174]
- Freier T, Koh HS, Kazazian K, Shoichet MS. Controlling cell adhesion and degradation of chitosan films by N-acetylation. *Biomaterials* 2005a;26:5872–5878. [PubMed: 15949553]
- Freier T, Montenegro R, Shan Koh H, Shoichet MS. Chitin-based tubes for tissue engineering in the nervous system. *Biomaterials* 2005b;26:4624–4632. [PubMed: 15722132]
- Friedman JA, Windebank AJ, Moore MJ, Spinner RJ, Currier BL, Yaszemski MJ. Biodegradable polymer grafts for surgical repair of the injured spinal cord. *Neurosurgery* 2002;51:742–752. [PubMed: 12188954]
- Gautier SE, Oudega M, Fragoso M, Chapon P, Plant GW, Bunge MB, Parel JM. Poly (alpha-hydroxyacids) for application in the spinal cord: resorbability and biocompatibility with adult rat Schwann cells and spinal cord. *J Biomed Mater Res* 1998;42:642–654. [PubMed: 9827690]
- Giannetti S, Lauretti L, Fernandez E, Salvinelli F, Tamburrini G, Pallini R. Acrylic hydrogel implants after spinal cord lesion in the adult rat. *Neurol Res* 2001;23:405–409. [PubMed: 11428522]
- Goldner JS, Bruder JM, Li G, Gazzola D. Neurite bridging across micropatterned grooves. *Biomaterials* 2006;27:460–472. [PubMed: 16115675]
- Grill R, Murai K, Blesch A, Gage FH, Tuszynski MH. Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury. *J Neurosci* 1997a;17:5560–5572. [PubMed: 9204937]
- Grill RJ, Blesch A, Tuszynski MH. Robust growth of chronically injured spinal cord axons induced by grafts of genetically modified NGF-secreting cells. *Exp Neurol* 1997b;148:444–452. [PubMed: 9417824]
- Gupta D, Tator CH, Shoichet MS. Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord. *Biomaterials* 2006;27:2370–2379. [PubMed: 16325904]
- Hadlock T, Sundback C, Hunter D, Cheney M, Vacanti JP. A polymer foam conduit seeded with Schwann cells promotes guided peripheral nerve regeneration. *Tissue Eng* 2000;6:119–127. [PubMed: 10941207]
- Han Q, Sun W, Lin H, Zhao W, Gao Y, Zhao Y, Chen B, Xiao Z, Hu W, Li Y, Yang B, Dai J. Linear ordered collagen scaffolds loaded with collagen-binding brain-derived neurotrophic factor improve the recovery of spinal cord injury in rats. *Tissue Eng A* 2009;16 (Epub March 16, 2009).
- Hejcl A, Urdzikova L, Sedy J, Lesny P, Pradny M, Michalek J, Burian M, Hajek M, Zamecnik J, Jendelova P, Sykova E. Acute and delayed implantation of positively charged 2-hydroxyethyl methacrylate scaffolds in spinal cord injury in the rat. *J Neurosurg Spine* 2008;8:67–73. [PubMed: 18173349]
- Hiroshi N, Tator CH, Shoichet MS. Bioengineered strategies for spinal cord repair. *J Neurotrauma* 2006;23:496–507. [PubMed: 16629632]
- Hofstetter CP, Holmstrom NA, Lilja JA, Schweinhardt P, Hao J, Spenger C, Wiesenfeld-Hallin Z, Kurpad SN, Frisen J, Olson L. Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. *Nat Neurosci* 2005;8:346–353. [PubMed: 15711542]
- Houweling DA, Lankhorst AJ, Gispens WH, Bär PR, Joosten EAJ. Collagen containing neurotrophin-3 (NT-3) attracts regrowing injured corticospinal axons in the adult rat spinal cord and promotes partial functional recovery. *Exp Neurol* 1998;153:49–59. [PubMed: 9743566]

- Hurtado A, Moon LD, Maquet V, Blits B, Jerome R, Oudega M. Poly (D,L-lactic acid) macroporous guidance scaffolds seeded with Schwann cells genetically modified to secrete a bi-functional neurotrophin implanted in the completely transected adult rat thoracic spinal cord. *Biomaterials* 2006;27:430–442. [PubMed: 16102815]
- Iannotti C, Li H, Yan P, Lu X, Wirthlin L, Xu XM. Glial cell line-derived neurotrophic factor-enriched bridging transplants promote propriospinal axonal regeneration and enhance myelination after spinal cord injury. *Exp Neurol* 2003;183:379–393. [PubMed: 14552879]
- Jain A, Kim YT, McKeon RJ, Bellamkonda RV. *In situ* gelling hydrogels for conformational repair of spinal cord defects, and local delivery of BDNF after spinal cord injury. *Biomaterials* 2006;27:497–504. [PubMed: 16099038]
- Jeng CB, Coggeshall RE. Permeable tubes increase the length of the gap that regenerating axons can span. *Brain Res* 1985;408:239–242.
- Jimenez Hamann MC, Tator CH, Shoichet MS. Injectable intrathecal delivery system for localized administration of EGF and FGF-2 to the injured rat spinal cord. *Exp Neurol* 2005;194:106–119. [PubMed: 15899248]
- Johnson P, Parker S, Sakiyama-Elbert S. Fibrin-based tissue engineering scaffolds enhance neural fiber sprouting and delay the accumulation of reactive astrocytes at the lesion in a subacute model of spinal cord injury. *J Biomed Mater Res A* 2009;22 (Epub January 22, 2009).
- Kang C, Poon P, Tator CH, Shoichet MS. A new paradigm for local and sustained release of therapeutic molecules to the injured spinal cord for neuroprotection and tissue repair. *Tissue Eng A* 2009;15:595–604.
- Kataoka K, Suzuki Y, Kitada M, Hashimoto T, Chou H, Bai H, Ohta M, Wu S, Suzuki K, Ide C. Alginate enhances elongation of early regenerating axons in spinal cord of young rats. *Tissue Eng* 2004;10:493–504. [PubMed: 15165466]
- Kataoka K, Suzuki Y, Kitada M, Ohnishi K, Suzuki K, Tanihara M, Ide C, Endo K, Nishimura Y. Alginate, a bioresorbable material derived from brown seaweed, enhances elongation of amputated axons of spinal cord in infant rats. *J Biomed Mater Res* 2001;54:373–384. [PubMed: 11189043]
- Katz JS, Burdick JA. Hydrogel mediated delivery of trophic factors for neuronal repair. *Rev Nanomed Nanobiotechnol* 2009;1:128–139.
- Khademhosseini A, Langer R. Microengineered hydrogels for tissue engineering. *Biomaterials* 2007;28:5087–5092. [PubMed: 17707502]
- Kim DH, Martin DC. Sustained release of dexamethasone from hydrophilic matrices using PLGA nanoparticles for neural drug delivery. *Biomaterials* 2006;27:3031–3037. [PubMed: 16443270]
- Kim H, Tator CH, Shoichet MS. Design of protein-releasing chitosan channels. *Biotechnol Prog* 2008;24:932–937. [PubMed: 18324828]
- Kim YT, Caldwell JM, Bellamkonda RV. Nanoparticle-mediated local delivery of methylprednisolone after spinal cord injury. *Biomaterials* 2009;30:2582–2590. [PubMed: 19185913]
- King VR, Henseler M, Brown RA, Priestley JV. Mats made from fibronectin support oriented growth of axons in the damaged spinal cord of the adult rat. *Exp Neurol* 2003;182:383–398. [PubMed: 12895449]
- Kohane D, Langer R. Polymeric biomaterials in tissue engineering. *Pediatr Res* 2008;63:487–491. [PubMed: 18427292]
- Krych AJ, Rooney GE, Chen B, Schermerhorn TC, Ameenuddin S, Gross LA, Moore MJ, Currier BL, Friedman JA, Spinner RJ, Yaszemski MJ, Windebank AJ. Relationship between scaffold channel diameter and number of regenerating axons in the transected rat spinal cord. *Acta Biomater*. 2009 (Epub March 27, 2009).
- Kwon BK, Roy J, Lee JH, Okon EB, Zhang H, Marx JC, Kindy MS. Magnesium chloride in a polyethylene glycol formulation as a neuroprotective therapy for acute spinal cord injury: preclinical refinement and optimization. *J Neurotrauma* 2009;24 (Epub March 24, 2009).
- Lali HS, Sekhon LHS, Fehlings MG. Epidemiology, demographics, and pathophysiology of acute spinal cord injury. *Spine* 2001;26:S2–12. [PubMed: 11805601]
- Lane MA, Fuller DD, White TE, Reier PJ. Respiratory neuroplasticity and cervical spinal cord injury. *Trends Neurosci* 2008;31:538–547. [PubMed: 18775573]

- Lavery PH, Leskova A, Breur GJ, Coates JR, Bergman RL, Widmer WR, Toombs JP, Shapiro S, Borgens RB. A preliminary study of intravenous surfactants in paraplegic dogs: polymer therapy in canine clinical SCI. *J Neurotrauma* 2004;21:1767–1777. [PubMed: 15684768]
- Lesny P, Pradny M, Jendelova P, Michalek J, Vacik J, Sykova E. Macroporous hydrogels based on 2-hydroxyethyl methacrylate. Part 4: growth of rat bone marrow stromal cells in three-dimensional hydrogels with positive and negative surface charges and in polyelectrolyte complexes. *J Mater Sci Mater Med* 2006;17:829–833. [PubMed: 16932865]
- Liu W, Cao Y. Application of scaffold materials in tissue reconstruction in immunocompetent mammals: our experience and future requirements. *Biomaterials* 2007;28:5078–5086. [PubMed: 17669487]
- Loh NK, Woerly S, Bunt SM, Wilton SD, Harvey AR. The regrowth of axons within tissue defects in the CNS is promoted by implanted hydrogel matrices that contain BDNF and CNTF producing fibroblasts. *Exp Neurol* 2001;170:72–84. [PubMed: 11421585]
- Lowry NA, Temple S. Making human neurons from stem cells after spinal cord injury. *PLoS Med* 2007;4:e48. [PubMed: 17298166]
- Lu J, Ashwell K. Olfactory ensheathing cells: their potential use for repairing the injured spinal cord. *Spine* 2002;27:887–892. [PubMed: 11935115]
- Lu P, Jones LL, Snyder EY, Tuszynski MH. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. *Exp Neurol* 2003;181:115–129. [PubMed: 12781986]
- Lu P, Tuszynski MH. Growth factors and combinatorial therapies for CNS regeneration. *Exp Neurol* 2008;209:313–320. [PubMed: 17927983]
- Ma W, Fitzgerald W, Liu QY, O'Shaughnessy TJ, Maric D, Lin HJ, Alkon DL, Barker JL. CNS stem and progenitor cell differentiation into functional neuronal circuits in three-dimensional collagen gels. *Exp Neurol* 2004;190:276–288. [PubMed: 15530869]
- Mackinnon SE, Dellon AL. Clinical nerve reconstruction with a bioabsorbable polyglycolic acid tube. *Plast Reconstr Surg* 1990;85:419–424. [PubMed: 2154831]
- Madaghiale M, Sannino A, Yannas IV, Spector M. Collagen-based matrices with axially oriented pores. *J Biomed Mater Res A* 2008;85:757–767. [PubMed: 17896767]
- Mahoney MJ, Krewson C, Miller J, Saltzman WM. Impact of cell type and density on nerve growth factor distribution and bioactivity in 3-dimensional collagen gel cultures. *Tissue Eng* 2006;12:1915–1927. [PubMed: 16889521]
- Marchand R, Woerly S, Bertrnad L, Valdes N. Evaluation of two crosslinked collagen gels implanted in the transected spinal cord. *Brain Res Bull* 1993;30:415–422. [PubMed: 8457891]
- Maysinger D, Krieglstein K, Filipovic-Grcic J, Sendtner M, Unsicker K, Richardson P. Microencapsulated ciliary neurotrophic factor: physical properties and biological activities. *Exp Neurol* 1996;138:177–188. [PubMed: 8620916]
- Menei P, Montero-Menei C, Whittemore SR, Bunge RP, Bunge MB. Schwann cells genetically modified to secrete human BDNF promote enhanced axonal regrowth across transected adult rat spinal cord. *Eur J Neurosci* 1998;10:607–621. [PubMed: 9749723]
- Moore K, MacSween M, Shoichet M. Immobilized concentration gradients of neurotrophic factors guide neurite outgrowth of primary neurons in macroporous scaffolds. *Tissue Eng* 2006a;12:267–278. [PubMed: 16548685]
- Moore MJ, Friedman JA, Lewellyn EB, Mantila SM, Krych AJ, Ameenuddin S, Knight AM, Lu L, Currier BL, Spinner RJ, Marsh RW, Windebank AJ, Yaszemski MJ. Multiple-channel scaffolds to promote spinal cord axon regeneration. *Biomaterials* 2006b;27:419–429. [PubMed: 16137759]
- Murray AJ, Shewan DA. Epac mediates cyclic AMP-dependent axon growth, guidance and regeneration. *Mol Cell Neurosci* 2008;38:578–588. [PubMed: 18583150]
- Namba RM, Cole AA, Bjugstad KB, Mahoney MJ. Development of porous PEG hydrogels that enable efficient, uniform cell-seeding and permit early neural process extension. *Acta Biomater* 2009;5(6):1884–1897. [PubMed: 19250891]
- Nash HH, Borke RC, Anders JJ. Ensheathing cells and methylprednisolone promote axonal regeneration and functional recovery in the lesioned adult rat spinal cord. *J Neurosci* 2002;22:7111–7120. [PubMed: 12177207]

- National Spinal Cord Injury Association (NSCIA). National Spinal Cord Injury Database. Statistical Center; Birmingham, Alabama: 2009.
- Nisbet DR, Kylie EC, Horne MK, Finkelstein DI, Forsythe JS. Neural tissue engineering of the CNS using hydrogels. *J Biomed Mater Res B: Appl Biomater* 2008;87B:251–263. [PubMed: 18161806]
- Nogues MA, Benarroch E. Abnormalities of respiratory control and the respiratory motor unit. *Neurologist* 2008;14 (5):273–288. [PubMed: 18784597]
- Nomura H, Baladie B, Katayama Y, Morshead CM, Shoichet MS, Tator CH. Delayed implantation of intramedullary chitosan channels containing nerve grafts promotes extensive axonal regeneration after spinal cord injury. *Neurosurgery* 2008a;63:127–141. [PubMed: 18728578]
- Nomura H, Katayama Y, Shoichet MS, Tator CH. Complete spinal cord transection treated by implantation of a reinforced synthetic hydrogel channel results in syringomyelia and caudal migration of the rostral stump. *Neurosurgery* 2006a;59:183–192. [PubMed: 16823315]
- Nomura H, Tator C, Shoichet M. Bioengineered strategies for spinal cord repair. *J Neurotrauma* 2006b;23:496–507. [PubMed: 16629632]
- Nomura H, Zahir T, Kim H, Katayama Y, Kulbatski I, Morshead CM, Shoichet MS, Tator CH. Extramedullary chitosan channels promote survival of transplanted neural stem and progenitor cells and create a tissue bridge after complete spinal cord transection. *Tissue Eng A* 2008b;14:649–665.
- Novikova LN, Mosahebi A, Wiberg M, Terenghi G, Kellerth JO, Novikov LN. Alginate hydrogel and matrigel as potential cell carriers for neurotransplantation. *J Biomed Mater Res A* 2006;77:242–252. [PubMed: 16392134]
- Oh J, Recknor JB, Recknor JC, Mallapragada SK, Sakaguchi DS. Soluble factors from neocortical astrocytes enhance neuronal differentiation of neural progenitor cells from adult rat hippocampus on micropatterned polymer substrates. *J Biomed Mater Res A* 2008;4 (Epub November 4, 2008).
- Olson HE, Rooney GE, Gross L, Nesbitt JJ, Galvin KE, Knight A, Chen B, Yaszemski MJ, Windebank AJ. Neural stem cell- and schwann cell-loaded biodegradable polymer scaffolds support axonal regeneration in the transected spinal cord. *Tissue Eng A* 2009;15 (7):1797–1805.
- Oudega M, Gautier SE, Chapon P, Frago M, Bates ML, Parel JM, Bunge MB. Axonal regeneration into Schwann cell grafts within resorbable poly(α -hydroxyacid) guidance channels in the adult rat spinal cord. *Biomaterials* 2001;22:1125–1136. [PubMed: 11352092]
- Oudega M, Hagg T. Nerve growth factor promotes regeneration of sensory axons into adult rat spinal cord. *Exp Neurol* 1996;140:218–229. [PubMed: 8690064]
- Oudega M, Hagg T. Neurotrophins promote regeneration of sensory axons in the adult rat spinal cord. *Brain Res* 1999;818:431–438. [PubMed: 10082829]
- Oudega M, Moon LD, de Almeida Leme RJ. Schwann cells for spinal cord repair. *Braz J Med Biol Res* 2005;38:825–835. [PubMed: 15933775]
- Patist CM, Mulder MB, Gautier SE, Maquet V, Jerome R, Oudega M. Freeze-dried poly(D,L-lactic acid) macroporous guidance scaffolds impregnated with brain-derived neurotrophic factor in the transected adult rat thoracic spinal cord. *Biomaterials* 2004;25:1569–1582. [PubMed: 14697859]
- Pearse DD, Pereira FC, Marcillo AE, Bates ML, Berrocal YA, Filbin MT, Bunge MB. cAMP and Schwann cells promote axonal growth and functional recovery after spinal cord injury. *Nat Med* 2004;10:610–616. [PubMed: 15156204]
- Pfister BJ, Iwata A, Taylor AG, Wolf JA, Meaney DF, Smith DH. Development of transplantable nervous tissue constructs comprised of stretch-grown axons. *J Neurosci Methods* 2006;153:95–103. [PubMed: 16337007]
- Phillips JB, King VR, Ward Z, Porter RA, Priestley JV, Brown RA. Fluid shear in viscous fibronectin gels allows aggregation of fibrous materials for CNS tissue engineering. *Biomaterials* 2004;25:2769–2779. [PubMed: 14962555]
- Piantino J, Burdick JA, Goldberg D, Langer R, Benowitz LI. An injectable, biodegradable hydrogel for trophic factor delivery enhances axonal rewiring and improves performance after spinal cord injury. *Exp Neurol* 2006;201:359–367. [PubMed: 16764857]
- Pinzon A, Calancie B, Oudega M, Noga BR. Conduction of impulses by axons regenerated in a Schwann cell graft in the transected adult rat thoracic spinal cord. *J Neurosci Res* 2001;64:533–541. [PubMed: 11391708]

- Piotrowicz A, Shoichet M. Nerve guidance channels as drug delivery vehicles. *Biomaterials* 2006;27:2018–2027. [PubMed: 16239029]
- Plant GW, Chirila TV, Harvey AR. Implantation of collagen IV/poly(2-hydroxyethyl methacrylate) hydrogels containing Schwann cells into the lesioned rat optic tract. *Cell Transplant* 1998;7:381–391. [PubMed: 9710307]
- Plant GW, Harvey AR, Chirila TV. Axonal growth within poly(2-hydroxyethyl methacrylate) sponges infiltrated with Schwann cells and implanted into the lesioned rat optic tract. *Brain Res* 1995;671:119–130. [PubMed: 7728523]
- Quencer R, Bunge RP. The injured spinal cord: imaging, histopathologic, clinical correlates, and basic science approaches to enhancing neural function after spinal cord injury. *Spine* 1996;21:2064–2066. [PubMed: 8893428]
- Ramon-Cueto A, Cordero MI, Santos-Benito FF, Avila J. Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia. *Neuron* 2000;25:425–435. [PubMed: 10719896]
- Ramon-Cueto A, Plant GW, Avila J, Bunge MB. Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. *J Neurosci* 1998;18:3803–3815. [PubMed: 9570810]
- Recknor JB, Sakaguchi DS, Mallapragada SK. Directed growth and selective differentiation of neural progenitor cells on micropatterned polymer substrates. *Biomaterials* 2006;27:4098–4108. [PubMed: 16616776]
- Reynolds LF, Bren MC, Wilson BC, Gibson GD, Shoichet MS, Murphy RJ. Transplantation of porous tubes following spinal cord transection improves hindlimb function in the rat. *Spinal Cord* 2008;46:58–64. [PubMed: 17420773]
- Richardson PM, McGuinness UM, Aguayo AJ. Axons from CNS neurons regenerate into PNS grafts. *Nature* 1980;284:264–265. [PubMed: 7360259]
- Rochkind S, Shahar A, Amon M, Nevo Z. Transplantation of embryonal spinal cord nerve cells cultured on biodegradable microcarriers followed by low power laser irradiation for the treatment of traumatic paraplegia in rats. *Neurol Res* 2002;24:355–360. [PubMed: 12069281]
- Rooney GE, Vaishya S, Ameenuddin S, Currier BL, Schiefer TK, Knight A, Chen B, Mishra PK, Spinner RJ, Macura SI, Yaszemski MJ, Windebank AJ. Rigid fixation of the spinal column improves scaffold alignment and prevents scoliosis in the transected rat spinal cord. *Spine* 2008;33:E914–E919. [PubMed: 19011531]
- Sachlos E, Czernuszka JT. Making tissue engineering scaffolds work. Review: the application of solid freeform fabrication technology to the production of tissue engineering scaffolds. *Eur Cell Mater* 2003;5:29–39. [PubMed: 14562270]
- Schnell E, Klinkhammer K, Balzer S, Brook G, Klee D, Dalton P, Mey J. Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-epsilon-caprolactone and a collagen/poly-epsilon-caprolactone blend. *Biomaterials* 2007;28:3012–3025. [PubMed: 17408736]
- Shoichet MS, Tator CH, Poon P, Kang C, Baumann MD. Intrathecal drug delivery is safe and efficacious for localized delivery to the spinal cord. *Prog Brain Res* 2007;161:385–392. [PubMed: 17618992]
- Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI. Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science* 2004;303:1352–1355. [PubMed: 14739465]
- Soderquist RG, Milligan ED, Sloane EM, Harrison JA, Douvas KK, Potter JM, Hughes TS, Chavez RA, Johnson K, Watkins LR, Mahoney MJ. PEGylation of brain-derived neurotrophic factor for preserved biological activity and enhanced spinal cord distribution. *J Biomed Mater Res A*. 2008 (Epub December 1, 2008).
- Stichel CC, Hermanns S, Lausberg F, Muller HW. Effects of schwann cell suspension grafts on axon regeneration in subacute and chronic CNS traumatic injuries. *Glia* 1999;28:156–165. [PubMed: 10533059]
- Stokols S, Sakamoto J, Breckon C, Holt T, Weiss J, Tuszynski MH. Templated agarose scaffolds support linear axonal regeneration. *Tissue Eng* 2006;12:2777–2787. [PubMed: 17518647]
- Stokols S, Tuszynski MH. The fabrication and characterization of linearly oriented nerve guidance scaffolds for spinal cord injury. *Biomaterials* 2004;25:5839–5846. [PubMed: 15172496]

- Stokols S, Tuszynski MH. Freeze-dried agarose scaffolds with uniaxial channels stimulate and guide linear axonal growth following spinal cord injury. *Biomaterials* 2006;27:443–451. [PubMed: 16099032]
- Tabesh H, Amoabediny G, Nik NS, Heydari M, Yosefifard M, Siadat SO, Mottaghy K. The role of biodegradable engineered scaffolds seeded with Schwann cells for spinal cord regeneration. *Neurochem Int* 2009;54:73–83. [PubMed: 19084565]
- Tadie M, Liu S, Robert R, Guiheneuc P, Pereon Y, Perrouin-Verbe B, Mathe JF. Partial return of motor function in paralyzed legs after surgical bypass of the lesion site by nerve autografts three years after spinal cord injury. *J Neurotrauma* 2002;19:909–916. [PubMed: 12225651]
- Talac R, Friedman JA, Moore MJ, Lu L, Jabbari E, Windebank AJ, Currier BL, Yaszemski MJ. Animal models of spinal cord injury for evaluation of tissue engineering treatment strategies. *Biomaterials* 2004;25:1505–1510. [PubMed: 14697853]
- Taylor SJ, Rosenzweig ES, McDonald JW, Sakiyama-Elbert SE. Delivery of neurotrophin-3 from fibrin enhances neuronal fiber sprouting after spinal cord injury. *J Control Release* 2006;113:226–235. [PubMed: 16797770]
- Taylor SJ, Sakiyama-Elbert SE. Effect of controlled delivery of neurotrophin-3 from fibrin on spinal cord injury in a long term model. *J Control Release* 2006;116:204–210. [PubMed: 16919351]
- Teng YD, Lavik EB, Qu X, Park KI, Ourednik J, Zurakowski D, Langer R, Snyder EY. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. *Proc Natl Acad Sci USA* 2002;99:3024–3029. [PubMed: 11867737]
- Tsai EC, Dalton PD, Shoichet MS, Tator CH. Synthetic hydrogel guidance channels facilitate regeneration of adult rat brainstem motor axons after complete spinal cord transection. *J Neurotrauma* 2004;21:789–804. [PubMed: 15253805]
- Tsai EC, Dalton PD, Shoichet MS, Tator CH. Matrix inclusion within synthetic hydrogel guidance channels improves specific supraspinal and local axonal regeneration after complete spinal cord transection. *Biomaterials* 2006;27:519–533. [PubMed: 16099035]
- Vleggeert-Lankamp CL, de Ruiter GC, Wolfs JF. Pores in synthetic nerve conduits are beneficial to regeneration. *J Biomed Mater Res A* 2007;80:965–982. [PubMed: 17106899]
- Wang HB, Mullins ME, Cregg JM, Hurtado A, Oudega M, Trombley MT, Gilbert RJ. Creation of highly aligned electrospun poly-L-lactic acid fibers for nerve regeneration applications. *J Neural Eng* 2009a;6:016001. [PubMed: 19104139]
- Wang S, Yaszemski MJ, Knight AM, Gruetzmacher JA, Windebank AJ, Lu L. Photo-crosslinked poly (epsilon-caprolactone fumarate) networks for guided peripheral nerve regeneration: material properties and preliminary biological evaluations. *Acta Biomater* 2009b;5(5):1531–1542. [PubMed: 19171506]
- Wang YC, Wu YT, Huang HY, Lin HI, Lo LW, Tzeng SF, Yang CS. Sustained intraspinal delivery of neurotrophic factor encapsulated in biodegradable nanoparticles following contusive spinal cord injury. *Biomaterials* 2008;29:4546–4553. [PubMed: 18774604]
- Willerth SM, Arendas KJ, Gottlieb DI, Sakiyama-Elbert SE. Optimization of fibrin scaffolds for differentiation of murine embryonic stem cells into neural lineage cells. *Biomaterials* 2006;27:5990–6003. [PubMed: 16919326]
- Willerth SM, Fixel TE, Gottlieb DI, Sakiyama-Elbert SE. The effects of soluble growth factors on embryonic stem cell differentiation inside of fibrin scaffolds. *Stem Cells* 2007;25:2235–2244. [PubMed: 17585170]
- Willerth SM, Sakiyama-Elbert SE. Approaches to neural tissue engineering using scaffolds for drug delivery. *Adv Drug Deliv Rev* 2007;59:325–338. [PubMed: 17482308]
- Willerth SM, Sakiyama-Elbert SE. Cell therapy for spinal cord regeneration. *Adv Drug Deliv Rev* 2008;60:263–276. [PubMed: 18029050]
- Woerly S, Pinet E, De Robertis L, Bousmina M, Laroche G, Roitback T, Vargova L, Sykova E. Heterogeneous PHPMA hydrogels for tissue repair and axonal regeneration in the injured spinal cord. *J Biomater Sci Polym Ed* 1998;9:681–711. [PubMed: 9686335]
- Woerly S, Pinet E, de Robertis L, Van Diep D, Bousmina M. Spinal cord repair with PHPMA hydrogel containing RGD peptides (NeuroGel). *Biomaterials* 2001;22:1095–1111. [PubMed: 11352090]

- Wong DY, Leveque JC, Brumblay H, Krebsbach PH, Hollister SJ, Lamarca F. Macro-architectures in spinal cord scaffold implants influence regeneration. *J Neurotrauma* 2008;25:1027–1037. [PubMed: 18721107]
- Xie J, Willerth SM, Li X, Macewan MR, Rader A, Sakiyama-Elbert SE, Xia Y. The differentiation of embryonic stem cells seeded on electrospun nanofibers into neural lineages. *Biomaterials* 2009;30:354–362. [PubMed: 18930315]
- Xu XM, Guenard V, Kleitman N, Bunge MB. Axonal regeneration into Schwann cell-seeded guidance channels grafted into transected adult rat spinal cord. *J Comp Neurol* 1995;351:145–160. [PubMed: 7896937]
- Yang F, Murugan R, Wang S, Ramakrishna S. Electrospinning of nano/micro scale poly(L-lactic acid) aligned fibers and their potential in neural tissue engineering. *Biomaterials* 2005a;26:2603–2610. [PubMed: 15585263]
- Yang Y, De Laporte L, Rives CB, Jang JH, Lin WC, Shull KR, Shea LD. Neurotrophin releasing single and multiple lumen nerve conduits. *J Control Release* 2005b;104:433–446. [PubMed: 15911044]
- Yao L, Damodaran G, Nikolskaya N, Gorman AM, Windebank AJ, Pandit A. The effect of laminin peptide gradient of enzymatically cross-linked collagen scaffold on neurite growth. *J Biomed Mater Res A* 2009a;11 (Epub February 11, 2009).
- Yao L, O'Brien N, Windebank A, Pandit A. Orienting neurite growth in electrospun fibrous neural conduits. *J Biomed Mater Res B Appl Biomater* 2009b;90(2):483–491. [PubMed: 19130615]
- Yao L, Wang S, Cui W, Sherlock R, O'Connell C, Damodaran G, Gorman A, Windebank A, Pandit A. Effect of functionalized micropatterned PLGA on guided neurite growth. *Acta Biomater* 2009c; 5:580–588. [PubMed: 18835227]
- Yoshii S, Ito S, Shima M, Taniguchi A, Akagi M. Functional restoration of rabbit spinal cord using collagen-filament scaffold. *J Tissue Eng Regen Med* 2009;3:19–25. [PubMed: 19012267]
- Yoshii S, Shima M, Taniguchi A, Akagi M. Restoration of function after spinal cord transection using a collagen bridge. *J Biomed Mater Res A* 2004;70:569–575. [PubMed: 15307161]
- Yu LM, Kazazian K, Shoichet MS. Peptide surface modification of methacrylamide chitosan for neural tissue engineering applications. *J Biomed Mater Res A* 2007;82:243–255. [PubMed: 17295228]
- Yu TT, Shoichet MS. Guided cell adhesion and outgrowth in peptide-modified channels for neural tissue engineering. *Biomaterials* 2005;26:1507–1514. [PubMed: 15522752]
- Yuan Y, Zhang P, Yang Y, Wang X, Gu X. The interaction of Schwann cells with Chitosan membranes and fibres *in vitro*. *Biomaterials* 2004;25:4273–4278. [PubMed: 15046917]
- Zahir T, Nomura H, Guo XD, Kim H, Tator C, Morshead C, Shoichet M. Bioengineering neural stem/progenitor cell-coated tubes for spinal cord injury repair. *Cell Transplant* 2008;17:245–254. [PubMed: 18522228]
- Zhong Y, Bellamkonda RV. Biomaterials for the central nervous system. *J R Soc Interface* 2008;5:957–975. [PubMed: 18477539]
- Zimmer MB, Nantwi K, Goshgarian HG. Effect of spinal cord injury on the neural regulation of respiratory function. *Exp Neurol* 2007;209:399–406. [PubMed: 17603041]

Fig. 1. Polymer scaffold *in situ*. Lateral radiograph showing a well-aligned scaffold within the spinal canal after 4 weeks. The spine has been fixed and the scaffold contains barium contrast within the polymer. From Rooney et al. (2008) with permission.

Fig. 2.

The effect of spine stabilization on scaffold alignment. Three-dimensional magnetic resonance microscopy (MRM) in coronal ((A) and (B)) and axial ((C) and (D)) images 4 weeks after scaffold placement into the transected cord with ((A) and (C)) and without ((B) and (D)) spine fixation. From Rooney et al. (2008) with permission.

Fig. 3.

Scaffold polymer porosity. Porosity is controlled in a polyethylene glycol based hydrogel by varying the size of the porogen. Scanning electron microscopy (SEM) of lyophilized hydrogel ((a)–(c)) shows a highly porous structure with open, interconnected pores surrounded by polymer walls. Magnetic resonance microscopy (MRM) of scaffolds in their hydrated state ((d)–(f)) are compared. The distribution of sodium chloride particles was in the range of 100–500 μm . Scaffold with no porosity ((a) and (d)), porous scaffold with 75% porogen content and 300 μm particle size, ((b) and (e)), and porous scaffold with 75% porogen content and 500 μm particle size ((c) and (f)). Figure from Dadsetan et al. (2008) with permission.

Fig. 4.

Macroarchitectural design. Various injection-molding strategies for spinal cord scaffolds to align fascicular bundles. (A) Templated agarose cast over polystyrene fibres produces linear aligned channels of 200 μm diameter for a hemisection model (from Stokols et al. (2006) with permission). (B) Cylinder, tube, multichannel and open-path designs, from Wong et al. (2008) with permission, for casting in poly (ϵ -caprolactone). (C) PLGA multichannel scaffolds cast over parallel metal wiring provide dorsal and ventral channels, (left, bar 500 μm , from Moore et al. (2006) which may be elaborated into molds of complex anatomical design, (right, Friedman et al. (2002) both figures with permission).

Fig. 5. Oriented axonal growth. Microengineering strategies with oriented extension of neurite outgrowth in relation to micropatterned grooves and nanofibres. SEM imaging of PC12 cell growth on a collagen type I-coated PLGA film (A) and on a laminin peptide-coated PLGA film (B) each with a laser-etched groove size of 10 µm. Bar 40 µm, from Yao et al. (2009). Neurite extension along the length of electrospun PCL fibres of a diameter ranging from 0.8 ± 0.7 µm (C) and 3.7 ± 0.5 µm (D), from Yao et al. (2009) with permission.

Fig. 6.

Freeze dried agarose scaffolds in a complete transection model, figure from Stokols and Tuszynski (2006) with permission. Upper panel: scanning electron microscopic images of scaffolds in (A) longitudinal or (B) cross-sectional orientation shows the arrangement of channels in a honeycomb structure. Scale bar is 100 µm. Lower panel: neurofilament labeling demonstrates penetration and linear growth of axons within channels of scaffolds. (A) Scaffold lacking growth factor. (B) Scaffold loaded with 2 µg recombinant human BDNF into walls and matrix-filled lumen of individual channels. Magnitude of linear axonal growth is significantly increased. (C) Best example of linear axonal growth through complete length of channel. Scale bars = 100 µm.

Fig. 7.

Scaffolds *in situ*. A dorsal hemisection injury (A) is filled with a fibrin matrix, as visualized with fibrinogen immunofluorescence (B). Diverse applications have been developed with this polymer, including affinity-based drug elution from heparin complexes and embryonic stem cell differentiation. Bar is 200 μm . Adapted from (Johnson et al., 2009) with permission. ((C)–(F)) A complete spinal cord transection is bridged with chitosan scaffolds loaded with brain-derived neural stem cells ((C) and (D)) or Schwann cells ((E) and (F)) as seen from a dorsal and lateral aspect. Neuronal tissue bridges have developed from the transected cord stumps. Figure from Zahir et al. (2008) with permission.

Fig. 8.

Neurofilament staining of axons in transverse sections through a PLGA scaffold after 1 month *in vivo*. Fluorescent microscopy image of one transverse section of a scaffold (loaded with NSCs) stained with an antibody against neurofilament with the region in which axons were counted encircled by a white line (A). Transverse sections of one channel from the NSC group (B), one from the SC group (C), and one from the control group (D) stained for neurofilament using an AEC chromogen. Magnification: 25 $\times$ (A); 100 $\times$ ((B)–(D)). Dot plots of axonal counts per cord in the NSC-, SC-, and control-treated groups (E). Data points represent individual animals. 95% Confidence interval was used for each group. *Medians vary significantly ($p < 0.05$). Figure from Olson et al. (2009) with permission.

Fig. 9.

(A) Cells of different types can be placed in a scaffold. PCL-fumarate scaffold of 2-mm diameter loaded with SpL201 cells (blue), Schwann cells from a GFP rat (green) and rat Mesenchymal stem cells labeled with red quantum dots. Subsequently the scaffold can be placed in a way that ventral and dorsal orientation is retained. (B) Neurospheres of neural stem cells suspended in scaffold channels at 2 h grow to fill the channel diameter (C) within 3 days, figure from Olson et al. (2009) with permission. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 10.

((A) and (B)) Light micrographs of the microsphere-loaded chitosan channels. The thickness of the channels when hydrated is approximately 200 μm , of which the secondary chitosan layer (indicated by arrows) contributes about 20 μm . (C) Scanning electron microscopy shows microspheres (arrowheads) embedded by the secondary chitosan coating. Figure from Kim et al. (2008) with permission.

Table 1

The major natural polymers in use in animal models of spinal cord injury and examples of their applications.

Natural polymers	Structure	Examples of applications for spinal cord repair
<i>Natural hydrogels</i>		
Collagen, type I	Glycine-X-Y polypeptide fibrils	Collagen gels as extracellular support growth matrix. (Marchand et al., 1993; Ma et al., 2004) Collagen gels with embedded neurotrophic factors [NT-3, BDNF] (Houweling et al., 1998) Collagen filament bridges as growth substrates (Yoshii et al., 2004; Pfister et al., 2006) Linear ordered collagen scaffolds with collagen binding BDNF (Han et al., 2009) Schwann cell delivery (Dewitt et al., 2009) Electrospun nanofibres and stem cell differentiation (Xie et al., 2009) Gels for drug delivery [EGF and FGF-2] (Jimenez Hamann et al., 2005) Growth factor infused matrix for synthetic polymer channels (Tsai et al., 2006) Collagen coating of PLGA for gene delivery (De Laporte et al., 2009) Guidance gradients with incorporated laminin (Yao et al., 2009)
Agarose/alginate	Linear polysaccharide, β -D-mannuronic acid and α -L-guluronic acid	Agarose gels as growth matrix (Bellamkonda et al., 1995; Balgude et al., 2001) Microencapsulation of neurotrophic factors (Maysinger et al., 1996) Agarose gels with neurotrophic gradients (Cao and Shoichet, 2001). Freeze dried alginate sponges as neurite growth substrate (Kataoka et al., 2004) Cell encapsulation into alginate hydrogel (Novikova et al., 2006) Linear guidance pore scaffolds (freeze dried agarose) (Stokols and Tuszynski, 2006) Uniaxial multichannel scaffolds with BDNF-secreting MSCs (Stokols et al., 2006) Scaffolds with neurotrophin integration (Jain et al., 2006; Stokols and Tuszynski, 2006) Guidance gradients with incorporated laminin (Dodla and Bellamkonda, 2006) Steroid delivery with agarose embedded PLGA nanoparticles (Chvatal et al., 2008)
Hyaluronic acid	Dissacharide units of glucuronic acid and <i>N</i> -acetylglucosamine	Intrathecal drug and growth factor delivery with a methylcellulose composite gel. (Gupta et al., 2006; Shoichet et al., 2007; Kang et al., 2009)
Chitosan	Co-polymer of <i>N</i> -acetylglucosamine and <i>N</i> -glucosamine	Chitosan scaffolds supporting cell adhesion and growth (Freier et al., 2005; Freier et al., 2005) Schwann cell encapsulation (Yuan et al., 2004) Neural stem cell differentiation (Zahir et al., 2008) Protein elution (Kim et al., 2008) Extra and intramedullary tissue bridge conduits with neural stem cell differentiation (Nomura et al., 2008)
Fibronectin	Plasma glycoprotein dimer	Fibronectin mats for oriented axonal growth (King et al., 2003) Neurotrophin and drug delivery (Phillips et al., 2004)
Fibrin	Fibrillar glycoprotein polymer	Neurotrophin elution from heparin complexes (Taylor et al., 2006) Embryonic stem cell differentiation (Willerth et al., 2007) Gliosis inhibition (Johnson et al., 2009)

Table 2

The main synthetic polymers in use in animal models of spinal cord injury and examples of their applications.

Synthetic Polymers	Structure	Examples of Applications for Spinal Cord Repair
<i>Biodegradable</i>		
PLA	Poly(D,L lactic acid)	Single and multichannel scaffolds seeded with Schwann cells.
PGA	Poly(glycolic acid)	PLA: single channel (Oudega et al., 2001)
PLGA	Poly(lactic-co-glycolic acid)	Freeze dried macroporous foam scaffold with incorporated BDNF (Patist et al., 2004) Freeze dried macroporous scaffold with BDNF-secreting Schwann cells (Hurtado et al., 2006) PLGA: multichannel scaffolds seeded with Schwann cells (Moore et al., 2006) Multichannel scaffolds seeded with neural stem cells (Teng et al., 2002) Neurotrophin-eluting micro and nanospheres within scaffolds (Yang et al., 2005; Wang et al., 2008) Protein release from chitosan scaffolds (Kim et al., 2008)
PCL	Poly- ϵ -caprolactone	Nanofibre spinning for axonal growth orientation (Schnell et al., 2007)
PCLF	Poly- ϵ -caprolactone fumarate	Multichannel scaffolds (Wang et al., 2009)
<i>Hydrogels</i>		
PEG	Polyethylene glycol	Intravenous solution (Lavery et al., 2004) Immunoprotective sealant gel (Borgens et al., 2002; Duerstock and Borgens, 2002) Injectable gel with PLA for NT-3 delivery (Piantino et al., 2006) and BDNF (Soderquist et al., 2008) Three-dimensional neurite growth matrix (Namba et al., 2009) Intravenous PEG with Magnesium Sulfate (Ditor et al., 2007; Kwon et al., 2009)
<i>Non-biodegradable hydrogels</i>		
pHEMA	Poly(2-hydroxyethyl methacrylate)	pHEMA sponges (Giannetti et al., 2001) Guidance channels (Tsai et al., 2004) Fibre templated scaffolds (Flynn et al., 2003) Cell adhesion gradients (Yu and Shoichet, 2005) Neurotrophic gradients (Moore et al., 2006) Co-polymer with chitosan including cell adhesion peptides (Yu et al., 2007) Coil (Nomura et al., 2006) or multilayered pHEMA (Carone and Hasenwinkel, 2006) scaffold reinforcement Surface charge modification for cell adhesion (Lesny et al., 2006) and axonal growth (Hejcl et al., 2008)
pHEMA-MMA	pHEMA-co-methyl methacrylate	NGF-release from PGLA microspheres and pHEMA-NGF polymer bilayer (Piotrowicz and Shoichet, 2006) Matrix filled channels for acidic fibroblast growth factor (FGF-1) and NT-3 delivery (Tsai et al., 2006)
pHPMA	Poly[N-(2-hydroxypropyl) methacrylamide]	pHPMA colloid gel (Woerly et al., 1998) Colloid gel with cell adhesion peptides (Woerly et al., 2001) (Neurogel™) Colloid gel with CNTF and BDNF (Loh et al., 2001)
PAN/PVC	Poly(acrylonitrile-co-vinylchloride)	Schwann cell seeded scaffolds with BDNF & NT-3 cord infusion (Bamber et al., 2001) Schwann cell seeded scaffolds with GDNF Matrigel™ (Iannotti et al., 2003)