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NEURAL TISSUE ENGINEERING: Strategies for Repair and Regeneration

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■ **Abstract** Nerve regeneration is a complex biological phenomenon. In the peripheral nervous system, nerves can regenerate on their own if injuries are small. Larger injuries must be surgically treated, typically with nerve grafts harvested from elsewhere in the body. Spinal cord injury is more complicated, as there are factors in the body that inhibit repair. Unfortunately, a solution to completely repair spinal cord injury has not been found. Thus, bioengineering strategies for the peripheral nervous system are focused on alternatives to the nerve graft, whereas efforts for spinal cord injury are focused on creating a permissive environment for regeneration. Fortunately, recent advances in neuroscience, cell culture, genetic techniques, and biomaterials provide optimism for new treatments for nerve injuries. This article reviews the nervous system physiology, the factors that are critical for nerve repair, and the current approaches that are being explored to aid peripheral nerve regeneration and spinal cord repair.

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INTRODUCTION

Physiology of the Nervous System

The physiology of the nervous system presents unique challenges to bioengineering research addressing nerve injuries. This section briefly describes the general organization and the cellular components of the nervous system as well as the anatomy of the peripheral nerve and spinal cord. Discussions of nerve injuries and the currently available clinical treatments are also presented.

ORGANIZATION OF THE NERVOUS SYSTEM The nervous system is classified into the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS, which includes the brain, spinal cord, optic, and olfactory and auditory systems, conducts and interprets signals as well as provides excitatory stimuli to the PNS. The PNS consists of the cranial nerves arising from the brain, the spinal nerves arising from the spinal cord, and sensory nerve cell bodies (dorsal root ganglia) and their processes. Peripheral nerves innervate muscle tissue, transmitting sensory and excitatory input to and from the spinal column.

CELLULAR COMPONENTS OF THE NERVOUS SYSTEM The nervous system is composed of two cell types: neurons and neuroglia. Neurons are the basic structural and functional elements of the nervous system and consist of a cell body (soma) and its extensions (axons and dendrites). Clusters of sensory nerve soma, known as ganglia, are located just outside the spinal column. Dendrites transmit electrical signals to the neuron cell body and the axon conducts impulses away. Glial cells, or neuroglia, are support cells that aid the function of neurons and include Schwann cells in the PNS and astrocytes and oligodendrocytes in the CNS. Glial cells are more abundant than neurons, and unlike neurons, which cannot undergo mitosis, glial cells have some capacity for cell division. Although neurons cannot divide by mitosis, they can regenerate a severed portion or sprout new processes under certain conditions.

In the PNS, sheaths of living Schwann cells surround all axons. On the outer surface of this Schwann cell layer is the neurilemma, a basement membrane similar

to that found in epithelial layers. In contrast to axons in the PNS, CNS axons do not possess this continuous basement membrane and sheath of Schwann cells. Many axons are instead surrounded by an insulating myelin sheath, which is formed from dense layers of successive wrappings of the cell membrane of Schwann cells (PNS) or oligodendrocytes (CNS). Myelin serves to increase the propagation velocity of the nerve impulse, which is particularly important for those axons that extend long distances (up to 1 m).

ANATOMY OF THE PERIPHERAL NERVE AND SPINAL CORD A peripheral nerve consists of motor and sensory axons bundled together by support tissue into an anatomically defined trunk (Figure 1). Endoneurium surrounds individual axons and their Schwann cell sheaths and is composed predominantly of oriented collagen fibers. Next, the perineurium, formed from many layers of flattened cells (i.e., fibroblasts) and collagen, surrounds groups of axons to form fascicles. Finally, epineurium, an outer sheath of loose fibrocollagenous tissue, binds individual nerve fascicles into a nerve trunk. Peripheral nerves are well vascularized by capillaries within the support tissue of the nerve trunk or by vessels that penetrate the nerve from surrounding arteries and veins.

The spinal cord is composed of dendrites, axons, and cell bodies (Figure 2). The center of the spinal cord, a butterfly-shaped region referred to as gray matter, contains the cell bodies of excitatory neurons, as well as glial cells and blood vessels. The gray matter is surrounded by white matter, which helps to protect and insulate the spinal cord. White matter consists of axons and glial cells, including

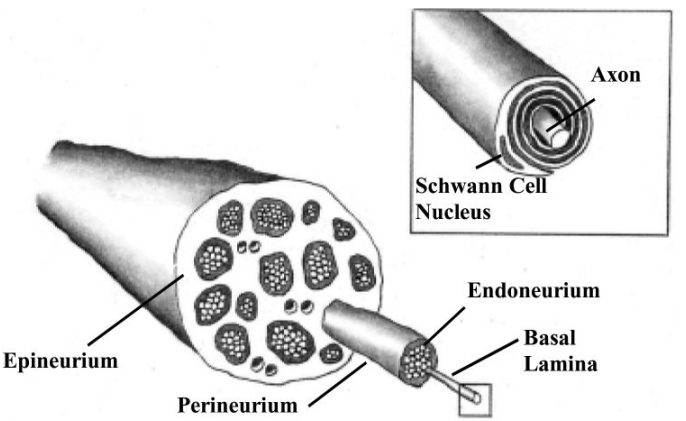


Figure 1 Anatomical overview of the PNS. Axons, surrounded by myelinating Schwann cell sheaths, are enclosed by endoneurium. Next, the perineurium binds individual axons together to form fascicles. Several axons are contained in each fascicle. Lastly, epineurium groups fascicles to one another, forming the nerve cable. Reprinted from Reference 343, pp. 375–415. Copyright (2002) with permission from Marcel Dekker, Inc.

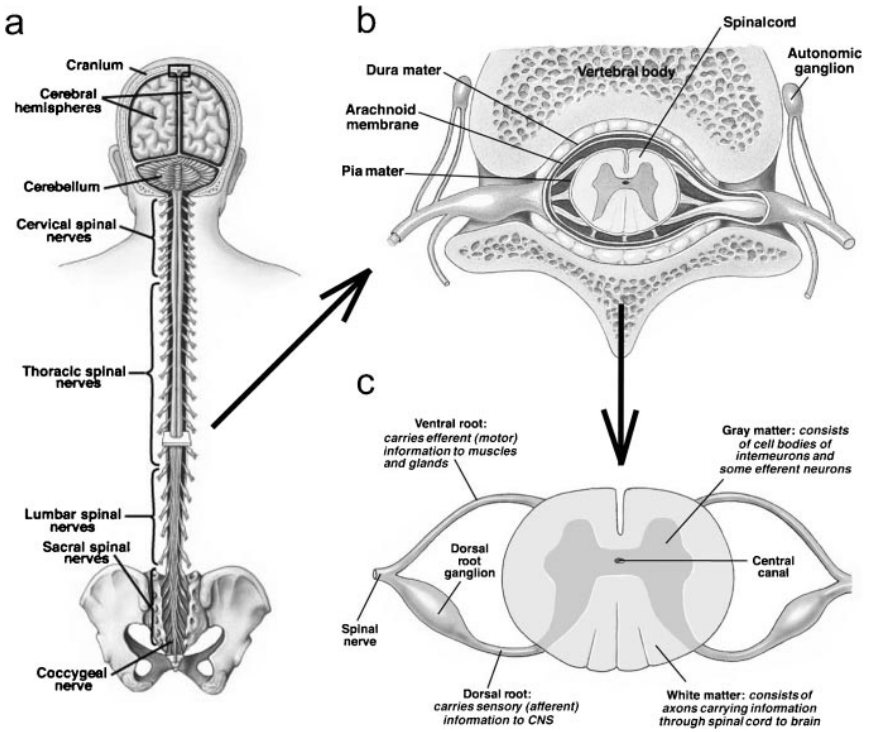


Figure 2 Anatomy overview of the spinal cord. (*a*) The spinal cord is composed of the cervical, thoracic, lumbar, sacral, and coccygeal spinal regions. (*b*) Nerves, well protected by vertebrae and protective membranes, project from each side of the cord and connect with innervation sites in the left and right sides of the body. (*c*) The nerves on each side are further subdivided into roots. The ventral root carries motor signals from the CNS to the muscles and glands, whereas the dorsal root carries sensory signals coming into the CNS. The dorsal root ganglia are located along the dorsal root and contain the cell bodies of sensory neurons. Reprinted from Reference 346. Copyright (2001) with permission from Pearson Education, Inc.

oligodendrocytes, astrocytes, and microglia (immune cells). Oligodendrocytes serve to myelinate the axons in the CNS, whereas astrocytes contribute to the blood-nerve barrier, separating the CNS from blood proteins and cells. Axons project from the white matter in bundles, known as fascicles, which exit the encasing bone of the spinal column, travel through the PNS-CNS transition zone, and enter the PNS. The transition zone is a clearly defined region where the glial cells in the CNS are separated from those in the PNS.

Nerve Injury and Regeneration

PERIPHERAL NERVOUS SYSTEM INJURY The most severe injury is a complete nerve transection. After a nerve is severed, the distal portion begins to degenerate as a

result of protease activity and separation from the metabolic resources of the nerve cell bodies (Figure 3*a*). The cytoskeleton begins to breakdown, followed by the dissolution of the cell membrane. The proximal end of the nerve stump swells, but experiences only minimal damage via retrograde degradation. After the cytoskeleton and membrane degrade, Schwann cells surrounding the axons in the distal end shed their myelin lipids. Phagocytotic cells, such as macrophages and Schwann cells, clear myelin and axonal debris (1). In addition to clearing myelin debris, macrophages and Schwann cells also produce cytokines, which enhance axon growth (2). Following debris clearance, regeneration begins at the proximal end and continues toward the distal stump. New axonal sprouts usually emanate from the nodes of Ranvier, nonmyelinated areas of axons located between Schwann cells. Functional reinnervation requires that axons extend until they reach their distal target, and in humans, axon regeneration occurs at a rate of about 2–5 mm/day; thus significant injuries can take many months to heal (3).

When a hollow nerve conduit is used to repair a severed peripheral nerve (discussed further below), an additional step for regeneration is required (4, 5). After injury, a fibrin bridge is formed through the conduit and across the defect site. This fibrin cable includes macrophages and other cells thought to be involved in debris clearance. The fibrin bridge retracts as Schwann cells and capillaries begin to grow across the gap, and regeneration proceeds as normal. It is not clear if the formation of a fibrin cable also occurs in the absence of a conduit or when a conduit contains an internal matrix.

CENTRAL NERVOUS SYSTEM INJURY A key difference between the PNS and CNS is the capacity for peripheral nerves to regenerate; CNS axons do not regenerate appreciably in their native environment. Several glycoproteins in the native extracellular environment (myelin) of the CNS are inhibitory for regeneration (6–8). The physiological response to injury in the CNS is also different compared to that of the PNS. After injury in the CNS, macrophages infiltrate the site of injury much more slowly compared to macrophage infiltration in the PNS, delaying the removal of inhibitory myelin (9). This is largely a result of the blood-spine barrier, which limits macrophage entry into the nerve tissue to just the site of injury, where barrier integrity is weakened. In addition, cell adhesion molecules in the distal end of the injured spinal cord are not upregulated appreciably as they are in the PNS, limiting macrophage recruitment. Finally, astrocytes proliferate in a manner similar to that of Schwann cells in the PNS, but instead become "reactive astrocytes," producing glial scars that inhibit regeneration (Figure 3*b*) (10).

Current Clinical Approaches for Treating Nerve Injuries

For peripheral nerve injury, treatment typically consists of either direct end-to-end surgical reconnection of the damaged nerve ends (Figure 4) or the use of an autologous nerve graft. Suturing the ends of the two nerve ends together can repair small defects or gaps in the nerve. For longer nerve gaps, this approach is not desired because any tension introduced into the nerve cable would inhibit nerve

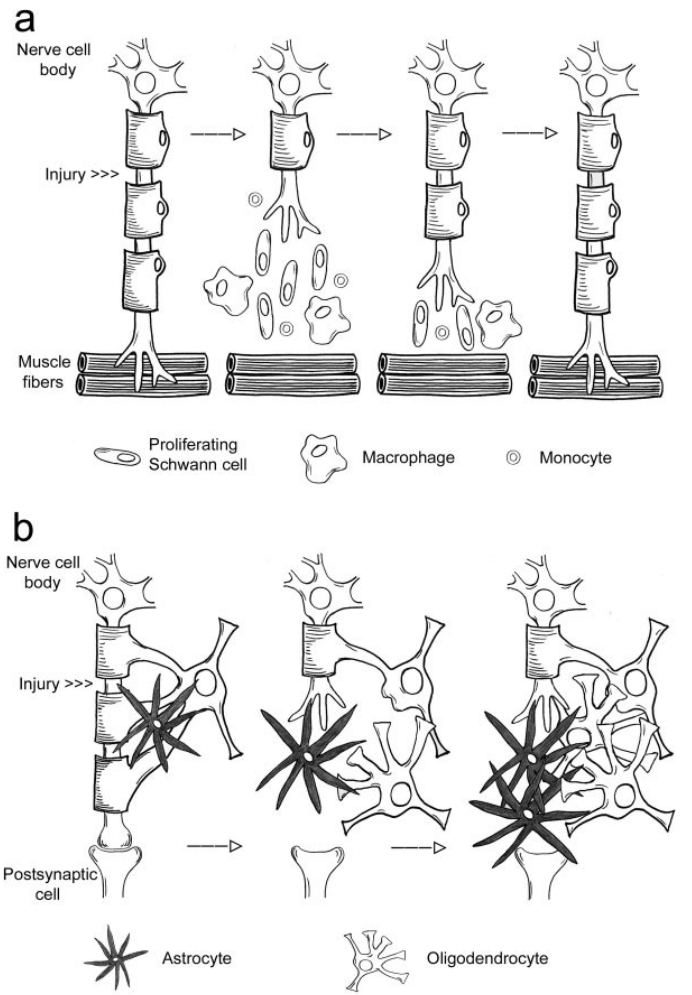


Figure 3 Responses to axotomy in the PNS and spinal cord. (*a*) In the PNS, support cells aid neuronal regeneration. Proliferating Schwann cells, macrophages, and monocytes work together to remove myelin debris, release neurotrophins, and lead axons toward their synaptic targets, resulting in restored neuronal function. (*b*) In the CNS, however, the few neurons that survive axotomy attempt regeneration and subsequently meet an impenetrable glial scar composed of myelin and cellular debris, as well as astrocytes, oligodendrocytes, and microglia. Fibroblasts, monocytes, and macrophages may also be present in the glial scar. Consequently, regenerating neurons in the spinal cord are blocked from reaching their synaptic target. Figure adapted from Bahr & Bonhoeffer (345).

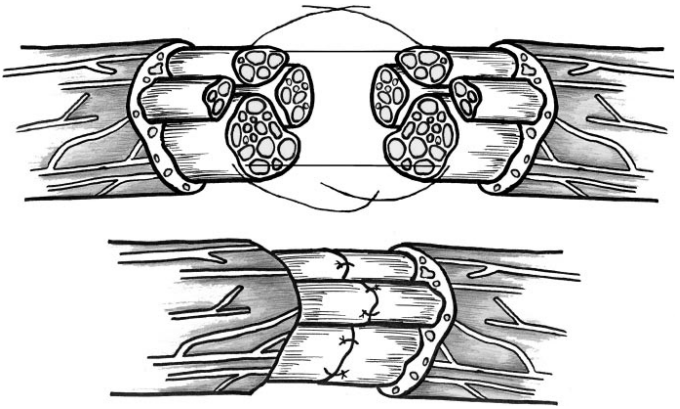


Figure 4 Surgical reconnection. One of the current clinical treatments for nerve transection is surgical end-to-end reconnection, which involves the suturing of individual fascicles within the nerve cable. End-to-end repair, however, is only effective if the nerve ends are directly adjacent and can be reconnected without causing tension. If the injury creates a gap in the nerve, autologous nerve grafts or autografts are used. Figure adapted from Lundborg (162).

regeneration (11). Thus, for a larger nerve defect, an autologous nerve graft that is harvested from another site in the body is used to span the injury site (12, 13). Disadvantages of this technique include loss of function at the donor site and the need for multiple surgeries. There are a few devices that are now FDA approved for relatively short nerve defects, including Integra Neurosciences Type I collagen tube (NeuraGen Nerve Guide) (14) and SaluMedica's SaluBridge Nerve Cuff (15). However, these treatments are reserved for small defects (several millimeters) and do not address larger peripheral nerve injuries.

For CNS injury, and particularly spinal cord injury, clinical treatment is less promising. If bone fragments exist near the site of injury, then surgery may be performed to reduce any risk of secondary injury. Antiinflammatory drugs, such as methylprednisone, are often also administered to reduce swelling and secondary injury (16). Unfortunately, there is currently no treatment available to restore nerve function. After swelling from the injury subsides, patients begin a long period of rehabilitation during which time they train remaining nerves to compensate for the loss due to injury.

Challenges and Bioengineering Strategies for Nerve Repair

In the PNS, the challenge is to find an alternative to the autologous nerve graft and thus eliminate the need for two surgeries and the removal of tissue from the patient. Also, clinical functional recovery rates typically approach only 80% for nerve injuries treated using autologous nerve grafts (17). Thus, bioengineering

strategies for the PNS have focused on developing alternative treatments to the nerve graft (e.g., nerve guidance channels), especially for larger defects, and improving recovery rates and functional outcome.

The CNS is a greater challenge for new therapies. The ability of spinal nerves to regenerate was not decisively shown until 1980 (18), and it was not until after this time that research in this area rapidly developed. In addition, results from various studies have been controversial (19, 20), complicating developments. It has been shown that both embryonic spinal cord grafts and peripheral nerve tissue grafts can support regenerating fibers in the CNS, but the fibers often do not successfully grow back across the PNS-CNS transition zone (21, 22). Thus, bioengineering efforts are focused on creating a permissive environment for regeneration and providing a seamless interface between the CNS and PNS.

These challenges provide fertile ground for the development of therapies and devices to enhance regeneration. Many researchers are presently focusing efforts on creating physical or chemical pathways for regenerating axons. These devices include physical or mechanical guidance cues, cellular components, and biomolecular signals, as reviewed individually below. Future therapies will incorporate multiple cues into unique devices that more closely mimic native nerve. They will also be interactive and programmable, and thus capable of seamless communication with surrounding tissues.

GUIDANCE THERAPIES

Historical Introduction to Guidance Therapies

It is commonly accepted that physical guidance of axons is a vital component of nerve repair. During the nineteenth century, many materials were used in an attempt to physically guide the regeneration of damaged peripheral nerves, including autologous nerve grafts (23), bone (24), metal tubes (25), and fat sheaths (26). It was not until the 1960s that Millesi pioneered microsurgical techniques to accurately align nerve fascicles in the direct resection of nerve ends, with improved functional outcomes (11). He also determined that the use of nerve grafts reduced tension on the damaged nerves in many cases and further enhanced functional recovery. These results also supported the need for physical guidance as an essential element in nerve regeneration. Later research demonstrated that biochemical signals (see Biomolecular Therapies, below) as well as physical guidance are critical for nerve regeneration (27–30).

Currently, the autologous nerve graft is the gold standard for repair of a peripheral nerve defect (12, 13). Current research is focused on developing improved scaffolds that can be used to physically guide regeneration of nerves across lesions. Similar techniques are also being explored for the repair of transected nerves in the spinal cord. These “nerve guides” or “nerve guidance channels” serve to direct axons sprouting from the proximal nerve end, provide a conduit for the diffusion of growth factors secreted by the injured nerve ends, and reduce the infiltration of scar tissue. Past research in this area has focused either on existing natural or

synthetic materials; however, none of the materials studied to date have matched or exceeded the performance of the nerve autograft. As a result, researchers are now focusing on the combination of materials and desired biomolecules to create new composite materials that can actively stimulate nerve regeneration. In addition, methods to minimize the immune response to nonautologous tissue could provide a source of natural material for nerve repair.

Note: For additional reviews on nerve regeneration, nerve grafts, and nerve guidance channels, refer to (31–38). Table 1 provides a summary of materials

TABLE 1 Nerve grafts and nerve conduit materials

Graft	Reference
Autologous tissue grafts	
1. Nerve grafts (gold standard)	(12, 13)
2. Vein grafts	(17, 43–45)
3. Muscle grafts	(41, 42)
4. Epineurial sheaths	(46)
5. Tendon grafts	(47)
Nonautologous/acellular grafts	
1. Immunosuppression with allografts	(373)
2. Acellular allografts and xenografts	
Thermal decellularization	(52, 53, 57)
Radiation treatment	(54, 58)
Chemical decellularization	(55, 56)
3. Small intestinal submucosa (SIS)	(65, 66, 70)
4. Human amnion	(71, 75, 76)
Natural-based materials	
1. ECM protein-based materials	
Fibronectin	(84, 85)
Laminin	(82, 88)
Collagen	(90–92)
2. Hyaluronic acid-based materials	(95)
3. Fibrin/fibrinogen	(96, 97)
4. Other materials (alginate, agarose . . .)	(99, 100, 102)
Synthetic materials	
1. Biodegradable synthetic materials	
Poly(lactic acid) (PLA)	(109, 110)
Poly(lactic-co-glycolic acid) PLGA	(107)
Poly(caprolactone)	(111, 113)
Poly(urethane)	(114)
Poly(organo)phosphazene	(112)
Poly(3-hydroxybutyrate)	(116)
Poly(ethylene glycol) “glue”	(128, 156)
Biodegradable glass	(117, 118)
2. Electrically active materials	
Piezoelectric	(119)
Electrically conducting	(120)
3. Nonbiodegradable synthetic materials	
Silicone	(122, 127)
Gore-Tex or ePTFE	(123–125)

in use or under investigation for nerve repair applications, as described in detail below.

Autologous Tissue Grafts

Natural tissues, including autologous tissue grafts, possess several advantages. Natural materials are more likely to be biocompatible than artificial materials, are less toxic, and provide a support structure to promote cell adhesion and migration. Drawbacks, on the other hand, include potential difficulties with isolation and controlled scale-up.

Autologous tissue grafts have been used extensively for nerve repair applications. Nerve autografts are typically derived from one of several cutaneous nerves, such as the sural or saphenous nerve, with an available length up to about 40 cm and a cable diameter of 2–3 cm (39, 40). For a more thorough synopsis of the early history of nerve repair using nerve grafts, refer to the review by Chiu (17), and for additional information on the surgical techniques used in nerve grafting, refer to (13, 38).

In addition to the nerve graft, other natural tissues, such as autologous muscle (41, 42) and vein grafts (17, 43–45), have been used to limited extents in the clinic. Furthermore, some current research efforts are focused on natural tissue grafts for peripheral nerve repair, including the use of epineurial sheaths (46), tendon grafts (47), muscle-vein combined grafts (48, 49), inside-out vein grafts (50), and vein grafts impregnated with autologous Schwann cells (51). All have exhibited encouraging results in research but still suffer from the key drawback that tissue must be removed from the patient.

Nonautologous Tissue and Acellular Grafts

As a result of the limitations with using autologous tissue, attention has turned toward nonautologous tissue and extracellular matrix (ECM)-based materials. Allogenic and xenogeneic tissues (donor tissue from cadavers and animals, respectively) have the advantages that supplies can be large and their use does not require harvest from the patient. However, these tissues possess some risk of disease transmission and must either be used in conjunction with immunosuppressants or must be processed to remove immunogenic components. Many efforts are being made to process intact nonautologous tissue, rendering it less immunogenic for clinical use. These methods focus on removal or destruction of the immunogenic cells and the preservation of the ECM components that are essentially conserved between species. Many different methods have been explored, including thermal techniques (52, 53), radiation (54), and chemical processes (55, 56).

The most common decellularization technique is thermal decellularization, which involves repeated freeze-thaw cycles to kill and fragment the cells. Nerve grafts processed using this approach have been shown to be generally nonimmunogenic (57); however, the structure of the ECM is typically damaged and the cellular remnants are not completely extracted, resulting in inflammation when implanted.

Radiation treatments destroy cells in tissues and produce relatively little damage to the matrix structure, but they also fail to extract all cellular components (58). Several chemical (e.g., detergent) treatments have also been developed that are more effective in the complete removal of cell debris. Recent approaches, using optimized combinations of detergents, show good cellular clearance and excellent structural preservation (Figure 5) (T.W. Hudson & C.E. Schmidt, unpublished results). Similar thermal and chemical decellularization methods have also been applied to muscle tissue for use in nerve repair applications (59–62). These efforts to develop acellular sources of tissue for nerve repair applications appear quite promising, especially in light of recent successes to create other acellular tissues for clinical applications, such as cardiovascular tissue [reviewed in (63)] and skin (64). Thus, the use of acellular tissues for clinical nerve repair may become a viable option in the future.

Other natural tissues explored for nerve repair applications include small intestinal submucosa (SIS) and amniotic tissue grafts. SIS is an acellular matrix derived from small intestine, typically of porcine origin. SIS is prepared from the mucosa and muscle layers of the small intestine, which are treated with a hypotonic solution to lyse and wash away the cells. The resultant ECM material is composed of collagen, fibronectin, growth factors, glycosaminoglycans, proteoglycans, and glycoproteins (65, 66). SIS has been used with encouraging results as a regenerative scaffold for a number of tissues including vascular grafts (67), urinary tract (68), and tendon (69). Recently, SIS derived from rats has been used in conjunction with Schwann cells to create nerve grafts that promote regeneration almost as well as the nerve autograft (70).

Amnion harvested from human placental tissues has also received attention for its potential use in nerve regeneration applications (71–76). The amnion is a natural, biodegradable tissue that exhibits low immunogenicity and stimulates new vascularization (77). This material is also readily available in large quantities and does not require surgical procedures for harvest. To process this tissue, the epithelial cell layer of the amnion membrane is removed while the basement membrane and stromal surfaces remain intact. After removing the epithelial cells, the resulting acellular connective tissue matrix can be manufactured into thin dry sheets and then subsequently processed into conduits. Amnion tubes have been shown to promote regeneration comparable to that of the nerve autograft across 1 cm defects in the sciatic nerves of rats, and the tubes completely degrade by 4 months (73).

Natural-Based Materials

In addition to intact acellular tissues, a great deal of research has focused on the use of purified natural ECM proteins and glycosaminoglycans, which can be modified to serve as appropriate scaffolding. ECM molecules, such as laminin, collagen, and fibronectin, have been shown to play a significant role in axonal development and repair in the body (78, 79). Furthermore, many other proteoglycans and glycosaminoglycans of the ECM are known to modulate neural activity and neurite

extension; some provide stimulatory cues, whereas others provide inhibitory cues (80, 81). Thus, ECM components are obvious candidates for use in nerve guides.

There are a number of examples in which the ECM proteins laminin, fibronectin, and collagen have been used for nerve repair applications (82–90). For example, silicone tubes filled with laminin, fibronectin, and collagen show improved regeneration over a 10 mm rat sciatic nerve gap compared to empty silicone controls (86). Oriented mats or strands of fibronectin have been used to bridge 10 mm nerve defects in rats, with results close to that of the nerve autograft (84). Collagen filaments have also been used to guide regenerating axons across 20–30 mm defects in rats (87, 90). Further studies have shown that oriented fibers of collagen within gels, aligned using magnetic fields, provide an improved template for neurite extension compared to randomly oriented collagen fibers (91, 92). Rates of regeneration comparable to those using a nerve autograft have been achieved using collagen tubes containing a porous collagen-glycosaminoglycan matrix (93, 94). It is believed that providing a suitable matrix for Schwann cell and neurite migration enhances nerve repair.

Other naturally derived molecules investigated for their application in nerve repair include hyaluronic acid (95), fibrinogen (96), fibrin gels (97), self-assembling peptide scaffolds (98), alginate (99), agarose (100, 101), and chitosan (102). Current studies are under way to further modify these materials for tissue engineering applications, such as the chemical cross-linking of hyaluronic acid, an ECM glycosaminoglycan, to allow it to be photopolymerized into porous, three-dimensional hydrogels and to stabilize it against rapid degradation (103). Other studies include the modulation of fibrin gels either using magnetic fields to align the polymer fibers (104) or with appropriate biomolecules to enhance neurite extension (105, 106).

Synthetic Materials

Research is also under way to identify synthetic materials that can be used for nerve repair applications. Synthetic materials are attractive because their chemical and physical properties (e.g., degradation rate, porosity, mechanical strength) can be specifically optimized for a particular application. However, the biocompatibility of synthetic materials poses a challenge because the body's inflammatory response can vary considerably from one material to another. In addition, some synthetic materials that are tolerated by the body's immune system are unfortunately incompatible with cell adhesion and tissue repair. These materials are often modified to render them more "cell friendly."

To select an appropriate synthetic material, there are several general properties that all nerve guidance channels should possess: (*a*) They must be readily formed into a conduit with desired dimensions, (*b*) they must be sterilizable, (*c*) they must be tear resistant, and (*d*) they must be easy to handle and suture. Permanent materials pose a higher risk for infection, are more likely to provoke a chronic inflammatory response, and have the potential to compress the nerve over time. Thus, a nerve guide that degrades as the nerve regenerates is preferred. Additionally,

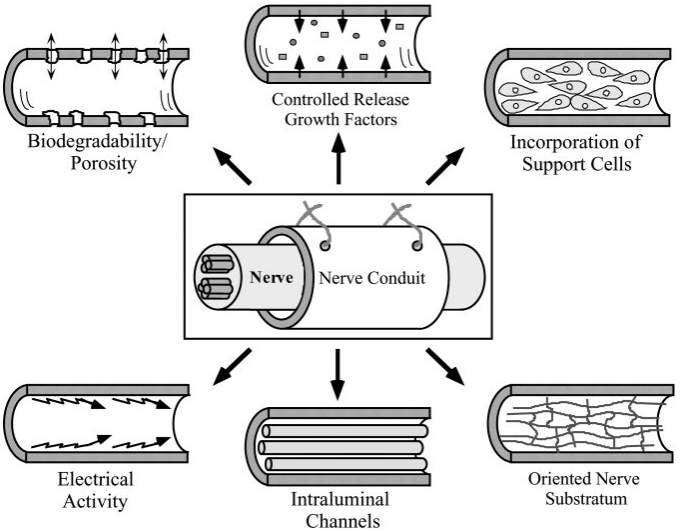


Figure 6 Properties of the ideal nerve guidance channel. The desired physical properties of a nerve conduit include (*clockwise from top left*): a biodegradable and porous channel wall; the ability to deliver bioactive factors, such as growth factors; the incorporation of support cells; an internal oriented matrix to support cell migration; intraluminal channels to mimic the structure of nerve fascicles; and electrical activity. Reprinted from Reference 34. Copyright (1999), with permission from Elsevier Science.

guidance channels should be pliable, but should maintain their shape and resist collapse during implantation and over the time course for regeneration. Research has also shown that guidance channels should be semipermeable and should have a smooth inner wall. Hudson et al. (34) review the desired physical properties of the nerve guidance channel (Figure 6).

A number of different synthetic materials have been explored for use in aiding nerve regeneration. Poly(esters), such as poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and poly(lactic-co-glycolic acid) (PLGA) (107, 108), were some of the first synthetic polymers studied because of their availability, ease of processing, biodegradation characteristics, and approval by the FDA. These materials continue to be researched to date and have been processed into foams (Figure 7) and seeded with Schwann cells to improve their regenerative potential (109, 110). Other biodegradable poly(esters), such as poly(caprolactones), have also demonstrated promise for nerve regeneration applications (111–113). In addition to poly(esters), biodegradable poly(urethane) (114), poly(organo phosphazene) (112), methacrylate-based hydrogels (115), and poly(3-hydroxybutyrate) (116) have shown a capacity for guiding regeneration. Biodegradable glass tubes have also been studied, but results have not been optimistic (117, 118). More advanced

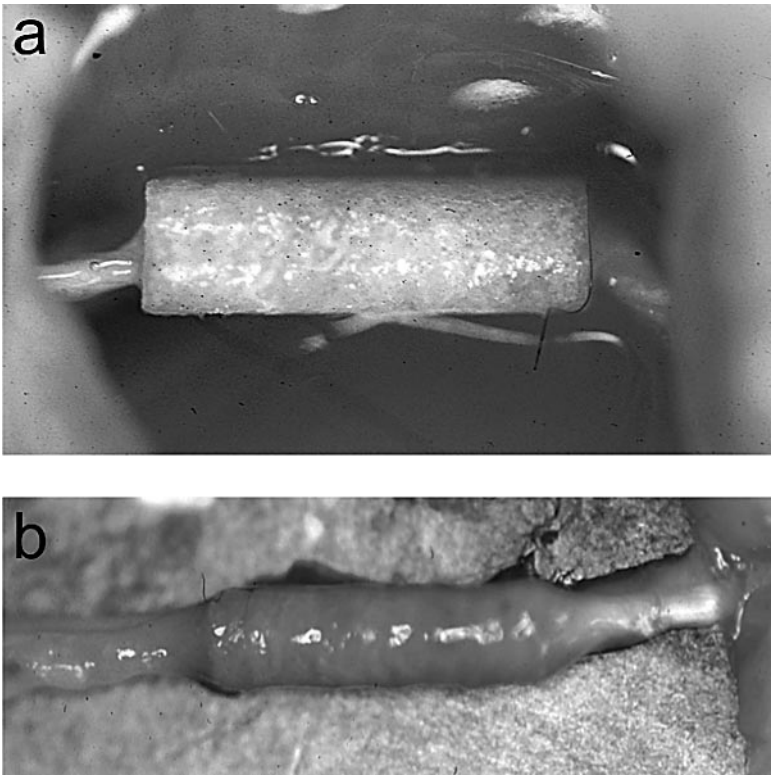


Figure 7 Poly(L-lactic acid) foam nerve guidance channels. Porous biodegradable poly(L-lactic acid) (PLL) conduits were synthesized using a solvent casting, extrusion, and particulate leaching technique. (*a*) Nerve guidance channels from 10 mm to 22 mm in length were used to repair transected rat sciatic nerves, a common model for studying peripheral nerve regeneration. (*b*) After 4 months, the PLL conduits remained structurally intact, supported tissue infiltration and vascularization, and resulted in structural and functional regeneration comparable to isografts, the current clinical gold standard (109). Figure courtesy of C.W. Patrick, Jr., Department of Plastic Surgery, The University of Texas M.D. Anderson Cancer Center and G.R. Evans, Division of Plastic Surgery, University of California-Irvine.

materials processing techniques to create three-dimensional channels and unique pores or fiber structures are discussed below in Advanced Therapies.

Research has also shown that electrical charges play a significant role in stimulating the cellular differentiation for several tissue types. Neurite extension, for example, is significantly enhanced on piezoelectric materials (i.e., materials that generate a surface charge with small deformations), such as poly(vinylidene fluoride) (PVDF) (119), and on electrically conducting polymers, such as poly(pyrrole)

(120). Further modification of these materials with biological stimuli (e.g., hyaluronic acid) may provide interactive biomaterials for use as nerve guidance channels (121).

Several nondegradable synthetic materials have been used in nerve repair applications, including silicone tubing (122), which has been applied in clinical settings as well as in research settings, and expanded poly(tetrafluoroethylene) or ePTFE (Gore-Tex) (123–125). Silicone, in particular, has been studied since the 1960s (126, 127), and much fundamental insight into nerve regeneration has come from the use of this model system. In general, inert silicone tubes can be used to bridge short gaps with some success. However, it is commonly accepted that impermeable, inert guidance channels, such as silicone, do not support regeneration across defects larger than 10 mm (in rats) without the presence of exogenous growth factors. Instead, research is now focused on developing semipermeable or degradable guidance channels that can actively stimulate improved regeneration over longer, more clinically relevant defect lengths. The development of nondegradable guidance channels is not under very active pursuit because of the limitations associated with permanent materials, as described earlier.

Poly(ethylene glycol) (PEG) has also been applied to nerve regeneration applications. In one unique approach, PEG has been used to "fuse" the membranes of severed nerve ends of sciatic and spinal nerves (128). These studies have shown that conduction of axon potentials can be restored immediately with this procedure. Unfortunately, this process can only be applied if the severed nerve ends are directly adjacent, and therefore is not useful for large nerve defects. Additionally, cross-linked PEG hydrogels that are modified with factors to mimic the ECM are under active development, particularly for cardiovascular applications (129, 130). Recent studies on these systems are also looking into their ability to aid nerve regeneration (131). For example, PC12 cells are able to extend neurites on a PEG hydrogel when the cell adhesion motif Arg-Gly-Asp-Ser (RGDS) is covalently incorporated into the material (Figure 8) (B.K. Mann, personal communication).

Applications in the Central Nervous System

Although regeneration of the mammalian CNS was once thought to be impossible (132), studies over the past two decades have shown that axonal growth after spinal cord injury can occur when provided with the correct substratum (133–135). Research has focused mainly on the use of peripheral nerve grafts and embryonic spinal cord grafts (18, 136–140), and recent studies have looked to embryonic neural progenitor or stem cells (141, 142). Research suggests that embryonic spinal cord grafts both rescue neurons from injury-induced cell death and serve as a substrate to support new axonal growth (143, 144). On the other hand, peripheral nerve grafts appear to only provide a permissive substrate for the ingrowth of CNS axons (145).

Cultured Schwann cells (146–148), ECM-based materials (149, 150), and synthetic polymers (128, 131, 151–158) have also been investigated for their ability to

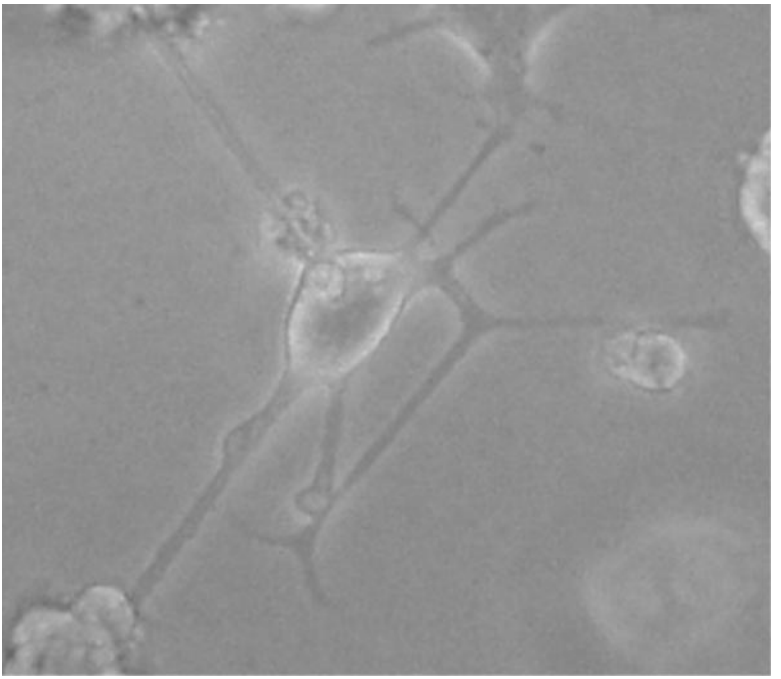


Figure 8 Biomimetic polyethylene glycol (PEG) hydrogels for nerve regeneration. PEG hydrogels are an alternative synthetic material that could be used for nerve regeneration. Bioactive factors, such as cell adhesion ligands, growth factors, and proteolytic degradation sites, can be incorporated into PEG hydrogels in order to render a more biomimetic scaffold (129, 130). PC12 cells are able to adhere to and extend neurites on PEG hydrogels with covalently incorporated cell adhesion ligands (RGDS, YIGSR, and IKVAV) but not on hydrogels with a nonadhesive control peptide RGES. This image shows PC12 cells extending neurites on a PEG hydrogel with RGDS covalently incorporated into the material. The amount of neurite extension was dependent on the type and concentration of adhesion ligand incorporated into the hydrogel. Figure courtesy of B.K. Mann, Keck Graduate Institute.

serve as templates for spinal nerve regeneration. For example, Woerly et al. (151) have shown that a poly[N-(2-hydroxypropyl)methacrylamide] (pHPMA) hydrogel containing the cell-adhesive region of fibronectin Arg-Gly-Asp (RGD) is able to support tissue development within a lesion created in a rat spinal cord. Areas of angiogenesis and axonal growth were observed within the tissue, and necrosis was reduced in the adjacent white and gray matter, suggesting that pHPMA hydrogel matrices could potentially serve as a substrate for spinal nerve regeneration across a defect. Additional synthetic matrices under investigation in the CNS include: poly(lactic acid) or poly(D,L-lactide) (154, 155), poly(2-hydroxyethyl

methacrylate) or pHEMA (131, 153), poly(lactic-co-glycolic acid) (PLGA) and block copolymer of poly(lactic-co-glycolic acid)-poly(lysine) (159), and poly (ethylene glycol) "glue" (128, 152, 156). Results to date from these various studies give great hope for the therapeutic reconstruction of neural connections after spinal cord injury; systems can be successfully designed to provide a permissive substrate for regenerating spinal nerve fibers. However, major challenges that remain include the growth of the nerve fibers back into the spinal cord and the functional integration of the nerve fibers with the host synaptic pathways. These hurdles may be addressed in the future by combining guidance therapy approaches with various biomolecular therapies, as described below. For reviews of tissue engineering and biomaterials strategies applied to spinal cord injury, see (157, 160).

BIOMOLECULAR THERAPIES

Neurotrophic Factors to Promote Regeneration

The role of neurotrophic factors in neural regeneration has been the focus of extensive research [for reviews see (161–165)]. The influence of these factors in neural development, survival, outgrowth, and branching has been explored on various levels, from molecular interactions to macroscopic tissue responses. One family of neurotrophic factors, the neurotrophins, has been heavily investigated in nerve regeneration studies. The neurotrophins include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). Outside of the neurotrophin family, other factors of importance are ciliary neurotrophic factor (CNTF), glial cell line-derived growth factor (GDNF), and acidic and basic fibroblast growth factor (aFGF, bFGF). As discussed below, these factors promote a range of neural responses (summarized in Table 2). A

TABLE 2 Neural responses to neurotrophic factors

Neural response promoted	Neurotrophic factors
Motor neuron survival	BDNF, NT-3, NT-4/5, CNTF, GDNF
Motor neuron outgrowth	BDNF, NT-3, NT-4/5, CNTF, GDNF
Sensory neuron survival	NGF, NT-4/5, GDNF
Sensory neuron outgrowth	NGF, BDNF, NT-3
Spinal cord regeneration	NGF, NT-3, CNTF, FGFs
Peripheral nerve regeneration	NGF, NT-3, NT-4/5, CNTF, GDNF, FGFs
Sensory nerve growth across the PNS-CNS transition zone	NGF, NT-3, GDNF, FGFs

Abbreviations: Brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), ciliary neurotrophic factor (CNTF), glial cell line-derived growth factor (GDNF), nerve growth factor (NGF), acidic and basic fibroblast growth factors (FGFs).

detailed discussion of all neurotrophic factors, their additive effects, and their direct influence on glial cells is not within the scope of this review; see (161–164, 166) for more information on these topics.

NGF is vital to the development and regeneration of the nervous system; consequently, NGF is the most thoroughly characterized neurotrophic factor (162, 167). NGF is expressed at low levels in healthy peripheral nerve and is upregulated in the distal stump upon injury (168). Similarly, following spinal cord transection, NGF accumulates in both the distal and proximal stumps (169). On the cellular level, NGF promotes survival, outgrowth, and branching in sensory neurons, but does not aid motor neuron regeneration (170–172). Nonetheless, much work has focused on delivering NGF to neuronal injuries. Studies using nerve guidance channels filled with NGF solutions have provided conflicting results (173–175), perhaps due to leakage from the channel or NGF inactivation. Continuous delivery devices (discussed in more detail below) offer a more reliable means of administering NGF, and such work has been associated with increased regeneration in both the PNS (176–178) and the spinal cord (171, 179, 180). Application of exogenous NGF has also been linked to increased sensory neuron regeneration from the dorsal root ganglia, through the PNS-CNS transition zone, and into the spinal cord (180–182). However, the use of NGF is not without disadvantages: the application of exogenous NGF to spinal cord injuries has been associated with significant sprouting of uninjured sensory axons (181). This sprouting has been linked to serious side effects, including chronic pain (181–183) and inappropriate neuronal reflexes (181, 184).

BDNF supports motor neuron survival (185–187) and promotes the axonal growth of motor (188) and sensory (189) neurons. However, research investigating the effects of BDNF on nerve regeneration has provided inconclusive results in both the PNS (178, 190, 191) and the spinal cord (179, 189, 192–196). Similar to NGF, these inconsistencies likely stem from different methods used to deliver BDNF, as it has been noted that BDNF must be delivered locally and at high concentrations to have an effect on nerve regeneration (165).

NT-3, like BDNF, promotes motor neuron survival (187) and outgrowth (188) as well as sensory axon growth (197). In vivo, NT-3 plays a vital role in aiding the regeneration of peripheral nerves (178, 198) and spinal cord (179, 189, 194–196). NT-3 has also been associated with the increased ability of sensory axons to grow from the dorsal root ganglia, across the PNS-CNS transition zone, and into the spinal cord (180, 182, 195). NT-4/5 has not been studied in as much detail as NT-3. However, NT-4/5 has been shown to promote the survival of motor neurons (187, 199) and sensory neurons (200). NT-4/5 also supports the axonal outgrowth of motor neurons (188) and has been associated with improved regeneration of severed peripheral nerve (201).

CNTF promotes motor neuron survival (202, 203), outgrowth (204), and sprouting (205). CNTF is thought to play a role in the response of spinal cord to injury, as CNTF mRNA is found at increased levels adjacent to spinal cord lesions (206). The application of exogenous CNTF has been associated with increased levels of

regeneration following injury in both the spinal cord (196) and peripheral nerve (207). A drawback, however, is that CNTF has been demonstrated to play a role in glial scarring (i.e., an injury response that results in a nonpermissive growth environment in the CNS; see below for more information) (208, 209).

GDNF promotes the survival of motor (210), sensory (172, 209), and autonomic (211) neurons. GDNF also promotes the growth of motor neurons in the CNS (212) and has been correlated with improved peripheral nerve regeneration (177). In comparison to NGF and NT-3, GDNF was shown to promote more extensive growth of sensory neurons from the dorsal root ganglia, through the PNS-CNS transition zone, and into the spinal cord (180, 182).

aFGF and bFGF have been associated with enhanced regeneration following injuries in the peripheral nerve (213, 214) and spinal cord (19, 179). The fibroblast growth factors are strong promoters of angiogenesis (215) and could, therefore, directly and indirectly aid in the healing of injured nerves. Like other neurotrophins (i.e., NGF, NT-3, and GDNF), bFGF has been associated with increased outgrowth of sensory neurons from the dorsal root ganglia, through the PNS-CNS transition zone, and into the spinal cord (181).

In summary, neurotrophic factors promote a variety of neural responses: survival and outgrowth of the motor and sensory nerves, spinal cord and peripheral nerve regeneration, and sensory nerve growth across the PNS-CNS transition zone. However, in vivo responses can vary due to the method of delivering the growth factor. Therefore, the continued use and development of highly controllable delivery devices are required for the study of these extremely complex systems.

Biomolecule Delivery Techniques

The delivery of biomolecules to support regeneration has several intrinsic challenges, including the toxicities and poor stability associated with many bioactive factors. A variety of techniques to deliver therapeutics to the nervous system have been established, including osmotic pumps (216) and silicone reservoirs (217). However, these methods are often associated with drawbacks, including device failure and higher potentials for inflammation and infection due to their nondegradable components (218). Polymer matrices, microspheres, and gene therapy, as described below, are effective delivery methods that overcome these challenges. For a recent review on these and other methods of delivering biomolecules to the nervous system, see Maysinger & Morinville (218).

Synthetic and naturally derived polymers are widely used in controlled release devices for protein delivery. These devices are designed such that bioactive factors are released in a spatially and temporally controlled manner; for example, release can occur as the polymer degrades or by diffusion through pores in the polymer matrix. In neural applications, two types of delivery devices have been primarily used: polymer matrices and microspheres. One example of a polymer matrix delivery device is the nerve guidance channel. These tubular conduits have been primarily used as a model system to study peripheral nerve repair (see Guidance

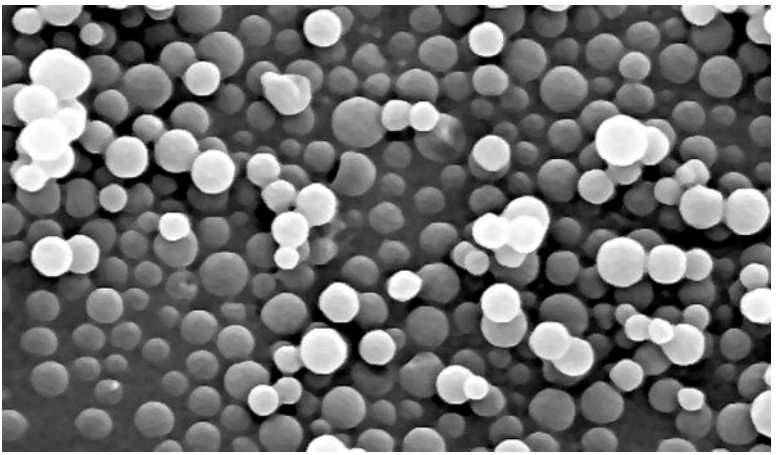


Figure 9 Poly(lactide-co-glycolide) microspheres. Poly(lactide-co-glycolide) microspheres prepared using a water/oil/water double emulsion solvent evaporation process. The number average size of these particles is about 1 mm. Particles were freeze-dried and sputter coated for observation under scanning electron microscopy. Figure courtesy of K. Roy, S.P. Kasturi, and J. Mendenhall, Department of Biomedical Engineering and Institute of Cellular and Molecular Biology, The University of Texas at Austin.

Therapies above for more detail). By incorporating growth factors into the conduit wall, the nerve guidance channel itself becomes a delivery device. Ethylene vinyl acetate (177, 178) and fibronectin mats (198) are examples of polymeric nerve guidance channel materials that have been successfully modified to deliver growth factors to regenerating peripheral nerve.

Microspheres (Figure 9) are suitable for a variety of delivery applications and are commonly used to deliver molecules to the CNS. Like polymeric matrices, microspheres aid in the controlled release of active biomolecules; however, these small devices typically have diameters in the range of 1 μm to 1 mm and can be applied less invasively (e.g., injection). Growth factors encapsulated in chitosan, alginate, poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), and poly(caprolactone) have been investigated and show promise for further study in models of spinal cord injury (219, 220).

Though polymers can be used for controlled release, they only provide a finite reservoir of active biomolecular agents. Thus, researchers are turning toward gene therapy techniques for the long-term production of active growth factors in situ (see Cellular Therapies below for a discussion of genetically modified cells for transplant applications). Recent reviews provide an excellent overview of gene therapy in the nervous system (221–223); therefore, this section only provides a brief summary.

A number of viral and nonviral gene delivery techniques are available (221, 222). Viral vectors have been investigated in a variety of tissue systems and include

methods based on retrovirus, herpes virus, adenovirus, and adeno-associated virus; efforts in the nervous system have primarily implemented herpes virus (224, 225) and adenovirus (197, 203). Viral gene transfer is able to promote high levels of gene expression from the vector (221). Nonetheless, some level of risk is associated with administering a therapy based on a viral agent. Before they can be used in the clinic, viral vectors must be proven to be safe and any inflammatory response must be minimized (222, 223).

Given the drawbacks inherent to viral vectors, researchers are also investigating nonviral transfection techniques. In the absence of viral protein machinery to enter cells, transfections with nonviral vectors rely on direct delivery or nonspecific internalization methods (221). Naked DNA can be injected directly, but this technique often results in low expression. Gene guns increase transfection efficiencies by attaching the DNA to a gold particle and then shooting the complex into the cell with a high-voltage arc or high-pressure gas. Therefore, the use of gene guns can result in higher transfection efficiencies, but at the cost of increased tissue damage.

Cationic lipids and polymers can also be used to assist transfection; these methods primarily depend upon nonspecific internalization to deliver DNA into neuronal cells (221). Lipoplexes, or complexes of DNA with cationic lipids, are one of the most common and successful nonviral gene delivery methods (221). However, the mechanisms by which nonviral vectors are able to transfect cells is not well understood; thus, the optimization of these systems is yet very challenging. [For an excellent review of the current challenges to nonviral gene therapy in the CNS, see Berry et al. (221)]. Nevertheless, through new means of delivering and targeting the complexes, scientists are able to increase the transfection efficiencies of nonviral vectors. For example, cell-specific targeting ligands (e.g., the nontoxic neuronal-specific fragment C of tetanus toxin) (226) allow transfection only in the desired cell types, and gene-activated matrices (GAMs; biodegradable matrices loaded with DNA) allow repeated transfection as neurites extend throughout the matrix (221).

Intrinsic Neuronal Factors to Promote Regeneration

As described above, the application of exogenous neurotrophic factors can be an effective means of promoting regeneration. Through an intricate and synergistic cascade of signaling events, neurotrophic factors affect the expression of genes that promote neural survival and axonal outgrowth. Because of the complexity associated with delivering active neurotrophic factors, researchers are also looking toward other means of manipulating the genes that control nerve regeneration. One approach gains insight from contrasting the intrinsic neuronal mechanisms of two systems: the mature response to injuries in adults and the embryonic development of the nervous system. A separate but related approach considers gene expression following axotomy in the PNS (where regeneration does occur) compared to the spinal cord (where regeneration typically does not occur). Based on the analysis of these systems, researchers are investigating methods of optimally controlling

regeneration-associated genes (RAGs), neuronal cytoskeletal components, and antiapoptosis factors.

RAGs are first expressed during the development of the nervous system [for reviews, see (227, 228)]. The developmental expression of RAGs is transient, and in healthy adult nerve, the expression of these genes is negligible. However, when peripheral nerves are damaged, a selection of these genes is re-expressed, often resulting in successful regeneration. This is not the case following spinal cord injury and may be one of the reasons that regeneration is not successful in this system. Many have hypothesized, therefore, that inducing the upregulation of RAGs may be an effective treatment of spinal cord injury. Two of the most abundant and well-studied RAGs are GAP-43 and CAP-23 (227, 229). The overexpression of each of these factors alone can induce axonal sprouting (230–232), but not to a degree suitable for the support of regeneration (233). Based on this information, it has been suggested that an effective therapy to treat spinal cord regeneration may not rely on the upregulation of a single RAG, but will require the coordinated overexpression of two or more RAGs acting together (227, 228). Such a therapy could be based on in situ transfection, such as the work with adenoviral vectors for the overexpression of GAP-43 (234), or rely on the administration of neurotrophins (e.g., BDNF or NT-4/5), which are known to stimulate RAG expression (193).

Another means of promoting the intrinsic neuronal mechanisms of regeneration relies on an understanding of the cytoskeletal dynamics driving axonal growth. Actin polymerization and rearrangement are crucial to the migration of cells, such as fibroblasts (235), and the elongation of nerve processes (236). However, the mechanism of this process is not well understood. Furnish et al. (237) suggest that actin accessory proteins, such as gelsolin, could play an important role in regulating axonal growth (Figure 10). Information gained from such studies could lead to a better understanding of the cytoskeleton's role in nerve regeneration and uncover new possible targets for clinical therapies.

Before regeneration and cytoskeletal reorganization begin, apoptosis is one of the main obstacles to overcome following nerve injury. Therefore, scientists have attempted overexpressing antiapoptosis factors in neurons as a means of aiding nerve regeneration. Work in this area has primarily focused on overexpressing bcl-2 (225, 238–242). Interestingly, the expression of bcl-2 has also been implicated in aiding axonal outgrowth (238, 240). However, the exact nature of this gene is not well understood, as some have suggested that bcl-2 expression does not directly enhance nerve regeneration (241, 242). While the majority of these studies were carried out in transgenic mice, promising work has demonstrated the suitability of viral vectors for the overexpression of antiapoptosis factors (225).

Studies of neuronal development have helped to expose other factors that could also aid adult nerve regeneration. These include adhesion molecules (L1, NCAM, N-cadherin) (223, 243), molecules for axon guidance and path-finding (semaphorins, Slits, netrins, ephrins) (244), and synaptogenic factors (agrin, s-laminin, and ARIA) (245). For example, several studies have associated the

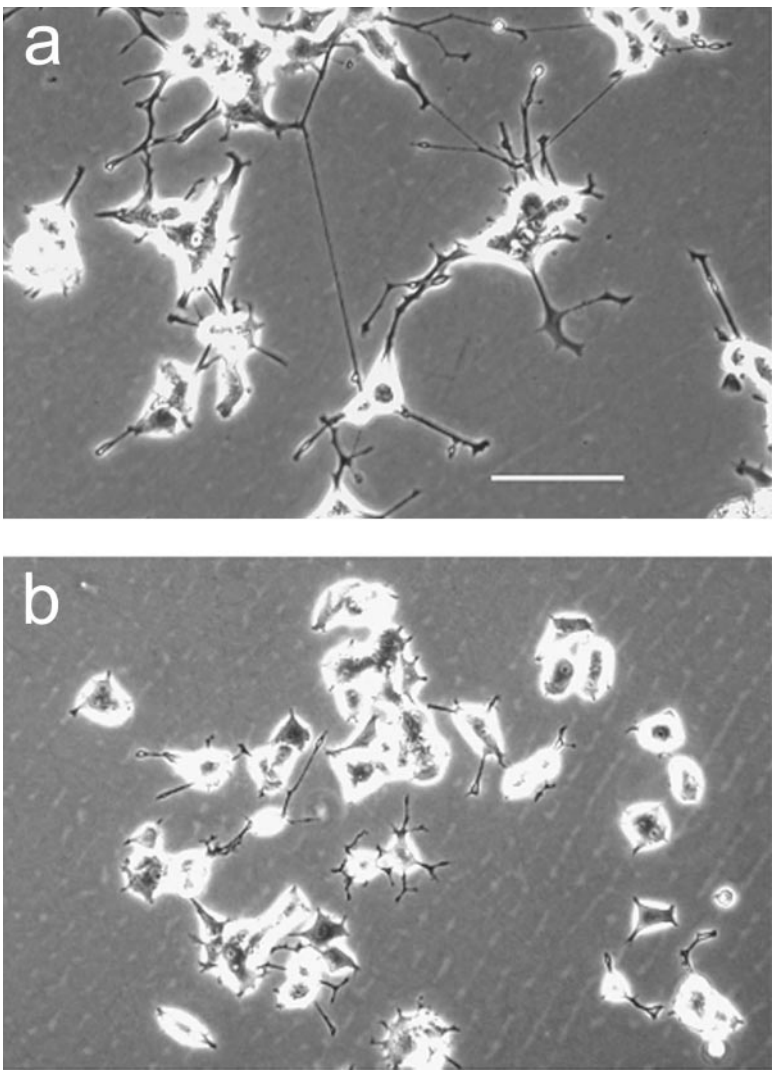


Figure 10 Enhancing the intrinsic neuronal mechanisms to improve regeneration: optimizing cytoskeletal dynamics for axonal outgrowth. The overexpression of gelsolin, an actin accessory protein, was associated with improved neurite outgrowth in PC12 cells. After three days, (*a*) PC12 cells transfected to overexpress gelsolin possessed longer neurites than (*b*) mock clones (cells that received the transfection vector without the gene for gelsolin) (237). Scale bar, 100 μm. Figure courtesy of E.J. Furnish & C.E. Schmidt, Departments of Chemical Engineering and Biomedical Engineering, The University of Texas at Austin.

overexpression of the adhesion molecule L1 with increased axonal regeneration following injury in the CNS (246, 247).

Blocking Inhibitory Biomolecules in the CNS

As described above, some nerve regeneration studies consider the differential injury responses found in the PNS and the spinal cord. Furthermore, as studies with neurotrophins, RAGs, and antiapoptosis factors have shown, neurons in the spinal cord have not necessarily lost their intrinsic ability to grow. However, one of the greatest challenges facing spinal cord regeneration still remains: glial scarring [see Nerve Injury and Regeneration above; for reviews, see (166, 248, 249–251)]. This response is characterized by the formation of a nonpermissive environment that inhibits axon growth and myelination. The main cell types involved are macrophages, microglia, oligodendrocytes, and astrocytes. The noncellular components of the glial scar include myelin-associated molecules (Nogo, myelin associated glycoprotein, oligodendrocyte-myelin glycoprotein), chondroitin sulfate proteoglycans (phosphacan, neurocan, brevican), axon guidance molecules (semaphorin, ephrin, netrin), and tenascin. Therefore, many bioengineering therapies for treating spinal cord injuries have focused upon attenuating the inhibitory glial scar components; these methods attempt to reduce the synthesis of the inhibitory components, to block their effects, or to remove them altogether with enzymatic degradation treatments.

The mechanisms that control the upregulation of inhibitory molecules following spinal cord injury are not well understood. However, factors, such as transforming growth factor-β2 (TGF-β2) (252) and ciliary neurotrophic factor (CNTF) (208, 209), have been associated with the stimulation of this process. Therapeutic treatments to block the effects of these growth factors are likely to aid in preventing the synthesis of glial scar components. For example, the administration of anti-TGF-β2 antibody was associated with decreased scarring following injury in the CNS (252).

Blocking the effects of glial scar critically relies upon identifying its inhibitory components. Studies by Caroni & Schwab (253) were the first to confirm that oligodendrocyte myelin inhibits nerve regeneration. In this work, IN-1, a monoclonal antibody raised against the myelin protein NI-35, was shown to block the myelin-associated inhibition of axonal growth. Later work indicated that the IN-1 antibody might indeed improve regeneration following spinal cord injury (254–256). NI-35, also called Nogo, was found to contain two inhibitory domains, Nogo-A (7, 257) and Nogo-66 (258). Shortly after the identification of NgR, the receptor for Nogo-66 (258), molecules were discovered that could block its interaction with Nogo. These include a soluble truncated form of NgR (259) and a competitive antagonist peptide that binds with high affinity to NgR (260). Both systems were subsequently shown to decrease the Nogo inhibition of neurite outgrowth in vitro (259, 260). Also, administration of the antagonist peptide was shown to improve functional recovery following spinal cord injury (260). Interestingly, NgR is a receptor for two

other growth inhibitors found in myelin, myelin-associated glycoprotein (MAG) (261), and oligodendrocyte-associated glycoprotein (OMgp) (262). This finding has prompted some to suggest that methods targeting NgR could lead to future clinical therapies (261, 263).

In addition to Nogo, MAG, and OMgp, there are likely a number of other inhibitory molecules in oligodendrocyte myelin left to be discovered. Therefore, therapeutic vaccination shows promise toward blocking multiple inhibitory components in one treatment. Vaccination stimulates the body's own immune system to produce polyclonal antibodies against an antigen. In such studies, myelin immunization in mice was associated with the long-distance regeneration of large numbers of axons and improved motor function following spinal cord injury (264). Many refinements will be required before this method can be translated into a therapy for humans; however, the polyclonal antibodies will likely prove useful for identifying any yet unknown inhibitory components of myelin.

Protease treatments degrade the inhibitory components in glial scar, facilitating their removal and increasing the ability of axons to grow through the injured area. Several extracellular matrix components, including chondroitin sulfate proteoglycans (CSPGs), are upregulated by oligodendrocytes and astrocytes following spinal cord injury, and are inhibitory towards axonal outgrowth (248). Treatment of spinal cord injuries with chondroitinase ABC, an enzyme that degrades the side chains of CSPGs, has been associated with improved regeneration (265) and improved functional recovery (266). CSPGs have also been found to be inhibitors of axonal outgrowth in the PNS (267), and chondroitinase ABC treatment was likewise linked to improved regeneration following sciatic nerve transection (268).

CELLULAR THERAPIES

As detailed in earlier sections, nerve regeneration can be greatly aided by the supplementation of supportive ECM components, neurotrophic factors, and cell adhesion molecules. Cells are effective and appropriate vehicles for supplying these factors. Glial cells (i.e., Schwann cells, astrocytes, and oligodendrocytes) and macrophages support regeneration by clearing debris and secreting neurotrophic factors to aid axonal outgrowth. In addition to these cells, olfactory ensheathing cells (OECs) and stem cells are being extensively investigated as transplants to support nerve regeneration. Transfection of these cells has further expanded their potential to aid repair in the nervous system.

Glial Cells and Macrophages

As stated earlier, peripheral nerve grafts have the ability to promote regeneration in PNS and spinal cord injuries (165). Schwann cells are primarily responsible for the supportive environment within this tissue, as they produce ECM, cell adhesion molecules, integrins, and neurotrophins (158, 243, 269). Schwann cells also play a critical role in leading peripheral axons to the distal nerve stump (Figure 11) and in

synapse formation (270). Furthermore, highly pure cultures of Schwann cells can be reliably cultured from nerve autografts (165), allowing for the transplantation of autologous support cells. For all of these reasons, the ability of Schwann cells to promote nerve regeneration has been a research area of intense focus [for reviews, see (165, 269)].

Recent studies of Schwann cells in central and peripheral nerve injuries have primarily centered on techniques to deliver these cells to the injury site. For example, Hadlock et al. demonstrated that rolled Schwann cell monolayer grafts implanted in a 7 mm gap in rat sciatic nerve increased functional regeneration after 10.5 weeks compared to acellular controls (70). Xu et al. have shown that Schwann cells, when implanted with Matrigel, can aid axonal regeneration across spinal cord transections in adult rats at levels above that of Matrigel alone (271). Kierstead et al. extended these studies to show that Schwann cell transplantation in combination with demyelination enhanced axonal growth in rat spinal cord injuries (272). In this study, demyelination allowed the Schwann cells to migrate beyond the site of implantation, facilitating longer regeneration distances. Moreover, research by Guest et al. (273) supports the extension of these studies to human therapies: grafts of human Schwann cells in the injured nude rat spinal cord were found to create a highly integrated cord-graft interface and allowed a small population of neurons to regenerate across the graft and reenter the spinal cord (273).

There are several challenges facing Schwann cell therapies for spinal cord repair. Notably, while regenerating axons grow into Schwann cell grafts, the axons fail to leave the hospitable environment, possibly due to unfavorable interactions with components in the glial scar (269). Schwann cells have likewise not been found to remyelinate axons beyond the injury site (274). Furthermore, Schwann cells may exacerbate chondroitin sulfate proteoglycan production (275) (a nonpermissive component of glial scar; see Biomolecular Therapies above) and may not aid nerve regeneration in astrocyte-rich environments (276). Thus, while Schwann cells have been associated with some success in promoting spinal cord repair, evidence indicates that Schwann cells hold their greatest promise when combined with other factors (such as Matrigel) or demyelination treatments. For comprehensive reviews of combination Schwann cell therapies, see Bunge (269) and Jones et al. (165).

Work with CNS glia (i.e., astrocytes, oligodendrocytes, and microglia) presents similarly conflicting data. For example, while one study has demonstrated associations between microglia and axonal regeneration (277), the effect of microglial activation and the role of these cells in neuronal function is not well understood (161). Furthermore, CNS glial cells are capable of contributing to the formation of glial scar tissue, which is inhibitory toward axonal growth (see the sections Nerve Injury and Regeneration in the Introduction and Biomolecular Therapies above for more information). Therefore, a greater understanding of CNS glia must be obtained before dependable therapies can be implemented for spinal cord repair. For a review of transplantation studies with CNS glia, see Houweling et al. (161).

Like Schwann cells, macrophages play a vital role in promoting peripheral nerve repair by clearing myelin debris. With this in mind, researchers have transplanted macrophages into peripheral nerve and spinal cord injuries (162). Such

transplants have been associated with a significant decrease in myelin-associated glycoproteins, as well as increased angiogenesis, Schwann cell infiltration, and axonal regeneration (278). However, other than clearing myelin debris, the degree to which macrophages aid regeneration by other means is unclear: Some suggest that macrophages produce factors that aid peripheral nerve regeneration (279), whereas others have found no evidence in rat spinal cord compression injuries that transplanted macrophages directly synthesize neurotrophins (278). Researchers have also found that macrophages can inhibit nerve repair following spinal cord injury (280). Popovich et al. hypothesized that macrophages contribute to a detrimental inflammatory response following spinal cord injury. In this work, enhanced regeneration was associated with systemic depletion of macrophages (280). By providing conflicting evidence for the role of macrophages in nerve regeneration, these results underscore the complexity of macrophage activation; thus, further work is required to develop a more thorough understanding of the role of macrophages in spinal cord injuries (280).

Olfactory Ensheathing Cells

One of the most promising new types of cellular transplants is OECs. The use of these cells in spinal cord regeneration has been extensively reviewed (276, 281, 282). OECs share phenotypic similarities to Schwann cells and astrocytes (281), but are only found in the olfactory system and are a distinct lineage from these cell types as well as oligodendrocytes (283). Normally, OECs aid axon outgrowth from the nasal epithelium, through the olfactory bulb PNS-CNS transition zone, to the central nerves in the olfactory glomeruli (281, 282). To do this, OECs migrate along with growing axons (284, 285) and support axonal outgrowth and survival by producing neurotrophins and cell adhesion molecules (282). Moreover, OECs provide a permissive substrate for axon growth (275), effectively supporting axonal outgrowth through glial scars (284–286). For all of these reasons, this unique glial cell has been found to benefit regeneration in both the PNS (287) and CNS (284, 285, 288). [For a broad list of OEC transplant studies, see Wewetzer et al. (289).]

Most work in this area has been carried out with centrally derived rat OECs, which are obtained from the olfactory bulb within the animal's skull (282). Transplants of centrally derived OECs support the regeneration of axons following spinal cord injury (286, 288) and dorsal root transection (290). Furthermore, Ramon-Cueto et al. found that centrally derived OECs enhance the regenerative effect of Schwann cells after complete spinal cord transection by enabling extensive regeneration through the glial scar and allowing long-distance axonal growth (284). In long-term studies, OECs injected into transected rat spinal cord were associated with extended axonal regeneration and regained sensory and motor function seven months after transplantation (291).

Promising studies have also shown that human OECs behave in a similar manner to that of rat OECs (292, 293). However, because of the invasiveness of the harvest procedure, which consequently compromises the host's sense of smell, it is

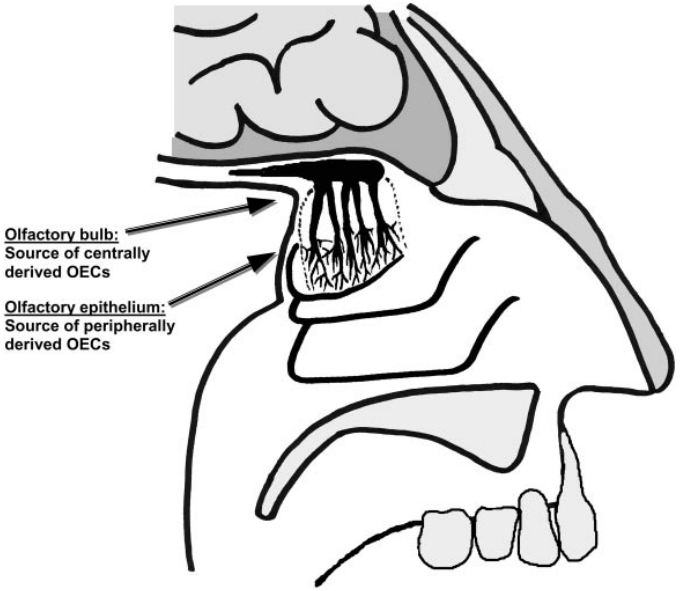


Figure 12 Centrally and peripherally derived olfactory ensheathing cells. Centrally derived olfactory ensheathing cells (OECs) are harvested from the relatively large olfactory bulb in rats through a procedure that would be not acceptable in humans (due to the invasive nature of the surgery that would leave the host with a compromised ability to smell). To address this problem, work with peripherally derived OECs, derived from human olfactory epithelium and harvested during a simple biopsy procedure through the nose, has indicated that these cells are a promising alternative to centrally derived OECs. Figure adapted from Lu & Ashwell (282).

not acceptable to remove the human olfactory bulb as a source of autografted OECs (282). Therefore, researchers have investigated an alternative source of OECs: peripherally derived OECs for autologous transplantation (Figure 12). Peripherally derived OECs can be obtained from biopsy of the olfactory epithelium in the nose (282), purified, and expanded in culture (294). Recent studies by Lu et al. have associated peripherally derived OEC transplants with partial functional recovery following spinal cord transection (282, 295). Clearly, this work must be corroborated and more thoroughly investigated, but these studies favorably indicate the future role of peripherally derived OEC transplants in nerve repair therapies.

OECs demonstrate a few distinct advantages over Schwann cell transplants in spinal cord injuries. As discussed above, Schwann cells have unfavorable interactions with astrocytes and have been associated with increased proteoglycan synthesis. On the other hand, OECs can favorably coexist with astrocytes (275, 276) and may aid in preventing an astrocytic response to injury (including proteoglycan synthesis) (276, 296, 297).

On the other hand, recent studies have challenged the claim that OECs offer advantages over Schwann cells. Takami et al. transplanted Schwann cells, OECs, or both cell types into contused rat spinal cord (298). At 12 weeks, the cords with Schwann cells had more myelinated axons and were associated with increased functional recovery compared to those with OECs (alone or in combination with Schwann cells). Moreover, Plant et al. suggest that OEC myelination studies could have been misinterpreted because of Schwann cell contaminants (299). In their work, purified OEC cultures do not form myelin and otherwise do not display Schwann cell-like associations with axons. Studies with clearly defined cell transplants are required to understand the abilities of Schwann cells and OECs (289). In the case of peripheral nerve repair, where OECs have shown promise in only one study (287), a detailed description of the differences between OECs and Schwann cells will be particularly helpful, as the transplantation of OECs only makes sense in cases where they pose distinct advantages over Schwann cells (289).

Stem Cells

Recently, researchers have begun to investigate the potential of stem cells in nerve regeneration applications (300). Neural stem cells have been isolated from rodent brain (301, 302), spinal cord (302, 303), skeletal muscle (304), and bone marrow (305). [However, recent studies have called into question the observations of bone marrow cells dedifferentiating into neurons (306, 307).] Interestingly, 2–5 weeks after transplant, stem cells implanted in injured rat spinal cord have survived; differentiated into neurons, astrocytes, and oligodendrocytes; and migrated up to 8 mm from the lesion; moreover, rats that had the transplanted stem cells showed improved functional recovery (141). Similarly, other studies have also found that stem cells implanted into injured spinal cord differentiate into neurons and glial cells (308, 309). It has consequently been suggested that the environment is a greater factor in neural stem cell fate than the intrinsic properties of the cell (302). Greater control over stem cell differentiation, by in vitro treatments (308, 309) or by using stem cells that are restricted to the neuronal lineage (310), may allow stem cell transplantation to yield more predictable results.

Glial progenitor cells have also been isolated from throughout the spinal cord (311) and have been shown to act as a source of astrocytes and oligodendrocytes in rat spinal cord following demyelination (312) or injury (313). Though glial progenitors have been used successfully in remyelination applications [for review, see Bartolomei & Greer (281)], they have not been extensively explored as a therapy following spinal cord injury (314, 315).

Genetically Modified Cells

The application of neurotrophins can result in significant increases in nerve regeneration (see Biomolecular Therapies above). However, it is difficult to deliver active growth factors controllably over the entire duration of regeneration (164). Transplanted genetically modified cells, on the other hand, pose advantages as a

means to deliver a continual supply of active neurotrophins (164, 218). Furthermore, if the gene expression in the modified cells can be turned on and off, it is probable that expression could be directed in a complex manner. For example, a cascade of neurotrophin expression could lead axons to grow into a graft, switch patterns of expression, and then lead axons out of the graft (164).

Genetically modified fibroblasts have been a highly studied model for the delivery of neurotrophins. For example, fibroblasts have been engineered to produce NGF (170, 179, 194), BDNF (179, 194, 317, 318), NT-3 (179, 194), CNTF (194, 316), GDNF (212), and bFGF (179, 194). Implants of transfected fibroblasts have provided information regarding the application of active neurotrophins locally to the nerve injury site. More advanced studies have included a molecular "on switch" to control the genetically modified expression of NGF in fibroblasts (Figure 13); such methods have included Muristerone A–inducible expression (321, 322) and tetracycline-responsive promoters (323, 324).

Schwann cells, OECs, and neural stem cells have also been transfected to overexpress neurotrophins, but have received less attention than fibroblasts. Schwann cells have been modified to release BDNF alone (192) or in combination with NT-3 (325), as well as modified to secrete NGF (326). Researchers have begun to transfect OECs to express colored markers for tracing experiments (328) or use transgenic animals expressing a xenogeneic protein as a source for modified OECs (328). Such studies indicate promise for future work using modified OECs to express neurotrophins. Finally, the neural stem cell clone C17.2 has been transfected to release NT-3 in spinal cord injury sites (329), further demonstrating the versatility of delivering neurotrophins through a variety of cell types. For a summary of genetically modified cells for transplantation, see Table 3.

ADVANCED THERAPIES

Advanced Guidance Channel Fabrication Techniques

To more accurately mimic natural repair in the body, recent studies have focused on the use of various advanced approached to create complex guidance channels and to combine multiple stimuli into a single therapy. With regard to advanced guidance channel fabrication, most techniques have focused on creating intricate internal structures that more accurately mimic the nerve architecture, such as the inclusion of fibers and channels to guide individual nerve fibers. These guidance channels are fabricated in a number of ways, including magnetic polymer fiber alignment, injection molding, phase separation, solid free-form fabrication, and ink-jet polymer printing. Each technique is desctibed in more detail below.

MAGNETIC ALIGNMENT, INJECTION MOLDING, AND PHASE SEPARATION Nerve guidance channels have been predominantly fabricated as hollow tubes or as porous foam rods because of the ease in manufacturing these devices. For hollow tubes, it is known that the body normally generates an oriented fibrin matrix after placement

TABLE 3 Genetically modified cells for transplantation

Implant site in rat	Cell type modified	Gene(s)	Results	Reference
Spinal cord lesion	Fibroblasts	BDNF, NT-3, NGF, bFGF	Dorsal root neurites penetrated grafts producing NT3, NGF or bFGF but not those producing BDNF	(179)
Spinal cord lesion	Fibroblasts	NGF	Aided axonal growth	(170)
Spinal cord lesion	Fibroblasts	NT3	Aided corticospinal axonal growth	(320)
Spinal cord hemisection	Fibroblasts	NGF	Aided axonal growth into (but not through) cell graft	(316)
Spinal cord contusion	Fibroblasts	BDNF, NT-3, NGF, bFGF, CNTF	Greatest axonal growth and myelination with BDNF or NT3	(194)
Spinal cord hemisection	Fibroblasts	BDNF	Aided axonal growth and recovery of motor function	(317)
Spinal cord lesion	Fibroblasts	NGF (tetracycline promoter)	Aided axonal survival and growth	(321)
Hypoglosseal transection	Fibroblasts	GDNF	Aided motor axonal growth	(212)
Optic tract cavity	Fibroblasts	BDNF and/or CNTF	Greatest axonal growth with combination of BDNF/CNTF	(316)
Spinal cord hemisection	Fibroblasts	BDNF	Aided axonal survival and growth	(319)
Spinal cord (uninjured)	Schwann cells	NGF	Aided axonal growth and myelination	(326)
Spinal cord transection	Schwann cells	BDNF	Aided axonal growth	(192)
Spinal cord lesion	Schwann cells	NGF	Aided axonal growth and myelination	(327)
Spinal cord lesion	OECs	β-galactosidase or GFP	Expression up to 30 days following implantation	(328)
Spinal cord (uninjured)	Neural stem cells	NT-3	Stem cell differentiation to neuronal and glial phenotypes	(329)

Abbreviations: Brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), nerve growth factor (NGF), basic fibroblast growth factor (bFGF), ciliary neurotrophic factor (CNTF), glial derived neurotrophic factor (GDNF), olfactory ensheathing cells (OECs), green fluorescent protein (GFP).

of a hollow conduit across a nerve defect, which serves as a critical precursor to axonal regeneration. With regard to foam rods, it is questionable whether an amorphous irregular structure optimally guides axonal regeneration. Thus, to mimic natural repair in the body and to shorten the time required for regeneration, more recent studies have focused on the modification of nerve guidance channels with internal matrices of longitudinally aligned fibers or channels. These devices often require more advanced processing techniques.

In one approach, guidance channels containing smaller chambers, or sublumina, to mimic the natural fascicular organization of the nerve have shown the capacity to support nerve regeneration (330). Tubes of poly(lactic acid) and poly(lactic-co-glycolic acid) were formed using a dip-molding technique. They were created to contain 1, 2, 4, or 5 sublumina, or "fascicular analogs." With this approach, it may be possible to incorporate particular neurotrophic factors into separate subluminal compartments in an effort to guide the elongation of specific subsets of nerve fibers (e.g., motor versus sensory fibers).

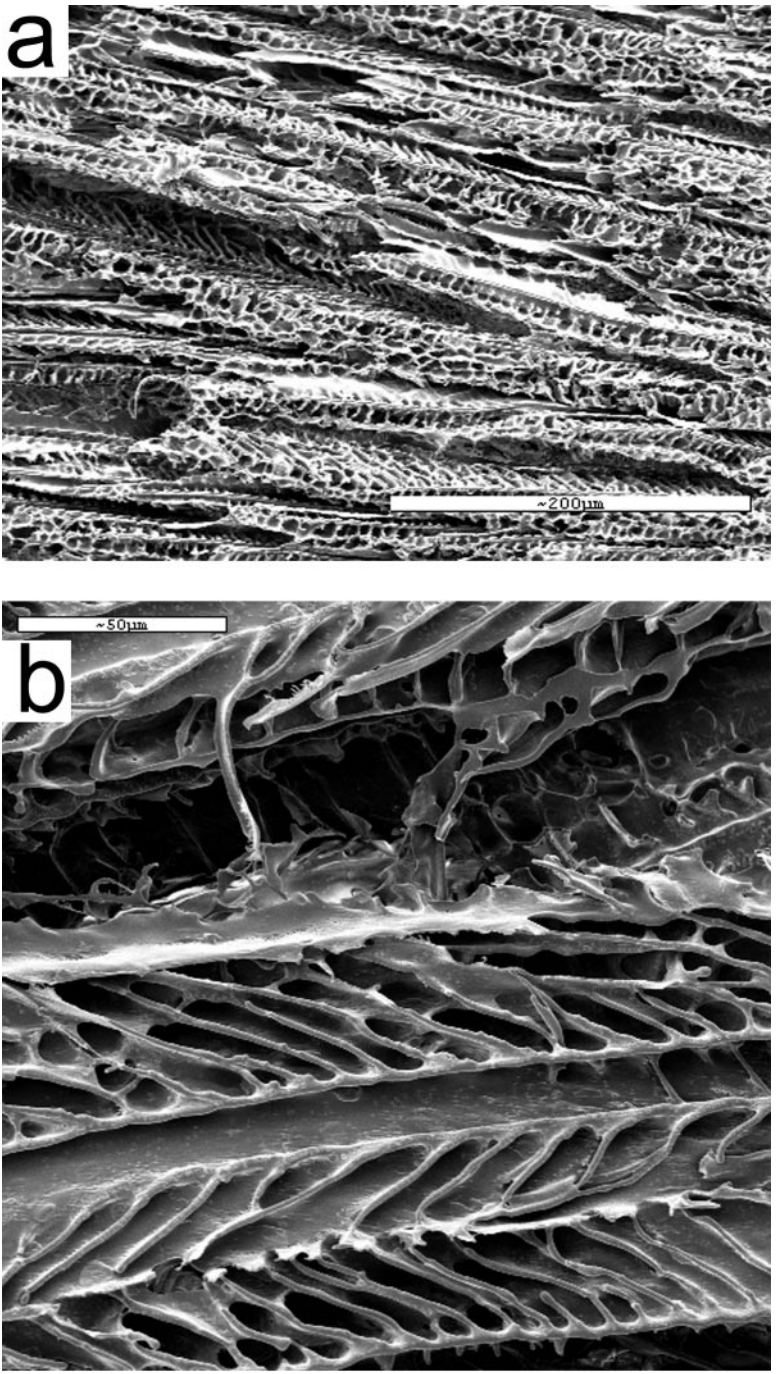
Several experiments have also investigated the use of extruded or spun fibers of collagen or other biodegradable materials that are then placed within the lumen of conduits. For example, Tong et al. (83) have shown good regeneration using collagen tubes that were filled with laminin and fibronectin double-coated collagen fiber bundles. Similarly, eight longitudinally oriented polyamide filaments (i.e., sutures) placed inside silicone tubes supported regeneration over 15 mm gaps in rats (15). Matsumoto et al. (331) showed that poly(glycolic acid)-collagen conduits (4 mm inner diameter, 50 μm wall thickness, and 90 mm in length) filled with collagen fibers (80 fibers/conduit; 50 μm in diameter, and 90 mm in length) permitted regeneration over 80 mm defect lengths in dogs. Previous reports illustrate regeneration up to a maximum length of 50 mm, suggesting that the internal collagen fibers indeed enhanced axonal elongation. Later, a study from this same laboratory showed that a conduit with an internal collagen sponge supported similar levels of regeneration compared to the collagen fiber-filled conduits (332). The authors claim that the collagen sponge is a preferred substrate because of its ease of preparation compared to the fibers. Indeed, these fiber approaches, as well as the sublumina approach mentioned earlier, are tedious and the fiber or chamber alignment may not be well controlled because of the handmade fabrication techniques. Thus, there is a push to devise other methods that can be used to create aligned internal structure and that are also not too tedious or difficult to prevent eventual scale-up.

As a means to provide the necessary matrix alignment and also improve upon processing conditions, magnetic fields have been used to orient protein polymers. Magnetically aligned fibrin and collagen matrices (Figure 14) have been shown to promote enhanced axonal outgrowth in vitro and in vivo compared to random fibrin and collagen matrices, respectively (91, 92, 104). In a different approach, a novel foam-processing technique, utilizing low-pressure injection molding, created highly porous conduits from poly(lactic-co-glycolic acid) with continuous longitudinal channels (333). Conduits were constructed containing 1, 5, 16, 45, or more longitudinally aligned channels. In similar studies, poly(lactic-co-glycolic acid) was subjected to injection molding followed by a thermally

induced phase transition process to produce conduits with longitudinally aligned internal channels (334). As part of a separate study from this same laboratory, a "two-phase" conduit was fabricated for spinal cord repair (159). The scaffold's inner portion emulated the gray matter and was composed of a porous polymer seeded with neural stem cells, which provided trophic support. The outer portion mimicked the white matter with long, axially oriented pores for axonal guidance and radial porosity to allow for fluid transport while inhibiting the ingrowth of scar tissue (Figure 15). The inner-oriented pores were created from poly(lactic co-glycolic acid) using a solid-liquid phase separation technique.

SOLID FREEFORM FABRICATION AND INK-JET LIQUID POLYMER PRINTING Recently, the emergence of advanced techniques for polymer processing, such as solid freeform fabrication (SFF) and ink-jet printing for liquid polymer solutions, has created even more opportunities for the design of intricate devices for peripheral nerve and spinal cord repair. There are several types of freeform fabrication devices, and currently these devices can produce features as small as 6 μm (157). Three-dimensional printing (3DP) is one type of solid freeform fabrication technique that employs powder processing in the construction of devices in a layer-wise manner (335–337). 3DP, like other SFF methods, is capable of fabricating a structure directly from a computer model and can handle complex features, such as internal walls, porosity gradients, tortuous channels, and multiple material regions. The process begins by spreading a thin layer of polymer powder onto a piston plate. A liquid binder solution is passed through a nozzle affixed to the fast-axis carriage, and the nozzle is rastered back and forth over the powder bed to selectively print droplets, which bind the powder particles together, generating a two-dimensional pattern. The piston is lowered, another thin layer of powder is spread, and the process is repeated. Polymers can be printed directly or one can fabricate a mold using SFF. The latter approach provides enhanced control over the type of material used to fabricate a desired device. Scaffolds created using SFF can contain biomimetic internal architectures that prove valuable for tissue engineering. For example, conduits with internal channels corresponding to key spinal cord tracts could be created using these approaches (157). Some limitations do exist, including the high cost of the instrument and the inability to incorporate biological components under some processing conditions.

MicroFab Inc., in Plano, Texas, has devised an ink-jet print station designed to precisely deliver solutions of polymers, such as poly(lactic acid), for tissue engineering applications. Three-dimensional structures can be created with desired thickness, dimensions, and incorporated biomolecules. In one study, they have generated bifurcated degradable polymer tubes that have ridges or support ribs (Figure 16*a*). The ridges provide strength to resist compressive forces, whereas the remaining polymer tube is thinner and allows for nutrient exchange and an optimum degradation rate. In a second study, MicroFab has created a nonbifurcated ridged tube that is impregnated with a gradient of fluorescent dye (Figure 16; note: the gradient is increasing from left to right). Similar gradients can be created with biomolecules and neurotrophic factors. These examples illustrate


a
~200µm
~50µm
b

the powerful opportunities provided with advanced instrumentation, such as SFF and ink-jet polymer printing.

Combination Approaches and Novel Stimuli

Many approaches to enhance nerve repair in the past have focused on mechanical, chemical, biological, or electrical stimuli. More research today is focused on using multiple stimuli in an effort to better mimic the complex milieu of signals normally found in the body. The challenge is assessing the optimal levels of different signals in such a complicated environment. For example, Miller et al. (338) have created micropatterned substrates for in vitro neurite elongation studies. Compression molded and solvent cast biodegradable polymer substrates made of poly(lactic acid) were micropatterned to form grooves on the substrate surfaces. Laminin was localized in the grooves and rat Schwann cells were seeded on the substrates. The micropatterns provide physical guidance, laminin provides chemical cues, and the Schwann cells provide biological cues to the axons. The synergistic combination of physical, chemical, and cellular guidance enabled greater than 98% alignment of neurites and accelerated outgrowth of nerve fibers in the direction of the microgrooves. In fact, the synergistic effect of physical and chemical guidance cues has been found in other studies to be more effective than individual cues in promoting directional outgrowth of neurites (339).

Beyond the typical signals found in the body, other stimuli are also being investigated for their ability to impact neuronal growth. For example, Ehrlicher et al. (340) have shown that weak optical forces can be used to guide the movement of an axon. To do this, a laser spot is placed in front of the actively growing neuron, enhancing growth into the beam focus and resulting in guided turns as well as enhanced growth. The power of the laser is chosen so that gradient forces are sufficiently powerful to bias actin polymerization and neuronal extension, but too weak to actually hold and move any part of the cell. This demonstrates the ability of light to control biological processes, which could prove useful in nerve regeneration (e.g., in laser-assisted surgical repair of damaged nerves).

Convergence of Neural Regeneration and Neural Prostheses

Neural prostheses are assistive devices that restore functions lost as a result of neural damage. These devices, which electrically stimulate nerves and are either

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Figure 15 Oriented poly(lactide-co-glycolide) scaffolds. Oriented scaffolds were created using solid-liquid phase separation (159). Poly(lactide-co-glycolide) was dissolved in dioxane. The dioxane was induced to crystallize in an oriented manner through the use of a thermal gradient and then was removed by sublimation. The scaffolds were sputter coated for observation under scanning electron microscopy. (*a*) Scale bar, 200 μm. (*b*) Scale bar, 50 μm. Images courtesy of R. Langer & E. Lavik, Departments of Chemical Engineering and Biomedical Engineering, Massachusetts Institute of Technology.

TABLE 4 Regeneration obstacles and strategies for repair

Peripheral nervous system	Central nervous system
Regeneration obstacles	
■ Cell body response	■ Cell body response
Some retrograde cell death	Retrograde cell death
Ample expression of regeneration associated genes	Low expression of regeneration-associated genes
■ Degeneration of the distal stump	■ Glial scar formation
■ Swelling of the proximal stump	■ Inhibitory molecules
■ Possible gap between nerve stumps	Myelin-associated glycoprotein
	Nogo
	Chondroitin sulfate proteoglycans
	Semaphorins, ephrins, netrins
	Tenascin
Strategies for repair	
■ Guidance therapies	■ Guidance therapies
Autologous tissue grafts	Peripheral nerve and embryonic spinal cord grafts
Acellular tissue grafts	Support matrices
Nerve conduits	■ Biomolecular therapies
■ Biomolecular therapies	Neurotrophic factors
Neurotrophic factors	Regeneration-associated genes
Regeneration-associated genes	Antiapoptosis genes
Antiapoptosis genes	Blocking inhibitory biomolecules
■ Cellular therapies	■ Cellular therapies
Schwann cells	Schwann cells
Macrophages	Macrophages
Olfactory ensheathing cells	Olfactory ensheathing cells
Stem cells	Stem cells
Genetically modified cells	Genetically modified cells

external or implanted devices, replace neural function rather than promote regeneration. Examples of such devices include the cochlear and retinal implants. In addition, epidural spinal cord stimulators and deep brain stimulators are implanted to control pain, tremor, and rigidity. Neural prostheses have also been developed to restore limb movements using electrodes tunneled under the skin to muscles and nerves. Spinal cord microstimulation is under study as an alternative method to restore movement and bladder control. Many researchers believe that even if neural regeneration after spinal cord injury becomes a clinical reality, functional recovery will probably remain incomplete (341). Thus, there may always exist a need for neural prostheses.

Many scientists also believe that key opportunities exist in merging the recent advances in the area of neural prostheses and electrical stimulation with those successes made in the area of neural regeneration (342). The common goal of

both research fields is the restoration of function after neurological damage. Areas of research that have been identified to have the greatest impact on achieving this goal include: enhancement of axonal regeneration with electric fields, development of hybrid neural interfaces combining silicon and biologically derived elements, and investigation of the role of patterned neural activity in regulating neuronal processes and neurorehabilitation (342). Some of this research, especially that of combining microelectronics technology and biologically derived elements, is reviewed in (343).

CONCLUSION

The requirements for functional nerve regeneration are complex. However, through the combined efforts of scientists and engineers from a variety of disciplines, experimental work in this field has made great progress. This review of nerve regeneration divided recent advances into guidance therapies, biomolecular therapies, and cellular therapies (summarized in Table 4). An additional section about advanced therapies outlined sophisticated guidance channel fabrication techniques and combination therapies. Yet, a single approach on its own has not allowed an effective clinical therapy. For example, although synthetic nerve guidance channels effectively guide axonal growth, these conduits have not been a widely used replacement for autografts, particularly in long defects. In spinal cord repair, Schwann cell transplantation to the site of injury has yielded improved recoveries, but challenges remain to implementing this treatment clinically.

On the other hand, many studies have been incrementally successful. New potential targets for novel therapies have been discovered through an increased understanding of the molecular biology of neural development and regeneration. Furthermore, advances in the areas of drug delivery, gene therapy, and biomaterials are able to apply these therapies with increased efficacy and biocompatibility. As the separate experimental approaches are melded together, future research will allow more dramatic successes in peripheral nerve regeneration and spinal cord repair. Multiple treatments, perhaps coordinated at different times or locations within the patient, will be needed to promote improved functional regeneration.

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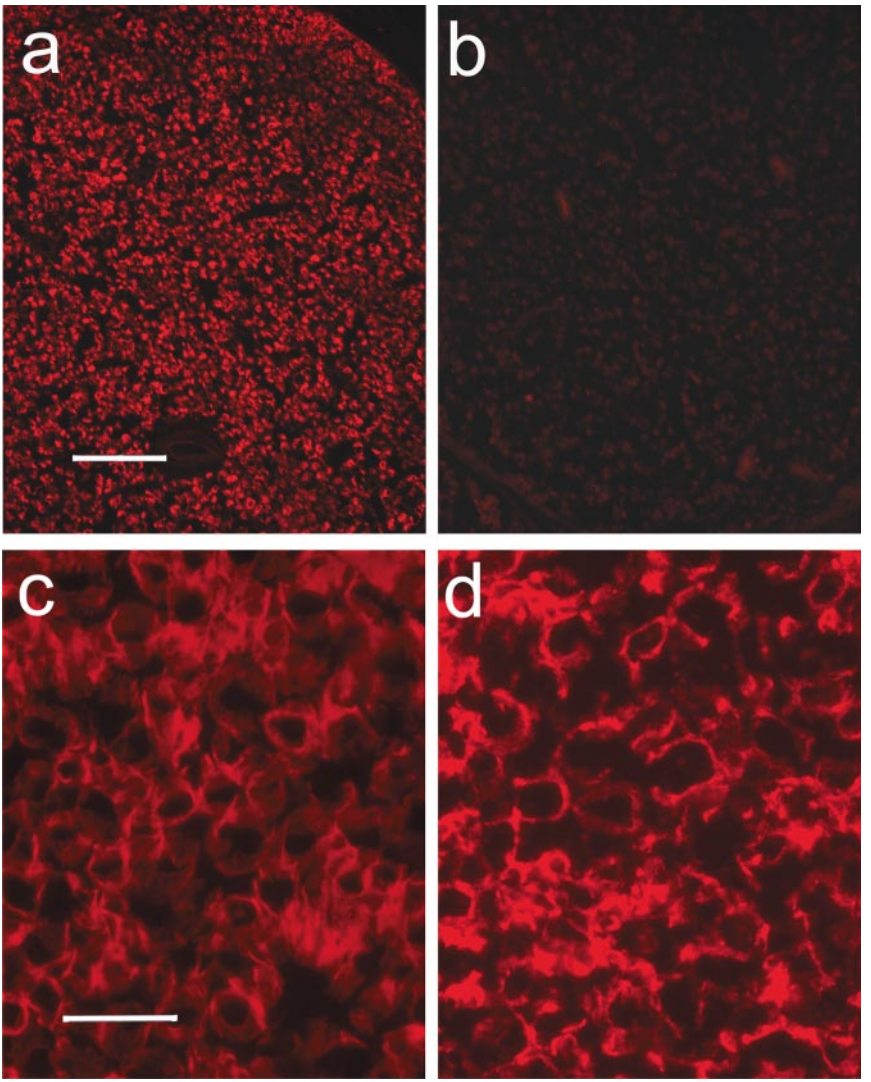

Figure 5 Acellular nerve grafts. Decellularization, or the removal of cellular components is an effective method of rendering nonautologous nerve tissue less immunogenic. One recent approach uses an optimized detergent treatment (including sulfobetaine-10, sulfobetaine-16, and Triton X-200) to remove cellular components while preserving the extracellular matrix structure. The result is an acellular nerve graft with a structure that mimics native nerve. Fresh (*a*) and treated (*b*) cross-sections of rat sciatic nerve, stained for lipids, demonstrate the removal of myelin and axon membranes (scale bar, 100 μm). Fresh (*c*) and treated (*d*) tissue sections, stained for laminin, show the preservation of the basal lamina tubes, the natural structural components that are thought to guide regenerating axons (scale bar, 25 μm). Figure courtesy of T.W. Hudson and C.E. Schmidt, Departments of Chemical Engineering and Biomedical Engineering, The University of Texas at Austin.

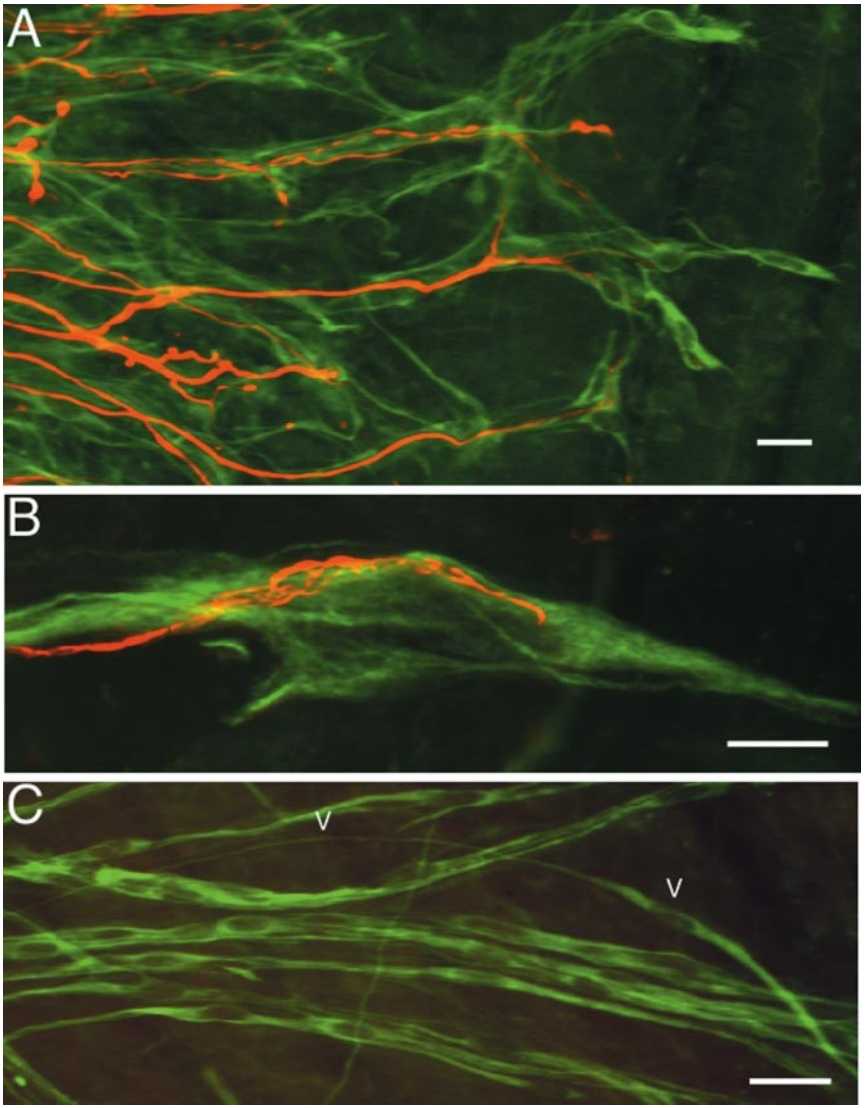

Figure 11 Schwann cells migrate from the severed nerve and assist axon outgrowth. The distal end of a nerve was transplanted onto the surface of a rat soleus muscle. (*a*) After 7 days, Schwann cells (green) and axons (red) have together grown out over the muscle surface (Scale bar, 20 μm). (*b*) Shown in higher magnification, axons regenerate along with Schwann cells (Scale bar, 10 μm). (*c*) In a nerve that was previously severed, no axons were present, as indicated by the lack of red staining. However, Schwann cells extend (V) processes from the end of the nerve, demonstrating that Schwann cell extension occurs independently from axonal regeneration (Scale bar, 40 μm). Figure reprinted from Reference 376, with permission from Elsevier Science.

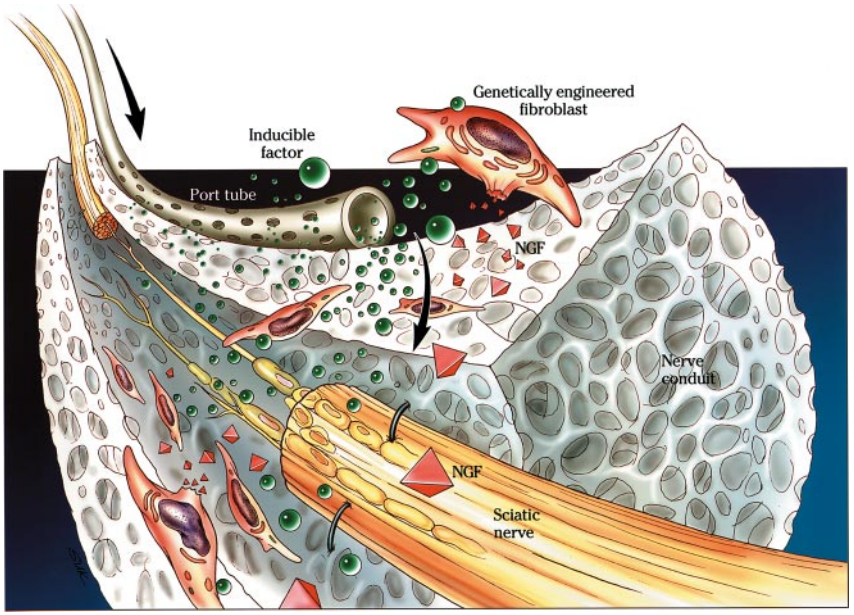

Figure 13 Transplant systems for inducible, genetically modified cells. Shown schematically, genetically modified fibroblasts can be implanted into a peripheral nerve defect to act as surrogate Schwann cells. Expression in this system can be "turned on" transiently by a locally delivered soluble induction factor. Muristerone A-inducible expression (349, 350) and tetracycline responsive promoters (351, 352) are examples of this type of system. Figure courtesy of C.W. Patrick, Jr., Department of Plastic Surgery, The University of Texas M.D. Anderson Cancer Center and G.R. Evans, Division of Plastic Surgery, University of California-Irvine.

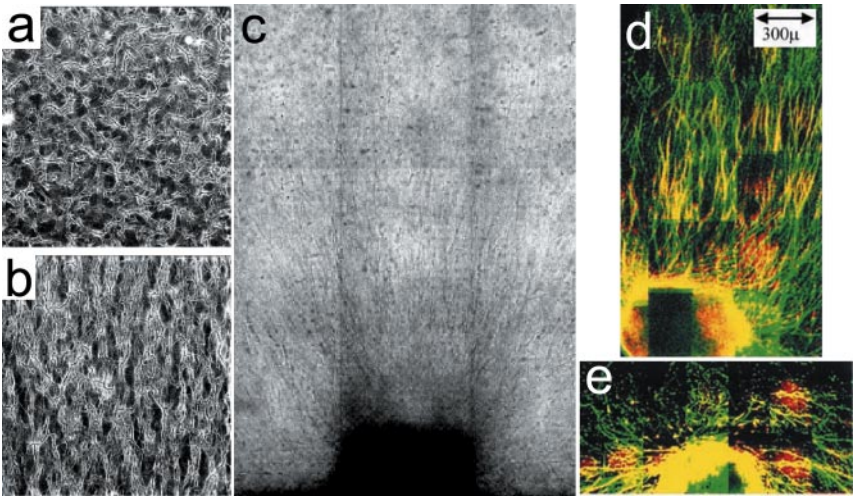

Figure 14 Magnetically aligned hydrogels. Magnetically aligned fibrin and collagen gels promote directed axonal outgrowth in vitro and in vivo compared to fibrin and collagen gels with isotropic (i.e., randomly oriented) fibers (92, 104). Fibrin hydrogels, made with fluorescently labeled fibrinogen, were imaged with confocal laser scanning microscopy. (*a*) Isotropic fibrin gels show no directional alignment, whereas (*b*) fibrin gels exposed to a 9.4 Tesla magnetic field were unidirectionally aligned. (*c*) As seen under bright field microscopy, neurite outgrowth from dorsal root ganglia is highly directional in magnetically aligned fibrin. Oriented neurite outgrowth from dorsal root ganglia also occurs in (*d*) aligned collagen gels, compared to (*e*) isotropic collagen gels; in (*d*) and (*e*), neurites are stained green, Schwann cells are stained red and colocalized neurites and Schwann cells appear yellow. Reprinted from Reference 104 and 92, with permission from Elsevier Science.

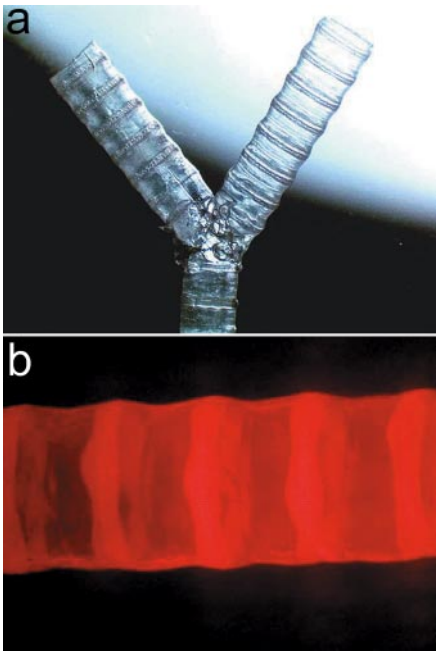

Figure 16 Ridged nerve guidance channels. The nerve guidance channels in (*a*) and (*b*) were fabricated using MicroFab Technologies Inc., Tissue Engineering ink-jet printing station. The image in (*a*) illustrates a bifurcated nerve guidance channel containing circumferential support ridges. Each segment is 1.8 mm in diameter and consists of a proprietary polycaprolactone and PLA polymer solution. As shown, each segment has external support ribs that allow the conduit to resist in vivo compression without adding excess bulk or mass transfer resistance. The guidance channel in (*b*) illustrates a single channel with support ribs. For this case, a red fluorescent dye molecule was incorporated into the channel in a gradient (the gradient is increasing from left to right). This illustrates the flexibility of this system to incorporate biomolecules into the guidance channels. Thus, using MicroFab's ink-jet printing capabilities, substrates can be custom designed to meet degradation rate, growth factor elution rate, and degree of compression resistance according to the needs of the application. Images courtesy of D.S. Silva and D. Hayes, MicroFab Technologies, Inc., Plano, Texas.