3 Advances in Treatment of Spinal Cord and Peripheral Nerve Injury

Ali Zomorodi and Roger D. Madison

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3.1 INTRODUCTION

An estimated 400,000 people in the U.S. have permanent paralysis as a consequence of spinal cord injury and an additional 10,000 are injured each year. Patients with spinal cord injuries (SCI) can live 25 to 30 years after their initial injuries. Each patient must cope with a lifetime of neurological dysfunction including paralysis, bowel and bladder dysfunction, sexual dysfunction, spasticity, deafferentation pain, loss of skin integrity, and autonomic dysfunction. Thus, SCI can be a devastating neurological disorder in terms of the years of disability caused and the associated physical and psychological complications. However, patients can remain highly functional with the use of modern aids, such as wheelchairs; they can participate fully in work, sports, and activities of daily living despite the obvious disability associated with the loss of function.
For thousands of years, physicians have been pessimistic in their approach to treating SCI because of the lack of innate recovery and secondary complications such as infections that usually ensue. Writing around 1700 BCE in the Edwin Smith Papyrus, an ancient Egyptian physician described SCI as a “disease not to be treated.” Now, almost 4000 years later, the treatment of SCI remains largely palliative: preventing injury progression; implementing bowel and bladder regimens; managing complications of sensory loss and skin breakdown; treating spasticity, dysautonomia, and deafferentation pain syndromes; and teaching patients how to cope with their disabilities. While such palliative care is highly successful and now results in nearly normal lifespans and functional capabilities, most affected patients still would like to enhance their mobility and regain more normal function. Fortunately, ongoing advances in neurobiology coupled with initiatives to facilitate the translation of this research into medical therapy promise to change this paradigm from palliation to cure.

Broadly speaking, current approaches to the treatment of SCI fall into one of four categories: (1) the prevention of secondary injury and delayed demyelination or axon loss (neuroprotection), (2) the repair or replacement of interrupted neural circuitry (spinal cord repair), (3) the use of aggressive rehabilitation techniques to optimize recovery through residual spinal cord plasticity (rehabilitation), and (4) the augmentation of function through prostheses (prosthetics). In this chapter, we will review advances in each discipline, the current hypotheses, and their future applications. Figure 3.1 outlines these potential treatment options.

**FIGURE 3.1** (See color insert following page 146.) Targets for the treatment of spinal cord and peripheral nerve injuries. A: Genetic or small molecule treatments to induce a proregeneration response in the cell body. B: Inhibition of myelin associated growth inhibitors and chondroitin sulfate proteoglycans at the site of injury. C: Neuroprotectants to prevent the spread of injury through secondary mechanisms. D: Transplantation of stem cells, olfactory ensheathing glia, or Schwann cell bridges to span the area of injury and replace lost cell populations; transplantation of macrophages or neurotrophin-secreting cells to prevent cell loss and promote regrowth. E: Use of synthetic grafts infused with Schwann cells, extracellular matrix, or neurotrophins to span the area of injury. F: Infusion of neurotrophins or use of electrical stimulation to improve the pace and accuracy of axonal regeneration.

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3.2 SPINAL CORD NEUROPROTECTION

3.2.1 MATURATION OF SPINAL CORD INJURY

SCI can occur when the spinal cord is lacerated or macerated by a sharp penetrating force, contused or compressed by a blunt force, or infarcted by a vascular insult. Blunt force injuries are the most common causes of SCI, accounting for up to 50% of cases.\(^1\) This form of SCI has been well modeled in animals, using weights dropped onto the exposed spinal cord. Initial histopathological studies suggested that secondary events that unfold after the mechanical injury enlarge the contusion and are responsible for a substantial portion of the ultimate functional deficit that results — the so-called secondary injury. From this came the hypothesis that identifying the components of secondary injury could provide rational targets for pharmaceutical interventions that could significantly limit the morbidity of SCI. This approach has dominated SCI research for much of this century and spawned many promising therapies. However, in reality, patients rarely lose additional function after they present with initial levels of injury, suggesting that, in practical terms, very little secondary injury occurs and most of the damage results from the initial impact.

In experimental models of blunt SCI, the initial mechanical force delivered to the cord results in a necrotic core that involves the spinal grey matter and spares a rim of white matter around the contusion site. Electrophysiological experiments have shown that neurons that survive the initial trauma become hyperexcitable and fire repeated salvos of action potentials. Intracellular and extracellular electrolyte concentrations are altered measurably as a consequence. Intracellular concentrations of calcium and sodium and extracellular concentrations of potassium increase significantly, making normal neuronal activity impossible. Clinically this is manifested as a flaccid motor paralysis below the level of injury and can last several months (“spinal shock”); it is eventually replaced by spasticity as the spinal cord slowly recovers innate tone. As a result of the flaccidity, a systemic hypotension (“neurogenic shock”) may ensue. Meanwhile, petechial hemorrhages and progressive edema develop around the injury site along with a collapse of the microcirculation with a measurable reduction in spinal cord perfusion. As cells lyse, excitatory neurotransmitters reach toxic levels in the extracellular fluid and free oxygen radicals are elaborated. The consequent lipid peroxidation hastens cell death and promotes formation of cytokines — major components of the inflammatory cascade. Neutrophils enter the contusion within 24 hours, followed closely by lymphocytes. These cells start cleaning the debris while elaborating more cytokines and chemokines that reach measurable levels within 48 hours and continue the inflammatory cascade. Whether this form of inflammation is restorative and necessary (to clean up debris) or destructive in some manner remains highly contentious.

Meanwhile, apoptosis occurs in cells surrounding the initial core of necrosis, causing the lesion to grow further. Neutrophils are eventually replaced by macrophages and fibroblasts. With time, areas of extensive necrosis are replaced by the classic glial scar. Areas of milder injury develop scars rich in astrocytes; areas of large hemorrhage are replaced by glial-lined areas of myelomalacia that can sometimes lead to late (years later) post-traumatic syrinx.
Secondary processes continue to play a role in the clinical features of SCI even chronically. Robust local sprouting of injured and uninjured axons within the spinal cord segments produces circuits implicated in spasticity. Changes in the distribution and excitability of ion channels along with changes in excitatory and inhibitory inputs cause permanent hyperexcitability in some cell populations, possibly leading to chronic pain syndromes and hyperactivity causing motor spasticity; chronic demyelination can block signaling in other pathways.

The response to SCI can be divided into acute and chronic phases. Acutely, cell loss occurs due to the mechanical injury associated with excitotoxicity, lipid peroxidation, and inflammation, as the lesion is cleaned up. Chronically, cells that survive the initial events may go on to regenerate in a limited and imprecise way or they may succumb to apoptosis or demyelination.3,4 These insights suggest several promising targets for pharmacologic intervention, assuming that the primary injury can be overcome.

3.2.2 SECONDARY NEUROPROTECTION TREATMENT SCHEMES

To date, the only clinical treatment to emerge from neuroprotection research is high dose methylprednisolone (MP) therapy. Since the 1960s, corticosteroids have been used in the treatment of SCI. Initially, these agents were used for their ability to limit inflammation and spinal cord edema. Optimal therapeutic schemes with steroids involved pretreatment prior to injury, which provided better benefits than treatment after injury (which obviously is beneficial for spinal cord surgery). However, the initial National Acute Spinal Cord Injury Study (NASCIS) a nonplacebo controlled comparison of high-dose versus low-dose MP failed to show any benefit in the treatment of SCI.5

In the early 1980s, it was shown that key components of secondary injury included post-traumatic alteration of spinal cord blood flow, elaboration of free radical oxygen, and peroxidation of membrane lipids.6,7 Trials in rodents, cats, and dogs demonstrated that MP can improve functional recovery from SCI by categorizing these processes, but it must be administered in intravenous doses of 30 mg/kg — much higher doses than those used in the NASCIS trial.8–10 Incorporating some of these findings, the second NASCIS trials found that sustained high doses of MP administered within 8 hours of injury caused a statistically significant improvement in neurologic function although new SCI scales were required to measure this improvement.11–14 Again, no placebo control was used and the initially determined outcome measures were abandoned and a new system for evaluating neurologic function in SCI was devised to show the benefits of treatment.

It is not apparent whether the statistically significant improvements translated into clinical benefit. Although the design and statistical analysis of the trials were widely challenged,15 the high dose “Solu-Medrol Protocol” is almost universally applied in the emergency room management of SCI.16 This is perhaps more reflective of physicians’ desperation to offer some treatment to SCI patients than of the scientific validity of the studies. However, this high dose, short-term protocol is now considered the standard of care.
These studies lent credence to the hypothesis that secondary injury mechanisms may be important in the clinical evolution of SCI and spurred the development of multiple agents, each of which has been shown in some animal models to be somewhat efficacious. Among these is the 21-aminosteroid, tirilazad mesylate (TM), that scavenges free radicals, inhibits lipid peroxidation, and maintains spinal cord blood flow in animal models. Because it lacks the glucocorticoid activity of MP, TM is a safer drug and considerable interest in its clinical efficacy was generated. \(^\text{17-19}\) Unfortunately, the NASCIS 3 trial concluded that TM does not appear beneficial in the treatment of SCI. \(^\text{12}\)

Based on the premise that acute inflammation is deleterious to nervous tissue, specific inhibitors of the inflammatory response have been evaluated for benefit in SCI. \(^\text{20}\) Among these, IL-10 has been shown to limit axonal loss, contusion size, and functional deficit following SCI in rats. \(^\text{21,22}\) So have the broad spectrum chemokine receptor antagonist, vMIP II, \(^\text{23}\) and the selective cyclooxygenase-2 inhibitors. \(^\text{24,25}\) These latter drugs are already approved for human use and are well tolerated; it would be relatively simple to verify their ability to provide benefit to human victims of SCI. However, in many instances, the initial inflammation after a CNS lesion may actually be considered favorable for axonal recovery and regrowth and for enhancement of cell survival, as demonstrated by placement of neural grafts into lesions at short postlesion time points (see Chapter 2). Other pathways considered for treatment options include blockade of excitotoxicity, \(^\text{26-33}\) treatment of apoptosis, \(^\text{34-40}\) and application of hypothermia. \(^\text{41}\)

Although most neuroprotection research produced promising results in animal models (similar to results shown for stroke research; see Chapter 4), the natural history of human SCI suggests a limited role for neuroprotectants. First, most patients with incomplete SCI and some patients with complete SCI at the time of presentation spontaneously regain some degree of neurological function over time. \(^\text{42}\) This spontaneous recovery creates difficulty for treatment study design because it is difficult to attribute an improvement to treatment without a randomized control group. It is also rare for a patient’s neurological injury to progress significantly after presentation, i.e., an injury is at its worst at the time of presentation. This suggests that secondary injury mechanisms do not play a major role in determining the clinical extent of injury. Furthermore, only a narrow window of opportunity exists for the administration of neuroprotectants. The best results with the different agents mentioned above were obtained when animals were pretreated. As in the case of stroke treatment, the degree of clinical improvement obtained by preventing secondary injury may be minor, suggesting that neuroprotection as a clinical field may represent a failure of application of animal models to the human setting.

This is not to suggest that secondary injury is not important. It simply may be that the mechanisms at work unfold so rapidly that a patient’s deficit is relatively fixed at the time of presentation. It is therefore important to develop treatments that can be administered by first responders or alternatively to develop treatments that deal with the sequelae of secondary injury mechanisms. One such treatment is 4-aminopyridine, a potassium channel blocker. It has been shown that many of the axons that escape the initial injury become demyelinated, possibly due to inflammation, excitotoxicity, and apoptotic death of oligodendrocytes. Demyelina-
tion causes redistribution of sodium channels and unmasks potassium channels, both of which interfere with the conduction of action potentials.\textsuperscript{43–45} In laboratory studies, 4-aminopyridine re-enabled signal conduction in demyelinated and partially myelinated axons.\textsuperscript{46–47} Following preliminary clinical evidence that 4-aminopyridine can improve motor and sensory functions in SCI patients, Accorda Therapeutics initiated Phase 3 clinical trials.\textsuperscript{48,49} Hopefully we will soon know whether this promising drug can be added to our meager armamentarium for treating SCI.

### 3.3 Spinal Cord Regeneration

Ever since the seminal observations of Ramon y Cajal early in the 20th century, it has been known that CNS neurons have very limited abilities to regenerate following injury and primarily generate local collaterals rather than long-distance axonal regrowth. This is the reason for such impetus for the development of neuroprotective agents. Allowing even a small number of neurons to escape the initial injury could produce profound functional benefit. Conversely, inducing even a small population of neurons to regenerate effectively could restore a significant amount of neurological function.

Both neuronal and non-neuronal factors limit CNS regeneration. The neurons confined to the CNS do not upregulate the expression of growth-associated genes unless they are injured close to their cell bodies.\textsuperscript{50–54} CNS neurons that also extend axons into the peripheral nervous system (e.g., dorsal root ganglia) can undergo proregeneration cell body responses if their peripheral processes are also injured.\textsuperscript{54–56} These findings suggest that CNS neurons possess the genetic machinery to regenerate, but they only express the necessary genes under very limited conditions.

One approach to repairing an injured spinal cord would be to find ways to turn on the regenerative machinery and effectively enhance axonal regrowth. A second approach would be to bridge the injury gap or replace cells with neural grafts, stimulating axon regrowth across the bridge or providing new cellular elements that could promote regeneration.

#### 3.3.1 Enhancement of Axonal Regrowth

One of the first genes shown to be involved in axonal regeneration was GAP-43.\textsuperscript{57–61} This gene, along with CAP-23, belongs to the MARCKS family of phosphoinositide-responsive protein kinases and is important in the organization and stabilization of growth cone components. Expression of the GAP-43 and CAP-23 genes in transgenic mice is sufficient to induce a regenerative response following isolated CNS injury.\textsuperscript{62} Our laboratory is working on gene therapy methods to deliver these proregeneration genes to adult neurons. Other researchers have found that inosine, perhaps through the activation of these same kinases, can induce the regeneration of layer 5 pyramidal axons and promote reinnervation following SCI in rats.\textsuperscript{63}

Other efforts aimed at inducing a proregeneration state in CNS neurons revolve around the use of neurotrophins, small molecules that promote neuronal outgrowth. The most promising of these appears to be NT-3, which not only promotes the
regeneration of neurons following axotomy, but also minimizes atrophy and cell loss following SCI.64–70 Clearly, the cell body response to injury in the CNS that usually does not promote regeneration can be manipulated to increase the chances for neurological recovery following SCI.

Many researchers have shown that constituents of CNS myelin inhibit the growth of neurons. Removing myelin from the CNS or using grafts lacking central myelin, for example, are two ways to promote regeneration.51,53,71–73 Another way to promote regeneration following CNS injury is to antagonize these inhibitory signals. Three inhibitory molecules identified thus far are all components of CNS myelin: nogo, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein.72,74–77 Strikingly, all these molecules bind the neuronal receptor nogo-66.77–79 This receptor has been shown to complex with p57 and activate the rho kinase pathway.78,80 Inhibiting the rho pathway in itself allows neurons to grow in otherwise nonpermissive environments.71,82

Several experiments with antibodies to nogo (IN-1) and peptide inhibitors of the nogo receptor (NEP1-40) induced axonal regeneration and provided some degree of functional recovery following CNS lesions.83–90 Interestingly, in one of these experiments, IN-1 treatment caused the unlesioned corticospinal tracts of rats to sprout and reinnervate targets on the contralateral side. Despite this clearly erroneous regeneration, the animals regained use of their affected limbs, suggesting a role for enhanced plasticity.84 In an important study, researchers showed administration of NEP1-40 up to 1 week after spinal cord hemisection induced growth of corticospinal tracts, upregulation of growth proteins, functional reformation of synaptic connections, and locomotor recovery.89 This has significant implications for the treatment of human SCI because the therapy can be administered at delayed (and much more convenient) times after the injury. Hopefully, the interests of pharmaceutical companies in inhibitors of nogo, will soon bring this mode of therapy to human trials.

Another class of inhibitory signals is the chondroitin sulfate proteoglycans (CSPG). These are expressed on astrocytes, oligodendrocyte precursors, and meningeal cells, which are all avidly recruited to the site of a CNS injury.91–94 The CSPGs commonly found in glial scars are versican, neurocan, and phosphacan. Each contains a glycosaminoglycan (GAG) domain that is essential to their function. Fortunately, several bacterial enzymes specifically target and digest sulfated sugar GAG chains. Recently, the intrathecal infusion of one such enzyme, chondroitinase ABC, following SCI in rats was shown to degrade CS-GAGs at the injury site, upregulate GAP-43 in injured neurons, and promote regeneration of both ascending sensory projections and descending corticospinal tract axons. Postsynaptic activity below the lesion was restored and significant recovery of locomotor and proprioceptive behaviors ensued.94 These findings have been validated in other injury models,95,96 suggesting that the antagonism of CSPGs has an important role in the treatment of SCI.
3.3.2 CELLULAR OR INERT BRIDGES AND NEURAL GRAFTING

An additional pathway around the problem of CNS inhibition is to use bridging materials or cellular implants to guide regenerating axons around areas of significant tissue loss and glial scarring. The peripheral nerve has been known to have growth-promoting properties since the early 20th century. The Schwann cell (SC), a key component of peripheral nerves, was the first graft material candidate. In an interesting set of experiments, researchers used a PVC polymer tube filled with SCs to reattach the two stumps of a completely transected spinal cord. After 1 month, they noticed significant growth of axons into the graft from both stumps. However, they also noticed that very few corticospinal axons had grown into the graft; that virtually no axons had grown out of the graft; and that noticeable tissue loss occurred at the graft–cord interfaces on both sides. Treating with MP prevented scaring and tissue loss at the interfaces and caused limited growth of axons back into the CNS environment. Very limited corticospinal fiber growth appeared in the graft.

Treatment with BDNF and NT-3 caused brainstem nuclei to extend axons into the graft and increased the total number of axons in the graft. However, again, very limited extension of graft axons was found in the cord. This may have been due in part to the lack of synaptic targets in the vicinity of the graft. For example, optic nerve axons have been shown to grow through an SC graft and extend into the superior colliculus where they can form synapses. The inhibitory mechanisms reviewed earlier may be the other causes of this seemingly unidirectional growth from host to graft. Axons are simply not easily persuaded to leave whatever growth-promoting environment that might be presented to them to enter the relatively inhospitable environment of the CNS. It will be important to study the combination of these grafts with nogo and CSPG inhibitors.

Other types of cellular grafts have shown exciting results in the treatment of SCI. One study with embryonal spinal cord implants showed that when neurotrophins were delivered with the implants, some host axons grew all the way through the implants. Furthermore, host axons formed synapses with the implanted cells and axons from the grafts extended for some distance into the host spinal cords. Thus it is possible that these grafts serve as relays for regenerating axons. This produced very impressive functional recovery from complete spinal cord transaction. Washington University is conducting an ongoing clinical trial with the transplantation of porcine fetal spinal cells to assess the hypothesis that grafted neural cells can either enhance regeneration (function as a bridge) or provide key cellular elements.

Recent advances in the understanding of the olfactory system have led to what might be the most promising approach to overcoming CNS growth inhibition. Neurons in the olfactory mucosa are constantly dying and are replaced by new neurons that must extend their axons into the CNS. A special group of cells known as olfactory ensheathing glia (OEG) form sheaths around these axons, express growth-promoting phenotypes, and accompany these growing axons into the CNS. In one study, OEG transplantation into the site of SCI was associated with the extension of corticospinal, raphe-spinal, and coeruleospinal axons through the
injury and into the caudal spinal cord for at least 1 cm. This was associated with recovery of both locomotor function and sensorimotor reflexes. Other researchers have seen similar results. One study showed significant recovery of function even when transplantation was delayed for 7 days following injury.\textsuperscript{101–103} OEGs can also be combined with SC grafts allowing further axonal growth into the host spinal cord.\textsuperscript{104}

Not all research with OEGs has been successful. Takami et al. transplanted SC, SC and OEG, and OEG alone into rat SCI sites. They found a higher number of myelinated axons and better functional outcomes in the SC-only grafts. However, more axons extended beyond the grafts in the OEG-containing transplants.\textsuperscript{105} These results may represent differences in the techniques for purifying and transplanting OEGs. The body of positive results with OEG transplants cannot be overlooked. Based on positive research findings, OEGs hold great promise for the future surgical treatment of human SCI.

One other cell implantation strategy for treating SCI bears mention. Schwartz et al. felt that the immune-privileged status of the CNS played a part in its poor regenerative properties.\textsuperscript{106} Noting the prominent role of macrophages in peripheral nervous system regeneration, they implanted homologous macrophages activated by exposure to segments of peripheral nerves into the transected spinal cords of rats. They found that when sufficient macrophages were transplanted, partial recovery of both functional and electrophysiological activities occurred.\textsuperscript{106} Based on these findings, Proneuron is in Phase I/II feasibility clinical trials with homologous activated macrophage transplantation in Belgium and at the Weizmann Institute in Israel. The results of these trials are eagerly awaited.

Much more work must be done before we achieve the goal of regenerating the injured spinal cord. For example, the problem remains of ensuring that correct synaptic patterns are reestablished after regeneration takes place. None of the existing studies have shown that axons are sufficiently elongated to reach targets. Achieving synaptic specificity upon reaching distal spinal cord targets may in and of itself be a very difficult challenge.

The work reviewed above highlights some of the important leads that are currently being pursued. From the preliminary evidence, it seems that the first practical applications will be with agents that remove CNS growth inhibition. After that, molecular approaches to replacing damaged cells and reestablishing severed connections will hopefully be perfected and will probably lead to new challenges in reestablishing appropriate functional connections.

### 3.4 REHABILITATION

It is important to consider possible noninvasive approaches to help functional recovery from SCI. Chief among these are aggressive neurorehabilitation and assisted ambulation. At least five clinical trials are currently assessing the utility of assisted ambulation with body weight-supported treadmill training in promoting locomotion after SCI.

The key concept in these trials is that the spinal cord contains local pattern generators that can function independently of descending input. This was
demonstrated in cats whose spinal cords were transected at the thoracic level. Edgerton showed that appropriate limb loading and manual stepping on a treadmill for 4 weeks enabled the cats to regain the ability to support their own body weights and walk on the treadmill over a range of speeds.\textsuperscript{107,108} It was later shown that the lumbosacral grey matter responded to locomotion-associated patterns of stimulation and started generating rhythmic patterns of activity that could initiate stepping and perhaps support ambulation.\textsuperscript{109} The human lumbosacral spinal cord also has the ability to respond to the sensory stimulation of locomotion and generate locomotion-like electromyographic (EMG) patterns after training.\textsuperscript{110–112} The basis of this training is Edgerton’s proposal that providing the specific sensory activity associated with a task and repetitively practicing the task can lead to motor learning and plasticity in the human spinal cord.\textsuperscript{109} Based on this research, the University of California at Los Angeles, the University of Florida, The Miami Project to Cure Paralysis, the Ohio State University, and others are enrolling patients in assisted ambulation studies.

This approach, if successful, could be combined with invasive interventions to treat SCI. For example, transplanted cells and nogo inhibitors both may increase neuronal plasticity. These treatments could be combined with aggressive physical therapy and may stimulate the reorganization of intrinsic spinal circuits and allow coordination among multiple segments to dramatically improve locomotor function. Unfortunately, budgetary constraints may limit application of aggressive physical therapy techniques, although all patients with SCI receive intensive rehabilitation currently, and as new techniques arise, this training could be redirected to different patterns.

### 3.5 Neuroprosthetics

Another therapeutic avenue that will play a prominent role in the treatment of SCI patients is functional electrical stimulation (FES) and the field of neuroprosthetics in general (see Chapter 7). By stimulating muscles, lower motor neurons, and peripheral nerves, FES aims to return some functional modalities to patients who have complete SCI. Surgeons are implanting phrenic nerve stimulators to preserve respiration in high cervical injuries, sacral nerve stimulators to aid bowel and bladder function, and ulnar and median nerve stimulators to allow grasping movements of the hands.

Clinical studies sponsored by the Veterans’ Administration and the FDA are also evaluating systems to restore arm function, enable patients to stand, and assist them in walking. These devices have the potential to significantly improve the lives of patients with SCIs and the results achieved with such devices will improve as progress is made in the field of electronics and new ways are developed to interface nervous systems and computers. The theoretical approaches to and current research and progress with CNS–machine interfaces are reviewed Chapter 7, but in general, these approaches use external actuators instead of a patient’s own musculature to provide enhanced motor function.
3.6 COMBINATION THERAPIES

Future treatment of SCI will probably involve a synthesis of the approaches described earlier. Specific interventions can activate regeneration-associated genes and antagonize inhibitory signals within the CNS milieu, allowing surviving neurons to start to reestablish severed connections (Figure 3.1). This can be augmented by the transplantation of embryonic cells, olfactory ensheathing glia, and neurotrophin secreting cells to support regenerating cells, act as relays, and replace lost cell populations. The residual functional deficit after optimal treatment could then be ameliorated further by advances in FES, aggressive rehabilitation, and improved neuroprosthesis. Thus, any improvement in axonal regrowth will likely require significant patient training and rehabilitation to achieve clinical improvement.

3.7 PERIPHERAL NERVE REPAIR

Attempts to surgically treat peripheral nerve injury have been more fruitful than attempts to repair the injured spinal cord. Additionally, the two conditions are closely related as insights into the behavior of regenerating neurons obtained from the former are being applied to the latter, and vice versa. The first reported surgical repair of injured peripheral nerve was in 1608. The wars of the last two centuries, starting with the studies of Mitchell during the American Civil War, provided much material for the study of peripheral nerve injuries and repair techniques. Suture repairs of severed nerves, directly or by autografting, became standard practice by World War II. Unfortunately, the results were disappointing. The key problems were inadequate realignment of fascicles, the formation of neuromas, and the difficulty of filling large gaps with autologous peripheral nerve cable grafts.

The development of surgical microscopes helped improve these results. With good microsurgical technique, it became apparent that direct repair with microsurgical alignment of fascicles provided the best results. However, if damage to the nerve was severe enough to leave a gap greater than 2 cm, an autologous nerve graft had to be used for a tension-free repair. Unfortunately, the use of normal donor nerves from another location can be limited by tissue availability, the risk of causing secondary deformities, the failure of graft survival, and the differences in graft diameter that could complicate the repair. Current research on peripheral nerve regeneration focuses on developing engineered graft materials and improving specificity of reinnervation and thus functional recovery following peripheral nerve repair.

3.7.1 NERVE GUIDE TUBES

The development of nerve guide tubes stands as a critical example of translational research in neurosurgery. The use of tubular conduits in peripheral nerve repair was proposed as early as 1964. By the late 1980s, researchers had tested polytetrafluoroethylene (PTFE), silicone, polyvinylidenefluoride (PVDF), arteries, preformed mesothelial tubes, collagen, polylactate, polyesters, and polylactate/polyglycolate
copolymers. From these studies emerged the following criteria for useful nerve conduits; collagen was one material that met all the criteria:

1. The nature of the material is important in determining whether axons can grow on it.
2. The rate of resorption of the material must be on the appropriate time scale for axon regeneration to take place.
3. The mechanical properties must be stable in vitro.
4. The material must have appropriate permeability properties.
5. The material must not induce a deleterious inflammatory reaction.
6. The material properties must allow for easy manufacturing of different sized conduits.

Initial studies on rodents were carried out to identify the specific permeability properties that the collagen tubules would need in order to promote nerve regeneration. Collagen derived from bovine Achilles tendon was purified, gelled, homogenized, and deposited by compression onto a rotating mandrel to form tubules. Varying the amount of compression allowed control of the amount of permeability. Researchers implanted different tubules into rodents and found that making the tubules permeable to molecules the size of bovine serum albumin allowed four times greater axonal regeneration than the less permeable tubules. These results were attributed to the fact that the tubule could concentrate molecules such as growth factors and adhesion molecules within its lumen, creating a “reaction chamber” that promoted axon growth.

After these initial promising results, based on funding from the National Institutes of Health and the Department of Veterans Affairs, the researchers planned to move ahead with trials in nonhuman primates. A New Jersey biomaterials company became interested in the product, assumed responsibility for manufacturing it, and also contributed funding for the trials. Fifteen median nerves and one ulnar nerve were transected above the wrists of eight *Macaca fasicularis* monkeys; a 5-mm section was removed from each nerve. One nerve in each monkey was repaired with the collagen tubule, and another with an autologous nerve graft. Four other nerves were repaired by direct suturing in standard clinical fashion. The nerves were studied for motor and sensory conduction, response to tactile stimulation, and morphology over a period of 42 months. Researchers found similar amplitudes and latencies of tactile-evoked potentials, similar recovery rates of compound muscle action potentials, and an increase in the number of myelinated axons in the distal stumps following both nerve graft and synthetic nerve conduit repairs. Thus, a synthetic material produced results similar to autologous grafting. Based on these findings, the company obtained approval for use in humans and has been marketing the conduit under the brand name of NeuraGen® (Integra LifeSciences Holding Company, Plainsboro, NJ).

This example illustrates true translational neuroscience research, beginning from a technical concept in a small laboratory to large animal research with the support of a biotechnology company, to human trials, and clinical application. However, as is the case with many FDA-approved products, additional postapproval clinical trials...
Current research in nerve conduits centers on many of the same interventions attempted for spinal cord regeneration. As noted earlier, SCs are critical components in peripheral nerve grafts for axonal regeneration. They express specific cell adhesion molecules and bind specific extracellular matrix molecules that allow axon extension; they produce and secrete neurotrophic factors for neuronal support and axonal growth; and they possess receptors for neurotrophic factors and may act as neurotrophin-presenting cells for axon pathfinding. Some researchers are thus attempting to incorporate SCs into nerve conduits to improve the current results.\textsuperscript{119–123}

Other researchers are experimenting with the incorporation of extracellular matrix components into nerve tubules to promote axonal outgrowth.\textsuperscript{124,125} Some research aimed at improving the growth of axons into nerve guide tubes and distal stumps focus on the delivery of neurotrophins within grafts\textsuperscript{126} or by genetic manipulation of SCs to express neurotrophins distal to grafts.\textsuperscript{127,128} Other translational research in peripheral nerve repair focuses on the technical aspects of nerve repair. Researchers are studying different types of fibrin glues, fasteners, and laser repairs for treating peripheral nerve lesions in animals.\textsuperscript{129–133}

### 3.7.2 Enhancement of Specificity of Regeneration

Merely increasing the number of axons that grow into the distal stump is not sufficient. Care must be taken to promote appropriate axonal pathfinding. If axons fail to reach the correct sensory or motor end organ, patients will not achieve clinical improvement, and even worse, may be left with painful consequences. The rat femoral nerve that divides into a motor branch to the quadriceps and a sensory branch to the skin serves as a useful model for studying axon pathfinding. Researchers have found that motor axons are better at finding appropriate motor fascicles in the distal stump than are sensory axons — a process called preferential motor reinnervation.\textsuperscript{134,135} Pruning may be the reason for this.

Following injury, regenerating axons form many (redundant) collateral sprouts, and these enter SC tubules in the distal stump in a random fashion. However, with motor axons the branches that enter distal sensory fascicles are pruned back. Sensory axon neurons, on the other hand, do not necessarily trim back branches that have inappropriately entered motor fascicles in a distal stump. The result is that over time more motor axons find their targets. This suggests that local signals within SC tubules influence axonal pathfinding and under specific conditions can significantly increase specificity of regeneration.\textsuperscript{136}

In support of this, it has been shown that SCs in contact with motor axons express different membrane glycolipids than do SCs in contact with sensory axons.\textsuperscript{137} Also, blocking certain myelin proteins in the distal stumps can increase preferential motor reinnervation.\textsuperscript{138} If the local determinants of axonal pathfinding were identified, it would then be possible to manipulate the expression of these signals to improve the specificity of regeneration across synthetic grafts.

Other promising interventions include noninvasive measures to enhance peripheral nerve regeneration. Electrical stimulation is felt to be beneficial in nerve repair.\textsuperscript{139}
Recently it was reported that stimulation of the rat femoral nerve proximal to its repair site increased the degree and specificity of motor axon regeneration. These effects were shown to occur by influencing the cell body to increase expression of BDNF and its trkB receptor. Since electrical stimulation is already used clinically in the treatment of orthopedic injuries (for bone regrowth), it would be easy to extend its application to the treatment of peripheral nerve injuries.

The success rate with current peripheral nerve repair techniques is still disappointing. A recent report of the largest clinical series using the latest microsurgical techniques to treat peripheral nerve injuries reported at best a 70% return of function in direct repair of the ulnar nerve. We have been able to produce synthetic graft material that can support regeneration. Future refinements of these materials will likely incorporate cells and signaling molecules to improve the pace and accuracy of axon regeneration (Figure 3.1). We still face significant challenges in the treatment of peripheral nerve injuries. One issue still to be addressed is the prevention of end organ atrophy prior to reinnervation. Aggressive physical therapy may also be useful in this context. If we can take control of the processes of axon regeneration and pathfinding, we can get closer to the goal of full functional recovery.

3.8 CONCLUSIONS

SCI and peripheral nerve injury share the problem of long-distance axon regrowth. These problems are in many ways distinct from upper CNS regeneration schemes, in which actual neuronal cell loss may be the critical event, leading to neural grafting schemes for cortical lesions in stroke or epilepsy (see Chapter 2) and Parkinson’s disease (see Chapter 8). A considerable number of research schemes are under consideration for translational approaches based on promising preclinical data. However, the major problem remaining, even after axonal regrowth is achieved clinically, will be the issues of specificity when axons reach their targets and appropriate synaptic connectivity. Perhaps rehabilitation or neuroprosthetic approaches may partially bridge this subsequent, very difficult problem.

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