Biomedical Engineering Strategies for Peripheral Nerve Repair: Surgical Applications, State of the Art, and Future Challenges

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ABSTRACT: Damage to the peripheral nervous system is surprisingly common and occurs primarily from trauma or a complication of surgery. Although recovery of nerve function occurs in many mild injuries, outcomes are often unsatisfactory following severe trauma. Nerve repair and regeneration presents unique clinical challenges and opportunities, and substantial contributions can be made through the informed application of biomedical engineering strategies. This article reviews the clinical presentations and classification of nerve injuries, in addition to the state of the art for surgical decision-making and repair strategies. This discussion presents specific challenges that must be addressed to realistically improve the treatment of nerve injuries and promote widespread recovery. In particular, nerve defects a few centimeters in length use a sensory nerve autograft as the standard technique; however, this approach is limited by the availability of donor nerve and comorbidity associated with additional surgery. Moreover, we currently have an inadequate ability to noninvasively assess the degree of nerve injury and to track axonal regeneration. As a result, wait-and-see surgical decisions can lead to undesirable and less successful "delayed" repair procedures. In this fight for time, degeneration of the distal nerve support structure and target progresses, ultimately blunting complete functional recovery. Thus, the most pressing challenges in peripheral nerve repair include the development of tissue-engineered nerve grafts that match or exceed the performance of autografts, the ability to noninvasively assess nerve damage and track axonal regeneration, and approaches to maintain the efficacy of the distal pathway and targets during the regenerative process. Biomedical engineering strategies can address these issues to substantially contribute at both the basic and applied levels, improving surgical management and functional recovery following severe peripheral nerve injury.

KEY WORDS: regeneration, nerve injury, tissue engineering, peripheral nerve, Schwann cell, nerve conduit, neurography, MRI, DTI

I. INTRODUCTION

I.A. Incidence of Peripheral Nerve Injury

The peripheral nervous system (PNS) is damaged primarily by traumatic injury, surgery, or repetitive compression (tunnel syndromes). Traumatic injuries can occur due to stretch, crush, laceration (sharps or bone fragments), and ischemia, and are more frequent in wartime, i.e., blast exposure. Peripheral nerve injuries occur with surprising frequency, as they are reported in up to 3% of *all* trauma patients, increasing to 5% if plexus and root avulsion cases are included.[1–3] In addition to unanticipated injury,

ABBREVIATIONS

PNS, peripheral nervous system; PLGA, poly(lactic-co-glycolic acid; TIB, tibial nerve; CP, common peroneal nerve; BDNF, brain-derived neurotrophic factor; GDNF, glial-derived neurotrophic factor; TGF-β, transforming growth factor β; MRI, magnetic resonance imaging; DTI, diffusion tensor imaging; Gf, gadofluorine-M; NAA, *N*-acetyl aspartate; DWI, diffusion-weighted imaging; DTI, diffusion tensor imaging; ADC, apparent diffusion coefficient; FA, fractional anisotropy; EMG, electromyography

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nerves are damaged due to surgical manipulation or unavoidable transection during tissue removal. For instance, nerves are often sacrificed during intra-abdominal and cervical surgical procedures such as tumor resection. Overall, a recent study revealed that PNS injuries were 87% from trauma and 12% due to surgery (one-third tumor related, two-thirds non–tumor related). Nerve injuries occurred 81% of the time in the upper extremities and 11% in the lower extremities, with the balance in other locations.[4] It is important to note however, the incidence of PNS injury is grossly underestimated due to the span of causes and the intervention from many clinical disciplines, including orthopedic surgery, plastic surgery, as well as neurosurgery.[1–4]

Injury to the PNS can range from severe, leading to major loss of function or intractable neuropathic pain, to mild, with some sensory and/or motor deficits affecting quality of life. When surgical repair of the nerve is required, the goal is to guide regenerating sensory, motor, and autonomic axons to the distal, degenerating nerve segment to maximize the chance of target reinnervation.[5,6] Despite best efforts and modern surgical techniques, functional restoration is often incomplete, with approximately 50% of surgical cases achieving normal to good restoration of function.[4,7] Accordingly, there is a clear need for biomedical engineering research to develop novel strategies and grafting options to improve outcomes following nerve damage.[4]

I.B. Executive Summary of Biomedical Engineering Challenges

When a direct repair of the two nerve ends is not possible, synthetic or biological nerve conduits are typically used for small nerve gaps of 1 cm or less. For extensive nerve damage over a few centimeters in length, the nerve autograft is the "gold standard" technique. The biggest challenges, however, are the limited number and length of available donor nerves, the additional surgery associated with donor site morbidity, and the few effective nerve graft alternatives.[3,8,9] A survey of clinicians indicated that a direct surgical repair of the nerve is performed in 78% of the cases, autografts are used in 15% of cases, alternative methods (i.e., conduits) are used 4% of the time, and the balance receives no repair.[4] Repair results varied greatly among clinicians and may reflect treatment decisions influenced by limited confidence in alternative repair options. Moreover, the literature is clear that autografting is superior to all grafting alternatives. Nonetheless, given the short supply and comorbidities associated with autografts, comprehensive engineered solutions that match or surpass the performance of autografts would be extremely beneficial to improve overall outcome following severe nerve injuries and/or multiple nerve trauma scenarios.

In certain injury cases it may take many months (typically 3–6 months, sometimes longer) to determine whether spontaneous restoration of function will occur, causing the most opportune timing for surgical augmentation to pass. If surgical repair is then attempted, the delay reduces the likelihood of success due to degeneration of the distal nerve support structure and target (e.g., muscle) atrophy. Biomedical engineers have a great opportunity to contribute strategies to assist and improve surgical decisions. In particular, there is currently a lack of precision in our ability to noninvasively assess the degree of nerve injury or to track the progress of axonal regeneration. The development and validation of advanced neuroimaging modalities capable of assessing axonal tract integrity and the progress of spontaneous regeneration would be beneficial to properly grading injuries and promptly identifying cases requiring surgical intervention with less ambiguity.[10–14]

Degeneration of the axonal segment in the distal nerve is an inevitable consequence of disconnection, yet the distal nerve support structure as well as the final target must maintain efficacy to guide and facilitate appropriate axonal regeneration. There is currently no clinical practice targeted at maintaining fidelity of the distal pathway/target, and only a small number of researchers are investigating ways to preserve the distal nerve segment, such as the use of electrical stimulation or localized drug delivery. Overall, biomedical engineering approaches could contribute solutions to the most pressing limitations in peripheral nerve repair, including the development of tissue-engineered nerve graft alternatives that match or exceed the performance

of autografts, the ability to noninvasively assess nerve damage and track axonal regeneration, and the ability to maintain the efficacy of the distal pathway and target.

I.C. Scope of this Article

For the biomedical engineer to improve upon current peripheral nerve repair strategies, a thorough knowledge of the anatomy, pathophysiology, and surgical reconstruction techniques is prerequisite.[2,3,7,8,15] Accordingly, we review the clinical presentations and classification of nerve injuries, in addition to the state of the art for surgical decision-making and repair strategies. This discussion is framed to present specific challenges that are required to substantially improve the treatment of nerve injuries and to promote recovery in currently intractable cases. Particular attention is given to tissue-engineered constructs to replace and/or augment the use of autografts, advanced neuroimaging and diagnostic modalities to assess axonal integrity and track regeneration, and strategies to maintain efficacy of the distal regenerative pathway and target. Biomedical engineering approaches are appropriate to address these issues and can substantially contribute at both the basic and applied levels, ultimately resulting in improved surgical management and functional recovery following peripheral nerve injuries.

II. PERIPHERAL NERVE ANATOMY AND INJURY CLASSIFICATION

II.A. Peripheral Nerve Anatomy

The anatomy of a peripheral nerve is shown in Fig. 1A. Axons are grouped into fascicles supported by a collagenous *endoneurium*. Each fascicle is delineated by a *perineurium* sheath—a perineural cell layer serving as a blood-nerve barrier. Together, the perineurium and endoneurium provide elasticity to the nerve. Depending on the nerve and location, the nerve can contain many fascicles (polyfascicular) or just a few (oligofascicular). The *epineurium* is a loose connective-tissue sheath that defines the nerve architecture. The external epineurium surrounds all fascicles, whereas the mainly collagenous internal epineurium provides mechanical support for the nerve fascicles and blood vessels. The *mesoneurium* is the outermost connective tissue of the nerve, spanning the epineurium to the surrounding tissue. Structurally, the mesoneurium allows for expansion and contraction of nerve related to extremity movement. For instance, maximal flexion and extension of the median nerve requires longitudinal movement up to 3 cm distally. The nerve blood supply enters through the mesoneurium; blood vessels run longitudinally within the epi- and perineurium and end as capillaries in the endoneurium.[6,16,17]

II.B. Injury Classification

Depending on the injury type and severity, surgical intervention may be required. Only a specific subset of cases, however, may require a guidance conduit, nerve graft, or tissue-engineered construct. Nerve injuries are classified in two fundamental ways: the broad pathological descriptions of H.J. Seddon (neurapraxia, axonotmesis, and neurotmesis) and degrees of anatomical disruption and regenerative potential (1st through 5th degree) by S. Sunderland (Fig. 1B and Table 1).[18,19] Neurapraxia (1st degree) is a blockage of nerve conduction at a discrete location. It is characterized by a short episode of myelin breakdown and related dysfunction without physical disruption of the nerve tissues or axons; therefore, regeneration is not involved in repair. These mild injuries are brought about by compression, lack of blood flow, or mild blows, and the loss of conduction returns within days to a few months. It is not treated surgically and there is no need for a tissue-engineered solution.

Axonotmesis (2nd degree) is a more severe nerve injury, characterized by axonal damage and Wallerian degeneration of the distal nerve. Injuries are typically due to a traumatic crush or stretch causing disruption in motor, sensory, and autonomic function. Here, damaged proximal axons attempt to regenerate and are guided by the distal nerve to reinnervate their targets. In 2nd degree injury, damage is purely axonal, where the distal architecture and Schwann cell basal lamina remain intact. No surgery is required as axons regenerate down intact endoneurial tubes and recovery of function is likely. Again, a tissue-engineered solution is not needed in these cases.

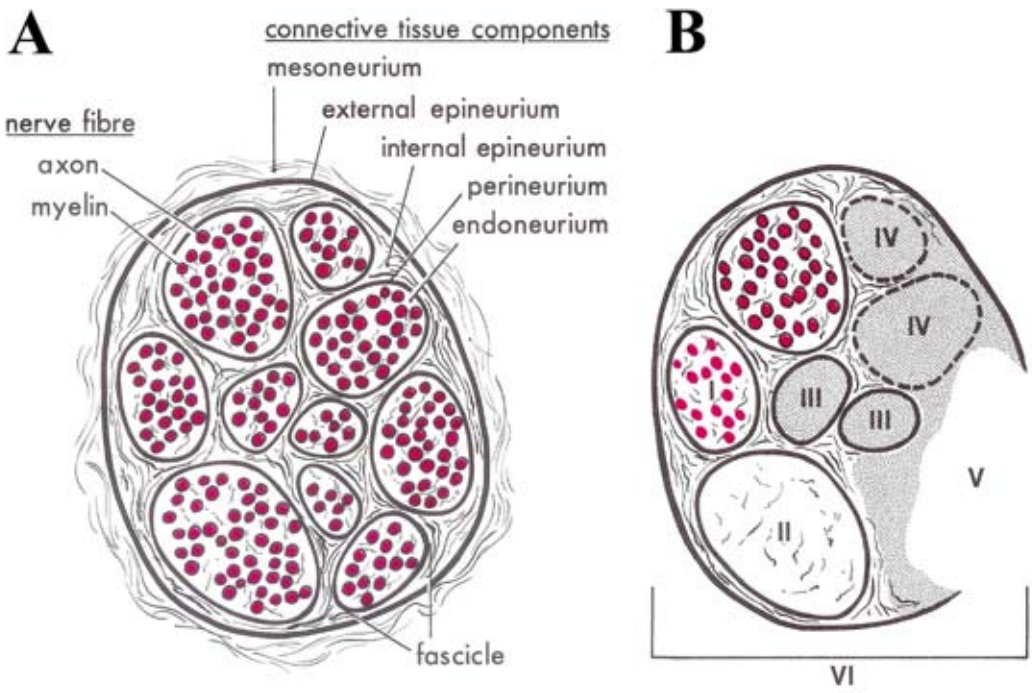

FIGURE 1. Nerve injury classification. (A) Cross-section of a normal nerve. (B) Illustration of injury classifications. Type I: myelin disruption with axons intact. Type II: axon disruption with intact perineurium. Type III: damaged Schwann cell basal lamina and endoneurial scarring inhibiting regeneration. Type IV: nerve fascicle disruption and loss of the perineurium sheath; repair required. Type V: disruption of the entire nerve; repair required. Type VI: mixed injury of all types along the damaged nerve. Reprinted with permission from Ref. 6.

In 3rd degree injury, there is disruption of the Schwann cell basal lamina and potential scarring of the endoneurium. Axons must grow through the damaged and scarred tissue, which may lead to axonal loss and misdirection. Regeneration remains within fascicles since the perineurium is intact. Surgery is typically not needed unless it localizes to a known area of nerve compression. In these cases a surgical decompression procedure will ensure there is not a superimposed component of compression.

The perineurium is disrupted in 4th degree injury and the nerve is typically nonfunctional. Continuity within the epineurium is comprised of scar tissue with little to no tissue architecture, which results in a blockage of regenerating axons. Recovery does not occur without surgical intervention to remove the lesioned area. Unfortunately, diagnosis requires a wait-and-see period, typically over three months, the time it takes for 2nd and some 3rd degree injuries to show signs of repair.

Neurotmesis (5th degree) is the most severe lesion, characterized by a complete transection of the epineurium and encapsulating connective tissue continuity. Surgical intervention is required for repair and to prevent neuroma formation at the proximal stump. An additional 6th degree injury, described by S.E. Mackinnon, characterizes a mixed pattern of injuries (1st to 5th degree) to the multiple fascicles in the nerve.[6,20]

III. PERIPHERAL NERVE REPAIR: SURGICAL GOALS AND STRATEGIES

PNS reconstructive repair strategies are focused on 3rd to 6th degree nerve injuries, whereas 1st and 2nd degree injuries are left to heal on their own. While 3rd degree injuries are not the most severe, they are the most challenging due to the diagnostic process. Patients present with functional loss; however, the

TABLE 1. Peripheral Nerve Injury Classification*

Injury Degree	Pathology	Treatment	TEC	Prognosis
I Neurapraxia	Axons not disrupted Possible segmented demyelination	None	Not needed	Full recovery Days up to 3 months
II Axonotmesis	Axon loss Endoneurium, perineurium, epineurium intact	None Slow regeneration 2–3 cm per month	Not needed	Good, rate is slow
III	Axon loss Endonurium disrupted Perineurium, epineurium intact	None Surgery only if no recovery in 2–3 months Slow regeneration 2–3 cm per month	?	Incomplete Axonal loss and misdirection
IV	Axon loss Endonurium, perineurium disrupted Epineurium intact	Surgery required to remove scar tissue. Autograft or conduit for gaps	Yes	Regeneration only after repair Availability of graft material
V Neurotmesis	Complete disruption of nerve	Surgical repair to proximate the two ends Direct repair, Autograft or conduit for gaps	Yes	Regeneration only after repair Availability of graft material
VI	Mixed injury	Surgical repair	Yes	

*Adapted from Refs. 6, 1; TEC = tissue engineered construct.

injury is intra-endoneurial and damage is not visible with conventional functional assessments or imaging modalities. Ultimately, the injury could undergo spontaneous regeneration similar to a 2nd degree injury, or develop inhibiting scar tissue and require surgical intervention to restore regeneration. A waiting period of three months is standard prior to surgery, during which 2nd degree injuries would see a return of function.[6]

There are three surgical reconstruction strategies: (1) *direct repair*, where the proximal and distal nerve ends are sutured back together, (2) *nerve grafting*, required to bridge a gap between nerve ends, and (3) *nerve transfer,* when the distal or proximal nerve segment is unusable or missing (Fig. 2). A direct repair is appropriate for reconnection of injured nerves where no gaps exist between the ends, and the stumps are sutured together in what is called an end-to-end neurorrhaphy. An important microsurgical technique is to identify, separate, and join each perineurial defined fascicle.[3,6] If there is no scar tissue at the suture line, proximal axons extend into a network of proliferating Schwann cells within the distal (degenerating) nerve segment, which promotes and directs regeneration. Difficulties with this strategy include reproducing the original alignment of nerve fascicles and a neurorrhaphy without inducing tension.[6,20,21]

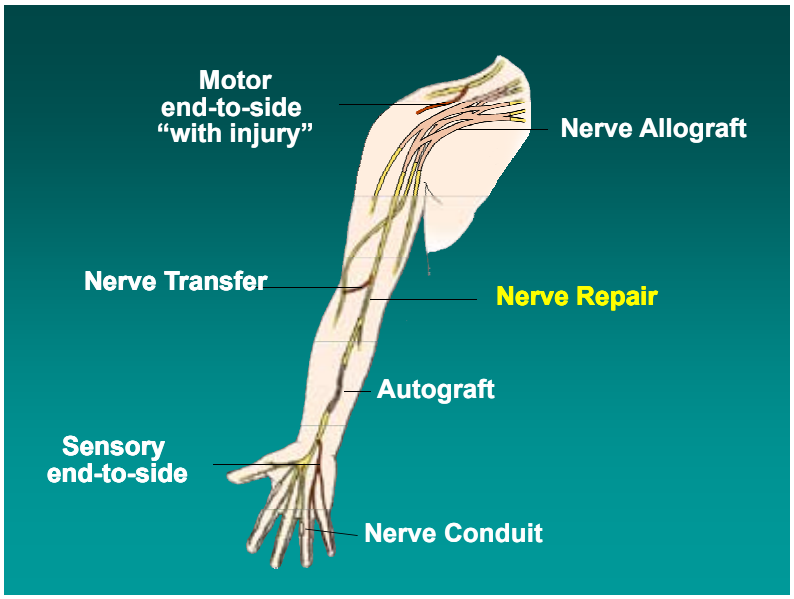

FIGURE 2. Options for surgical nerve repair. The method of repair depends upon the classification and location of the injured nerve. Note that more proximal injuries require strategies other than grafting because the distance is too long for regeneration to occur before the distal nerve and target lose the ability to support regeneration. Figure adapted with permission from Ref. 20.

Importantly, a direct repair must be outside of the zone of injury, meaning the entire damaged nerve segment must be removed to prevent scar tissue formation and inhibition of regeneration.[21] This and other surgical procedures such as tumor excision can leave a gap between nerve endings. In these cases an end-to-end neurorrhaphy would induce longitudinal tension, known to lead to poor outcome. In particular, tension has been shown to attenuate or stop epineurial blood flow that is believed to cause tissue necrosis from chronic ischemia. Under high tension, the perineurium may become permeable and endoneurial structures damaged.[3,16,17,22,23] To avoid tension when joining the nerve ends, the preferred bridging material is an autograft. Similar to the distal nerve segment, an autograft provides a Schwann cell loaded scaffold and tissue architecture primed for regenerating axons emanating from the proximal nerve.

Challenges with grafting include graft phenotype (sensory versus motor), donor site morbidity, and limited grafting material.[3,6,20,24,25] In addition, axons can be easily misguided with increasing growth distance through grafts or a distal nerve that loses its supportive capacity before regeneration is complete. The importance of graft phenotype is highlighted here. First, superior motor axon regeneration and recovery is achieved when using motor nerve rather than sensory nerve grafts. Specifically, motor axon growth appears to prefer a motor pathway, whereas sensory nerves are less specific (Fig. 3).[25–27] Motor grafts may also be preferred over sensory due to their larger endoneurial tube diameter (which can yield greater axon number). However, sensory nerves are the preferred sources for autografts, as the primary complication is localized numbness (which is often temporary) rather than a motor deficit.

In cases where autografts are not possible, allografts and nerve conduits are the alternatives. Allografts necessitate systemic immunosuppressive therapy for up to two years and are typically reserved for patients with extensive or otherwise irreparable nerve injuries. Acellularized allografts have been used with success and experimentally shown to be superior to nerve conduits, but are relatively cost-prohibitive and not the primary means of repair in nerve graft-

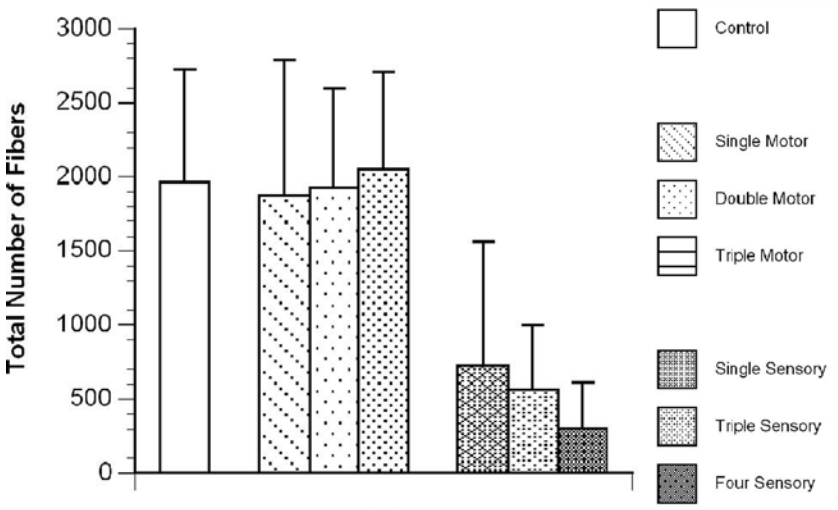

FIGURE 3. Effect of nerve phenotype on regenerative capacity. Nerve grafts consisting of primarily motor fibers allow for more robust regeneration than from grafts consisting primarily of sensory fibers. This is because motor axons regenerate preferentially through motor grafts, whereas sensory axons will regenerate down either phenotype. Control was an isograft. Reprinted with permission from Ref. 27.

ing.[20,28] Accordingly, a good substitute to nerve grafting for short defects is a *nerve conduit*, a short cylinder that approximates the nerve stumps and constrains aberrant regeneration. Conduits can be either biological (e.g., vein grafts) or synthetic (e.g., PLGA or collagen tubes).[3,6,7,29,30] Indeed, synthetic conduits are appealing since they can be easily fabricated and stored until they are needed. Nerve conduits are used clinically for smaller, noncritical nerve repair (gaps <3 cm) in small-caliber nerves. Unfortunately, conduits fail to promote adequate nerve regeneration in critical large-diameter nerve gaps longer than 1 cm or small-diameter nerve gaps longer than 3 cm in length.[9,31] Since empty conduits do not contain factors that may directly facilitate axon regeneration, such as extracellular matrix, growth factors, or support cells, nerve grafting remains superior overall. Nerve conduits have also had success as a protective wrap, particularly in surgical areas.

In some cases, the proximal segment of the nerve is not available or the gap between the proximal and distal ends is too large to graft. When the two ends cannot be connected or the injury is too proximal (too far) for axons to regenerate, axons are recruited from a nearby donor nerve to reinnervate the distal nerve.[20] One strategy is to connect the distal end to an adjacent uninjured nerve in an end-to-side neurorrhaphy (Fig. 4). When motor recovery is necessary, a redundant motor nerve is sought and injured by epineurotomy or compression proximal to the suture site. Motor axons will sprout only in an end-to-side fashion with injury (Fig. 4A). This injury induces axons to extend into the newly coapted distal nerve segment. The disadvantages of this method are inducing an additional injury and the "stolen nerves" causing a reduction of innervation at the original healthy nerve target. Sensory axons, on the other hand, will sprout spontaneously without injury (Fig. 4B).[32,33]

A growing practice in motor nerve repair is a *nerve transfer*, the redirection of a nearby motor nerve. The goal is to maximize functional recovery with fast reinnervation of denervated motor targets. First, an expendable motor nerve must be located near the target denervated muscle. In a high ulnar transection, for instance, the distal anterior interosseous motor nerve can be redirected to the denervated ulnar motor target. This method provides fast and superior muscle reinnervation compared to other techniques, which rely more heavily on slowly regenerating nerves. The

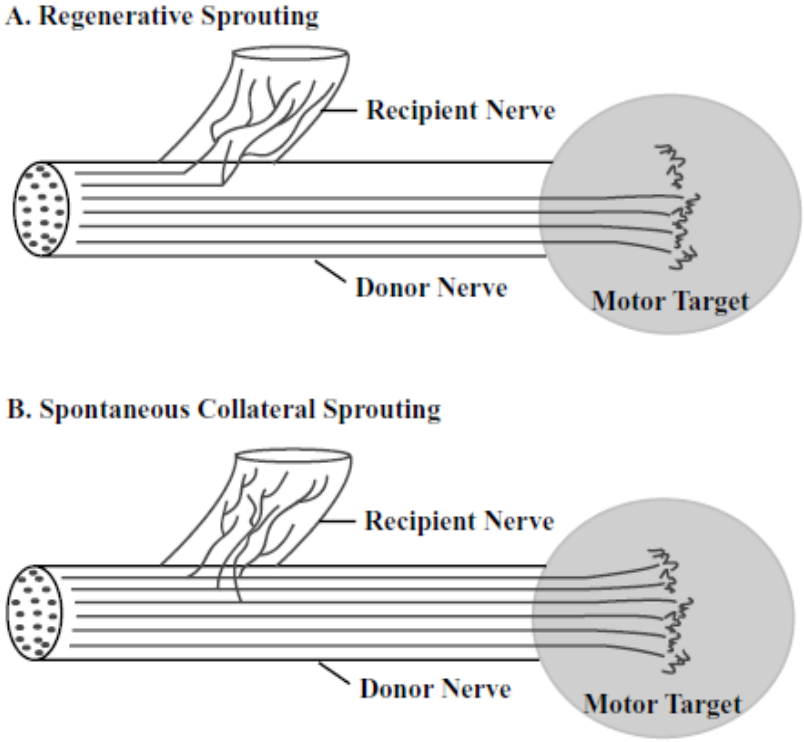

FIGURE 4. Regeneration schemes from an end-to-side neurorrhaphy. When the proximal nerve is unavailable, the distal segment is attached to a neighboring redundant nerve in an end-to-side neurorrhaphy. (A) To redirect motor and sensory fibers, the donor nerve must be injured to induce regeneration into the distal segment of the damaged nerve. (B) Unlike motor axons, sensory axons will spontaneously sprout without inducing an injury. Reprinted with permission from Ref. 33.

disadvantages are finding an expendable donor nerve near the target muscle with a large enough motor fiber population from which to "borrow." Importantly, the donor nerve target should be synergistic with the redirected target for the brain to accommodate the rewiring of the newly redirected fibers.[20] Currently there are only a very limited number of surgeons that perform nerve transfers.

IV. NEUROBIOLOGICAL SEQUELAE AFFECTING PERIPHERAL NERVE REGENERATION

IV.A. Acute Cellular and Molecular Events That Support Nerve Regeneration

Axonal regeneration after peripheral nerve injury may be reasonably good after surgical repair. Many cellular and molecular events take place after nerve injury that ultimately support nerve regeneration and target reinnervation.[24,34,35] Briefly, injured neurons typically survive if the injury is not too close to the cell body. After injury the neuronal cell body undergoes chromatolysis in which changes in gene expression prepare the neurons for regeneration of their axons.[36] The nerve stump distal to the injury undergoes Wallerian degeneration with loss of myelin and axons followed by the proliferation of the Schwann cells within the endoneurium. The latter cells play a critical role in regeneration of axons through the distal nerve stump to reinnervate the denervated and atrophic muscle.[35] In particular, a choreographed organization of Schwann cells forms aligned columns, referred to as the Bands of Bungner, which provide neurotrophic support and contact guidance to direct axonal regeneration towards appropriate targets. Thus, neurons commence regeneration of their axons in the growth-

permissive environment of the Schwann cells in the distal nerve stumps.[35]

However, despite these pro-regenerative changes in damaged axons and Schwann cells, functional outcomes in patients are frequently poor, especially for injuries requiring great lengths for target reinnervation, such as the brachial and lumbar plexi. This has generally been attributed to deterioration of denervated targets.[24] This view, however, is being revised with evidence that deterioration of the regenerative power of injured nerves and the growth environment of the distal nerve stumps accounts for regenerative failure with time and distance.[34,35]

IV.B. Chronic Nerve Regeneration and Target Reinnervation

Motoneurons are normally in contact with the muscle fibers they supply. This neuron-muscle pair is called the *motor unit*. The motor unit was referred to as the common final pathway of the nervous system by C.S. Sherrington in the last century because all of the processing in the nervous system ultimately results in movement. Considering the problems of poor functional recovery after peripheral nerve injuries, both time and distance of axon regeneration are critical. At the wrist, for example, median and ulnar nerve injuries involve distances of about 100 mm over which axons must regenerate to reach many of the hand muscles. At the average regeneration rate of 1 mm/day in humans, recovery requires at least 100 days. More proximal nerve injuries, such as a brachial plexus injury, involve distances of up to a meter and require periods of more than 2–3 years for regenerating axons to reach and reinnervate the hand muscles (Fig. 5A). In such cases, it is well recognized clinically that there may be little or no restoration of function. During this long period of time, neurons remain without target connections (axotomized) and the target organ and distal nerve remain denervated until reached by regenerating axons. Although this failure of functional recovery has been attributed to irreversible atrophy of muscle targets and their replacement by fat, animal experiments are now indicating that it is the progressive failure of the neurons and Schwann cells to sustain axon regeneration over distance and time.[24,35]

A classic study by Fu and Gordon (1995) was performed to determine the independent effects of prolonged axotomy and chronic denervation of the Schwann cells in the distal nerve using a cross-suture technique in a rat model of nerve injury (Fig. 5B).[37] For chronic axotomy of the tibial neurons, the tibial nerve (TIB) was transected and the proximal nerve stump sutured to an innervated muscle and left alone (Fig. 5C). At specific time-points ranging from 0 to 12 months (chronic axotomy), the redirected TIB nerve was recut and sutured to the freshly denervated common peroneal (CP) nerve to encourage regeneration into freshly denervated tibialis anterior muscle (Fig. 5D).[38] To consider the effects of prolonged denervation of the Schwann cells in the distal nerve stump, the CP nerve was transected. Regeneration of axons through the chronically denervated CP nerve stump was prevented by ligating and suturing the proximal CP nerve stump to a nearby innervated muscle (Fig. 5E). After 0–12 months, the TIB nerve was cut and sutured to the chronically denervated CP distal nerve stump to encourage regeneration of motor axons into the distal nerve stump containing the chronically denervated Schwann cells (Fig. 5F).[37]

For both the chronically axotomized and denervated animals, at least 5 months were allowed for axonal regeneration. The number of motoneurons that had regenerated their axons and how well the reinnervated muscles recovered were determined. Ventral nerve roots (L3 to L5) were isolated to tease out single axons to stimulate and record the isometric contractile forces of the muscle fibers supplied by the single motor axon (motor unit force) as well as the contractile forces developed by all the reinnervated tibialis anterior muscle fibers (Fig. 6A). The ratio of the muscle and motor unit forces provides a good estimate of how many motor axons regenerate and reinnervate target muscle after prolonged axotomy or after prolonged denervation of the Schwann cells (Fig. 6B).

This study found that the regenerative capacity of neurons declines with time due to both prolonged

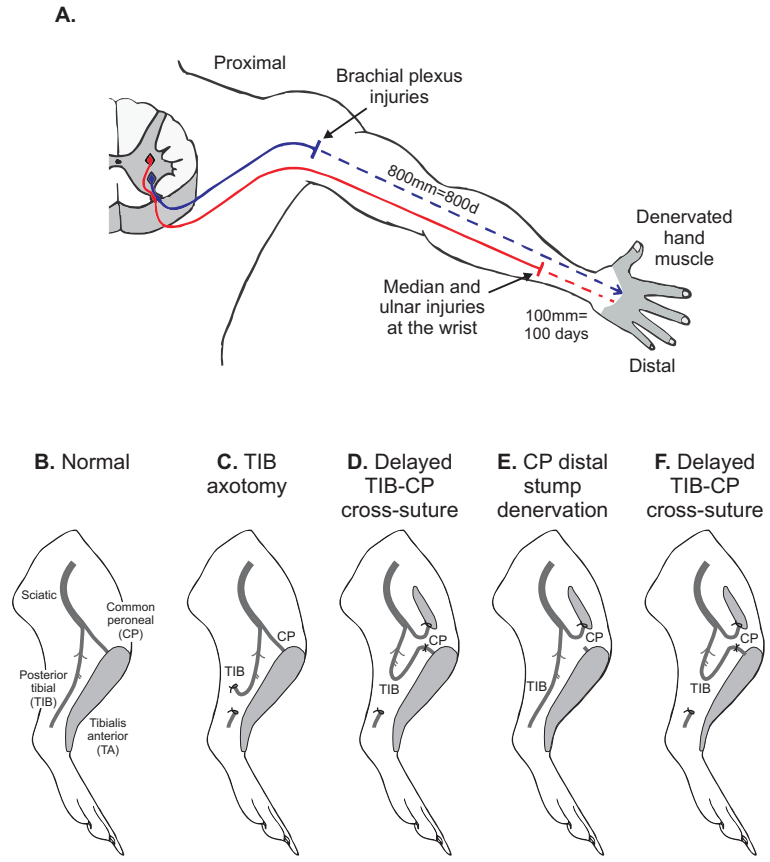

FIGURE 5. Illustrations of (A) injuries to large nerves in the arm and the distances that must be traversed by regenerating nerves to reinnervate denervated hand muscles at a rate of 1 mm/day in humans; (B) the rat hindlimb, showing the branching of the sciatic nerve into the common peroneal (CP), nerve innervating the tibialis anterior muscle of the ankle flexor muscle group, and the tibial (TIB) nerve innervating the ankle extensor muscles; the sural nerve innervating skin is not shown; (C) TIB nerve transection by cutting all the TIB neuronal axons to separate their axons from target connections and thereby to axotomize the TIB neurons; (D) delayed suture of the proximal nerve stump of axotomized TIB neurons to freshly denervated CP distal nerve stump to encourage nerve regeneration; (E) CP nerve transection to promote Wallerian degeneration and denervation of Schwann cells, prior to (F) delayed suture of freshly axotomized TIB nerve to chronically denervated CP nerve.

axotomy and the Schwann cell denervation. As the period of prolonged axotomy increased, the number of motoneurons that regenerated decreased. After delayed repair of more than 4 months, regeneration declined to ~33% of the number of axons that could regenerate after an immediate nerve repair.[38] Of considerable importance was that recordings of maximal contractile ability indicated full recovery despite the reduction in numbers of motor nerves that reinnervated denervated muscle. This apparent paradox of full recovery of muscle was accounted for by findings that the reduced numbers of regenerating nerves that supplied the muscle reinnervated three times as many denervated muscle fibers as they normally do. The enlarged motor units compensated for the poor regenerative ability of regenerating nerves after prolonged axotomy. These findings demonstrated the detrimental effects of time and distance

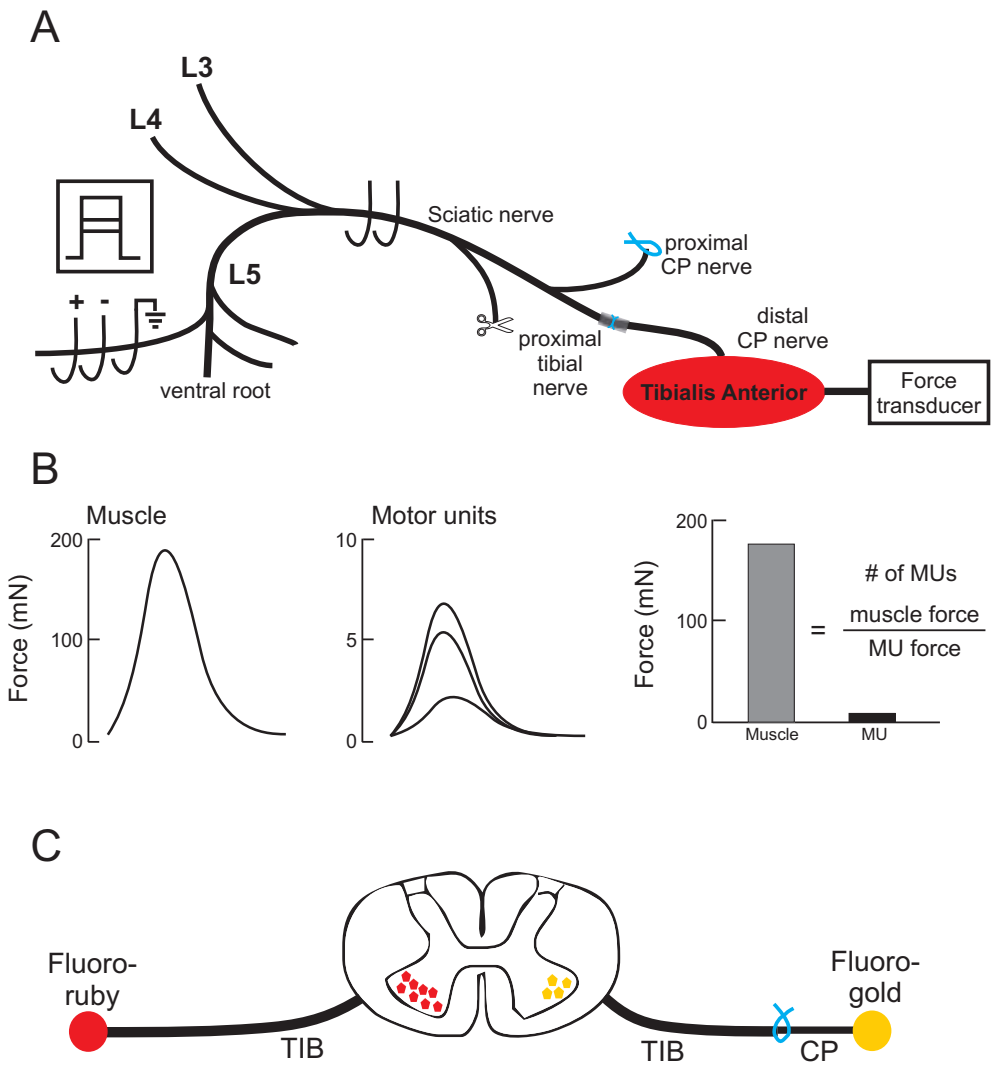

FIGURE 6. Illustration of (A) experimental setup to stimulate either all (in the sciatic nerve) or single axons (in ventral root filaments) that regenerated to supply the tibialis anterior muscle in the rat; (B) the muscle twitch contraction in response to stimulation of all the axons, motor unit twitch contractions in response to stimulation of single axons, and calculation of numbers of TIB axons that reinnervated the muscle from the ratio of the muscle twitch force and average motor unit force; (C) application of retrograde dyes, Fluoro-Ruby and Fluoro-Gold, to TIB axons and CP axons to count Fluoro-Ruby-labeled TIB motoneurons that normally send axons through the TIB nerve and Fluoro-Gold TIB motoneurons that regenerate their axons through the Schwann cells in the CP distal nerve stump.

on regenerative capacity of injured nerves that had not been appreciated previously.

The effect of prolonged denervation of Schwann cells on the ability of motoneurons to regenerate their axons was even more profound. After periods of more than 4 months of prolonged denervation, less than 10% of the motoneurons were able to regenerate their axons successfully through the atrophic Schwann cell environment. This poor regenerative capacity could not be compensated by the previously

seen threefold increase in numbers of muscle fibers reinnervated by each motoneuron. Accordingly, many muscle fibers were not reinnervated, resulting in denervation atrophy. The poor functional reinnervation was due to the chronic denervation alone and not chronic axotomy because the tibial nerve was cut and immediately cross-sutured to the chronically denervated CP distal nerve stump.

Many could still argue that these findings reflect the inability of denervated muscle to accept reinnervation after prolonged periods of denervation. The surgical paradigm was therefore repeated with the additional experimental method of retrograde dye labeling of neurons to count motoneurons that had regenerated their axons (Fig. 6C).[39] The results of this study demonstrated conclusively that indeed the prolonged neuron axotomy and the prolonged denervation of Schwann cells progressively reduce regenerative success and explain why peripheral nerve regeneration so frequently fails to achieve functional recovery.[39,40] In summary, axon regeneration after peripheral nerve injury progressively fails due to chronic axotomy of the neurons, chronic Schwann cell denervation, and is not due solely to irreversible atrophy of muscle as was previously believed. Indeed, chronically denervated muscles can be reinnervated and in turn, will function.

IV.C. Treatments to Improve Outcome Following Chronic Axotomy and Denervation

Based on these seminal findings, several experimental manipulations to obviate the negative effects of chronic axotomy and prolonged denervation have been explored in attempts to improve peripheral manipulations to overcome the effects of chronic axotomy include: electrical stimulation to both (1) accelerate expression of neurotrophic factors within the neurons, including brain-derived neurotrophic factor (BDNF), and (2) accelerate axon outgrowth across the lesion site, (3) the use of exogenous sources of neurotrophic factors, including BDNF and glial-derived neurotrophic factor (GDNF),[40,47,48] and (4) FK506 to reverse effects of chronic axotomy on neurons.[49] In the case of chronic denervation of Schwann cells, some manipulations can improve axon regeneration, including activation of atrophic dormant Schwann cells with the cytokine transforming growth factor β (TGF-β) or enhancing their numbers by injection of skin-derived progenitor cells that differentiate into Schwann cells.[50,51]

All of these techniques were found to promote axon regeneration. In particular, one has been recently brought to fruition in human patients in a small pilot clinical trial.[43,52–54] Patients suffering severe carpal tunnel syndrome were selected with documented loss of at least 50% of the functional motor units in the thenar eminence of the hand (innervated by the injured median nerve). Wallerian degeneration was verified electrophysiologically. All of the patients underwent surgical release of the carpal tunnel by cutting through the overlying ligament. In half of the patients, the median nerve proximal to the compression injury was electrically stimulated at a frequency of 20Hz for 1 hour. The protocol used was previously established to be effective in accelerating axon outgrowth across the surgical site of reunion of a cut femoral nerve in rats.[54] In addition, a motor unit number estimation technique using electromyographic rather than contractile force recordings was used before surgery to establish numbers of remaining motor units and at 3-month intervals after surgery to evaluate muscle reinnervation. Without electrical stimulation, there was only a small increase in the number of innervated motor units over 12 months after carpal tunnel release. In contrast, those patients whose median nerve was stimulated proximal to the site of injury for 1 hour demonstrated significant increases in motor unit numbers within 6 months and complete restoration of numbers of motor units in the thenar eminence by 12 months. These promising results indicate the clinical potential for use of electrical stimulation to promote functional recovery after surgical repair in humans. The effectiveness of this method for ulnar nerve compression at the elbow is being investigated with promising results (Ming Chan, unpublished observations).

In summary, the regenerative capacity of the peripheral nervous system inherent to sensory and motor neurons depends critically on the growth

response in the neurons and the growth support of Schwann cells in the distal nerve stumps of the injured nerve. The growth response of neurons, including upregulation of growth-permissive genes, cytoskeletal proteins, and neurotrophic factors, is relatively short-lived and declines exponentially with time. Similarly the growth permissive state of the Schwann cells deteriorates such that the cells progressively fail to support axon regeneration with declining expression of neurotrophic factors and the low-affinity p75 receptor for the factors. Several techniques have been explored to obviate the negative effects of time and distance, many of which show promising potential.

V. BIOMEDICAL ENGINEERING CHALLENGE AND FOCUS AREAS

V.A. Importance of Biomedical Engineering Contribution to PNS Repair

Biomedical engineers have made significant contributions to PNS repair, yet clearly there are unmet needs and future opportunities. Indeed, surgical techniques will continue to improve, potentially necessitating advancement in tissue engineering, biomaterials, surgical tools, and aids. However, current best practices of autograft surgery require stealing healthy nerves to fix damaged nerves, a practice that needs alternatives. In particular, more effective off-the-shelf alternatives and ultimately, an equal replacement for the autograft are desired. Biomedical engineering will be a major player in the design, manufacture, storage, and implementation of advanced synthetic conduits, incorporation and delivery of neurotrophic factors, or the processing and storage of biological conduits such as acellularized allografts. In addition, the decision of surgical intervention remains ambiguous in some cases, resulting in undesirable delayed repair associated with a poor outcome. Thus, advanced neuroimaging and/or functional assessment of nerve injury and regeneration would be beneficial.

Biomedical engineers must identify the components essential to fulfilling the needs of the clinician. Categorically, the primary current unmet clinical needs lie in three interrelated areas: (1) tissue-engineered nerve grafts, (2) advanced diagnostics, and (3) pathway/target maintenance. In addition, the field would benefit from advanced models capable of replicating important facets of peripheral nerve injury and regeneration both in vivo and in vitro.

V.B. Applications for Growth Conduits, Nerve Grafts, and Tissue-Engineered Constructs

Currently, the most active biomedical research is directed at developing better synthetic nerve conduits, with the goal of producing adequate nerve regeneration across lengths near or slightly exceeding 3 cm. This will satisfy only the subset of short and small-caliber injuries that are commonly repaired via grafting.[4] Long nerve gaps (>3 cm) and proximal nerve injuries such as brachial plexus injuries will continue to be difficult because nerve regeneration progressively fails with distance and time, and the Schwann cells in the distal nerve stumps progressively fail to support axon outgrowth.[35,37,38] While biomedical engineers are eager to exceed the regenerative potential of nerve autografts, work could also be done to create options or enhancements for nerve transfers or develop more economical means of processing and storing acellularized allografts.

Nonetheless, given the limitations in supply and comorbidities associated with autografts, engineered solutions that match or surpass the performance of autografts would be extremely beneficial to improve overall outcome following severe nerve injuries and/or multiple nerve trauma scenarios. A particular area of need is the surgical repair of 4th to 6th degree injuries that necessitate removal of a segment of nerve, often leaving a substantial gap between the proximal and distal ends. Unfortunately, the tension created by pulling the ends together results in the interruption of intraneural blood flow. This is believed to be responsible for tension-induced neuropathy and conduction blockage from the disruption of axons and endoneural tubes or separation of the suture line.[16,22,23] Accordingly, these nerve gaps require a bridging material or graft. Engineered biomaterials and degradable conduits have offered an alternative to autograft-

ing in small-caliber nerves over short 1- to 3-cm lengths. The most pressing unmet clinical need, deserving of substantial biomedical research focus, is improved conduits to support axon growth over longer distances and with the goal of matching and eventually exceeding the efficacy of the autograft.

While autografts remain the gold standard of care, limited donor nerve, donor site morbidity, and the need for an additional surgery have surgeons calling for alternatives. The use of autografts is limited by the size of defect because they are nonvascularized and are subject to central necrosis in large-diameter grafts.[3,9,16,17,29] In addition, the donor nerve needs to be architecturally matched to the anatomical fascicular patterns (number and diameter) of the nerve in repair. Finally, grafts involve two suture lines, which can promote intraneural fibrosis and lead to constriction and compression on the regenerated nerve.[6,9,20] It is clear, however, that for any alternative strategy to be clinically applied, it needs to work as well or better than the autograft. Currently employed alternative strategies are allografts and nerve conduits. Allografts are immunogenic and are typically avoided as discussed above, but the use of de-cellularized allografts is gaining attention.[55] While the nerve architecture is preserved, they require the same cellular infiltration, signaling, and vascularization as nerve conduits, which may limit their use.[20]

More commonly, the surgeon will use an open lumen nerve conduit to constrain axon growth to the distal stump while preventing neuroma formation and infiltration of fibrous tissue. After transection, axoplasm is lost from the nerve and the fibroblasts and Schwann cells secrete several neurotrophic factors.[35] Conduits are thought to localize Schwann cell migration and allow trophic factors to accumulate. A fibrin matrix is formed within the lumen of the conduit accommodating Schwann cells, fibroblasts, and macrophage migration.[2,6,9,15,29] Importantly, conduits must be degradable, as nondegradable conduits must be removed to avoid scar tissue accumulation that leads to nerve compression.[8]

Engineered nerve conduits are considered clinically useful only for noncritical, small-diameter sensory nerves 3 cm or less. First, the volume of the conduit lumen appears to be critical to maintain a high concentration of growth factors.[9] Second, a small diameter is important for the diffusion of nutrients into a nonvascularized area. Third, conduit length needs to be short to allow for complete infiltration of Schwann cells.[9,20] When used on small-caliber sensory nerves up to 3 cm, conduits are better than end-to-end repair. In fact, in some cases results are better than an autograft in gaps less than 1 cm.[3,29] Unfortunately, regenerating nerves do not maintain specificity when using a conduit, and axons cross-innervate the targets.[9,29,31,56]

The goal of a peripheral nerve graft is to direct axon growth towards the disconnected distal nerve, ideally down the correct endoneurial tubes and to the original target.[6,15] For biomedical engineering efforts to be successful there must be consideration of the molecular interactions of normal nerve injury and repair. The autograft has Schwann cells and basal lamina, endoneurial, perineurial, and epineurial architecture, and even unknown phenotypic factors influencing sensory versus motor regeneration.[57] Elucidating these properties will provide enormous potential for growth in the field of nerve tissue engineering. In particular, Schwann cells in an autograft proliferate within the basal lamina lined endoneurial tubes and form the Bands of Bungner, the aligned columns that create a scaffold to guide regenerating axons.[6,15]

The engineering challenges for nerve repair are to accommodate larger deficits (diameter and length), maximize the number of regenerating axons, and guide axons with target specificity. An effective nervous tissue construct may require some combination of three primary components: a scaffold, cells, and signaling factors. Scaffolds provide a temporary structure necessary for Schwann cell migration and axon outgrowth, and are eventually replaced with host cells and extracellular matrix. In nerve conduits, the wound healing response forms a fibrin matrix within the lumen but only over short lengths.[15,58–60] Ideally, an engineered scaffold should serve to mimic the architectural anatomy and extracellular matrix of the injured nerve segment.

Table 2 provides a summary of engineered

constructs developed and tested in animal models using a variety of conduit materials and luminal components. The conduit refers to the cylindrical tube used to approximate the nerve ends, whereas the luminal contents support and guide regenerating axons. The efficacy and test methodology can be found in the original articles cited in the table. The presence of a luminal biomaterial scaffold is essential as a substrate on which cell migration and axon outgrowth can proceed down the conduit length. Many conduit luminal scaffolds have been attempted, from collagen and laminin hydrogels to synthetic and collagen filaments and channels.[2,7,8,15,61–65] However, these modifications have not produced results better than the autograft and therefore do not offer a substantial benefit over the autograft at this time.[3,8,63] Clearly, there are critical factors associated with autografts, or even decellularized allografts, which are yielding superior performance compared to engineered solutions. The systematic determination of these critical success factors may reveal key design criteria for next-generation nervous tissue constructs.

The addition of Schwann cells to nerve conduits is sometimes overlooked and may be an increasingly important component in larger nerve constructs.[8,63,66–68] Axon communication with Schwann cells is not yet fully understood, though it is clear that Schwann cells are a critical component for nerve regeneration.[35,69–71] Schwann cell migration into nerve conduits or acellularized allografts is insufficient beyond 2 cm and is therefore one of the major limiting factors to axonal advancement over large gaps.[6,9,31] To overcome this limitation many studies investigated using exogenous cells within the nerve construct (Table 2). While they have shown great promise, Schwann cells are immunogenic and their use in a nerve conduit requires immunosuppressive therapy unless they are derived from the patient themselves. Further study is needed on autologous Schwann cell isolation and expansion (e.g., proliferation) before they become clinically useful.[71–74] In parallel, techniques that increase host Schwann cell migration should be vigorously pursued, for nerve conduits, acellularized allografts, and ultimately engineered constructs designed specifically for that purpose. Finally, as nerve constructs become larger, mass transport issues will become increasingly important, and pre- or pro-vascularized grafts may be required to maintain viability of transplanted and/or infiltrating cells.

Neuroscience research has produced numerous studies on axon growth and pathfinding throughout embryogenesis and development.[75–77] In addition, there have been investigations on alterations in signaling following nerve injury.[8,35,48,69,75,78–80] Accordingly, research in axon regeneration has considered these factors and has begun to incorporate purified neurotrophins and other signaling factors in nerve conduits.[8,15,63,81] The biggest challenges have been how to incorporate the factor into the conduit and studying the effects of more than one factor at a time. Table 2 also lists many trophic factors that have been investigated in nerve conduits; for reviews, see Refs. 8, 82–84. Currently there are three general biomaterial approaches for local factor delivery: (1) incorporation of factors into a conduit filler such as a hydrogel,[8,15,63,64,67,68] (2) designing a drug release system from the conduit biomaterial such as microspheres, and (3) immobilizing factors on the scaffold that are sensed in place or liberated upon matrix degradation.[61,62,65,82–87]

Solving the complexity of nerve repair can also greatly benefit from creative design. Long nerve gap lengths have been among the most difficult injuries to repair, demonstrating slow rates of regeneration and often incomplete recovery. Thus, the continued development of novel concepts to accommodate longer nerve deficits must be encouraged. One creative approach to bridge larger gaps is the combination of nerve grafts and open conduits in an alternating "stepping stone" assembly, which may perform better than an empty conduit alone.[56] Another is the addition of minced nerve to the lumen of a conduit, with outcomes that exceed those with an empty conduit.[88] In a fundamentally different approach, functional axon fascicles grown in vitro have been used as a persistent pathway to guide regeneration.[89–92]

It is clear that countless specific parameters associated with nerve conduits and/or tissue-engineered grafts need to be considered. Computational

TABLE 2. Engineered Nerve Conduits Tested in Animal and Human Models*

Natural Degradable Conduits

Conduit Material	Luminal Matrix	Cells	GF	Model	Reference
Chitin		SC, BMSC		Rat sciatic	Zhang 2005[258]
Chitosan	PGA filaments			Rat sciatic	Hu 2008[185]
Chitosan	Interposed nerve segments			Rat sciatic	Zhang 2008[259]
Collagen	Collagen or empty	SC		Rat sciatic	Stang 2005[232]
Collagen (Collagen Matrix NeuroMatrix / Neuroflex)				FDA Approved*	Yuen D 2003[256]
Collagen (Integra™ NeuraGen™ Nerve Guide)	Magnetically aligned collagen fibril gel			Mouse sciatic	Ceballos 1999[165]
Collagen (Integra™ NeuraGen™ Nerve Guide)				Human / FDA Approved	Archibald 1991;[138] Li 1992;[193] Ashley 2006;[158] Lohmeyer 2007[194]
Collagen (Kevlar reinforced)	Saline or empty	SC		Rat sciatic	Ansselin 1997[155]
Collagen	Collagen filaments			Rat sciatic	Yoshii 2001,[252] 2002,[253] 2003[254]
Collagen with laminin coat				Rat sciatic	Kauppila 1993[190]
Fibronectin mats (Oriented)	Fibronectin mats, hyrogel		NT-3	Rat sciatic	Sterne 1997[233] used NT-3; Whitworth 1995[245]
Gelatin	Gelatin fibers, laminin, fibronectin		NGF	Rat sciatic	Gamez 2004[176]
Heparin and alginate hydrogel			bFGF	Rat sciatic	Ohta 2004[218]
Human amnionic membrane	Hyaluronic acid		NGF	Rabbit peripheral	Mohammad 2000[209]
None	Alginate sponge			Rat sciatic	Hashimoto 2002[182]
Silk fibroin (SF)	Oriented silk fibroin filaments			Rat sciatic	Yang 2007[251]

TABLE 2. Engineered Nerve Conduits Tested in Animal and Human Models* (continued)

Synthetic Degradable Conduits

Outer Conduit Material	Luminal Matrix	Cells	GF	Model	Reference
Biodegradable (not specified)	Laminin gel			Mouse sciatic	Madison 1985,[200] 1987[201]
Biodegradable glass				Sheep facial	Gilchrist 1998[178]
Glycolide trimethylene carbonate (GTMC, Maxon®) or collagen				Primate ulnar, radial sensory	Mackinnon 1990[199]
Glycolide trimethylene carbonate (GTMC)	Collagen gel			Rat peroneal	Rosen 1992[224]
PGA and collagen	Laminin-coated collagen fibers or sponge			Canine peroneal	Matsumoto 2000;[203] Toba 2002[234]
Poly-3-hydroxybutyrate (PHB)	Alginate, fibronectin hydrogel	SC	rhLIF	Rat sciatic	Mosahebi 2001,[213] 2002,[211] & 2003[212] used SC; McKay Hart 2003[204] used rhLIF
Poly-3-hydroxybutyrate (PHB)	Alginate, fibronectin hydrogel		rhGGF2	Rabbit peroneal	Mohanna 2005[210]
Poly-3-hydroxybutyrate (PHB)	Fibrin gel	SC, dMSC		Rat sciatic	Kalbermatten 2008[189]
Poly-D/L-lactic acid (PDLLA)			bFGF	Rat sciatic	Wang 2003[241]
	Micropatterned lumen	SC		Rat sciatic	Rutkowski 2004[225]
Poly-D/L-lactide-ε-caprolactone (PDLLA/CL, Polyganics Neurolac®)				Human / FDA Approved	Meek 2004[206]
Poly-D/L-lactide-ε-caprolactone (PDLLA/CL)	Skeletal muscle-enriched			Rat sciatic	Varejao 2003[237]
Poly-D/L-lactide-ε-caprolactone (PDLLA/CL)	Matrigel	SC		Mouse sciatic	Rodriguez 2000[222]
Poly-L-lactide-ε-caprolatone (PLL/CL) or polyphosphazene				Rat sciatic	Nicoli Aldini 2000[215]

TABLE 2. Engineered Nerve Conduits Tested in Animal and Human Models* (continued)

Synthetic Degradable Conduits

Outer Conduit Material	Luminal Matrix	Cells	GF	Model	Reference
Poly-ε-caprolactone (PCL)				Rat sciatic, peroneal	Vleggeert-Lankamp 2007[239]
Polycaprolactone-co-ethyl ethylene phosphate (PCLEEP)	PCLEEP fibers		GDNF	Rat sciatic	Chew 2007[171]
Polyglycolic acid (PGA, Synovis Neurotube®)	Saline			Human digital / FDA Approved	Mackinnon 1990;[199] Weber 2000[243]
Polyglycolic acid (PGA)				Human digital	Casanas J 2000[164]
Polyglycolic acid (PGA)	Collagen gel			Rat peroneal	Rosen 1990[223]
Polyglycolic acid (PGA) and collagen	Collagen sponge			Human digital, superficial peroneal, intrapelvic	Hagiwara 2002;[181] Inada 2005[186]
Polyglycolic acid (PGA, collagen coated)	Laminin-soaked collagen sponge or fibers			Canine peroneal	Toba 2001[235]
Polylactic acid (PLA) or Silicone	Collagen gel	SC		Rat sciatic	Evans 2002[173]
	PLLA fibers, Matrigel			Rat sciatic	Cai 2005[163]
Polylactic-co-glycolic acid (PLGA)	Rat tail glue, laminin	SC		Rat sciatic	Chang 2006,[166] Cheng 2002[170] used rat tail glue & laminin; Chang 2007[167] used SC
Polylactic-co-golycolic acid (PLGA)	Collagen gel	EMSC		Rat sciatic	Nie 2006[216]
Polylactic-co-glycolic acid (PLGA, foam)	PLGA channels, laminin	SC		Rat sciatic	Hadlock 2000[180]

TABLE 2. Engineered Nerve Conduits Tested in Animal and Human Models* (continued)

Synthetic Degradable Conduits

Outer Conduit Material	Luminal Matrix	Cells	GF	Model	Reference
Polylactide-glycolide-ε-caprolactone (PLG/CL)	Fibrin gel			Rat sciatic	Nakayama 2007[214]
Polyterephthalate-ethyl phosphoester (PPE)				Rat sciatic	Wang 2001[242]
Trimethylenecarbonate-ε-caprolactone (TMC/CL)	Fibrin gel, Matrigel	SC		Rat median	Sinis 2005[229]

Synthetic Nondegradable Conduit

Outer Conduit Material	Luminal Matrix	Cells	GF	Model	Reference
Expanded Polytetrafluoro-ethylene (e-PTFE, Gore-tex)				Human inferior alveolar, lingual	Pogrel 1998[220]
Expanded Polytetrafluoro-ethylene (e-PTFE, Gore-tex)	Heparin			Human ul-nar, median	Stanec 1998,[230] 1998[231]
Poly-2-hydroxyethyl methacrylate-co-methyl methacrylate (PHEMA-MMA)	Collagen gel		aFGF, BDNF, NT-3	Rat sciatic	Midha 2003[208]
Polyacylonitrile / polyvinyl-chloride (PAN/PVC)	Matrigel	SC		Rat sciatic	Guenard 1992[179]
Polyacylonitrile methacry-late (PAN-MA)	Aligned PAN-MA fibers			Rat sciatic	Kim 2008[191]
Polyethylene (PE) or bio-degradable conduit	Laminin gel			Mouse	Madison 1987[201]
Polyethylene (PE), Polyvi-nyl (PV) and rubber tubes				Human radial	Garrity 1955[177]

TABLE 2. Engineered Nerve Conduits Tested in Animal and Human Models* (continued)

Synthetic Nondegradable Conduits

Outer Conduit Material	Luminal Matrix	Cells	GF	Model	Reference
Polyethylene-co-vinyl acetate (PEVA, Dupont Elvax®) / BSA rods			GDNF, NGF, NT-3	Rat facial	Fine 2002;[174] Barras 2002[159]
Polysulfone	Agarose hydrogel, laminin		NGF	Rat sciatic	Yu 2003;[255] Dodla 2008[61]
Polysulfone	Biomatrix(~Matrigel), collagen, or methylcellulose		PDGF-BB, IGF-I	Rat sciatic	Wells 1997[244]
Silastic® (Dow Corning) / Silicone	Bioglass® 45S5 fibers			Rat sciatic	Bunting 2005[162]
Silicone	Keratin hydrogel			Mouse tibial	Sierpinski 2008;[228] Apel 2008[156]
Silicone	Aligned collagen fibrils	Fibro-blasts, SC		Rat sciatic	Phillips 2005[219]
Silicone	Aligned polyamide, cat gut, polydioxanone, polyglactin filaments			Rat sciatic	Arai 2000[157]
Silicone	Blood plasma	SC		Rat sciatic	Nilsson 2005[217]
Silicone	Collagen or laminin gel			Rat sciatic	Satou 1986;[226] Madison 1988[202]
Silicone	Collagen or PLA fila-ments			Rat sciatic	Itoh 2001[187]
Silicone	Collagen, laminin, & fibronectin gel			Rat sciatic	Chen 2000[169]
Silicone	Fibrin gel			Rat sciatic	Williams 1987[59, 246]
Silicone	Fibrin gel		GDNF, NGF	Rat sciatic	Wood 2009[248]
Silicone	Gelatin	BMSC		Rat sciatic	Chen 2007[168]
Silicone	Heparin, fibrin gel		NGF	Rat sciatic	Lee 2003[192]

TABLE 2. Engineered Nerve Conduits Tested in Animal and Human Models* (continued)

Synthetic Nondegradable Conduits

Outer Conduit Material	Luminal Matrix	Cells	GF	Model	Reference
Silicone	Hyaluronic acid (fibrin)			Rat sciatic	Seckel 1995[227]
Silicone	Interposed nerve segments			Rat sciatic	Maeda 1993;[56] Francel 1997[175]
Silicone	Collagen - magnetically aligned or Matrigel			Mouse sciatic	Verdu 2002[238]
Silicone	Matrigel		VEGF	Rat sciatic	Hobson 2000[184]
Silicone	Motor, sensory nerve fragments			Rat sciatic	Lloyd 2007[88]
Silicone	Polyamide filaments			Human median, ulnar, Rat sciatic	Lundborg 1997[195, 198]
Silicone			NGF, CNTF	Rat sciatic	Rich 1989;[221] He 1992;[183] Zhang 2004[257]
Silicone			βNGF, GDNF	Mouse sciatic	Unezaki 2009[236]
Silicone	Valproic acid (VPA)			Rat sciatic	Wu 2008[249]
Silicone				Human median, ulnar	Merle 1989;[207] Lundborg 1991,[196] 1994;[197] Braga-Silva 1999[161]
Silicone or Polyphosphoester (PPE)	PPE microspheres		NGF	Rat sciatic	Xu 2003[250]
Synthetic (not specified)			aFGF	Rat sciatic	Walter 1993[240]

*Efficacy and test methodology can be found in the original articles. Conduits were sorted alphabetically by conduit material, then luminal filler. The data for this table were combined and modified using the reviews of Jiang 2010,[188] Schmidt & Leach 2003,[8] Meek & Coert 2002[29] and 2008,[205] and Yan 2009,[63] and missing studies have been added. Outer conduit material refers to the cylindrical tube used to approximate the nerve ends. The luminal matrix is the material contained within the conduit serving as a regeneration scaffold. Cells and growth factors placed in the lumen are identified: SC = Schwann cells; BMSC = bone marrow stromal cells; dMSC = differentiated mesenchymal stem cells (SC-like); EMSC = ectomesenchymal stem cells; NT-3 = neurotrophin 3; NGF = nerve growth factor; FGF = fibroblast growth factor; LIF = murine leukemia inhibitory factor; GGF2 = human glial growth factor 2; GDNF = glial-derived neurotrophic factor; BDNF = brain-derived growth factor; PDGF = human platelet–derived growth factor BB; IGF-I = insulin-derived growth factor type 1; VEGF = vascular endothelial growth factor; CNTF = ciliary neurotrophic factor.

models can be useful in helping to organize and prioritize the importance of various design criteria.[93–98] For example, models could provide insight into increasing conduit length demands, improving mass transport, and enhancing vascularization and cellular migration. Computational models could also be used to predict how these parameters change with varied diameter conduits and identify critical limitations.

V.C. Advanced Injury Diagnostics and Regenerative Tracking

Current methods to assess the extent of nerve injury or potential of recovery in human patients are not accurate enough to make early surgical decisions in every case.[99–101] Peripheral nerve injuries are typically diagnosed by clinical examination and in some cases with the aid of electrophysiological data. Gross and fine function and evoked potentials are effective in correctly distinguishing minor neurapraxia from axonotmesis and neurotmesis. Using current diagnostic techniques, however, it is difficult to precisely discriminate between 2nd, 3rd, and 4th degree lesions as classified on the Sunderland scale, making the necessity of surgical intervention unclear. In particular, 2nd degree injuries typically recover spontaneously and should not receive surgery. Third degree injuries can recover in a similar spontaneous fashion; however, if there is scarring of the endoneurium, effective regeneration cannot proceed without surgical decompression and removal of the scar tissue. Fourth degree injuries, where the perineurium is disrupted, will require surgery in almost all cases for functional restoration to be achieved.

In cases of surgical uncertainty, it often takes several months to years as physicians monitor signs of recovery. Upon determining that regenerative restoration of function will not occur, the most opportune time for surgical intervention has passed. Since acute repair leads to better functional restoration, delays introduced by "wait-and-see" diagnostics can be costly. When surgical intervention occurs months after injury, regeneration will ensue in an environment not optimized for axonal regeneration that is marked by degeneration of the distal nerve support structure and target atrophy. Moreover, in 3rd degree injuries that eventually require surgery, the primary hindrance to regeneration is the scar tissue that develops after the injury—underscoring the need to periodically evaluate the regenerative environment and make surgical decisions as quickly as possible. When restoration of function does not occur and incomplete healing of a nerve injury is suspected, the current state of the art for diagnosis is invasive exploration. Thus, surgical decision-making can be greatly improved by noninvasive diagnostic methods that can accurately assess peripheral nerve injury severity as well as track regenerative progress.

Many potential noninvasive diagnostics with the ability to track axonal regeneration are still in experimental phases. In particular, advanced neuroimaging strategies are being developed with routines capable of accurate assessment of the initial degree of nerve injury and tracking axonal regeneration either directly or indirectly. Specifically, advanced magnetic resonance imaging (MRI) routines are providing promising solutions to this problem, but have only recently been used in this capacity.[102–106] These techniques build on the seminal work of Howe et al., who developed MRI routines to specifically image nerves.[107]

A summary of seminal studies applying MRI with various protocols to grade injury severity and assess regeneration is presented in Table 3. These studies include both clinical (human) applications and animal studies, with the latter using controlled injuries and histopathological, electrophysiological, and/or behavioral correlations. It is important to note that in a few animal studies, damaged nerves were excised prior to imaging to acquire sufficient resolution and remove motion artifacts associated with respiration. The principles applied in these cases, however, provide valuable proof of concept. Particularly promising techniques to differentiate between healthy and injured nerves exploit the anisotropy in the longitudinally aligned axons and nerve sheaths, such as diffusion tensor imaging (DTI) and tractography.[11–14,108] Notably, DTI has also been use to image white matter tracts in the brain.[10,109] Adjunct technologies such as axonal

tracers and contrast agents that are detectable via advanced or standard MRI, could also be used as guides to improve nerve diagnostics and regenerative tracking.

Several recent studies have demonstrated the use of MR neurography to indirectly or directly assess nerve degeneration and, in some cases, track myelin reorganization and/or axonal regeneration.[110–119] Despite initial concerns that MRI does not provide high-resolution images of nerve, several innovations, including fat suppression, optimized pulse sequence echo times, and T2-weighted scans, have provided high-fidelity images of peripheral nerves. Using these MR protocols, damaged nerves present a hyperintense signal on T2-weighted MR images that is often associated with axons undergoing Wallerian degeneration in the distal nerve segment.[110–114,117] Moreover, this hyperintensity attenuates following successful regeneration. Although increased signal intensity in T2-weighted images is a good indicator of degeneration, the image can also be affected by edema and inflammation. Accordingly, the actual cause of increased signal intensity could be multifold. First, it may be the result of obstruction of axoplasm leading to increased water content of the nerve. Second, it could be due to compression, which in turn causes Wallerian degeneration and a breakdown of myelin. Finally, it may be from impeded venous blood flow causing greater epineurial water content. Thus, one or more of several pathologies, including inflammation, axonal damage/degeneration, and/or demyelination may result in the observed MR signal changes. Histological analysis is often necessary to determine the actual cause.

MRI performed with the use of specialized contrast agents and/or axonal tracers has additional promise to increase the specificity in assessing and tracking axonal changes in damaged nerves.[120,121] For instance, the experimental contrast agent gadofluorine-M (Gf) has been used with T1 scans to identify peripheral nerve degeneration and regeneration.[120,122] Interestingly, Gf was taken up only by damaged portions of the nerve that were undergoing Wallerian degeneration and/or loss of myelination. The contrast enhancement was seen for a shorter period of time proximal to the injury than distal to the injury and therefore could be used to trace regeneration. However, this contrast agent was delivered systemically, and concerns about invasiveness and potential toxicity may limit clinical applicability. In another approach, injection of Mn^{+2} into the distal portion of the injured nerve is retrogradely transported.[121] Correlation was found between the MR signal intensity and retrograde tracing of Mn^{+2} in this experiment, indicative of regenerating axons; however, further investigations are needed to establish this agent for clinical PN injury. In the future, these strategies could be deployed in conjunction with other cutting-edge technologies such as molecular imaging and image enhancers that can be specifically engineered to track nerve degeneration and axonal regeneration.

Proton MR spectroscopy may also prove to be useful in diagnosing nerve degeneration and regeneration through the ability to measure the concentration and diffusion of specific metabolites such as *N*-acetyl aspartate (NAA). NAA is exclusively expressed in neurons and their axons. Reduction of NAA levels would be indicative of demyelination and axonal loss (suggesting degeneration), whereas restoration of NAA levels may be useful to track regeneration by following the leading front of regenerating axons. Using this technique, the anisotropic diffusion of metabolites, including NAA, was investigated in excised frog peripheral nerve.[123] Concerns over this technique include lack of specificity, as NAA levels in the axon could fluctuate for a number of reasons, not just physical compression or transection injuries. Currently, sufficient resolution can only be attained using excised nerves.

Diffusion-weighted imaging (DWI) and diffusion tensor imaging (DTI) are perhaps the most effective MRI methods for tracking peripheral-nerve degeneration and regeneration by taking advantage of both the diffusion of water and the anisotropic properties of axons.[13,108] In a given environment without any impediments, water will exhibit Brownian motion and diffuse randomly. In the presence of axons, the myelin sheath hinders the diffusion of water across the nerve fiber, creating a preferential path for diffusion longitudinally along

TABLE 3. Noninvasive Imaging Studies to Identify Axonal Degeneration and Assess/Track Regeneration

Reference	Injury Model	Imaging Method and Routine	Experimental Groups	Findings	Summary
Lehmann 2010[13] EP + Histology+ Behavior –	Mice (multiple strains) Sciatic nerve crush (30sec w/ forceps) or transection	MRI: 11.7T, 15-mm coil DTI and tractography	Nerve crush Four different mouse strains (n = 4–12 each) Nerve transection (n = 10) Noninjured (n = 8)	• Nerve crush: decrease in FA that subsequently normalized with regeneration • Nerve transection: greater decrease in FA that did not recover • Recovery and regeneration in the nerve crush group corresponded to CMAP measurements of muscle activity • FA and λ1 values correlated to histological evidence of myelinated axons 17d post injury • FA can be used to monitor regeneration: decrease in FA at given location indicative of degeneration. Normalization indicative of regeneration • DTI findings were consistent across mice strains	Identify degeneration: Y Assess regeneration: Y Track regeneration: Y
Matsuda 2010[21] EP – Histology + Behavior +	Sprague-Dawley rats Sciatic nerve crush (30 sec w/ forceps) or transection	MRI: 4.7T Protocol: T1W Manganese used as retrograde axonal tracer	Nerve crush 3 d, 2 w, 4 w, 12 w time-points (n = 6 each) Nerve transection (n = 4) Noninjured (n = 6)	• Reduced MR signal intensity proximal to injury site • Gradually increased with recovery in nerve crush group; however, remained low in transection group • MR signal intensity correlated to both SFI and retrograde axonal tracing using fluorogold	Identify degeneration: Y Assess regeneration: Y Track regeneration: N
Swanger 2010[19] Case Report EP – Histology –	36 yr old male Transected ulnar nerve following chainsaw injury Surgical repair	MRI: 1.5T Protocol: T1W, T2W	N/A	• MRI at site of transection: nerve fascicles not distinct; signs of edema. • MRI above and below injury: nerve fascicles distinctly visible • MRI demonstrated anatomic regeneration of nerve post-surgery	Identify degeneration: Y Assess regeneration: Y Track regeneration: N.I.

TABLE 3. Noninvasive Imaging Studies to Identify Axonal Degeneration and Assess/Track Regeneration (continued)

Reference	Injury Model	Imaging Method and Routine	Experimental Groups	Findings	Summary
Takagi 2009[108] EP – Histology + Behavior +	Sprague-Dawley rats Sciatic nerve crush (brain aneurysm clip 150g x 5min)	MRI: 7.0T, 38-mm coil DTI + tractography	Time-points: 3 h, 1 d, 4 d, 1 w, 2 w, 3 w, 4 w, 6 w, 8 w, 12 w (n = 10 each) Noninjured (n = 10)	• FA decreased at different time periods post injury depending on the distance from the site of injury • Reduction in FA seen sooner post injury in sites closer to the lesion. λ1 value high, λ2 and λ3 values did not have any influence • Findings correlated to histological, behavioral, and quantitative analysis indicating demyelination, functional impairment, reduced axon size/density and myelin sheath inflammation	Identify degeneration: Y Assess regeneration: Y Track regeneration: Y
Behr 2009[111] EP – Histology + Behavior –	Lewis rats Sciatic nerve transection	MRI: 4.7T, 70-mm coil Protocol: T2W	Surgical Repair Coaptation (n = 33) No repair (n = 33) Noninjured (n = 4) Time-points: 3 d, 6 d, 10 d, 14 d, 21 d, 28 d, 42 d, 63 d, 84 d	• Both groups displayed increased signal intensity initially • These levels normalized faster in rats with coapted nerves (21d post operation) as opposed to rats with nerves left unrepaired (42d post operation)	Identify degeneration: Y Assess regeneration: Y Track regeneration: Y
Li 2008[117] EP – Histology + Behavior +	New Zealand rabbits Sciatic nerve crush (microvessel clamp 3.6 kg x 5 min or 10.5 kg x 5 min	MRI: 1.5T, 80-mm circular surface coil Protocol: T1W, 3DT2W, T2W, STIR, SPIR, B-FFE	Crush strength 3.6 kg (n = 16) 10.5 kg (n = 16) Noninjured (n = 10) Contralateral nerves (n = 22) Time-points: 1 w, 2 w, 4 w, 8 w	• SIR higher at distal site than proximal site • SIR increased post-injury until 2 wks, normalized from 4–8 wks • Finding corresponded to histological and behavioral indications of degeneration and functional impairment of hind limb, followed by Schwann cell proliferation and restoration of function • T2W provided best diagnostic rate of 93.75%	Identify degeneration: Y Assess regeneration: Y Track regeneration: Y

TABLE 3. Noninvasive Imaging Studies to Identify Axonal Degeneration and Assess/Track Regeneration (continued)

Reference	Injury Model	Imaging Method and Routine	Experimental Groups	Findings	Summary
Bendszus 2005[120] EP – Histological + Behavioral -	Wistar rats Sciatic nerve crush (60sec w/ forceps; unilateral or bilateral), transection, or CCI	MRI: 1.5T Protocol: fat suppressed T1W with contrast agent: Gadoflourine M	Robust experimental design varying injury, time-points Gf was administered, and MRI time-points (n = 66 total; see chart in original paper)	• Only degenerating nerve took up Gf • Proximal site no longer exhibited contrast enhancement by 2 wks post-injury • Distal site no longer took up contrast at 5 wks • Technique promising to track regeneration	Identify degeneration: Y Assess regeneration: Y Track regeneration: Y
Bendzus 2004[112] EP + Histological + Behavioral -	CD rats Sciatic nerve ligation (suture for 1 wk)	MRI: 1.5T, 40-mm round surface coil Protocol: T1W, T2W, TIRM, fat suppressed T1W	MRI before, after surgery: 0 d, 1 d, 2 d, 4 d, 7 d, then weekly until 90 d (n = 5) EMG and CMAP before, after surgery: 1–4 d, then weekly from 7–90 d (n = 9) Histology: 1 d, 2 d, 1 w, 4 w, 6 w, 13 w post-injury (n = 4 each)	• Hyperintense T2W signal distal to injury within 24hrs • Hyperintense signal gradually receded proximally to distally • CMAP in foot recovered at 12 wks, 2 wks after hyperintense signal was resolved • MR signal changes histologically correlated with axonal degeneration and nerve edema • MR findings parallel EP findings, with time shift	Identify degeneration: Y Assess regeneration: Y Track regeneration: Y
Aagaard 2003[110] EP -Histological + Behavioral +	Lewis rats Sciatic nerve crush (10 sec w/ forceps) or transection w/ cap	MRI: 1.5T Protocol: T2W, T1W, STIR Contrast agent: gadofluorine-M (crush, n = 6; transection, n = 6)	Nerve crush (n = 18) Nerve transection (n = 17) Noninjured (n = 10) Time-points: 7 d, 10 d, 14 d, 21 d, 28 d, 35 d, 42 d, 60 d, 90 d	• Longer hyperintense signal in cut vs. crushed nerves • Corresponded to behavioral and histological evidence of hastened regeneration in crushed nerves • Denervated muscles showed normal signals after recovery; chronically denervated muscles displayed consistently elevated signals and atrophy	Identify degeneration: Y Assess regeneration: Y Track regeneration: Y

TABLE 3. Noninvasive Imaging Studies to Identify Axonal Degeneration and Assess/Track Regeneration (continued)

Reference	Injury Model	Imaging Method and Routine	Experimental Groups	Findings	Summary
Lacour-Petit 2003[116] Case Reports EP + Histological +	6 human patients (3 male, 3 female; ages 16–63) with nerve palsy	MRI: 1.5T Protocol: T2W, STIR, T1W	Unilateral peroneal nerve palsy (n = 5) Unilateral tibial nerve palsy (n = 1)	• Two patients had high T2 signal intensity indicative of hypertrophy • Biopsy in one of these patients displayed minimal axon degeneration • MRI may be useful to identify the location and length of abnormal nerve	Identify degeneration: Y Assess regeneration: N.I. Track regeneration: N/A
Cudlip 2002[113] EP – Histological – Behavior +	Wistar rats Sciatic nerve crush (20 sec w/ forceps)	MRI: 4.7T, 50-mm coil Protocol: T2W	Nerve crush (n = 12) Non-injured (contralateral nerves, n = 12) Time-points: 7 d, 14 d, 30 d, 70 d	• Increased signal intensity following nerve crush, peaking at 14 d and normalized by 70 d • Corresponded to functional walking track analysis	Identify degeneration: Y Assess regeneration: Y Track regeneration: N.I.
Stanisz 2001[118] EP – Histology + Behavior -	Lewis rats Sciatic nerve crush (1 min w/ forceps) Sciatic nerve transection w/ suture	MRI: 1.5 T Protocol: T1W, T2W, MTW, DW	Nerve crush (n = 15) Nerve transection (n = 15) Noninjured (n = 3) Time-points: 1 w, 2 w, 3 w, 4 w, 6 w	• Increased T1/T2 relaxation times, reduced MT, increased ADC perpendicular to axon and reduced diffusion anisotropy • Correlated with histology revealing distal demyelination, axonal loss and inflammation • MR changes more prominent in cut (non-regenerating) vs. crushed (regenerating) nerves • MR changes resolved within 4 wks following crush, but not following cut	Identify degeneration: Y Assess regeneration: Y Track regeneration: N.I.

TABLE 3. Noninvasive Imaging Studies to Identify Axonal Degeneration and Assess/Track Regeneration (continued)

Reference	Injury Model	Imaging Method and Routine	Experimental Groups	Findings	Summary
Dailey 1997[114] Case Study EP + Histology –	29-year-old male w/ laceration of right sciatic nerve; complete peroneal nerve transection	MRI: 1.5T Protocol: T1W, T2W with fat suppression	N/A	• Acute surgical anastomosis failed to result in reinnervation • At 4 and 6 mos post operation, T2W signal was increased distal to transection • At 8 mos, 2nd operation performed to remove scar tissue and bridge gap w/ sural nerve grafts • Over next 8–16 mos, T2 signal began to normalize, muscle strength improved, and EMG showed functional restoration • T2 signal at graft remained elevated	Identify degeneration: Y Assess regeneration: Y Track regeneration: N.I.
Does and Snyder 1996[115] EP – Histology + Behavior -	African clawed toads Nerve crush (30 sec w/ nylon thread)	MRI: 2.35T Protocol: T2W	Time points: 4 d, 7 d, 11 d, 14–16 d, 21–25 d, 28–35 d (n = 18 total)	• Components of T2 relaxation spectra changed following crush • Changes correlated with Wallerian degeneration • Specific components of the relaxation spectra correlated with loss of myelinated fibers, interstitial edema, and myelin loss.	Identify degeneration: Y Assess regeneration: N.I. Track regeneration: N/A

Key: 1st column: EP: Electrophysiology; "+" indicates study contains this outcome; "–" indicates study does not contain this outcome.
3rd–6th columns: ADC: Apparent Diffusion Coefficient; B-FFE: Balanced Fast-Field Echo; CCI: Chronic Constriction Injury; CMAP: Compound Muscle Action Potential; DW: Diffusion Weighted; DTI: Diffusion Tensor Imaging; DTT: Diffusion Tensor Tractography; EMG: Electromyography; FA: Fractional Anisotropy; Gf: Gadofluorine-M; MR: Magnetic Resonance; MTW: Magnetization Transfer Weighted; N: No—indicates that study did not successfully demonstrate this ability; NAA: *N*-Acetyl Aspartate; N/A: Not applicable to study; N.I.: Not investigated in study; NMR: Nuclear Magnetic Resonance; SFI: Sciatic Function Index; SPIR: T2-Weighted turbo spin echo images with spectral presaturation with inversion recovery; SIR: Signal Intensity Ratio; STIR: Short-Time Inversion Recovery; T1W: T1-Weighted; T2W: T2-Weighted; 3DT2WI: 3 Dimension Turbo Spin-Echo T2-Weighted; $\lambda 1$: Eigenvalue for parallel diffusivity; $\lambda 2$ and $\lambda 3$: Eigenvalues for perpendicular diffusivity; Y: Yes—indicates study successfully demonstrated this ability

the fiber. DWI measures the rate of water diffusion in tissue to determine the apparent diffusion coefficient (ADC), a measure of diffusivity. Following nerve injury, the ADC increases perpendicular to the nerve (axon) orientation. Alternatively, the state of the target muscle may also be analyzed to indirectly assess axonal degeneration. The ADC of denervated muscles is higher than that of normal muscles due to greater disorder, making this measure a useful diagnostic for denervation.[124]

DTI builds upon this principle, but provides a tensor that includes both the magnitude and direction of water diffusivity in multiple dimensions. DTI is the most powerful technique to image tissue with anisotropic organization, such as peripheral nerve. The tensor will produce a sphere if the tissue is isotropic and become ellipsoidal with tissue anisotropy. The direction of diffusion along an ellipsoid is determined from the tensor eigenvalues. Eigenvalue $\lambda 1$ is representative of the parallel diffusion along the ellipsoid, and $\lambda 2$ and $\lambda 3$ are representative of the perpendicular directions. Combining sequential tensor measurements mathematically allows fibers to be traced through tractography. Currently, fractional anisotropy (FA) is being tested as a measure of degeneration and regeneration in peripheral nerves. FA is a measure of relative anisotropy from the eigenvalues, where 0 is isotropic and 1 is anisotropic. Recently, this technology has been applied to track the regeneration of peripheral nerves following crush injury in mice.[13,108] Here, lesions result in decreased FA corresponding to destruction of the myelin sheath and therefore greater disorder in the motion of water diffusion. This was found to be primarily dependent upon the parallel diffusivity ($\lambda 1$ eigenvalue). Thus, FA values at different points from the site of injury combined with diffusion tensor tractography served as mechanisms to trace regeneration within a given nerve.

Until advances in imaging technology move to the clinic, many valuable improvements can be made in traditional electrophysiological diagnosis of nerve injury and regenerative tracking. Electromyography (EMG) with recording of evoked compound action potentials is the most commonly used method to determine nerve connectivity. This technique is more useful for superficial nerves than for deep nerves. Unfortunately, recordings of the compound action potentials are somewhat limiting since they do not directly reveal details of the nerve injury such as the length or number of nerve fibers damaged. Recordings of single unit action potentials on the other hand provide information on the number of innervated and functional motor units in the muscle of interest.[54,125] Biomedical engineering strategies may be developed to contribute to better diagnostics and can improve upon the ubiquitous measurements of nerve conduction and muscle stimulation. For instance, novel nervous tissue interfaces and algorithms could potentially be transformed into diagnostic devices such as miniature and multielectrode stimulators and recorders.

Key challenges need to be addressed before noninvasive imaging or electrophysiological evaluation can become the standard of care in nerve injury diagnosis and regenerative tracking. Currently, these techniques are experimental and require future refinement; thus additional studies are necessary to set thresholds and determine clinical applicability. Improvements in MR capabilities may also be required: a key challenge is the imaging resolution capabilities versus the size of the features to be measured. Axons typically measure 5–20 microns in diameter and with myelination typically measure 10–25 microns in diameter. Typical voxel sizes are on the order of millimeters, with highest MR resolution on the order of hundreds of microns. With older MR technology, common in many hospitals, the voxel size may be larger than the nerve of interest, so sufficient resolution becomes exceedingly difficult. The future development of easily implemented, highly specific contrast agents with little or no side effects may mitigate these issues. However, currently these techniques require specialized expertise and often-expensive, state of the art imaging technology. Thus, in the near term, this technology may be limited to larger nerves such as the brachial plexus to find broad application. Despite these challenges, the future development and validation of advanced imaging modalities capable of assessing axonal tract integrity and/or regen-

erative rate would be beneficial to properly grading nerve injuries, to assess the progress of spontaneous nerve regeneration, and to establish those cases of nerve injury that require surgical intervention with less ambiguity and much earlier.

V.D. Pathway and Target Maintenance

Degeneration of the axonal segment in the distal nerve is an unavoidable consequence of disconnection. However, the distal nerve support structure as well as the final target must maintain efficacy to guide and facilitate appropriate axonal regeneration. Although the distal pathway initially transforms into a pro-regenerative environment, the pathway ultimately loses the capacity to support robust regeneration on the order of several months post-injury (i.e., post-axonal loss). However, there are currently few strategies directly targeted at maintaining distal pathway and/or target fidelity. Biomedical engineering strategies may be applicable to target pathway degeneration, for instance, through localized delivery of factors that may maintain the pro-regenerative capacity, including the de-axonized distal nerve structure required to support targeted axonal regeneration as well as the sensory/motor targets that must retain the ability to function and re-integrate with the nervous system. Such targeted delivery of neurotrophic agents may maintain the efficacy of the distal pathway over extended periods of time, thus increasing the degree of axonal regeneration, innervation, and functional recovery.[50,51]

A myriad of factors affect relevant cell signaling pathways and should be considered as adjunct treatment for injuries near the midline that require many months (or years) for functional restoration. In addition, many of these factors affect cell extension and organization and thus should be considered as critical components to the advancement of nerve tissue constructs. Chemotrophic factors are needed to promote cell survival and enhance axonal growth.[35] Chemotrophic factors can also be used to enhance Schwann cell migration, which in turn guides axon advancement. The interplay, however, between endogenously loaded trophins and those produced by Schwann cells is complex and merits careful consideration. Moreover, the effects of neurotrophic factors on nerve regeneration are vast and complex, with sometimes dichotomous effects based on situation-specific parameters, including injury type or severity, timing, and co-delivered factors (see Refs. 8, 35, 48, 69, and 126 for reviews).

Of specific interest is how end targets, such as muscle, provide strong specificity signaling for regeneration of nerve fibers. Stimulation or mimicry of end targets may play an important role in keeping extension on a preferred path. For example, the chemotrophin BDNF has been shown to promote motor neuron survival and outgrowth. In muscle, it may serve as a stop signal for regenerating axons.[127] Thus, an engineered chemotrophic gradient of BDNF would be better suited than a uniform concentration to the advancement of nerve fibers across long gaps. Since scaffolding and extracellular matrix also play a large role in chemotaxis, controlled delivery of neurotrophins may be advanced through incorporation in biomaterial scaffolds.

V.E. Modeling Peripheral Nerve Injury and Repair

The complex, multifaceted nature of peripheral nerve injury makes it difficult to use a single in vivo model due to a wide variety of scenarios, such as repetitive compression injuries (e.g., carpal tunnel), transection injuries, and/or traumatic crush injuries. Additionally, modeling of peripheral pain, neuroma, and scar tissue is often extremely complex and thus purposely limited in studies. The sciatic nerve is the most commonly used model. While a convenient nerve to use, the sciatic is not optimal due to heterogeneity—innervating multiple sensory, synergistic, and opposing muscle targets. For these reasons, some researchers use an upper-extremity nerve, such as the median nerve in rats, which is primarily a motor nerve and thus amenable to forearm reach/grip behavioral tasks.[128–131] Moreover, to overcome inherent length issues in this model, a cross-chest repair and innervation model was developed.[132] However, for the particular task of testing repair strategies for long (>3 cm) nerve defects, larger animal models are required, and typically include rabbits, canines,[133–137] and nonhuman

primates.[28,31,138–141] However, large animal studies are expensive and time-consuming. This is particularly the case in modeling long nerve defects and/or near-midline injuries, which unfortunately are two of the most pressing needs in clinical nerve repair. Taken together, a more reliable, standardized, translatable animal injury model is needed to assess mechanisms associated with nerve injury and to evaluate the usefulness of repair strategies. Accordingly, there is a growing effort to reconsider the availability and use of animal models of PNS injury.[35,142,143]

Our understanding of peripheral nerve injury and repair would benefit immensely from the development of a standardized in vitro model that replicates critical components of the in vivo situation in a reduced, yet systematically controlled environment. To date, many critical components have been isolated in vitro, such as elements of Schwann cell–axon interactions in culture,[144,145] axon outgrowth on various biomaterials scaffolds,[8,62,146–148] enhancement effects of growth factors,[8,48,69,126] and injury-induced alterations in gene expression.[80,149] Much utility would be gained from three-dimensional (3-D) models capable of evaluating haptotaxic and chemotaxic factors governing Schwann cell migration, proliferation, and/or organization in support of axonal regeneration. Moreover, factors promoting expeditious and targeted axonal regeneration through such 3-D cellular scaffolds could be systematically identified. Indeed, neural tissue engineering techniques have evolved to create long lengths of fasciculated axons that could mimic a nerve. For instance, the process of axon stretch growth has the potential to create bundles of axons in vitro that could then be myelinated to create functional nerves in culture.[90–92] These systems may be useful to study nerve injury in a 3-D, multicell-type environment that recreates key anatomical features, thus potentially providing a more physiologically relevant yet exquisitely accessible and controlled platform. Indeed, new in vitro models are being explored to provide testing platforms for mechanistic studies that are difficult to perform in vivo and for proof-of-concept ideas that would otherwise be costly and complicated in an animal model.[143,150–154]

The ability to promote nerve regeneration and provide better therapeutic options will be driven by our understanding of the fundamental neurobiological mechanisms. Accordingly, relationships between nerve injury and neurodegenerative disorders could reveal complementary therapeutic mechanisms. For instance, amyotrophic lateral sclerosis exhibits a preferential degeneration of motor neurons, yet the effects may be reversed through crush injury.[78] Rather than solely focusing on axon outgrowth, biomedical engineers should also consider and study the mechanisms of neuropathy (as well as growth and development) to gain insight into injury and repair. In addition, newly identified injury mechanisms are needed as markers for clinical diagnosis and studying and comparing injury models.

VI. CLOSING: CHALLENGES AND OPPORTUNITIES

In summary, peripheral nerve repair is a growing field with substantial progress being made in more effective repairs. Biomedical engineers have made significant contributions, and the associated techniques and approaches have a great deal more to offer. Contributions range from surgical instrumentation to the development of tissue engineered grafting substitutes. Tissue engineering has great potential, as evidenced by the rapid combination of facets of neuroscience and biomedical engineering research into the subdiscipline of neural tissue engineering. However, to date the field of neural tissue engineering has not progressed much past the conduit bridging of small gaps and has not come close to matching the autograft.[2,8,9,15] Still, a recent survey of clinical departments serving peripheral nerve injuries concluded, "Tissue engineering offers the best promise of improved outcome at the moment" and called for alternative/novel strategies, tissue engineering research, and potentially xenographic grafting options.[4] Indeed, neural tissue engineering must continue to evolve to directly address pressing clinical needs while factoring in neurobiological realities. Thus, interactions between biomedical engineers, neurobiologists, and clinicians must increase to address these challenges.

We conclude that the most pressing current needs in peripheral nerve repair include the development of tissue engineered nerve graft alternatives that match or exceed the performance of autografts, the ability to noninvasively assess nerve damage and track axonal regeneration, and the ability to maintain the efficacy of the distal pathway and target. In combination with a lack of effective diagnostic techniques, the choices in assessment and repair of peripheral nerve unfortunately remain limited. The question of whether or not to perform surgery on an injured nerve is a difficult choice; some 3rd degree injuries heal without intervention but others may not. Clearly, surgical intervention for treatment of major nerve injury of 4th to 6th degree is needed, but currently we lack optimal repair methods and tools to predict and track recovery progress. These challenges are compounded by a current shortage of trained peripheral-nerve surgeons and scientists who specialize in peripheral-nerve anatomy and repair strategies.

Tissue-engineered graft alternatives have yet to reach the effectiveness of the autograft. The incremental improvements that have been made in developing a nervous tissue construct, individually, have not produced results that can be useful clinically. The concept of a nerve conduit is still limited to small-diameter, short-gap repairs. Next-generation tissue-engineered constructs that combine many aspects of the nerve architecture (cells, scaffold, signaling, and vasculature) may be required to offer a true alternative to the autograft, yet must also be designed to accommodate mass transport and mitigate immune rejection. Moreover, such comprehensive tissue-engineered constructs must be multifaceted in purpose, simultaneously facilitating natural host reparative processes (e.g., Schwann cell migration and organization), promoting expeditious and targeted axonal outgrowth, as well as providing trophic support to maintain the efficacy of the distal pathway beyond the graft/lesion site. The judicious application of biomedical engineering practices and principles, with utmost cognizance of neurobiological sequelae, clinical needs, and surgical limitations, will be needed to substantially improve patient outcomes following severe peripheral nerve injury.

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REFERENCES

1. Robinson LR. Traumatic injury to peripheral nerves. Muscle Nerve. 2000;23(6):863–73.
2. Evans GR. Peripheral nerve injury: a review and approach to tissue engineered constructs. Anat Rec. 2001;263(4):396–404.
3. Siemionow M, Brzezicki G. Chapter 8: Current techniques and concepts in peripheral nerve repair. Int Rev Neurobiol. 2009;87:141–72.
4. Scholz T, Krichevsky A, Sumarto A, Jaffurs D, Wirth GA, Paydar K, Evans G. Peripheral nerve injuries: an international survey of current treatments and future perspectives. J Reconstr Microsurg. 2009;25(6):339–44.
5. Brushart TM, editor. The mechanical and humoral control of specificity in nerve repair. Philadelphia: JB Lippincott; 1991.
6. Mackinnon SE, Dellon AL. Surgery of the peripheral nerve. New York: Thieme Medical Publishers; 1988.
7. Battiston B, Raimondo S, Tos P, Gaidano V, Audisio C, Scevola A, Perroteau I, Geuna S. Chapter 11: Tissue engineering of peripheral nerves. Int Rev Neurobiol. 2009;87:227–49.
8. Schmidt CE, Leach JB. Neural tissue engineering: strategies for repair and regeneration. Annu Rev Biomed Eng. 2003;5:293–347.
9. Moore AM, Kasukurthi R, Magill CK, Farhadi HF, Borschel GH, Mackinnon SE. Limitations of conduits in peripheral nerve repairs. Hand (NY). 2009;4(2):180–6.
10. Assaf Y, Pasternak O. Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review. J Mol Neurosci. 2008;34(1):51–61.
11. Hiltunen J, Suortti T, Arvela S, Seppa M, Joensuu R, Hari R. Diffusion tensor imaging and tractography of distal peripheral nerves at 3 T. Clin Neurophysiol. 2005;116(10):2315–23.

12. Khalil C, Budzik JF, Kermarrec E, Balbi V, Le Thuc V, Cotten A. Tractography of peripheral nerves and skeletal muscles. Eur J Radiol. 2010;76(3):391–7.
13. Lehmann HC, Zhang J, Mori S, Sheikh KA. Diffusion tensor imaging to assess axonal regeneration in peripheral nerves. Exp Neurol. 2010;223(1):238–44.
14. Vargas MI, Viallon M, Nguyen D, Delavelle J, Becker M. Diffusion tensor imaging (DTI) and tractography of the brachial plexus: feasibility and initial experience in neoplastic conditions. Neuroradiology. 2010;52(3):237–45.
15. Belkas JS, Shoichet MS, Midha R. Peripheral nerve regeneration through guidance tubes. Neurol Res. 2004;26(2):151–60.
16. Smith JW. Factors influencing nerve repair. II. Collateral circulation of peripheral nerves. Arch Surg. 1966;93(3):433–7.
17. Smith JW. Factors influencing nerve repair. I. Blood supply of peripheral nerves. Arch Surg. 1966;93(2):335–41.
18. Seddon HJ. Surgical disorders of the peripheral nerves. Second ed. New York: Churchill Livingstone; 1975.
19. Sunderland S. Nerves and nerve injuries. Second ed. New York: Churchill Livingstone; 1978.
20. Ray WZ, Mackinnon SE. Management of nerve gaps: autografts, allografts, nerve transfers, and end-to-side neurorrhaphy. Exp Neurol. 2010;223(1):77–85.
21. Prasad AR, Steck JK, Dellon AL. Zone of traction injury of the common peroneal nerve. Ann Plast Surg. 2007;59(3):302–6.
22. Lundborg G, Rydevik B. Effects of stretching the tibial nerve of the rabbit. A preliminary study of the intraneural circulation and the barrier function of the perineurium. J Bone Joint Surg Br. 1973;55(2):390–401.
23. Millesi H, Meissl G, Berger A. The interfascicular nerve-grafting of the median and ulnar nerves. J Bone Joint Surg Am. 1972;54(4):727–50.
24. Kline DG, Hudson AR. Nerve injuries: operative results for major nerve injuries, entrapments and tumors. Philadelphia: W.B. Saunders; 1995.
25. Nichols CM, Brenner MJ, Fox IK, Tung TH, Hunter DA, Rickman SR, Mackinnon SE. Effects of motor versus sensory nerve grafts on peripheral nerve regeneration. Exp Neurol. 2004;190(2):347–55.
26. Brenner MJ, Dvali L, Hunter DA, Myckatyn TM, Mackinnon SE. Motor neuron regeneration through end-to-side repairs is a function of donor nerve axotomy. Plast Reconstr Surg. 2007;120(1):215–23.
27. Brenner MJ, Hess JR, Myckatyn TM, Hayashi A, Hunter DA, Mackinnon SE. Repair of motor nerve gaps with sensory nerve inhibits regeneration in rats. Laryngoscope. 2006;116(9):1685–92.
28. Hess JR, Brenner MJ, Fox IK, Nichols CM, Myckatyn TM, Hunter DA, Rickman SR, Mackinnon SE. Use of cold-preserved allografts seeded with autologous Schwann cells in the treatment of a long-gap peripheral nerve injury. Plast Reconstr Surg. 2007;119(1):246–59.
29. Meek MF, Coert JH. Clinical use of nerve conduits in peripheral-nerve repair: review of the literature. J Reconstr Microsurg. 2002;18(2):97–109.
30. Chiu DT, Strauch B. A prospective clinical evaluation of autogenous vein grafts used as a nerve conduit for distal sensory nerve defects of 3 cm or less. Plast Reconstr Surg. 1990;86(5):928–34.
31. Mackinnon SE, Dellon AL. A study of nerve regeneration across synthetic (Maxon) and biologic (collagen) nerve conduits for nerve gaps up to 5 cm in the primate. J Reconstr Microsurg. 1990;6(2):117–21.
32. Hayashi A, Pannucci C, Moradzadeh A, Kawamura D, Magill C, Hunter DA, Tong AY, Parsadanian A, Mackinnon SE, Myckatyn TM. Axotomy or compression is required for axonal sprouting following end-to-side neurorrhaphy. Exp Neurol. 2008;211(2):539–50.
33. Pannucci C, Myckatyn TM, Mackinnon SE, Hayashi A. End-to-side nerve repair: review of the literature. Restor Neurol Neurosci. 2007;25(1):45–63.
34. Burnett MG, Zager EL. Pathophysiology of peripheral nerve injury: a brief review. Neurosurg Focus. 2004;16(5):E1.
35. Fu SY, Gordon T. The cellular and molecular basis of peripheral nerve regeneration. Mol Neurobiol. 1997;14(1–2):67–116.
36. Gordon T. Dependence of peripheral nerves on their target organs. In: Burnstock G, O'Brien R, Vrbove G, editors. Somatic and autonomic

nerve-muscle interactions. Amsterdam: Elsevier; 1983. p. 289–316
37. Fu SY, Gordon T. Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. J Neurosci. 1995;15(5 Pt 2):3886–95.
38. Fu SY, Gordon T. Contributing factors to poor functional recovery after delayed nerve repair: prolonged axotomy. J Neurosci. 1995;15(5 Pt 2):3876–85.
39. Sulaiman OA, Gordon T. Effects of short- and long-term Schwann cell denervation on peripheral nerve regeneration, myelination, and size. Glia. 2000;32(3):234–46.
40. Boyd JG, Gordon T. A dose-dependent facilitation and inhibition of peripheral nerve regeneration by brain-derived neurotrophic factor. Eur J Neurosci. 2002;15(4):613–26.
41. Al-Majed AA, Brushart TM, Gordon T. Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. Eur J Neurosci. 2000;12(12):4381–90.
42. Eberhardt KA, Irintchev A, Al-Majed AA, Simova O, Brushart TM, Gordon T, Schachner M. BDNF/TrkB signaling regulates HNK-1 carbohydrate expression in regenerating motor nerves and promotes functional recovery after peripheral nerve repair. Exp Neurol. 2006;198(2):500–10.
43. Al-Majed AA, Neumann CM, Brushart TM, Gordon T. Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. J Neurosci. 2000;20(7):2602–8.
44. Brushart TM, Hoffman PN, Royall RM, Murinson BB, Witzel C, Gordon T. Electrical stimulation promotes motoneuron regeneration without increasing its speed or conditioning the neuron. J Neurosci. 2002;22(15):6631–8.
45. Brushart TM, Jari R, Verge V, Rohde C, Gordon T. Electrical stimulation restores the specificity of sensory axon regeneration. Exp Neurol. 2005;194(1):221–9.
46. Geremia NM, Gordon T, Brushart TM, Al-Majed AA, Verge VM. Electrical stimulation promotes sensory neuron regeneration and growth-associated gene expression. Exp Neurol. 2007;205(2):347–59.
47. Boyd JG, Gordon T. Glial cell line-derived neurotrophic factor and brain-derived neurotrophic factor sustain the axonal regeneration of chronically axotomized motoneurons in vivo. Exp Neurol. 2003;183(2):610–9.
48. Boyd JG, Gordon T. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. Mol Neurobiol. 2003;27(3):277–324.
49. Sulaiman OA, Voda J, Gold BG, Gordon T. FK506 increases peripheral nerve regeneration after chronic axotomy but not after chronic Schwann cell denervation. Exp Neurol. 2002;175(1):127–37.
50. Midha R, Munro CA, Chan S, Nitising A, Xu QG, Gordon T. Regeneration into protected and chronically denervated peripheral nerve stumps. Neurosurgery. 2005;57(6):1289–99; discussion –99.
51. Walsh SK, Gordon T, Addas BM, Kemp SW, Midha R. Skin-derived precursor cells enhance peripheral nerve regeneration following chronic denervation. Exp Neurol. 2010;223(1):221–8.
52. Gordon T, Brushart TM, Amirjani N, Chan KM. The potential of electrical stimulation to promote functional recovery after peripheral nerve injury—comparisons between rats and humans. Acta Neurochir Suppl. 2007;100:3–11.
53. Gordon T, Brushart TM, Chan KM. Augmenting nerve regeneration with electrical stimulation. Neurol Res. 2008;30(10):1012–22.
54. Gordon T, Amirjani N, Edwards DC, Chan KM. Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. Exp Neurol. 2010;223(1):192–202.
55. Whitlock EL, Tuffaha SH, Luciano JP, Yan Y, Hunter DA, Magill CK, Moore AM, Tong AY, Mackinnon SE, Borschel GH. Processed allografts and type I collagen conduits for repair of peripheral nerve gaps. Muscle Nerve. 2009;39(6):787–99.
56. Maeda T, Mackinnon SE, Best TJ, Evans PJ, Hunter DA, Midha RT. Regeneration across 'stepping-stone' nerve grafts. Brain Res. 1993;618(2):196–202.
57. Hoke A, Redett R, Hameed H, Jari R, Zhou C, Li ZB, Griffin JW, Brushart TM. Schwann cells express motor and sensory phenotypes that regulate axon regeneration. J Neurosci. 2006;26(38):9646–55.

58. Williams LR, Longo FM, Powell HC, Lundborg G, Varon S. Spatial-temporal progress of peripheral nerve regeneration within a silicone chamber: parameters for a bioassay. J Comp Neurol. 1983;218(4):460–70.
59. Williams LR, Danielsen N, Muller H, Varon S. Exogenous matrix precursors promote functional nerve regeneration across a 15-mm gap within a silicone chamber in the rat. J Comp Neurol. 1987;264(2):284–90.
60. Varon S, Williams LR, Gage FH. Exogenous administration of neuronotrophic factors in vivo protects central nervous system neurons against axotomy induced degeneration. Prog Brain Res. 1987;71:191–201.
61. Dodla MC, Bellamkonda RV. Differences between the effect of anisotropic and isotropic laminin and nerve growth factor presenting scaffolds on nerve regeneration across long peripheral nerve gaps. Biomaterials. 2008;29(1):33–46.
62. Bellamkonda RV. Peripheral nerve regeneration: an opinion on channels, scaffolds and anisotropy. Biomaterials. 2006;27(19):3515–8.
63. Yan H, Zhang F, Chen MB, Lineaweaver WC. Chapter 10: Conduit luminal additives for peripheral nerve repair. Int Rev Neurobiol. 2009;87:199–225.
64. Dubey N, Letourneau PC, Tranquillo RT. Guided neurite elongation and Schwann cell invasion into magnetically aligned collagen in simulated peripheral nerve regeneration. Exp Neurol. 1999;158(2):338–50.
65. Rosner BI, Siegel RA, Grosberg A, Tranquillo RT. Rational design of contact guiding, neurotrophic matrices for peripheral nerve regeneration. Ann Biomed Eng. 2003;31(11):1383–401.
66. Hudson TW, Evans GR, Schmidt CE. Engineering strategies for peripheral nerve repair. Clin Plast Surg. 1999;26(4):617–28, ix.
67. de Ruiter GC, Malessy MJ, Yaszemski MJ, Windebank AJ, Spinner RJ. Designing ideal conduits for peripheral nerve repair. Neurosurg Focus. 2009;26(2):E5.
68. Kemp SW, Walsh SK, Midha R. Growth factor and stem cell enhanced conduits in peripheral nerve regeneration and repair. Neurol Res. 2008;30(10):1030–8.
69. Hall S. Nerve repair: a neurobiologist's view. J Hand Surg [Br]. 2001;26(2):129–36.
70. Vrbova G, Mehra N, Shanmuganathan H, Tyreman N, Schachner M, Gordon T. Chemical communication between regenerating motor axons and Schwann cells in the growth pathway. Eur J Neurosci. 2009;30(3):366–75.
71. Bunge RP. The role of the Schwann cell in trophic support and regeneration. J Neurol. 1994;242(1 Suppl 1):S19–21.
72. Bunge MB, Bunge RP, Kleitman N, Dean AC. Role of peripheral nerve extracellular matrix in Schwann cell function and in neurite regeneration. Dev Neurosci. 1989;11(4–5):348–60.
73. Bunge RP. Expanding roles for the Schwann cell: ensheathment, myelination, trophism and regeneration. Curr Opin Neurobiol. 1993;3(5):805–9.
74. Morrissey TK, Kleitman N, Bunge RP. Isolation and functional characterization of Schwann cells derived from adult peripheral nerve. J Neurosci. 1991;11(8):2433–42.
75. Giger RJ, Hollis ER, 2nd, Tuszynski MH. Guidance molecules in axon regeneration. Cold Spring Harb Perspect Biol. 2010;2(7):a001867.
76. Dickson BJ. Molecular mechanisms of axon guidance. Science. 2002;298(5600):1959–64.
77. Yu TW, Bargmann CI. Dynamic regulation of axon guidance. Nat Neurosci. 2001;4 Suppl:1169–76.
78. Franz CK, Quach ET, Krudy CA, Federici T, Kliem MA, Snyder BR, Raore B, Boulis NM. A conditioning lesion provides selective protection in a rat model of Amyotrophic Lateral Sclerosis. PLoS One. 2009;4(10):e7357.
79. O'Donnell M, Chance RK, Bashaw GJ. Axon growth and guidance: receptor regulation and signal transduction. Annu Rev Neurosci. 2009;32:383–412.
80. Willis D, Li KW, Zheng JQ, Chang JH, Smit A, Kelly T, Merianda TT, Sylvester J, van Minnen J, Twiss JL. Differential transport and local translation of cytoskeletal, injury-response, and neurodegeneration protein mRNAs in axons. J Neurosci. 2005;25(4):778–91.
81. Dahlin L, Johansson F, Lindwall C, Kanje M. Chapter 28: Future perspective in peripheral nerve reconstruction. Int Rev Neurobiol. 2009;87:507–30.
82. Nomura H, Tator CH, Shoichet MS. Bioengineered strategies for spinal cord repair. J Neurotrauma. 2006;23(3–4):496–507.
83. Willerth SM, Sakiyama-Elbert SE. Approaches

to neural tissue engineering using scaffolds for drug delivery. Adv Drug Deliv Rev. 2007;59(4–5):325–38.
84. Pfister LA, Papaloizos M, Merkle HP, Gander B. Nerve conduits and growth factor delivery in peripheral nerve repair. J Peripher Nerv Syst. 2007;12(2):65–82.
85. Yu LM, Wosnick JH, Shoichet MS. Miniaturized system of neurotrophin patterning for guided regeneration. J Neurosci Methods. 2008;171(2):253–63.
86. Geuna S, Nicolino S, Raimondo S, Gambarotta G, Battiston B, Tos P, Perroteau I. Nerve regeneration along bioengineered scaffolds. Microsurgery. 2007;27(5):429–38.
87. Piotrowicz A, Shoichet MS. Nerve guidance channels as drug delivery vehicles. Biomaterials. 2005;26 (29) : 5872–78.
88. Lloyd BM, Luginbuhl RD, Brenner MJ, Rocque BG, Tung TH, Myckatyn TM, Hunter DA, Mackinnon SE, Borschel GH. Use of motor nerve material in peripheral nerve repair with conduits. Microsurgery. 2007;27(2):138–45.
89. Huang JH, Cullen DK, Browne KD, Groff R, Zhang J, Pfister BJ, Zager EL, Smith DH. Long-term survival and integration of transplanted engineered nervous tissue constructs promotes peripheral nerve regeneration. Tissue Eng Part A. 2009;15(7):1677–85.
90. Pfister BJ, Bonislawski DP, Smith DH, Cohen AS. Stretch-grown axons retain the ability to transmit active electrical signals. FEBS Lett. 2006;580(14):3525–31.
91. Pfister BJ, Iwata A, Meaney DF, Smith DH. Extreme stretch growth of integrated axons. J Neurosci. 2004;24(36):7978–83.
92. Pfister BJ, Iwata A, Taylor AG, Wolf JA, Meaney DF, Smith DH. Development of transplantable nervous tissue constructs comprised of stretch-grown axons. J Neurosci Methods. 2006;153(1):95–103.
93. Lexell J, Downham D, Sjostrom M. Distribution of different fibre types in human skeletal muscles. A statistical and computational model for the study of fibre type grouping and early diagnosis of skeletal muscle fibre denervation and reinnervation. J Neurol Sci. 1983;61(3):301–14.
94. Tyler MJ, Cameron DA. Cellular pattern formation during retinal regeneration: a role for homotypic control of cell fate acquisition. Vision Res. 2007;47(4):501–11.
95. Sakumura Y, Tsukada Y, Yamamoto N, Ishii S. A molecular model for axon guidance based on cross talk between rho GTPases. Biophys J. 2005;89(2):812–22.
96. Lagerlund TD, Low PA. Mathematical modeling of time-dependent oxygen transport in rat peripheral nerve. Comput Biol Med. 1993;23(1):29–47.
97. Lagerlund TD, Low PA. Axial diffusion and Michaelis-Menten kinetics in oxygen delivery in rat peripheral nerve. Am J Physiol. 1991;260(2 Pt 2):R430–40.
98. Sharma GC, Jain M. A computational solution of mathematical model for oxygen transport in peripheral nerve. Comput Biol Med. 2004;34(7):633–45.
99. Lundborg G. Nerve injury and repair: regeneration, reconstruction, and cortical remodeling, 2nd ed. Philadelphia : Elsevier Science Health Science Div.; 2005.
100. Gordon T, Sulaiman O, Midha R. Pathophysiology of surgical nerve disorders. In: Winn HR, Youmans JR, editors. Neurological Surgery, 6th ed. Philadelphia: W.B. Saunders Company; 2010.
101. Filler AG, Kline DG. General principles in evaluating and treating peripheral nerve pathology, injuries, and entrapments and their historical content. In: Winn HR, Youmans JR, editors. Neurological Surgery, 6th ed. Philadelphia: W.B. Saunders Company; 2010.
102. Bendszus M, Stoll G. Technology insight: visualizing peripheral nerve injury using MRI. Nat Clin Pract Neurol. 2005;1(1):45–53.
103. Jambawalikar S, Baum J, Button T, Li H, Geronimo V, Gould ES. Diffusion tensor imaging of peripheral nerves. Skeletal Radiol. 2010;39(11):1073–9.
104. Khachi G, Skirgaudes M, Lee WP, Wollstein R. The clinical applications of peripheral nerve imaging in the upper extremity. J Hand Surg Am. 2007;32(10):1600–4.
105. Koltzenburg M, Bendszus M. Imaging of peripheral nerve lesions. Curr Opin Neurol. 2004;17(5):621–6.
106. Sheikh KA. Non-invasive imaging of nerve regeneration. Exp Neurol. 2010;223(1):72–6.

107. Howe FA, Filler AG, Bell BA, Griffiths JR. Magnetic resonance neurography. Magn Reson Med. 1992;28(2):328–38.
108. Takagi T, Nakamura M, Yamada M, Hikishima K, Momoshima S, Fujiyoshi K, Shibata S, Okano HJ, Toyama Y, Okano H. Visualization of peripheral nerve degeneration and regeneration: monitoring with diffusion tensor tractography. Neuroimage. 2009;44(3):884–92.
109. Huang H. Delineating neural structures of developmental human brains with diffusion tensor imaging. ScientificWorld J. 2010;10:135–44.
110. Aagaard BD, Lazar DA, Lankerovich L, Andrus K, Hayes CE, Maravilla K, Kliot M. High-resolution magnetic resonance imaging is a noninvasive method of observing injury and recovery in the peripheral nervous system. Neurosurgery. 2003;53(1):199–203; discussion –4.
111. Behr B, Schnabel R, Mirastschijski U, Ibrahim B, Angenstein F, Schneider W. Magnetic resonance imaging monitoring of peripheral nerve regeneration following neurotmesis at 4.7 Tesla. Plast Reconstr Surg. 2009;123(6):1778–88.
112. Bendszus M, Wessig C, Solymosi L, Reiners K, Koltzenburg M. MRI of peripheral nerve degeneration and regeneration: correlation with electrophysiology and histology. Exp Neurol. 2004;188(1):171–7.
113. Cudlip SA, Howe FA, Griffiths JR, Bell BA. Magnetic resonance neurography of peripheral nerve following experimental crush injury, and correlation with functional deficit. J Neurosurg. 2002;96(4):755–9.
114. Dailey AT, Tsuruda JS, Filler AG, Maravilla KR, Goodkin R, Kliot M. Magnetic resonance neurography of peripheral nerve degeneration and regeneration. Lancet. 1997;350(9086):1221–2.
115. Does MD, Snyder RE. Multiexponential T2 relaxation in degenerating peripheral nerve. Magn Reson Med. 1996;35(2):207–13.
116. Lacour-Petit MC, Lozeron P, Ducreux D. MRI of peripheral nerve lesions of the lower limbs. Neuroradiology. 2003;45(3):166–70.
117. Li X, Shen J, Chen J, Wang X, Liu Q, Liang B. Magnetic resonance imaging evaluation of acute crush injury of rabbit sciatic nerve: correlation with histology. Can Assoc Radiol J. 2008;59(3):123–30.
118. Stanisz GJ, Midha R, Munro CA, Henkelman RM. MR properties of rat sciatic nerve following trauma. Magn Reson Med. 2001;45(3):415–20.
119. Swanger RS, Maldjian C, Buckley K. MRI appearance of nerve regeneration in a surgically repaired ulnar nerve. Eur J Trauma Emerg Surg. 2010;36(1):73–5.
120. Bendszus M, Wessig C, Schutz A, Horn T, Kleinschnitz C, Sommer C, Misselwitz B, Stoll G. Assessment of nerve degeneration by gadofluorine M-enhanced magnetic resonance imaging. Ann Neurol. 2005;57(3):388–95.
121. Matsuda K, Wang HX, Suo C, McCombe D, Horne MK, Morrison WA, Egan GF. Retrograde axonal tracing using manganese enhanced magnetic resonance imaging. Neuroimage. 2010;50(2):366–74.
122. Wessig C, Bendszus M, Stoll G. In vivo visualization of focal demyelination in peripheral nerves by gadofluorine M-enhanced magnetic resonance imaging. Exp Neurol. 2007;204(1):14–9.
123. Ellegood J, McKay RT, Hanstock CC, Beaulieu C. Anisotropic diffusion of metabolites in peripheral nerve using diffusion weighted magnetic resonance spectroscopy at ultra-high field. J Magn Reson. 2007;184(1):20–8.
124. Holl N, Echaniz-Laguna A, Bierry G, Mohr M, Loeffler JP, Moser T, Dietemann JL, Kremer S. Diffusion-weighted MRI of denervated muscle: a clinical and experimental study. Skeletal Radiol. 2008;37(12):1111–7.
125. Bromberg MB. Motor unit number estimation: new techniques and new uses. Suppl Clin Neurophysiol. 2004;57:120–36.
126. Gordon T. The physiology of neural injury and regeneration: The role of neurotrophic factors. J Commun Disord. 2010;43(4):265–73.
127. Gordon T, Sulaiman O, Boyd JG. Experimental strategies to promote functional recovery after peripheral nerve injuries. J Peripher Nerv Syst. 2003;8(4):236–50.
128. Galtrey CM, Fawcett JW. Characterization of tests of functional recovery after median and ulnar nerve injury and repair in the rat forelimb. J Peripher Nerv Syst. 2007;12(1):11–27.
129. Karabeg R, Jakirlic M, Dujso V. Sensory recovery after forearm median and ulnar nerve grafting. Med Arh. 2009;63(2):97–9.
130. Ronchi G, Nicolino S, Raimondo S, Tos P, Battiston B, Papalia I, Varejão AS, Giacobini-

Robecchi MG, Perroteau I, Geuna S. Functional and morphological assessment of a standardized crush injury of the rat median nerve. J Neurosci Methods. 2009;179(1):51–7.
131. Wang H, Spinner RJ, Sorenson EJ, Windebank AJ. Measurement of forelimb function by digital video motion analysis in rat nerve transection models. J Peripher Nerv Syst. 2008;13(1):92–102.
132. Sinis N, Schaller HE, Becker ST, Lanaras T, Schulte-Eversum C, Müller HW, Vonthein R, Rösner H, Haerle M. Cross-chest median nerve transfer: a new model for the evaluation of nerve regeneration across a 40 mm gap in the rat. J Neurosci Methods. 2006;156(1–2):166–72.
133. Ichihara S, Inada Y, Nakada A, Endo K, Azuma T, Nakai R, Tsutsumi S, Kurosawa H, Nakamura T.. Development of new nerve guide tube for repair of long nerve defects. Tissue Eng Part C Methods. 2009;15(3):387–402.
134. Suzuki K, Kawauchi A, Nakamura T, Itoi S, Ito T, So J, Ukimura O, Hagiwara A, Yamagishi H, Miki T. Histologic and electrophysiological study of nerve regeneration using a polyglycolic acid-collagen nerve conduit filled with collagen sponge in canine model. Urology. 2009;74(4):958–63.
135. Young RC, Wiberg M, Terenghi G. Poly-3-hydroxybutyrate (PHB): a resorbable conduit for long-gap repair in peripheral nerves. Br J Plast Surg. 2002;55(3):235–40.
136. Tung WL, Zhao C, Yoshii Y, Su FC, An KN, Amadio PC. Comparative study of carpal tunnel compliance in the human, dog, rabbit, and rat. J Orthop Res. 2010;28(5):652–6.
137. Mligiliche N, Tabata Y, Endoh K, Ide C. Peripheral nerve regeneration through a long detergent-denatured muscle autografts in rabbits. Neuroreport. 2001;12(8):1719–22.
138. Archibald SJ, Krarup C, Shefner J, Li ST, Madison RD. A collagen-based nerve guide conduit for peripheral nerve repair: an electrophysiological study of nerve regeneration in rodents and nonhuman primates. J Comp Neurol. 1991;306(4):685–96.
139. Zhang P, Zhang C, Kou Y, Yin X, Zhang H, Jiang B. The histological analysis of biological conduit sleeve bridging rhesus monkey median nerve injury with small gap. Artif Cells Blood Substit Immobil Biotechnol. 2009;37(2):101–4.
140. Mackinnon SE, Dellon AL, Lundborg G, Hudson AR, Hunter DA. A study of neurotrophism in a primate model. J Hand Surg Am. 1986;11(6):888–94.
141. Krarup C, Archibald SJ, Madison RD. Factors that influence peripheral nerve regeneration: an electrophysiological study of the monkey median nerve. Ann Neurol. 2002;51(1):69–81.
142. Tos P, Ronchi G, Nicolino S, Audisio C, Raimondo S, Fornaro M, Battiston B, Graziani A, Perroteau I, Geuna S. Employment of the mouse median nerve model for the experimental assessment of peripheral nerve regeneration. J Neurosci Methods. 2008;169(1):119–27.
143. Tos P, Ronchi G, Papalia I, Sallen V, Legagneux J, Geuna S, Giacobini-Robecchi MG. Chapter 4: Methods and protocols in peripheral nerve regeneration experimental research: part I—experimental models. Int Rev Neurobiol. 2009;87:47–79.
144. Crawford AT, Desai D, Gokina P, Basak S, Kim HA. E-cadherin expression in postnatal Schwann cells is regulated by the cAMP-dependent protein kinase a pathway. Glia. 2008;56(15):1637–47.
145. Yang DP, Zhang DP, Mak KS, Bonder DE, Pomeroy SL, Kim HA. Schwann cell proliferation during Wallerian degeneration is not necessary for regeneration and remyelination of the peripheral nerves: axon-dependent removal of newly generated Schwann cells by apoptosis. Mol Cell Neurosci. 2008;38(1):80–8.
146. Tresco PA. Tissue engineering strategies for nervous system repair. Prog Brain Res. 2000;128:349–63.
147. Wen X, Tresco PA. Effect of filament diameter and extracellular matrix molecule precoating on neurite outgrowth and Schwann cell behavior on multifilament entubulation bridging device in vitro. J Biomed Mater Res A. 2006;76(3):626–37.
148. Seidlits SK, Lee JY, Schmidt CE. Nanostructured scaffolds for neural applications. Nanomedicine (London). 2008;3(2):183–99.
149. Yoo S, van Niekerk EA, Merianda TT, Twiss JL. Dynamics of axonal mRNA transport and implications for peripheral nerve regeneration. Exp Neurol. 2010;223(1):19–27.
150. Pfister BJ, Huang JH, Kameswaran N, Zager EL, Smith DH. Neural engineering to produce in vitro nerve constructs and neurointerface. Neu-

rosurgery. 2007;60(1):137–41; discussion 41–2.
151. Vyas A, Li Z, Aspalter M, Feiner J, Hoke A, Zhou C, O'Daly A, Abdullah M, Rohde C, Brushart TM. An in vitro model of adult mammalian nerve repair. Exp Neurol. 2010;223(1):112–8.
152. Stecker MM, Baylor K, Stevenson M. In-vitro stability of peripheral nerve preparations: relation to ischemic responses. Brain Res. 2010;1337:1-7.
153. Yu Z, Graudejus O, Lacour SP, Wagner S, Morrison B, 3rd. Neural sensing of electrical activity with stretchable microelectrode arrays. Conf Proc IEEE Eng Med Biol Soc. 2009;2009:4210–3.
154. Cullen DK, A RP, Doorish JF, Smith DH, Pfister BJ. Developing a tissue-engineered neural-electrical relay using encapsulated neuronal constructs on conducting polymer fibers. J Neural Eng. 2008;5(4):374–84.
155. Ansselin AD, Fink T, Davey DF. Peripheral nerve regeneration through nerve guides seeded with adult Schwann cells. Neuropathol Appl Neurobiol. 1997;23(5):387–98.
156. Apel PJ, Garrett JP, Sierpinski P, Ma J, Atala A, Smith TL, Koman LA, Van Dyke ME. Peripheral nerve regeneration using a keratin-based scaffold: long-term functional and histological outcomes in a mouse model. J Hand Surg Am. 2008;33(9):1541–7.
157. Arai T, Lundborg G, Dahlin LB. Bioartificial nerve graft for bridging extended nerve defects in rat sciatic nerve based on resorbable guiding filaments. Scand J Plast Reconstr Surg Hand Surg. 2000;34(2):101–8.
158. Ashley WW, Jr., Weatherly T, Park TS. Collagen nerve guides for surgical repair of brachial plexus birth injury. J Neurosurg. 2006;105(6 Suppl):452–6.
159. Barras FM, Pasche P, Bouche N, Aebischer P, Zurn AD. Glial cell line-derived neurotrophic factor released by synthetic guidance channels promotes facial nerve regeneration in the rat. J Neurosci Res. 2002;70(6):746–55.
160. Borgens RB, Shi R, Bohnert D. Behavioral recovery from spinal cord injury following delayed application of polyethylene glycol. J Exp Biol. 2002;205(Pt 1):1–12.
161. Braga-Silva J. The use of silicone tubing in the late repair of the median and ulnar nerves in the forearm. J Hand Surg Br. 1999;24(6):703–6.
162. Bunting S, Di Silvio L, Deb S, Hall S. Bioresorbable glass fibres facilitate peripheral nerve regeneration. J Hand Surg Br. 2005;30(3):242–7.
163. Cai J, Peng X, Nelson KD, Eberhart R, Smith GM. Permeable guidance channels containing microfilament scaffolds enhance axon growth and maturation. J Biomed Mater Res A. 2005;75(2):374–86.
164. Casanas J, Serra J, Orduna M, Garcia-Portabella M, Mir X. Repair of digital sensory nerves of the hand using polyglycolic acid conduits. J Hand Surg Br. 2000;25(44).
165. Ceballos D, Navarro X, Dubey N, Wendelschafer-Crabb G, Kennedy WR, Tranquillo RT. Magnetically aligned collagen gel filling a collagen nerve guide improves peripheral nerve regeneration. Exp Neurol. 1999;158(2):290–300.
166. Chang CJ, Hsu SH. The effect of high outflow permeability in asymmetric poly(DL-lactic acid-co-glycolic acid) conduits for peripheral nerve regeneration. Biomaterials. 2006;27(7):1035–42.
167. Chang CJ, Hsu SH, Yen HJ, Chang H, Hsu SK. Effects of unidirectional permeability in asymmetric poly(DL-lactic acid-co-glycolic acid) conduits on peripheral nerve regeneration: an in vitro and in vivo study. J Biomed Mater Res B Appl Biomater. 2007;83(1):206–15.
168. Chen CJ, Ou YC, Liao SL, Chen WY, Chen SY, Wu CW, Wang CC, Wang WY, Huang YS, Hsu SH. Transplantation of bone marrow stromal cells for peripheral nerve repair. Exp Neurol. 2007;204(1):443–53.
169. Chen YS, Hsieh CL, Tsai CC, Chen TH, Cheng WC, Hu CL, Yao CH. Peripheral nerve regeneration using silicone rubber chambers filled with collagen, laminin and fibronectin. Biomaterials. 2000;21(15):1541–7.
170. Cheng B, Chen Z. Fabricating autologous tissue to engineer artificial nerve. Microsurgery. 2002;22(4):133–7.
171. Chew SY, Mi R, Hoke A, Leong KW. Aligned protein-polymer composite fibers enhance nerve regeneration: a potential tissue-engineering platform. Adv Funct Mater. 2007;17(8):1288–96.
172. Cui L, Jiang J, Wei L, Zhou X, Fraser JL, Snider BJ, Yu SP. Transplantation of embryonic stem cells improves nerve repair and functional recovery after severe sciatic nerve axotomy in rats. Stem Cells. 2008;26(5):1356–65.
173. Evans GR, Brandt K, Katz S, Chauvin P, Otto

L, Bogle M, Wang B, Meszlenyi RK, Lu L, Mikos AG, Patrick CW Jr. Bioactive poly(L-lactic acid) conduits seeded with Schwann cells for peripheral nerve regeneration. Biomaterials. 2002;23(3):841–8.
174. Fine EG, Decosterd I, Papaloizos M, Zurn AD, Aebischer P. GDNF and NGF released by synthetic guidance channels support sciatic nerve regeneration across a long gap. Eur J Neurosci. 2002;15(4):589–601.
175. Francel PC, Francel TJ, Mackinnon SE, Hertl C. Enhancing nerve regeneration across a silicone tube conduit by using interposed short-segment nerve grafts. J Neurosurg. 1997;87(6):887–92.
176. Gamez E, Goto Y, Nagata K, Iwaki T, Sasaki T, Matsuda T. Photofabricated gelatin-based nerve conduits: nerve tissue regeneration potentials. Cell Transplant. 2004;13(5):549–64.
177. Garrity R. The use of plastic and rubber tubing in the management of irreparable nerve injuries. Surg Forum. 1955;6:517–20.
178. Gilchrist T, Glasby MA, Healy DM, Kelly G, Lenihan DV, McDowall KL, Miller IA, Myles LM. In vitro nerve repair—in vivo. The reconstruction of peripheral nerves by entubulation with biodegradeable glass tubes—a preliminary report. Br J Plast Surg. 1998;51(3):231–7.
179. Guenard V, Kleitman N, Morrissey TK, Bunge RP, Aebischer P. Syngeneic Schwann cells derived from adult nerves seeded in semipermeable guidance channels enhance peripheral nerve regeneration. J Neurosci. 1992;12(9):3310–20.
180. Hadlock T, Sundback C, Hunter D, Cheney M, Vacanti JP. A polymer foam conduit seeded with Schwann cells promotes guided peripheral nerve regeneration. Tissue Eng. 2000;6(2):119–27.
181. Hagiwara A, Nakashima S, Itoh T, Sakakura C, Otsuji E, Yamagishi H, Okajima S, Kusuzaki K, Hase H, Kubo S, Soh J, Miki T, Toba T, Nakamura T, Shimizu Y. [Clinical application of PGA-tube for regeneration of intrapelvic nerves during extended surgery for intrapelvic recurrent rectal cancer]. Gan To Kagaku Ryoho. 2002;29(12):2202–4.
182. Hashimoto T, Suzuki Y, Kitada M, Kataoka K, Wu S, Suzuki K, Endo K, Nishimura Y, Ide C. Peripheral nerve regeneration through alginate gel: analysis of early outgrowth and late increase in diameter of regenerating axons. Exp Brain Res. 2002;146(3):356–68.
183. He C, Chen Z. Enhancement of motor nerve regeneration by nerve growth factor. Microsurgery. 1992;13(3):151–4.
184. Hobson MI, Green CJ, Terenghi G. VEGF enhances intraneural angiogenesis and improves nerve regeneration after axotomy. J Anat. 2000;197 Pt 4:591–605.
185. Hu W, Gu J, Deng A, Gu X. Polyglycolic acid filaments guide Schwann cell migration in vitro and in vivo. Biotechnol Lett. 2008;30(11):1937–42.
186. Inada Y, Morimoto S, Moroi K, Endo K, Nakamura T. Surgical relief of causalgia with an artificial nerve guide tube: Successful surgical treatment of causalgia (Complex Regional Pain Syndrome Type II) by in situ tissue engineering with a polyglycolic acid-collagen tube. Pain. 2005;117(3):251–8.
187. Itoh S, Takakuda K, Ichinose S, Kikuchi M, Schinomiya K. A study of induction of nerve regeneration using bioabsorbable tubes. J Reconstr Microsurg. 2001;17(2):115–23.
188. Jiang X, Lim SH, Mao HQ, Chew SY. Current applications and future perspectives of artificial nerve conduits. Exp Neurol. 2010;223(1):86–101.
189. Kalbermatten DF, Kingham PJ, Mahay D, Mantovani C, Pettersson J, Raffoul W, Balcin H, Pierer G, Terenghi G. Fibrin matrix for suspension of regenerative cells in an artificial nerve conduit. J Plast Reconstr Aesthet Surg. 2008;61(6):669–75.
190. Kauppila T, Jyvasjarvi E, Huopaniemi T, Hujanen E, Liesi P. A laminin graft replaces neurorrhaphy in the restorative surgery of the rat sciatic nerve. Exp Neurol. 1993;123(2):181–91.
191. Kim YT, Haftel VK, Kumar S, Bellamkonda RV. The role of aligned polymer fiber-based constructs in the bridging of long peripheral nerve gaps. Biomaterials. 2008;29(21):3117–27.
192. Lee AC, Yu VM, Lowe JB 3rd, Brenner MJ, Hunter DA, Mackinnon SE, Sakiyama-Elbert SE. Controlled release of nerve growth factor enhances sciatic nerve regeneration. Exp Neurol. 2003;184(1):295–303.
193. Li ST, Archibald SJ, Krarup C, Madison RD. Peripheral nerve repair with collagen conduits. Clin Mater. 1992;9(3–4):195–200.
194. Lohmeyer J, Zimmermann S, Sommer B, Machens HG, Lange T, Mailander P. [Bridging

peripheral nerve defects by means of nerve conduits]. Chirurg. 2007;78(2):142–7.
195. Lundborg G, Dahlin L, Dohi D, Kanje M, Terada N. A new type of «bioartificial» nerve graft for bridging extended defects in nerves. J Hand Surg Br. 1997;22(3):299–303.
196. Lundborg G, Dahlin LB, Danielsen N. Ulnar nerve repair by the silicone chamber technique. Case report. Scand J Plast Reconstr Surg Hand Surg. 1991;25(1):79–82.
197. Lundborg G, Rosen B, Abrahamson SO, Dahlin L, Danielsen N. Tubular repair of the median nerve in the human forearm. Preliminary findings. J Hand Surg Br. 1994;19(3):273–6.
198. Lundborg G, Rosen B, Dahlin L, Danielsen N, Holmberg J. Tubular versus conventional repair of median and ulnar nerves in the human forearm: early results from a prospective, randomized, clinical study. J Hand Surg Am. 1997;22(1):99–106.
199. Mackinnon SE, Dellon AL. Clinical nerve reconstruction with a bioabsorbable polyglycolic acid tube. Plast Reconstr Surg. 1990;85(3):419–24.
200. Madison R, da Silva CF, Dikkes P, Chiu TH, Sidman RL. Increased rate of peripheral nerve regeneration using bioresorbable nerve guides and a laminin-containing gel. Exp Neurol. 1985;88(3):767–72.
201. Madison RD, da Silva C, Dikkes P, Sidman RL, Chiu TH. Peripheral nerve regeneration with entubulation repair: comparison of biodegradeable nerve guides versus polyethylene tubes and the effects of a laminin-containing gel. Exp Neurol. 1987;95(2):378–90.
202. Madison RD, Da Silva CF, Dikkes P. Entubulation repair with protein additives increases the maximum nerve gap distance successfully bridged with tubular prostheses. Brain Res. 1988;447(2):325–34.
203. Matsumoto K, Ohnishi K, Kiyotani T, Sekine T, Ueda H, Nakamura T, Endo K, Shimizu Y. Peripheral nerve regeneration across an 80-mm gap bridged by a polyglycolic acid (PGA)-collagen tube filled with laminin-coated collagen fibers: a histological and electrophysiological evaluation of regenerated nerves. Brain Res. 2000;868(2):315–28.
204. McKay Hart A, Wiberg M, Terenghi G. Exogenous leukaemia inhibitory factor enhances nerve regeneration after late secondary repair using a bioartificial nerve conduit. Br J Plast Surg. 2003;56(5):444–50.
205. Meek MF, Coert JH. US Food and Drug Administration/Conformit Europe–approved absorbable nerve conduits for clinical repair of peripheral and cranial nerves. Ann Plast Surg. 2008;60(4):466–72.
206. Meek MF, Jansen K, Steendam R, van Oeveren W, van Wachem PB, van Luyn MJ. In vitro degradation and biocompatibility of poly(DL-lactide-epsilon-caprolactone) nerve guides. J Biomed Mater Res A. 2004;68(1):43–51.
207. Merle M, Dellon AL, Campbell JN, Chang PS. Complications from silicon-polymer intubulation of nerves. Microsurgery. 1989;10(2):130–3.
208. Midha R, Munro CA, Dalton PD, Tator CH, Shoichet MS. Growth factor enhancement of peripheral nerve regeneration through a novel synthetic hydrogel tube. J Neurosurg. 2003;99(3):555–65.
209. Mohammad JA, Warnke PH, Pan YC, Shenaq S. Increased axonal regeneration through a biodegradable amnionic tube nerve conduit: effect of local delivery and incorporation of nerve growth factor/hyaluronic acid media. Ann Plast Surg. 2000;44(1):59–64.
210. Mohanna PN, Terenghi G, Wiberg M. Composite PHB-GGF conduit for long nerve gap repair: a long-term evaluation. Scand J Plast Reconstr Surg Hand Surg. 2005;39(3):129–37.
211. Mosahebi A, Fuller P, Wiberg M, Terenghi G. Effect of allogeneic Schwann cell transplantation on peripheral nerve regeneration. Exp Neurol. 2002;173(2):213–23.
212. Mosahebi A, Wiberg M, Terenghi G. Addition of fibronectin to alginate matrix improves peripheral nerve regeneration in tissue-engineered conduits. Tissue Eng. 2003;9(2):209–18.
213. Mosahebi A, Woodward B, Wiberg M, Martin R, Terenghi G. Retroviral labeling of Schwann cells: in vitro characterization and in vivo transplantation to improve peripheral nerve regeneration. Glia. 2001;34(1):8–17.
214. Nakayama K, Takakuda K, Koyama Y, Itoh S, Wang W, Mukai T, Shirahama N. Enhancement of peripheral nerve regeneration using bioabsorbable polymer tubes packed with fibrin gel. Artif Organs. 2007;31(7):500–8.

215. Nicoli Aldini N, Fini M, Rocca M, Giavaresi G, Giardino R. Guided regeneration with resorbable conduits in experimental peripheral nerve injuries. Int Orthop. 2000;24(3):121–5.
216. Nie X, Zhang YJ, Tian WD, Jiang M, Dong R, Chen JW, Jin Y. Improvement of peripheral nerve regeneration by a tissue-engineered nerve filled with ectomesenchymal stem cells. Int J Oral Maxillofac Surg. 2007;36(1):32–8.
217. Nilsson A, Dahlin L, Lundborg G, Kanje M. Graft repair of a peripheral nerve without the sacrifice of a healthy donor nerve by the use of acutely dissociated autologous Schwann cells. Scand J Plast Reconstr Surg Hand Surg. 2005;39(1):1–6.
218. Ohta M, Suzuki Y, Chou H, Ishikawa N, Suzuki S, Tanihara M, Suzuki Y, Mizushima Y, Dezawa M, Ide C. Novel heparin/alginate gel combined with basic fibroblast growth factor promotes nerve regeneration in rat sciatic nerve. J Biomed Mater Res A. 2004;71(4):661–8.
219. Phillips JB, Bunting SC, Hall SM, Brown RA. Neural tissue engineering: a self-organizing collagen guidance conduit. Tissue Eng. 2005;11(9–10):1611–7.
220. Pogrel MA, McDonald AR, Kaban LB. Gore-Tex tubing as a conduit for repair of lingual and inferior alveolar nerve continuity defects: a preliminary report. J Oral Maxillofac Surg. 1998;56(3):319–21; discussion 21–2.
221. Rich KM, Alexander TD, Pryor JC, Hollowell JP. Nerve growth factor enhances regeneration through silicone chambers. Exp Neurol. 1989;105(2):162–70.
222. Rodriguez FJ, Verdu E, Ceballos D, Navarro X. Nerve guides seeded with autologous schwann cells improve nerve regeneration. Exp Neurol. 2000;161(2):571–84.
223. Rosen JM, Padilla JA, Nguyen KD, Padilla MA, Sabelman EE, Pham HN. Artificial nerve graft using collagen as an extracellular matrix for nerve repair compared with sutured autograft in a rat model. Ann Plast Surg. 1990;25(5):375–87.
224. Rosen JM, Padilla JA, Nguyen KD, Siedman J, Pham HN. Artificial nerve graft using glycolide trimethylene carbonate as a nerve conduit filled with collagen compared to sutured autograft in a rat model. J Rehabil Res Dev. 1992;29(2):1–12.
225. Rutkowski GE, Miller CA, Jeftinija S, Mallapragada SK. Synergistic effects of micropatterned biodegradable conduits and Schwann cells on sciatic nerve regeneration. J Neural Eng. 2004;1(3):151–7.
226. Satou T, Nishida S, Hiruma S, Tanji K, Takahashi M, Fujita S, Mizuhara Y, Akai F, Hashimoto S. A morphological study on the effects of collagen gel matrix on regeneration of severed rat sciatic nerve in silicone tubes. Acta Pathol Jpn. 1986;36(2):199–208.
227. Seckel BR, Jones D, Hekimian KJ, Wang KK, Chakalis DP, Costas PD. Hyaluronic acid through a new injectable nerve guide delivery system enhances peripheral nerve regeneration in the rat. J Neurosci Res. 1995;40(3):318–24.
228. Sierpinski P, Garrett J, Ma J, Apel P, Klorig D, Smith T, Koman LA, Atala A, Van Dyke M. The use of keratin biomaterials derived from human hair for the promotion of rapid regeneration of peripheral nerves. Biomaterials. 2008;29(1):118–28.
229. Sinis N, Schaller HE, Schulte-Eversum C, Schlosshauer B, Doser M, Dietz K, Rösner H, Müller HW, Haerle M. Nerve regeneration across a 2-cm gap in the rat median nerve using a resorbable nerve conduit filled with Schwann cells. J Neurosurg. 2005;103(6):1067–76.
230. Stanec S, Stanec Z. Ulnar nerve reconstruction with an expanded polytetrafluoroethylene conduit. Br J Plast Surg. 1998;51(8):637–9.
231. Stanec S, Stanec Z. Reconstruction of upper-extremity peripheral-nerve injuries with ePTFE conduits. J Reconstr Microsurg. 1998;14(4):227–32.
232. Stang F, Fansa H, Wolf G, Reppin M, Keilhoff G. Structural parameters of collagen nerve grafts influence peripheral nerve regeneration. Biomaterials. 2005;26(16):3083–91.
233. Sterne GD, Brown RA, Green CJ, Terenghi G. Neurotrophin-3 delivered locally via fibronectin mats enhances peripheral nerve regeneration. Eur J Neurosci. 1997;9(7):1388–96.
234. Toba T, Nakamura T, Lynn AK, Matsumoto K, Fukuda S, Yoshitani M, Hori Y, Shimizu Y. Evaluation of peripheral nerve regeneration across an 80-mm gap using a polyglycolic acid (PGA)–collagen nerve conduit filled with laminin-soaked collagen sponge in dogs. Int J Artif Organs. 2002;25(3):230–7.

235. Toba T, Nakamura T, Shimizu Y, Matsumoto K, Ohnishi K, Fukuda S, Yoshitani M, Ueda H, Hori Y, Endo K. Regeneration of canine peroneal nerve with the use of a polyglycolic acid-collagen tube filled with laminin-soaked collagen sponge: a comparative study of collagen sponge and collagen fibers as filling materials for nerve conduits. J Biomed Mater Res. 2001;58(6):622–30.
236. Unezaki S, Yoshii S, Mabuchi T, Saito A, Ito S. Effects of neurotrophic factors on nerve regeneration monitored by in vivo imaging in thy1-YFP transgenic mice. J Neurosci Methods. 2009;178(2):308–15.
237. Varejao AS, Cabrita AM, Meek MF, Fornaro M, Geuna S. Nerve regeneration inside fresh skeletal muscle-enriched synthetic tubes: a laser confocal microscope study in the rat sciatic nerve model. Ital J Anat Embryol. 2003;108(2):77–82.
238. Verdu E, Labrador RO, Rodriguez FJ, Ceballos D, Fores J, Navarro X. Alignment of collagen and laminin-containing gels improve nerve regeneration within silicone tubes. Restor Neurol Neurosci. 2002;20(5):169–79.
239. Vleggeert-Lankamp CL, de Ruiter GC, Wolfs JF, Pego AP, van den Berg RJ, Feirabend HK, Malessy MJ, Lakke EA. Pores in synthetic nerve conduits are beneficial to regeneration. J Biomed Mater Res A. 2007;80(4):965–82.
240. Walter MA, Kurouglu R, Caulfield JB, Vasconez LO, Thompson JA. Enhanced peripheral nerve regeneration by acidic fibroblast growth factor. Lymphokine Cytokine Res. 1993;12(3):135–41.
241. Wang S, Cai Q, Hou J, Bei J, Zhang T, Yang J, Wan Y. Acceleration effect of basic fibroblast growth factor on the regeneration of peripheral nerve through a 15-mm gap. J Biomed Mater Res A. 2003;66(3):522–31.
242. Wang S, Wan AC, Xu X, Gao S, Mao HQ, Leong KW, Yu H. A new nerve guide conduit material composed of a biodegradable poly(phosphoester). Biomaterials. 2001;22(10):1157–69.
243. Weber RA, Breidenbach WC, Brown RE, Jabaley ME, Mass DP. A randomized prospective study of polyglycolic acid conduits for digital nerve reconstruction in humans. Plast Reconstr Surg. 2000;106(5):1036–45; discussion 46–8.
244. Wells MR, Kraus K, Batter DK, Blunt DG, Weremowitz J, Lynch SE, Antoniades HN, Hansson HA. Gel matrix vehicles for growth factor application in nerve gap injuries repaired with tubes: a comparison of biomatrix, collagen, and methylcellulose. Exp Neurol. 1997;146(2):395–402.
245. Whitworth IH, Brown RA, Dore C, Green CJ, Terenghi G. Orientated mats of fibronectin as a conduit material for use in peripheral nerve repair. J Hand Surg Br. 1995;20(4):429–36.
246. Williams LR. Exogenous fibrin matrix precursors stimulate the temporal progress of nerve regeneration within a silicone chamber. Neurochem Res. 1987;12(10):851–60.
247. Woerly S, Pinet E, de Robertis L, Van Diep D, Bousmina M. Spinal cord repair with PHPMA hydrogel containing RGD peptides (NeuroGel). Biomaterials. 2001;22(10):1095–111.
248. Wood MD, Moore AM, Hunter DA, Tuffaha S, Borschel GH, Mackinnon SE, Sakiyama-Elbert SE. Affinity-based release of glial-derived neurotrophic factor from fibrin matrices enhances sciatic nerve regeneration. Acta Biomater. 2009;5(4):959–68.
249. Wu F, Xing D, Peng Z, Rao T. Enhanced rat sciatic nerve regeneration through silicon tubes implanted with valproic acid. J Reconstr Microsurg. 2008;24(4):267–76.
250. Xu X, Yee WC, Hwang PY, Yu H, Wan AC, Gao S, Boon KL, Mao HQ, Leong KW, Wang S. Peripheral nerve regeneration with sustained release of poly(phosphoester) microencapsulated nerve growth factor within nerve guide conduits. Biomaterials. 2003;24(13):2405–12.
251. Yang Y, Ding F, Wu J, Hu W, Liu W, Liu J, Gu X. Development and evaluation of silk fibroin-based nerve grafts used for peripheral nerve regeneration. Biomaterials. 2007;28(36):5526–35.
252. Yoshii S, Oka M. Peripheral nerve regeneration along collagen filaments. Brain Res. 2001;888(1):158–62.
253. Yoshii S, Oka M, Shima M, Taniguchi A, Akagi M. 30 mm regeneration of rat sciatic nerve along collagen filaments. Brain Res. 2002;949(1–2):202–8.
254. Yoshii S, Oka M, Shima M, Taniguchi A, Akagi M. Bridging a 30-mm nerve defect using collagen filaments. J Biomed Mater Res A. 2003;67(2):467–74.
255. Yu X, Bellamkonda RV. Tissue-engineered scaffolds are effective alternatives to autografts for bridging peripheral nerve gaps. Tissue Eng.

2003;9(3):421–30.
256. Yuen D, Jensen JR, Rodriguez G, and Li ST. A semipermeable, kink resistant type I collagen-based nerve guide for PNS repair. Trans Soc Biomaterials. 2003:p. 228.
257. Zhang J, Lineaweaver WC, Oswald T, Chen Z, Zhang F. Ciliary neurotrophic factor for acceleration of peripheral nerve regeneration: an experimental study. J Reconstr Microsurg. 2004;20(4):323–7.
258. Zhang P, He X, Zhao F, Zhang D, Fu Z, Jiang B. Bridging small-gap peripheral nerve defects using biodegradable chitin conduits with cultured schwann and bone marrow stromal cells in rats. J Reconstr Microsurg. 2005;21(8):565–71.
259. Zhang P, Xue F, Kou Y, Fu Z, Zhang D, Zhang H, Jiang B. The experimental study of absorbable chitin conduit for bridging peripheral nerve defect with nerve fasciculu in rats. Artif Cells Blood Substit Immobil Biotechnol. 2008;36(4):360–71.