HHS Public Access

Author manuscript

Muscle Nerve. Author manuscript; available in PMC 2021 June 01.

Published in final edited form as:

Muscle Nerve. 2020 June ; 61(6): 726–739. doi:10.1002/mus.26797.

Advances in the repair of segmental nerve injuries and trends in reconstruction

Deng Pan, BS[1], **Susan E. Mackinnon, MD**[1], **Matthew D. Wood, PhD**[1,*]

[1]Division of Plastic Surgery, Department of Surgery, Washington University School of Medicine, St. Louis, MO, 63110, U.S.A.

Abstract

Despite advances in surgery, the reconstruction of segmental nerve injuries continues to pose challenges. In this review, current neurobiology regarding regeneration across a nerve defect is discussed in detail. Recent findings include the complex roles of non-neuronal cells in nerve defect regeneration, such as the role of the innate immune system in angiogenesis and how Schwann cells migrate within the defect. Clinically, the repair of nerve defects is still best served by using nerve autografts with the exception of small, non-critical sensory nerve defects, which can be repaired using autograft alternatives, such as processed or acellular nerve allografts. Given current clinical limits for when alternatives can be utilized, advanced solutions to repair nerve defects demonstrated in animals are highlighted. These highlights include alternatives designed with novel topology and materials, delivery of drugs specifically known to accelerate axon growth, and greater attention to the role of the immune system.

Keywords

acellular nerve allograft; autograft; nerve gap; nerve guidance conduit; peripheral nerve

Introduction

Traumatic peripheral nerve injuries are common and caused by factors ranging from acts of violence, motor vehicle accidents, and recreational activities, to iatrogenic injuries during surgery. The majority of nerve injuries occur in the upper extremity[1,2]. It is estimated that 1–3% of all upper extremity trauma patients are diagnosed with nerve injuries during the first few months after trauma[3–5]. These injuries are often severely debilitating, resulting in lifestyle disruptions from loss of function, both at work and in leisure[6–12]. Moreover, traumatic nerve injuries frequently affect relatively young individuals, resulting in lifelong reductions in quality of life and income[13–15,4].

[*]**Address correspondence to:** Matthew Wood, PhD, Division of Plastic and Reconstructive Surgery, Washington University in St. Louis, 660 South Euclid Avenue, St Louis, MO 63110, woodmd@wustl.edu, Telephone number: 314-362-1275.

Disclosure of Conflicts of Interest: The authors declare no competing financial interests.

Ethical Publication Statement: We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Despite advances in surgery and neuroscience, the improvement of patient outcomes by the surgical reconstruction of nerve injuries continues to pose challenges. The most severe nerve injuries, in which trauma to the nerve generates a defect between the nerve ends, remain an area in significant need of improvement and further research. This review will discuss the current known neurobiology of regeneration across a nerve defect, and present current clinically available options for nerve defect repair. Additionally, we will provide a brief overview highlighting select areas of the large body of experimental animal work towards advanced solutions to repair nerve defects.

Biology of nerve regeneration across a defect

Peripheral nerve is capable of robust regeneration following injury. The molecular and cellular mechanisms have primarily been studied in rodent models. Following injury, neurons and their axons and the non-neuronal cellular environment distal to the injury undergo immediate morphological and molecular changes. Within the axon, there is a rapid influx of ions, principally calcium, as well as a disruption of transport proteins signaling a disruption to homeostasis with its end-organ. This multifactorial injury response from axon damage is rapidly transported to the neuron cell body leading to an upregulation of a regeneration associated gene (RAG) program[16]. This RAG program includes the upregulation of inflammatory genes, neurotrophic factors, and cytoskeletal protein-related genes serving to promote axon outgrowth from the damaged end of the proximal axon[16,17]. While this RAG program is upregulated in surviving neurons, some neurons will not survive the injury. Specifically, motor neurons are generally spared from cell death if the axonal injury is distal enough from the cell bodies[18], while sensory neuron death can be as high as 40% regardless of injury location[19–22].

Distal to the injury site, the process of Wallerian degeneration is initiated, which involves the fragmentation of axons disconnected from the neuron, and non-neuronal cell activation that primes the distal nerve environment for new axon outgrowth. Schwann cells (SCs) sensing the axon damage dedifferentiate and adopt a unique “repair” phenotype[23–26]. SC signaling involved in this dedifferentiation relies on Notch signaling and ERK-mediated signaling pathways[27–29], and is orchestrated by the phosphorylation of c-Jun[30,23]. Following c-Jun phosphorylation, myelination genes are down-regulated while growth promoting genes are up-regulated[30]. These activated repair SCs serve multiple functions. First, SCs begin to phagocytose axon and myelin debris around the site of injury to permit future axon growth. This debris is also mitogenic and thereby plays a role in SC proliferation post-injury[31,32]. Secondly, SCs begin to express neurotrophic factors and inflammatory factors, such as chemokines and cytokines[33–36], through activation of signaling pathways, such as Sox-2[37], which serve to recruit cells of the innate immune response, such as neutrophils[38] and macrophages[39]. Both hematogenous and resident macrophages are recruited to the site of injury. These innate immune cells provide critical phagocytic functions[40]. But additionally, these innate immune cells provide other functions essential to nerve regeneration, including re-myelination and functional recovery, which are not yet entirely understood[41,42,40]. After this Wallerian degeneration process is complete, SCs progressively assume long processes and align on the basal lamina of the intact distal nerve environment (Bands of Bungner), providing a permissive growth environment for the regenerating axons that emerge from the

proximal nerve stump[24,43]. As axon growth proceeds from proximal to distal nerve, remyelination of the axons is initiated primarily by axon-derived neuregulin-1 signaling through SCs' ErbB receptors[44,45]. At the target end organs, specialized SCs have already begun priming this environment for reestablishment of axonal connection[46]. For example, in muscle a unique type of SC, terminal SCs, extend cytoplasmic processes within days after injury, which serve as platforms for incoming axonal growth[47,48,46].

Our understanding of the regenerative processes enabling nerve regeneration across a small nerve defect or cell-free nerve graft, where the entire nerve tissue must regenerate, is incomplete (Figure 1). To first allow for cell migration and infiltration into this defect, an endogenous matrix must form. The repair of nerve defects using a pseudosynovial sheath demonstrated that plasma exudates from the proximal and distal nerve stumps fill the empty tube volume, and provide a deposition of extracellular matrix (ECM), including a fibrin matrix, that allows for innate immune system cell migration[49–56]. While the earliest (<4 days) macrophages are derived from tissue resident macrophages, subsequent macrophages are primarily hematogenous-derived[40]. Due to the hypoxic nature of this environment, macrophages support a substantial amount of angiogenesis enabling endothelial cell recruitment and vessel formation[56] (Figure 1A). These blood vessels become polarized allowing for the migration of SCs[56], which have a distinct phenotype similar to a stem cell in this [57,26], and in turn form cellular cords through their interactions with fibroblasts[58] (Figure 1B). After axons cross this bridge (Figure 1C), the regenerative processes described in the distal nerve promote the growth of axons to their down-stream targets.

Identifying and managing traumatic nerve injuries

In the clinic, nerve injuries present on a spectrum, which includes damage to the many axons contained within nerve that may be recoverable or non-recoverable depending on the type and degree of injury. Therefore, the extent of nerve injury must first be determined before any surgical decisions are made. For severe nerve injuries requiring repair, this identification may be immediately obvious due to substantial soft tissue damage resulting in loss of nerve, or less obvious and result from damage to nerve that generates a "zone of injury." This zone of injury, which refers to the scarring of nerve following damage, is not fully present until approximately 3 weeks after the initial injury[59,60]. It is critical to identify this zone, as this scar inhibits the endogenous processes for regeneration. Nerve repair performed before this is present has the risk that the proximal or distal end of the nerve still contains scar that would block endogenous regeneration facilitated by the repair from proceeding. To identify this zone of injury, the use of electrodiagnostics, clinical examination, and in the operating room, a technique termed "bread-loafing," as it dissects the nerve back to the start of scarred tissue, are commonly used[59,60]. Imaging modalities can be used as well, but these imaging modalities are generally of minimal clinical utility. Current clinical imaging modalities are unable to provide direct correlation to axonal injury and cannot adequately evaluate peripheral nerve injury in which the damaged tissue recovers over time[61].

After identifying the zone of injury together with any original gap, the effective defect size is revealed. A defect of even a few millimeters will often prevent the previously described endogenous mechanisms for regeneration, and therefore, nerve repair acts primarily to

facilitate this regenerative process. However, even with a nerve defect, nerve grafting to bridge the defect is not the only option to repair the nerve and restore function. After identifying the injury, the location of the injury is also considered. Nerve transfers, whereby the proximal portion of an expendable nerve is sutured to the damaged distal nerve end, are frequently used for most proximal nerve injuries. Transfers avoid not only the regenerative processes involved to bridge the nerve defect, but also reduce the long period of axon growth needed to reestablish axon reinnervation of end-organs[62–64]. It also obviates the need for grafts. Therefore, distal nerve injuries are typically reconstructed by direct coaptation of the proximal and distal nerve ends, either directly or with a grafting material. Direct epineurial or grouped fascicular repair is possible when a defect is small, only a few millimeters, so as to avoid inducing longitudinal tension[65,66]. For other injuries, nerve grafting is then employed[67–69].

Bridging materials to repair nerve defects

While the repair of nerve defects using a bridging material dates to the 1800s, it was not until the 1970s that the current standard of nerve defect management was initiated with the advent of the nerve autograft. The efforts of Huber and Bunnell established nerve autografting for nerve defect repair, while further understanding of anatomy and microsurgical techniques from Sunderland, Millesi, and Buncke advanced this technique[60]. Since then, several alternatives have become clinically-approved for repair of nerve defects. But, while this section will detail the clinically available options to repair a segmental nerve injury, the autologous nerve graft, nerve tissue harvested from the patient, remains the gold standard of nerve gap repair.

Nerve autografts

From all the biological principles just described in the previous sections, the nerve autograft serves in theory as an ideal bridging material. Nerve autograft is nerve tissue harvested from the patient taken from an area in the body where the loss of function from its harvest is thought to be minimal. Donor nerves that are commonly used as autografts are expendable sensory nerves, such as the sural nerve or the medial antebrachial cutaneous nerve[60]. The nerve autograft provides not only a suitably matched scaffolding structure for regenerating nerve, principally ECM arranged to include endoneurial tubes, but also a diverse cell supply, including a vascular network and support cells of nerve, critically SCs. While blood flow is initially disrupted during the grafting procedure, functional blood flow is rapidly restored within days via inosculation, or spontaneous end to end repair of existing vasculature, which occurs from both the tissue bed and reconnected nerve ends[70–72]. Furthermore, these graft undergo remodeling in the weeks that follow, including Wallerian degeneration, thus mimicking the scenario just described following injury for the distal portion of an injured nerve. These processes include an invasion of macrophages, proliferation of the donor graft SCs, degeneration of axons and myelin debris within the graft, and even a robust migration of SCs from the graft to the repaired nerve ends[73]. While similar in many ways to the processes affecting nerve distal to an injury site, there is one critical difference. It is highly likely that a portion of non-neuronal support cells within the graft die because of disrupted blood flow and tissue oxygenation before inosculation is complete. But, this cell death does

not appear to be a major issue that disrupts its capabilities to facilitate robust regeneration. Therefore, an autograft currently represents the best available, while imperfect, option for a grafting material.

Despite the inherent advantages of providing a cellular supplemented scaffold that promotes robust nerve regeneration across a defect, autografting comes with significant drawbacks. The donor harvest will entail morbidity at the site, including loss of donor nerve function, and its harvest entails additional operations that can include complications, scarring, and even the potential for neuroma formation at this additional surgical site[74,75]. Furthermore, there is a limited supply of expendable donor nerves, and the harvesting of these nerves adds to the overall operative time for the patient. Therefore, there has been a desire for alternatives, and a continued push to use these alternatives even if the outcomes do not yet match the autograft.

Cadaveric nerve allografts

Following the advent of adequate immunosuppressive regimens in the 1980s, cadaveric nerve allografts became a feasible clinical alternative to autografts. Nerve allografts, i.e. nerve tissue from organ donors, represent an analogous form of bridging material to the autograft while sparing the donor from loss of function. However, as their use requires immunosuppression to avoid rejection and regeneration failure[76], and the use of immunosuppression is associated with significant clinical morbidity[77], the use of allografts in peripheral nerve repair is quite rare, and limited to the repair of only the most severe cases of nerve injuries, with a considerably long nerve defect length[78].

Nerve guidance conduits

Conduits, also referred to as nerve guidance conduits, are the most diverse and deeply researched category of nerve autograft alternatives. Conduits consist of tubular structures to encapsulate and facilitate cellular and axon growth across the nerve ends, as these tubes serve to provide a protective environment for nerve regeneration. The idea of repairing a nerve using a conduit was largely driven from the previously-discussed animal studies that determined that encapsulating a nerve can promote a robust endogenous mechanism that drives an entire nerve to regenerate its structure. These experiments provided a framework demonstrating that even in the absence of a scaffold or cells, peripheral nerves are capable of producing their own scaffolding, and in turn, use that scaffolding to support cell migration and ultimately axon regeneration[50,49,79,53].

From this animal work, conduits were the first translated work providing “off the shelf” alternatives to treat nerve defects[80]. However, non-degradable conduits demonstrated significant limitations arising from the side-effects to nerve. Silicone conduits have been shown to cause significant chronic nerve compression and irritation at the implantation sites, requiring removal[81–83]. This incompatibility from the long-term presence of a material surrounding the nerve drove research into alternative materials that could act as more natural or temporary biodegradable conduits. Naturally-derived conduits, such as arteries, muscles and tendons, have been studied for bridging nerve defects, but vein tubes have received the most attention among researchers and even are used clinically[84]. Far and away,

manufactured conduits synthesized with properties that yield biodegradable structures represent the majority of modern products available in the clinic. At least seven synthetic nerve conduits have been approved by the US Food and Drug Administration (FDA) for clinical use in nerve reconstruction[85]. However, these conduits are generally similar in their principles and features, as they are biodegradable through various mechanisms and promote regeneration via first the formation of endogenous ECM within the empty tube structure. Extensive reviews of the wide range of conduit material properties are available[68,85–87].

Processed or acellular nerve allografts

More recently, processed or acellular tissue scaffolds have gained prominence for clinical reconstructions specifically in the United States (U.S.). For example, a recent survey estimated that ~70% of U.S. hand surgeons use processed nerve allografts in nerve defect repair[88]. Acellular tissue scaffolds are generated using techniques to retain a large portion of native ECM proteins while minimizing cellular debris and undesired immunological response (i.e. rejection). Acellular tissues, despite their lack of cells and processing techniques, typically maintain a highly organized extracellular matrix structure providing an ideal scaffold structure for regeneration. As such, acellular nerve allografts (ANAs) or processed nerve allografts (PNAs), which are generally synonymous, have become a prominent nerve autograft alternative choice due to these features[89,69,88]. Acellular nerve grafts are conceptually appealing not only because they provide a scaffold for immediate cell migration and angiogenesis, making them distinct from clinically-available conduits[90–92], but their ECM structure is similar to native nerve.

Numerous experimental methods have been used to generate these nerve grafts, which all entail some technique to remove cells and antigens from the nerve. This topic was reviewed extensively by Szynkaruk et al [92], but examples of decellularizing techniques include repeated freeze-thaw cycles, exposure to radiation, lyophilization, extended storage in cryopreservation solution, and decellularization with detergents[93–99]. However, a detergent based protocol developed by Hudson et al.[100] has been the only processing technique to translate to a clinical nerve product with FDA approval. A variation of this protocol was used in 2008 to develop the first commercially available acellular peripheral nerve allograft for clinical use. These PNAs are produced from harvested cadaveric human nerves, processed to remove cells using a human variation of Hudson et al.[100], as well as enzymatic removal of chondroitin-6-sulfate proteoglycan, a known inhibitor of axonal regeneration[101–103].

Guidelines for nerve graft repairs

Any form of nerve repair is affected by a range of variables that ultimately limits the quantity of axons regenerating and reaching their target. These include the distance for axon growth, a general decline in the capacity to promote axon growth over time, axonal misdirection from their appropriate end-organ targets, as well as atrophy of the end-organ targets[91,104–108]. For nerve grafting repairs, outcomes are also additionally affected by the bridged defect size (i.e. both diameter and length) and whether the nerve defect repaired is

supporting primarily sensory or motor functions. Furthermore, the choice of material to repair the defect affects the outcome.

The nerve autograft provides generally superior and more consistent outcomes compared to any currently available clinical alternatives. Animal studies have demonstrated the superiority of nerve autografting over alternatives to support axon regeneration and functional recovery. Two independent research groups have demonstrated in rodent models that autografts support a greater extent of axon regeneration across a defect compared to any clinically available alternative, as well as more rapid axon growth across the graft reaching the distal nerve[109,110]. As autografts supported axon regeneration across a defect more rapidly and to a greater extent than alternatives, this outcome has critical implications for functional recovery. Reducing the time of distal nerve and end-organ denervation is the best known strategy to achieve reinnervation of end-organ targets and functional recovery[104,91]. From these specific research studies, it was also determined that a hierarchy between grafting procedures exists. Autografts supported the greatest extent of axon regeneration across a defect, followed by ANAs, and then empty conduits[109,110].

Rather critically, there has yet to be a head-to-head prospective clinical study comparing the results of autografts to any existing nerve graft alternatives, which would provide the best evidence to support or oppose a change in practice. A comparison of nerve autografting to current clinical autograft alternatives has only been directly demonstrated in animal studies, as just described. Thus, any evidence demonstrating alternatives are comparable to autografts in outcomes has been derived from individual studies of alternatives used in nerve repair that are then compared to historical autograft data. Not only do these study designs introduce additional confounding factors, such as differences in surgical techniques and patient population, but comparisons to historic data can make the outcome comparisons appear biased.

While autografts remain the best option for managing nerve defect repairs, there are criteria that specify situations in which the use of autograft alternatives is preferred. First, the use of nerve graft alternatives in the reconstruction of small and short segmental sensory nerve injuries can be supported for logical reasons regardless of evidence of their efficacy. There are situations where harvest of a nerve autograft would be counterintuitive: for example, sacrificing a non-critical, small sensory nerve to fix a single non-critical, small sensory nerve defect. Another example is autograft harvest in a patient with an established pain syndrome, where pain at a nerve donor site is more likely to occur. If the reconstructed nerve is non-critical, more harm may be done than good with autograft harvest. In clinical situations such as these, alternatives are better justified so long as the risks of complications are minimized.

Short or small sensory nerve defects

A growing body of evidence has now demonstrated that nerve graft alternatives support adequate recovery (i.e. comparable recovery to an autograft based on historic data on outcomes) to treat short and small diameter segmental sensory nerve injuries. While defining these parameters can be slightly subjective, in general there is a consensus that short length defects are less than 30 mm[111,60,112]. The most common metric used for these comparisons has been meaningful recovery based on the Medical Research Council (MRC) scale (Table

1), set for sensory function[113] at ≥S3 and motor function[114] at ≥M4[115]. For digital nerves repaired with an autograft, the reported ranges of meaningful recovery are nearly 100%[116,117]. For comparison, commercial conduits yielded meaningful recovery in 44–75% of repairs[81,118,119]. Alternatively, the data for PNAs suggests superior clinical results when compared to conduits for the reconstruction of digital nerve defects. Multiple independent studies demonstrated meaningful recovery in 80–89% of digital nerve defect repairs using PNAs meeting similar criteria[120–122]. And finally, while there is limited data demonstrating a major difference in the degree of recovery when regeneration is successful, evidence demonstrates that autografts and PNAs provide more consistent recovery compared to conduits for treating short length or small diameter sensory nerve defects[123,111,124].

Short or small motor / mixed nerve defects

In general, regardless of grafting option (autograft or alternative), anticipated motor functional recovery rates will be appreciably reduced compared to sensory recovery when repairing motor and mixed segmental nerve injuries. Furthermore, the evidence to use any nerve graft alternative for their repair is much more contentious, and there are no evidence-based guidelines to use nerve graft alternatives instead of nerve autograft to reconstruct motor or mixed nerve defects[111]. For the repair of mixed or motor nerves, such as the median and ulnar nerves, studies have demonstrated that autograft repair results in meaningful recovery in 60–80% of radial and median nerves[125,126] and 57–60% of ulnar nerves[127]. For conduits, while few studies exists, the outcomes are dismal. In one study, the repair of median or ulnar nerve (defects less than 30 mm) resulted in only 8% meaningful recovery[119]. For the other prominent category of alternatives (PNAs), the outcomes from studies have been more promising. For median nerve repairs up to 50 mm, 75% of patients experienced meaningful recovery, and for ulnar nerve repairs of similar criteria, up to 67% of patients experienced recovery[120]. Furthermore, in a more recent study, a motor recovery rate of ~67% was appreciated across a variety of repaired nerve defects up to 30 mm[122]. Therefore, recent data suggest that even mixed or motor nerve defects less than 30 mm could be repaired equally well using PNAs compared to autografts. But, these outcomes represent few studies with select surgeons. As reviewed in greater detail by Rbia and Shin, there is still insufficient evidence at this time to support the widespread use of alternatives to repair mixed or motor nerve defects[111].

Long or large nerve defects

There is still a critical unmet need for improved nerve grafting options to treat long (>30 mm) segmental nerve injuries. Regardless of considerations for sensory or motor functions, neither is anticipated to recover well. And, these anticipated poor outcomes result even from repair using a nerve autograft, while it remains the best available option. There is no consensus on the maximum gap that can be bridged by a nerve autograft, and in fact, widely varying degrees of success have been reported in autografts up to 200 mm[128]. However, there is a general consensus that any regenerative success declines as autografts go beyond 60 mm[60]. As an example, meaningful sensory recovery from a digital nerve defect repair using autografts was observed in 100% of patients with defect lengths less than 21 mm, while this recovery rate fell to 67% for lengths between 21 and 49 mm and only 9% for lengths greater than 49 mm[117]. This relationship regarding defect length and recovery rates

has also been observed for conduits[81] and PNAs[122]. Similar to data on autograft repairs for long defects, studies using PNAs to repair long nerve defects demonstrate that sensory and motor recovery can vary considerably among studies. In one study, a high level (~86%) of meaningful sensory recovery was observed in patients with nerve defects repaired using PNAs at lengths between 40 and 50 mm[129]. Yet, more recent studies from Leckenby et al significantly temper these promising outcomes. In their studies, PNAs used to repair nerve defects between 30–49 mm yielded ~75% meaningful sensory recovery but only 38% motor recovery. Similarly, defects greater than 50 mm yielded 53% sensory recovery, but only 10% recovery ofmeaningful motor function[122].

Concerns regarding alternatives for repairing defects

A prevailing concern for caution regarding the use of alternatives is due to the general variability and inconsistency of results with alternatives compared to autografts. Alternatives can fail to facilitate any nerve regeneration across a bridged nerve defect, which is distinct from autograft repair. Specifically, a failure to regenerate across an alternative can result in no appreciable functional recovery, while a poor outcome from autografting will still yield some recovery, even if not ≥S3+ or M4. This phenomenon whereby nerve fails to regenerate any axons across a defect repaired using an alternative is well-documented in the animal literature,[130–132] but now there is an increasing body of evidence of these issues present in the clinical literature. Indeed, Moore et al, found that use of conduits can result in inferior quality nerve regeneration, which is most prevalent when conduits are used to repair nerve defects beyond their length indications. This failure can not only lead to lack of recovery, but can even result in formation of a painful neuroma in the repaired nerve defect[133]. Specific examples of this issue have been published on PNAs as well. Nietosvaara et al. presented three cases of PNAs ranging from 20 mm to 50 mm failing to facilitate any measurable axon regeneration across these PNAs. In fact, they document that the PNAs resorbed leaving behind scar tissue, and in one case, had thickened into a neuroma-like stump[134]. Overall, this evidence is perhaps the strongest reason to take a conservative approach to utilizing alternatives in the clinic until these circumstances are better understood.

Experimental nerve graft alternatives

Since the development of biodegradable conduits and identification of their limitations, experimental work to develop improved nerve graft alternatives has been ongoing. As the body of work on experimental alternatives is substantial, this section will highlight specific recent areas that show promise for translation. For a more comprehensive review of bioengineered nerve graft alternatives, see Pfister *et al.*[68], Boecker *et al*[86], and Kornfeld *et al*[87].

Scaffolding and topology

The use of synthetic conduits containing internal scaffolding that has included ECM, such as collagen, laminin, and fibrin[131,135,136], has demonstrated advantages in improving regeneration compared to empty conduits. However, there are limits to these internal scaffolds compared to acellular nerve. These scaffolds develop with a random arrangement of the molecular fibers, which differs significantly from the organized and longitudinal

arranged structure of nerve. Instead, recent advances that arrange the topology of the scaffold fibers holds promise, and the most interesting developments are the use of scaffolds with a longitudinal organization. This scaffolding can be achieved through a variety of means, including the use of electromagnetic fields and photolithography, but electrospinning techniques are becoming increasingly well-developed for this approach[137,138]. These techniques allow for aligned fibers, synthetic or "naturally-derived", to be deposited, resulting in longitudinally oriented pores or channels as small as 1 μm, which are comparable to endoneurial tubes, ranging from 1–20 μm[137]. These designs allow for scaffolds that not only more closely mimic the nerve's structure[139,140] but could lead to more rapid cell migration and polarization of cells[141,142]. As endothelial cell polarization is important for both SC migration and then axon outgrowth[56], this could lead to a major translational development. In addition, the use of synthetic fibers and/or conduits also holds an advantage in that drugs for improving regeneration (detailed in the next section) could be readily incorporated into the underlying scaffold.

Local drug delivery

The delivery of biologically active molecules locally during regeneration is another strategy that has been extensively pursued for several decades. Nerve scaffolds or the conduit itself can be incorporated with bioactive molecules via chemical interactions, via crosslinking or affinity-based interactions, or physically encapsulated. While chemical interactions can provide drugs immediately to cells or as cells proceed to grow within a scaffold, physical encapsulation of drugs provides a greater range of delivery options, such as long-term or sustained release. Numerous molecules or drugs have been delivered to nerve for regeneration across a nerve defect[143,144], but the most researched category for delivery has been neurotrophic factors. Of these, glial cell line-derived neurotrophic factor (GDNF) is a promising example that promotes regeneration because it targets both axons and SCs. Both sensory and motor neurons express receptors for GDNF (Ret/GFRα1)[145], where GDNF signaling promotes axon outgrowth and neuronal survival[146–151]. In addition, SCs also express receptors for GDNF (NCAM/GFRα1)[152], where GDNF signaling activates pathways in SCs implicated in cell migration, differentiation, and trophic factor production[152–163]. Exogenous GDNF delivered to nerve has been shown to improve not only axon regeneration but functional recovery[131,164].

More recently, the local delivery of drugs that accelerate axon outgrowth have been investigated. FK-506, an immunosuppressive drug, enhances nerve regeneration, as it increases the axonal growth rate in animal models[165–172]. Now, approaches have been developed to provide FK506 locally to enhance axon regeneration given its abilities to stimulate more rapid axon outgrowth[173–177]. This strategy is quite intriguing given that it reduces the potential for any systemic toxicity from its immunosuppression. Furthermore, while this delivery strategy has demonstrated its potential to improve nerve regeneration in animal models[174], the sustained local delivery of FK506 from a nerve graft alternative bridging a nerve defect could translate to benefits even after axons cross the bridged region, as it could continue to stimulate accelerated axon growth through the distal nerve.

Alternatively, based on our increasing knowledge of the immune response during regeneration across nerve defects, recruiting immune cells, such as macrophages, is a promising strategy. While angiogenic factors, such as vascular endothelial growth factor (VEGF), have been locally delivered from conduits to promote improved angiogenesis within bridged nerve gap[178,179], modulating the local immune system in order to recruit cells to promote endogenous angiogenesis could have advantages, such as a greater degree of endothelial cell polarization. Furthermore, the immune system has a critical role in resolving inflammation following injury, where macrophages also have additional roles in this aspect of tissue regeneration. Macrophages alter their secreted cytokines based on their phenotype. While a simplification, macrophages are broadly classified as classically activated (M1) or alternatively activated (M2) phenotypes. The M1 macrophage response predominates during the onset of injury while the M2 polarization or subtypes generally promotes healing, remodeling, and resolution of regeneration[180,181]. Studies using conduits releasing factors promoting a more M1-like phenotype (IFNγ) versus a more M2-like phenotype (IL-4, collagen VI, or fractalkine) have demonstrated improved nerve regeneration when factors promoting a greater accumulation of M2-like phenotype macrophages were used to repair a nerve defect compared to conduits lacking this ability[182–184]. Furthermore, the nerve autograft alternative itself could be designed to promote macrophage polarization to the more M2-like phenotype through its inherent biomaterial properties, which has been observed upon conduit materials such as chitosan[185]. Indeed, these conduits are now undergoing clinical trials to determine their efficacy in nerve repair[186].

Supplementing cells

Match and surpassing the outcomes of the autograft will likely entail alternatives that include a cellular component. SCs and stem cells have been shown to be the most promising sources for this supplementation[187]. However, several challenges remain: improving the survival of transplanted cells and determining the cells to transplant. Supplementing ANAs with SCs has been shown to result in very low survival of the transplanted cells, which can be as low as 2% of transplanted cells surviving up to 7 days[188,189]. And while SCs in culture do not transform or reach a proliferative limit[190], the difficulty in SC isolation and culture has impeded clinical translation[191,95,192]. Instead, stem cell transplantation seems a more promising avenue. A more extensive review of stem cells used for nerve graft alternatives can be found in Johnson et al.[69]. Recent work from Shin and colleagues is noteworthy. They developed an approach to seed mesenchymal stromal (stem) cells upon ANAs with improved viability and greater reproducibility. A bioreactor was used to seed the mesenchymal stromal cells onto ANA[193], a technique that has led to cells surviving up to 29 days[194]. Overall, supplementation of cells to nerve graft alternatives seems an inevitable translational advance once the processes to reliably seed these cells to the alternatives are robust.

Future research needed to improve alternative designs

While these experimental strategies are promising, unaddressed issues remain, and it is unclear whether these will be adequately addressed by new designs. The goal of these new

designs should be not only to meet the autograft “gold standard” but to also understand what drives and inhibits nerve regeneration in order to surpass the results of an autograft. To do this requires looking beyond new designs to also consider what are the factors limiting the current designs.

How is consistent regeneration and motor recovery promoted?

As demonstrated by clinical evidence, sensory recovery can generally be achieved using nerve graft alternatives, but motor recovery is highly inconsistent. Specifically, repairing motor nerve defects with alternatives yields meaningful motor function recovery at rates ranging from 8–75%[133,69,111,195]. We need to understand how to promote *consistent* regeneration across alternatives with robust recovery. A critical component to achieving any level of functional recovery is regeneration of an adequate number of axons to their target (i.e. skin or muscle) in a timely manner[196–201,17], but the promotion of functional recovery also requires other processes. Axonal guidance and non-neuronal cell signaling, including that occurring through the immune system, all contribute to regeneration leading to functional recovery[197,198,200,104,42]. The essential mechanisms involved in promoting nerve regeneration across alternatives yielding robust motor functional recovery are unresolved, despite many animal studies demonstrating return of motor recovery. Uncovering what factors are missing in the translation of animal to human research will be critical to advancing the field.

Why is regeneration functionally limited by graft length?

Animal studies have yet to demonstrate that nerve graft alternatives can reliably facilitate axon regeneration across longer defects (>30mm) despite their robust abilities to promote regeneration across shorter defects[202–206]. A review of this specific topic is available: see Kornfeld *et al*[87]. However, there is limited understanding as to why few axons regenerate across these long graft alternatives despite cues to promote axon growth and regeneration that succeed at shorter lengths. Effectively, alternatives are functionally limited by length.

While the cause of limited axon regeneration across long nerve graft alternatives is still controversial, a series of recent studies has illuminated some novel and insightful findings. Using ANAs as a model nerve graft alterative, these studies compared the regenerative processes within short (non-critical length) vs long (critical-length) ANAs revealing that cell repopulation of ANAs due to length was the primary reason for limited axon regeneration[202,203,206] (Figure 2). In these studies, to establish whether the environment that develops in long nerve graft alternatives – not the ability of neurons to regenerate their axons over long distances – is responsible for axon growth arrest[203], experiments were performed whereby long alternatives were shortened <30mm in length. These shortened alternatives, which should normally facilitate axon regeneration across a short defect, still contained this altered cellular environment and ECM structure. These now “stressed” short grafts halted axon growth and regeneration[203]. Furthermore, it was also established that this poor regenerative outcome across long grafts is not caused by a failure of neurons to regenerate their axons. Using a sciatic nerve defect repair with a long acellular nerve, regenerating axon growth was first arrested and then proceeded by replacing the remaining scaffold with an isograft (animal equivalent to an autograft). In this scenario, axon growth resumed[203].

Overall, these results demonstrated the environment of long nerve graft alternatives generates a barrier to axon growth.

Recently, immune cells other than macrophages were determined to have additional, undescribed processes that contributed to regeneration across ANAs based on length. Specifically, a novel role for T cells was elucidated. T cells accumulated within short (20mm) ANAs after macrophages, but before appreciable SC accumulation or axon growth. T cell accumulation within short ANAs was associated with robust nerve regeneration. Conversely, few T cells accumulated within long (40mm) ANAs, which were associated with minimal axon regeneration across these grafts. Furthermore, a direct causal relationship between T cells and nerve regeneration across ANAs was demonstrated. Nerve regeneration across short (20mm) ANAs, which normally support robust axon regeneration across the gap, was decreased by ~50% in T cell deficient rats[206]. Overall, research regarding the role of the immune system represents an important area that needs further examination in this context (Figure 3). Given the prominent role that the immune system plays in tissue regeneration, there is still limited knowledge on its role during regeneration across a nerve defect.

Acknowledgements and Sources of Financial Support

This work was supported in part by the National Institutes of Neurological Disorders and Stroke of the National Institutes of Health (NIH) under award numbers R01 NS086773 and R01 NS115960 to Washington University and by a Pilot Project Award from the Hope Center for Neurological Disorders at Washington University. The content is solely the responsibility of the authors and does not represent the views of the NIH or Washington University.

Abbreviations

ANA	Acellular nerve allograft
ECM	Extracellular matrix
FDA	Food and Drug Administration
FK506	Tacrolimus
GDNF	Glial cell line-derived neurotrophic factor
MRC	Medical Research Council
PNA	Processed nerve allograft
RAG	Regeneration associated gene
SC	Schwann cell
VEGF	Vascular endothelial growth factor

References

1. Ootes D, Lambers KT, Ring DC. The epidemiology of upper extremity injuries presenting to the emergency department in the United States. Hand 2012;7(1):18–22. [PubMed: 23449400]

2. Wenzinger E, Rivera-Barrios A, Gonzalez G, Herrera F. Trends in upper extremity injuries presenting to US emergency departments. Hand 2017:1558944717735943.

3. Noble J, Munro CA, Prasad VS, Midha R. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. Journal of Trauma and Acute Care Surgery 1998;45(1):116–122.

4. Taylor CA, Braza D, Rice JB, Dillingham T. The incidence of peripheral nerve injury in extremity trauma. American journal of physical medicine & rehabilitation 2008;87(5):381–385. [PubMed: 18334923]

5. Huckhagel T, Nüchtern J, Regelsberger J, Lefering R. Nerve injury in severe trauma with upper extremity involvement: evaluation of 49,382 patients from the TraumaRegister DGU® between 2002 and 2015. Scandinavian journal of trauma, resuscitation and emergency medicine 2018;26(1):76.

6. Chemnitz A, Dahlin LB, Carlsson IK. Consequences and adaptation in daily life–patients' experiences three decades after a nerve injury sustained in adolescence. BMC musculoskeletal disorders 2013;14(1):252. [PubMed: 23968274]

7. Novak CB, Anastakis DJ, Beaton DE, Mackinnon SE, Katz J. Relationships among pain disability, pain intensity, illness intrusiveness, and upper extremity disability in patients with traumatic peripheral nerve injury. The Journal of hand surgery 2010;35(10):1633–1639. [PubMed: 20888499]

8. Novak CB, Anastakis DJ, Beaton DE, Mackinnon SE, Katz J. Biomedical and psychosocial factors associated with disability after peripheral nerve injury. JBJS 2011;93(10):929–936.

9. Bailey R, Kaskutas V, Fox I, Baum CM, Mackinnon SE. Effect of upper extremity nerve damage on activity participation, pain, depression, and quality of life. The Journal of hand surgery 2009;34(9):1682–1688. [PubMed: 19896011]

10. Dijkers M Quality of life of individuals with spinal cord injury: a review of conceptualization, measurement, and research findings. Journal of rehabilitation research and development 2005;42(3):87. [PubMed: 16195966]

11. Dolan R, Butler J, Murphy S, Hynes D, Cronin K. Health-related quality of life and functional outcomes following nerve transfers for traumatic upper brachial plexus injuries. Journal of Hand Surgery (European Volume) 2012;37(7):642–651.

12. Domeshek LF, Krauss EM, Snyder-Warwick AK, Laurido-Soto O, Hasak JM, Skolnick GB, Novak CB, Moore AM, Mackinnon SE. Surgical treatment of neuromas improves patient-reported pain, depression, and quality of life. Plastic and reconstructive surgery 2017;139(2):407–418. [PubMed: 28125533]

13. Ciaramitaro P, Mondelli M, Logullo F, Grimaldi S, Battiston B, Sard A, Scarinzi C, Migliaretti G, Faccani G, Cocito D. Traumatic peripheral nerve injuries: epidemiological findings, neuropathic pain and quality of life in 158 patients. Journal of the Peripheral Nervous System 2010;15(2):120–127. [PubMed: 20626775]

14. Jaquet J-B, Luijsterburg AJ, Kalmijn S, Kuypers PD, Hofman A, Hovius SE. Median, ulnar, and combined median-ulnar nerve injuries: functional outcome and return to productivity. Journal of Trauma and Acute Care Surgery 2001;51(4):687–692.

15. Lee B, Cripps R, Fitzharris M, Wing P. The global map for traumatic spinal cord injury epidemiology: update 2011, global incidence rate. Spinal cord 2014;52(2):110. [PubMed: 23439068]

16. Abe N, Cavalli V. Nerve injury signaling. Current opinion in neurobiology 2008;18(3):276–283. [PubMed: 18655834]

17. 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. [PubMed: 12845152]

18. Xu QG, Forden J, Walsh SK, Gordon T, Midha R. Motoneuron survival after chronic and sequential peripheral nerve injuries in the rat. Journal of neurosurgery 2010;112(4):890–899. [PubMed: 19764828]

19. Schmalbruch H Loss of sensory neurons after sciatic nerve section in the rat. Anat Rec 1987;219(3):323–329. [PubMed: 3425951]

20. McKay Hart A, Brannstrom T, Wiberg M, Terenghi G. Primary sensory neurons and satellite cells after peripheral axotomy in the adult rat: timecourse of cell death and elimination. Exp Brain Res 2002;142(3):308–318. [PubMed: 11819038]
21. West CA, McKay Hart A, Terenghi G, Wiberg M. Sensory neurons of the human brachial plexus: a quantitative study employing optical fractionation and in vivo volumetric magnetic resonance imaging. Neurosurgery 2012;70(5):1183–1194; discussion 1194. [PubMed: 22095219]
22. Terenghi G, Hart A, Wiberg M. The nerve injury and the dying neurons: diagnosis and prevention. The Journal of hand surgery, European volume 2011;36(9):730–734.
23. Arthur-Farraj PJ, Latouche M, Wilton DK, Quintes S, Chabrol E, Banerjee A, Woodhoo A, Jenkins B, Rahman M, Turmaine M, Wicher GK, Mitter R, Greensmith L, Behrens A, Raivich G, Mirsky R, Jessen KR. c-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. Neuron 2012;75(4):633–647. [PubMed: 22920255]
24. Jessen KR, Mirsky R. The Success and Failure of the Schwann Cell Response to Nerve Injury. Frontiers in cellular neuroscience 2019;13:33. [PubMed: 30804758]
25. Jessen KR, Mirsky R. The repair Schwann cell and its function in regenerating nerves. The Journal of physiology 2016;594(13):3521–3531. [PubMed: 26864683]
26. Jessen KR, Arthur-Farraj P. Repair Schwann cell update: Adaptive reprogramming, EMT, and stemness in regenerating nerves. Glia 2019;67(3):421–437. [PubMed: 30632639]
27. Napoli I, Noon LA, Ribeiro S, Kerai AP, Parrinello S, Rosenberg LH, Collins MJ, Harrisingh MC, White IJ, Woodhoo A, Lloyd AC. A central role for the ERK-signaling pathway in controlling Schwann cell plasticity and peripheral nerve regeneration in vivo. Neuron 2012;73(4):729–742. [PubMed: 22365547]
28. Yang DP, Kim J, Syed N, Tung YJ, Bhaskaran A, Mindos T, Mirsky R, Jessen KR, Maurel P, Parkinson DB, Kim HA. p38 MAPK activation promotes denervated Schwann cell phenotype and functions as a negative regulator of Schwann cell differentiation and myelination. The Journal of neuroscience : the official journal of the Society for Neuroscience 2012;32(21):7158–7168. [PubMed: 22623660]
29. Cervellini I, Galino J, Zhu N, Allen S, Birchmeier C, Bennett DL. Sustained MAPK/ERK Activation in Adult Schwann Cells Impairs Nerve Repair. The Journal of neuroscience : the official journal of the Society for Neuroscience 2018;38(3):679–690. [PubMed: 29217688]
30. Parkinson DB, Bhaskaran A, Arthur-Farraj P, Noon LA, Woodhoo A, Lloyd AC, Feltri ML, Wrabetz L, Behrens A, Mirsky R, Jessen KR. c-Jun is a negative regulator of myelination. The Journal of cell biology 2008;181(4):625–637. [PubMed: 18490512]
31. Beuche W, Friede RL. The role of non-resident cells in Wallerian degeneration. Journal of neurocytology 1984;13(5):767–796. [PubMed: 6512566]
32. Scheidt P, Friede RL. Myelin phagocytosis in Wallerian degeneration. Properties of millipore diffusion chambers and immunohistochemical identification of cell populations. Acta Neuropathol 1987;75(1):77–84. [PubMed: 3434217]
33. Rotshenker S Wallerian degeneration: the innate-immune response to traumatic nerve injury. Journal of neuroinflammation 2011;8:109. [PubMed: 21878125]
34. Martini R, Willison H. Neuroinflammation in the peripheral nerve: Cause, modulator, or bystander in peripheral neuropathies? Glia 2016;64(4):475–486. [PubMed: 26250643]
35. Fischer S, Weishaupt A, Troppmair J, Martini R. Increase of MCP-1 (CCL2) in myelin mutant Schwann cells is mediated by MEK-ERK signaling pathway. Glia 2008;56(8):836–843. [PubMed: 18383340]
36. Zigmond RE. Cytokines that promote nerve regeneration. Experimental neurology 2012;238(2):101–106. [PubMed: 22981450]
37. Roberts SL, Dun XP, Doddrell RDS, Mindos T, Drake LK, Onaitis MW, Florio F, Quattrini A, Lloyd AC, D'Antonio M, Parkinson DB. Sox2 expression in Schwann cells inhibits myelination in vivo and induces influx of macrophages to the nerve. Development 2017;144(17):3114–3125. [PubMed: 28743796]
38. Lindborg JA, Mack M, Zigmond RE. Neutrophils Are Critical for Myelin Removal in a Peripheral Nerve Injury Model of Wallerian Degeneration. The Journal of neuroscience : the official journal of the Society for Neuroscience 2017;37(43):10258–10277. [PubMed: 28912156]

39. Bruck W The role of macrophages in Wallerian degeneration. Brain Pathol 1997;7(2):741–752. [PubMed: 9161725]
40. Zigmond RE, Echevarria FD. Macrophage biology in the peripheral nervous system after injury. Prog Neurobiol 2019;173:102–121. [PubMed: 30579784]
41. Dun XP, Carr L, Woodley PK, Barry RW, Drake LK, Mindos T, Roberts SL, Lloyd AC, Parkinson DB. Macrophage-Derived Slit3 Controls Cell Migration and Axon Pathfinding in the Peripheral Nerve Bridge. Cell reports 2019;26(6):1458–1472 e1454. [PubMed: 30726731]
42. Boivin A, Pineau I, Barrette B, Filali M, Vallieres N, Rivest S, Lacroix S. Toll-like receptor signaling is critical for Wallerian degeneration and functional recovery after peripheral nerve injury. The Journal of neuroscience : the official journal of the Society for Neuroscience 2007;27(46):12565–12576. [PubMed: 18003835]
43. Witzel C, Rohde C, Brushart TM. Pathway sampling by regenerating peripheral axons. The Journal of comparative neurology 2005;485(3):183–190. [PubMed: 15791642]
44. Stassart RM, Fledrich R, Velanac V, Brinkmann BG, Schwab MH, Meijer D, Sereda MW, Nave KA. A role for Schwann cell-derived neuregulin-1 in remyelination. Nature neuroscience 2013;16(1):48–54. [PubMed: 23222914]
45. Fricker FR, Lago N, Balarajah S, Tsantoulas C, Tanna S, Zhu N, Fageiry SK, Jenkins M, Garratt AN, Birchmeier C, Bennett DL. Axonally derived neuregulin-1 is required for remyelination and regeneration after nerve injury in adulthood. The Journal of neuroscience : the official journal of the Society for Neuroscience 2011;31(9):3225–3233. [PubMed: 21368034]
46. Santosa KB, Keane AM, Jablonka-Shariff A, Vannucci B, Snyder-Warwick AK. Clinical relevance of terminal Schwann cells: An overlooked component of the neuromuscular junction. J Neurosci Res 2018;96(7):1125–1135. [PubMed: 29536564]
47. Woolf CJ, Reynolds ML, Chong MS, Emson P, Irwin N, Benowitz LI. Denervation of the motor endplate results in the rapid expression by terminal Schwann cells of the growth-associated protein GAP-43. The Journal of neuroscience : the official journal of the Society for Neuroscience 1992;12(10):3999–4010. [PubMed: 1403096]
48. Reynolds ML, Woolf CJ. Terminal Schwann cells elaborate extensive processes following denervation of the motor endplate. Journal of neurocytology 1992;21(1):50–66. [PubMed: 1346630]
49. Lundborg G, Hansson HA. Nerve regeneration through preformed pseudosynovial tubes. A preliminary report of a new experimental model for studying the regeneration and reorganization capacity of peripheral nerve tissue. J Hand Surg [Am] 1980;5(1):35–38.
50. Lundborg G, Hansson HA. Regeneration of peripheral nerve through a preformed tissue space. Preliminary observations on the reorganization of regenerating nerve fibres and perineurium. Brain Res 1979;178(2–3):573–576. [PubMed: 509219]
51. 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. The Journal of comparative neurology 1983;218(4):460–470. [PubMed: 6619324]
52. Lundborg G, Dahlin LB, Danielsen N, Hansson HA, Johannesson A, Longo FM, Varon S. Nerve regeneration across an extended gap: a neurobiological view of nerve repair and the possible involvement of neuronotrophic factors. The Journal of hand surgery 1982;7(6):580–587. [PubMed: 7175129]
53. Lundborg G, Dahlin LB, Danielsen N, Gelberman RH, Longo FM, Powell HC, Varon S. Nerve regeneration in silicone chambers: influence of gap length and of distal stump components. Experimental neurology 1982;76(2):361–375. [PubMed: 7095058]
54. Williams LR. Exogenous fibrin matrix precursors stimulate the temporal progress of nerve regeneration within a silicone chamber. Neurochemical research 1987;12(10):851–860. [PubMed: 3683735]
55. Cattin AL, Lloyd AC. The multicellular complexity of peripheral nerve regeneration. Current opinion in neurobiology 2016;39:38–46. [PubMed: 27128880]
56. Cattin AL, Burden JJ, Van Emmenis L, Mackenzie FE, Hoving JJ, Garcia Calavia N, Guo Y, McLaughlin M, Rosenberg LH, Quereda V, Jamecna D, Napoli I, Parrinello S, Enver T, Ruhrberg

C, Lloyd AC. Macrophage-Induced Blood Vessels Guide Schwann Cell-Mediated Regeneration of Peripheral Nerves. Cell 2015;162(5):1127–1139. [PubMed: 26279190]

57. Clements MP, Byrne E, Camarillo Guerrero LF, Cattin AL, Zakka L, Ashraf A, Burden JJ, Khadayate S, Lloyd AC, Marguerat S, Parrinello S. The Wound Microenvironment Reprograms Schwann Cells to Invasive Mesenchymal-like Cells to Drive Peripheral Nerve Regeneration. Neuron 2017;96(1):98–114 e117. [PubMed: 28957681]
58. Parrinello S, Napoli I, Ribeiro S, Wingfield Digby P, Fedorova M, Parkinson DB, Doddrell RD, Nakayama M, Adams RH, Lloyd AC. EphB signaling directs peripheral nerve regeneration through Sox2-dependent Schwann cell sorting. Cell 2010;143(1):145–155. [PubMed: 20869108]
59. Isaacs J Treatment of acute peripheral nerve injuries: current concepts. The Journal of hand surgery 2010;35(3):491–497; quiz 498. [PubMed: 20138714]
60. Boyd KU, Fox IK. Nerve Repair and Grafting In: Mackinnon SE, editor. Nerve Surgery. 1st ed. Volume 1 New York: Thieme; 2015 p 75–100.
61. Rangavajla G, Mokarram N, Masoodzadehgan N, Pai SB, Bellamkonda RV. Noninvasive imaging of peripheral nerves. Cells, tissues, organs 2014;200(1):69–77. [PubMed: 25766202]
62. Mackinnon SE. Donor Distal, Recipient Proximal and Other Personal Perspectives on Nerve Transfers. Hand clinics 2016;32(2):141–151. [PubMed: 27094887]
63. Tung TH, Mackinnon SE. Nerve transfers: indications, techniques, and outcomes. The Journal of hand surgery 2010;35(2):332–341. [PubMed: 20141906]
64. Brown JM, Shah MN, Mackinnon SE. Distal nerve transfers: a biology-based rationale. Neurosurgical focus 2009;26(2):E12.
65. Houschyar KS, Momeni A, Pyles MN, Cha JY, Maan ZN, Duscher D, Jew OS, Siemers F, van Schoonhoven J. The Role of Current Techniques and Concepts in Peripheral Nerve Repair. Plastic surgery international 2016;2016:4175293. [PubMed: 26904282]
66. Siemionow M, Brzezicki G. Current Techniques and Concepts in Peripheral Nerve Repair. International Review of Neurobiology 2009;87:141–172. [PubMed: 19682637]
67. Terzis J, Faibisoff B, Williams BH. The Nerve Gap: Suture Under Tension vs. Graft. Plastic and Reconstructive Surgery 1975;56(2):166–170. [PubMed: 1096197]
68. Pfister BJ, Gordon T, Loverde JR, Kochar AS, Mackinnon SE, Cullen DK. Biomedical engineering strategies for peripheral nerve repair: surgical applications, state of the art, and future challenges. Critical reviews in biomedical engineering 2011;39(2):81–124. [PubMed: 21488817]
69. Johnson PJ, Wood MD, Moore AM, Mackinnon SE. Tissue engineered constructs for peripheral nerve surgery. European surgery : ACA : Acta chirurgica Austriaca 2013;45(3).
70. Best TJ, Mackinnon SE, Midha R, Hunter DA, Evans PJ. Revascularization of peripheral nerve autografts and allografts. Plast Reconstr Surg 1999;104(1):152–160. [PubMed: 10597688]
71. Best TJ, Mackinnon SE, Evans PJ, Hunter D, Midha R. Peripheral nerve revascularization: histomorphometric study of small- and large-caliber grafts. Journal of reconstructive microsurgery 1999;15(3):183–190. [PubMed: 10226953]
72. Farber SJ, Hoben GM, Hunter DA, Yan Y, Johnson PJ, Mackinnon SE, Wood MD. Vascularization is delayed in long nerve constructs compared with nerve grafts. Muscle & nerve 2016;54(2):319–321. [PubMed: 27144718]
73. Tomita K, Hata Y, Kubo T, Fujiwara T, Yano K, Hosokawa K. Effects of the in vivo predegenerated nerve graft on early Schwann cell migration: quantitative analysis using S100-GFP mice. Neurosci Lett 2009;461(1):36–40. [PubMed: 19500656]
74. Mackinnon SE. Surgical management of the peripheral nerve gap. Clinics in plastic surgery 1989;16(3):587–603. [PubMed: 2673638]
75. Mackinnon SE. Technical use of synthetic conduits for nerve repair. The Journal of hand surgery 2011;36(1):183. [PubMed: 21193138]
76. Evans PJ, Midha R, Mackinnon SE. The peripheral nerve allograft: a comprehensive review of regeneration and neuroimmunology. Prog Neurobiol 1994;43(3):187–233. [PubMed: 7816927]
77. Tung TH. Tacrolimus (FK506): Safety and Applications in Reconstructive Surgery. Hand 2009.
78. Mackinnon SE, Doolabh VB, Novak CB, Trulock EP. Clinical outcome following nerve allograft transplantation. Plast Reconstr Surg 2001;107(6):1419–1429. [PubMed: 11335811]

79. Lundborg G, Dahlin LB, Danielsen NP, Hansson HA, Larsson K. Reorganization and orientation of regenerating nerve fibres, perineurium, and epineurium in preformed mesothelial tubes - an experimental study on the sciatic nerve of rats. J Neurosci Res 1981;6(3):265–281. [PubMed: 7299843]
80. Dellon AL, Mackinnon SE. An alternative to the classical nerve graft for the management of the short nerve gap. Plast Reconstr Surg 1988;82(5):849–856. [PubMed: 2845455]
81. Battiston B, Geuna S, Ferrero M, Tos P. Nerve repair by means of tubulization: literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair. Microsurgery 2005;25(4):258–267. [PubMed: 15934044]
82. Dellon AL. Use of a silicone tube for the reconstruction of a nerve injury. Journal of hand surgery 1994;19(3):271–272.
83. Merle M, Dellon AL, Campbell JN, Chang PS. Complications from silicon-polymer intubulation of nerves. Microsurgery 1989;10(2):130–133. [PubMed: 2770512]
84. Chiu DT, Janecka I, Krizek TJ, Wolff M, Lovelace RE. Autogenous vein graft as a conduit for nerve regeneration. Surgery 1982;91(2):226–233. [PubMed: 7058501]
85. Gaudin R, Knipfer C, Henningsen A, Smeets R, Heiland M, Hadlock T. Approaches to Peripheral Nerve Repair: Generations of Biomaterial Conduits Yielding to Replacing Autologous Nerve Grafts in Craniomaxillofacial Surgery. BioMed research international 2016;2016:3856262. [PubMed: 27556032]
86. Boecker A, Daeschler SC, Kneser U, Harhaus L. Relevance and Recent Developments of Chitosan in Peripheral Nerve Surgery. Frontiers in cellular neuroscience 2019;13:104. [PubMed: 31019452]
87. Kornfeld T, Vogt PM, Radtke C. Nerve grafting for peripheral nerve injuries with extended defect sizes. Wien Med Wochenschr 2019;169(9–10):240–251. [PubMed: 30547373]
88. Azouz SM, Lucas HD, Mahabir RC, Noland SS. A Survey of the Prevalence and Practice Patterns of Human Acellular Nerve Allograft Use. Plastic and reconstructive surgery Global open 2018;6(8):e1803. [PubMed: 30254826]
89. FF IJ, Van De Graaf RC, Meek MF. The early history of tubulation in nerve repair. The Journal of hand surgery, European volume 2008;33(5):581–586.
90. Fu SY, Gordon T. The cellular and molecular basis of peripheral nerve regeneration. Mol Neurobiol 1997;14(1–2):67–116. [PubMed: 9170101]
91. Wood MD, Kemp SW, Weber C, Borschel GH, Gordon T. Outcome measures of peripheral nerve regeneration. Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft 2011;193(4):321–333. [PubMed: 21640570]
92. Szynkaruk M, Kemp SW, Wood MD, Gordon T, Borschel GH. Experimental and Clinical Evidence for Use of Decellularized Nerve Allografts in Peripheral Nerve Gap Reconstruction. Tissue engineering Part B, Reviews 2012.
93. Evans PJ, Mackinnon SE, Levi AD, Wade JA, Hunter DA, Nakao Y, Midha R. Cold preserved nerve allografts: changes in basement membrane, viability, immunogenicity, and regeneration. Muscle & nerve 1998;21(11):1507–1522. [PubMed: 9771677]
94. Gulati AK, Cole GP. Nerve graft immunogenicity as a factor determining axonal regeneration in the rat. Journal of neurosurgery 1990;72(1):114–122. [PubMed: 2294170]
95. 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):246–259. [PubMed: 17255680]
96. Hudson TW, Zawko S, Deister C, Lundy S, Hu CY, Lee K, Schmidt CE. Optimized acellular nerve graft is immunologically tolerated and supports regeneration. Tissue Eng 2004;10(11–12):1641–1651. [PubMed: 15684673]
97. Mackinnon SE, Hudson AR, Falk RE, Kline D, Hunter D. Peripheral nerve allograft: an immunological assessment of pretreatment methods. Neurosurgery 1984;14(2):167–171. [PubMed: 6608699]
98. Moradzadeh A, Borschel GH, Luciano JP, Whitlock EL, Hayashi A, Hunter DA, Mackinnon SE. The impact of motor and sensory nerve architecture on nerve regeneration. Experimental neurology 2008;212(2):370–376. [PubMed: 18550053]

99. Mackinnon SE, Hudson AR, Falk RE, Kline D, Hunter D. Peripheral nerve allograft: an assessment of regeneration across pretreated nerve allografts. Neurosurgery 1984;15(5):690–693. [PubMed: 6334246]
100. Hudson TW, Liu SY, Schmidt CE. Engineering an improved acellular nerve graft via optimized chemical processing. Tissue Eng 2004;10(9–10):1346–1358. [PubMed: 15588395]
101. Graham JB, Neubauer D, Xue QS, Muir D. Chondroitinase applied to peripheral nerve repair averts retrograde axonal regeneration. Experimental neurology 2007;203(1):185–195. [PubMed: 16970940]
102. Neubauer D, Graham JB, Muir D. Chondroitinase treatment increases the effective length of acellular nerve grafts. Experimental neurology 2007;207(1):163–170. [PubMed: 17669401]
103. Yang LJ, Lorenzini I, Vajn K, Mountney A, Schramm LP, Schnaar RL. Sialidase enhances spinal axon outgrowth in vivo. Proc Natl Acad Sci U S A 2006;103(29):11057–11062. [PubMed: 16847268]
104. Wood MD, Mackinnon SE. Pathways regulating modality-specific axonal regeneration in peripheral nerve. Experimental neurology 2015;265:171–175. [PubMed: 25681572]
105. Wojtkiewicz DM, Saunders J, Domeshek L, Novak CB, Kaskutas V, Mackinnon SE. Social impact of peripheral nerve injuries. Hand 2015;10(2):161–167. [PubMed: 26034424]
106. Gordon T, Chan KM, Sulaiman OA, Udina E, Amirjani N, Brushart TM. Accelerating axon growth to overcome limitations in functional recovery after peripheral nerve injury. Neurosurgery 2009;65(4 Suppl):A132–144. [PubMed: 19927058]
107. Chan KM, Gordon T, Zochodne DW, Power HA. Improving peripheral nerve regeneration: from molecular mechanisms to potential therapeutic targets. Experimental neurology 2014;261:826–835. [PubMed: 25220611]
108. Kubiak CA, Kung TA, Brown DL, Cederna PS, Kemp SWP. State-of-the-Art Techniques in Treating Peripheral Nerve Injury. Plast Reconstr Surg 2018;141(3):702–710. [PubMed: 29140901]
109. 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):787–799. [PubMed: 19291791]
110. Giusti G, Willems WF, Kremer T, Friedrich PF, Bishop AT, Shin AY. Return of motor function after segmental nerve loss in a rat model: comparison of autogenous nerve graft, collagen conduit, and processed allograft (AxoGen). The Journal of bone and joint surgery American volume 2012;94(5):410–417. [PubMed: 22398734]
111. Rbia N, Shin AY. The Role of Nerve Graft Substitutes in Motor and Mixed Motor/Sensory Peripheral Nerve Injuries. The Journal of hand surgery 2017;42(5):367–377. [PubMed: 28473159]
112. Moore AM, Ray WZ, Johnson PJ. Nerve Autograft Substitutes: Conduits and Processed Allografts In: Mackinnon SE, editor. Nerve Surgery. 1st ed. Volume 1 New York: Thieme; 2015 p 169–192.
113. Novak CB, Kelly L, Mackinnon SE. Sensory recovery after median nerve grafting. The Journal of hand surgery 1992;17(1):59–68. [PubMed: 1311345]
114. Brandsma JW, Schreuders TA, Birke JA, Piefer A, Oostendorp R. Manual muscle strength testing: intraobserver and interobserver reliabilities for the intrinsic muscles of the hand. J Hand Ther 1995;8(3):185–190. [PubMed: 8535479]
115. Wang Y, Sunitha M, Chung KC. How to measure outcomes of peripheral nerve surgery. Hand clinics 2013;29(3):349–361. [PubMed: 23895715]
116. Nunley JA, Ugino MR, Goldner RD, Regan N, Urbaniak JR. Use of the anterior branch of the medial antebrachial cutaneous nerve as a graft for the repair of defects of the digital nerve. The Journal of bone and joint surgery American volume 1989;71(4):563–567. [PubMed: 2703516]
117. Kallio PK. The results of secondary repair of 254 digital nerves. Journal of hand surgery 1993;18(3):327–330.
118. Lohmeyer JA, Siemers F, Machens HG, Mailander P. The clinical use of artificial nerve conduits for digital nerve repair: a prospective cohort study and literature review. Journal of reconstructive microsurgery 2009;25(1):55–61. [PubMed: 19037847]

119. Chiriac S, Facca S, Diaconu M, Gouzou S, Liverneaux P. Experience of using the bioresorbable copolyester poly(DL-lactide-epsilon-caprolactone) nerve conduit guide Neurolac for nerve repair in peripheral nerve defects: report on a series of 28 lesions. The Journal of hand surgery, European volume 2012;37(4):342–349.
120. Cho MS, Rinker BD, Weber RV, Chao JD, Ingari JV, Brooks D, Buncke GM. Functional outcome following nerve repair in the upper extremity using processed nerve allograft. The Journal of hand surgery 2012;37(11):2340–2349. [PubMed: 23101532]
121. Brooks DN, Weber RV, Chao JD, Rinker BD, Zoldos J, Robichaux MR, Ruggeri SB, Anderson KA, Bonatz EE, Wisotsky SM, Cho MS, Wilson C, Cooper EO, Ingari JV, Safa B, Parrett BM, Buncke GM. Processed nerve allografts for peripheral nerve reconstruction: a multicenter study of utilization and outcomes in sensory, mixed, and motor nerve reconstructions. Microsurgery 2012;32(1):1–14. [PubMed: 22121093]
122. Leckenby JI, Furrer C, Haug L, Juon Personeni B, Vogelin E. A retrospective case series reporting the outcomes of Avance nerve allografts in the treatment of peripheral nerve injuries. Plast Reconstr Surg 2019.
123. Braga Silva J, Marchese GM, Cauduro CG, Debiasi M. Nerve conduits for treating peripheral nerve injuries: A systematic literature review. Hand surgery & rehabilitation 2017;36(2):71–85. [PubMed: 28325431]
124. Safa B, Buncke G. Autograft Substitutes: Conduits and Processed Nerve Allografts. Hand clinics 2016;32(2):127–140. [PubMed: 27094886]
125. Kim DH, Kam AC, Chandika P, Tiel RL, Kline DG. Surgical management and outcome in patients with radial nerve lesions. Journal of neurosurgery 2001;95(4):573–583. [PubMed: 11596951]
126. Kim DH, Kam AC, Chandika P, Tiel RL, Kline DG. Surgical management and outcomes in patients with median nerve lesions. Journal of neurosurgery 2001;95(4):584–594. [PubMed: 11596952]
127. Kim DH, Han K, Tiel RL, Murovic JA, Kline DG. Surgical outcomes of 654 ulnar nerve lesions. Journal of neurosurgery 2003;98(5):993–1004. [PubMed: 12744359]
128. Lenoble E, Sokolow C, Ebelin M, Rigot J, Dabos N, Lemerle JP, Vilain R. [Results of the primary repair of 28 isolated median nerve injuries in the wrist]. Annales de chirurgie de la main : organe officiel des societes de chirurgie de la main 1989;8(4):347–351. [PubMed: 2688574]
129. Rinker B, Zoldos J, Weber RV, Ko J, Thayer W, Greenberg J, Leversedge FJ, Safa B, Buncke G. Use of Processed Nerve Allografts to Repair Nerve Injuries Greater Than 25 mm in the Hand. Annals of plastic surgery 2017;78(6S Suppl 5):S292–S295. [PubMed: 28328632]
130. 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):959–968. [PubMed: 19103514]
131. Wood MD, MacEwan MR, French AR, Moore AM, Hunter DA, Mackinnon SE, Moran DW, Borschel GH, Sakiyama-Elbert SE. Fibrin matrices with affinity-based delivery systems and neurotrophic factors promote functional nerve regeneration. Biotechnol Bioeng 2010;106(6):970–979. [PubMed: 20589674]
132. Yu X, Bellamkonda RV. Tissue-engineered scaffolds are effective alternatives to autografts for bridging peripheral nerve gaps. Tissue Eng 2003;9(3):421–430. [PubMed: 12857410]
133. Moore AM, Kasukurthi R, Magill CK, Farhadi HF, Borschel GH, Mackinnon SE. Limitations of conduits in peripheral nerve repairs. Hand 2009;4(2):180–186. [PubMed: 19137378]
134. Nietosvaara Y, Grahn P, Sommarhem A. Failed peripheral nerve reconstruction with processed nerve allografts in three patients. The Journal of hand surgery, European volume 2019;44(3):318–320.
135. 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. Experimental neurology 1987;95(2):378–390. [PubMed: 3803518]

136. 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):325–334. [PubMed: 3390701]

137. Mu Y, Wu F, Lu Y, Wei L, Yuan W. Progress of electrospun fibers as nerve conduits for neural tissue repair. Nanomedicine 2014;9(12):1869–1883. [PubMed: 25325242]

138. Xie J, MacEwan MR, Schwartz AG, Xia Y. Electrospun nanofibers for neural tissue engineering. Nanoscale 2010;2(1):35–44. [PubMed: 20648362]

139. Xie J, MacEwan MR, Liu W, Jesuraj N, Li X, Hunter D, Xia Y. Nerve guidance conduits based on double-layered scaffolds of electrospun nanofibers for repairing the peripheral nervous system. ACS applied materials & interfaces 2014;6(12):9472–9480. [PubMed: 24806389]

140. Jiang J, Li Z, Wang H, Wang Y, Carlson MA, Teusink MJ, MacEwan MR, Gu L, Xie J. Expanded 3D Nanofiber Scaffolds: Cell Penetration, Neovascularization, and Host Response. Advanced healthcare materials 2016;5(23):2993–3003. [PubMed: 27709840]

141. Xie J, MacEwan MR, Li X, Sakiyama-Elbert SE, Xia Y. Neurite outgrowth on nanofiber scaffolds with different orders, structures, and surface properties. ACS nano 2009;3(5):1151–1159. [PubMed: 19397333]

142. Xie J, Liu W, MacEwan MR, Bridgman PC, Xia Y. Neurite outgrowth on electrospun nanofibers with uniaxial alignment: the effects of fiber density, surface coating, and supporting substrate. ACS nano 2014;8(2):1878–1885. [PubMed: 24444076]

143. Kemp SW, Walsh SK, Midha R. Growth factor and stem cell enhanced conduits in peripheral nerve regeneration and repair. Neurol Res 2008;30(10):1030–1038. [PubMed: 19079977]

144. Sarker MD, Naghieh S, McInnes AD, Schreyer DJ, Chen X. Regeneration of peripheral nerves by nerve guidance conduits: Influence of design, biopolymers, cells, growth factors, and physical stimuli. Prog Neurobiol 2018;171:125–150. [PubMed: 30077776]

145. Naveilhan P, ElShamy WM, Ernfors P. Differential regulation of mRNAs for GDNF and its receptors Ret and GDNFR alpha after sciatic nerve lesion in the mouse. The European journal of neuroscience 1997;9(7):1450–1460. [PubMed: 9240402]

146. Gavazzi I, Kumar RD, McMahon SB, Cohen J. Growth responses of different subpopulations of adult sensory neurons to neurotrophic factors in vitro. The European journal of neuroscience 1999;11(10):3405–3414. [PubMed: 10564348]

147. Leclere PG, Norman E, Groutsi F, Coffin R, Mayer U, Pizzey J, Tonge D. Impaired axonal regeneration by isolectin B4-binding dorsal root ganglion neurons in vitro. The Journal of neuroscience : the official journal of the Society for Neuroscience 2007;27(5):1190–1199. [PubMed: 17267575]

148. Matheson CR, Carnahan J, Urich JL, Bocangel D, Zhang TJ, Yan Q. Glial cell line-derived neurotrophic factor (GDNF) is a neurotrophic factor for sensory neurons: comparison with the effects of the neurotrophins. Journal of neurobiology 1997;32(1):22–32. [PubMed: 8989660]

149. Tucker BA, Rahimtula M, Mearow KM. Laminin and growth factor receptor activation stimulates differential growth responses in subpopulations of adult DRG neurons. The European journal of neuroscience 2006;24(3):676–690. [PubMed: 16930399]

150. Trupp M, Ryden M, Jornvall H, Funakoshi H, Timmusk T, Arenas E, Ibanez CF. Peripheral expression and biological activities of GDNF, a new neurotrophic factor for avian and mammalian peripheral neurons. The Journal of cell biology 1995;130(1):137–148. [PubMed: 7790368]

151. Bennett DL, Michael GJ, Ramachandran N, Munson JB, Averill S, Yan Q, McMahon SB, Priestley JV. A distinct subgroup of small DRG cells express GDNF receptor components and GDNF is protective for these neurons after nerve injury. The Journal of neuroscience : the official journal of the Society for Neuroscience 1998;18(8):3059–3072. [PubMed: 9526023]

152. Iwase T, Jung CG, Bae H, Zhang M, Soliven B. Glial cell line-derived neurotrophic factor-induced signaling in Schwann cells. Journal of neurochemistry 2005;94(6):1488–1499. [PubMed: 16086701]

153. Ellerbroek SM, Wennerberg K, Burridge K. Serine phosphorylation negatively regulates RhoA in vivo. The Journal of biological chemistry 2003;278(21):19023–19031. [PubMed: 12654918]

154. Klemke RL, Cai S, Giannini AL, Gallagher PJ, de Lanerolle P, Cheresh DA. Regulation of cell motility by mitogen-activated protein kinase. The Journal of cell biology 1997;137(2):481–492. [PubMed: 9128257]

155. Lang P, Gesbert F, Delespine-Carmagnat M, Stancou R, Pouchelet M, Bertoglio J. Protein kinase A phosphorylation of RhoA mediates the morphological and functional effects of cyclic AMP in cytotoxic lymphocytes. The EMBO journal 1996;15(3):510–519. [PubMed: 8599934]

156. Meintanis S, Thomaidou D, Jessen KR, Mirsky R, Matsas R. The neuron-glia signal beta-neuregulin promotes Schwann cell motility via the MAPK pathway. Glia 2001;34(1):39–51. [PubMed: 11284018]

157. Kim HA, DeClue JE, Ratner N. cAMP-dependent protein kinase A is required for Schwann cell growth: interactions between the cAMP and neuregulin/tyrosine kinase pathways. J Neurosci Res 1997;49(2):236–247. [PubMed: 9272646]

158. Morgan L, Jessen KR, Mirsky R. The effects of cAMP on differentiation of cultured Schwann cells: progression from an early phenotype (04+) to a myelin phenotype (P0+, GFAP-, N-CAM-, NGF-receptor-) depends on growth inhibition. The Journal of cell biology 1991;112(3):457–467. [PubMed: 1704008]

159. Grimm L, Holinski-Feder E, Teodoridis J, Scheffer B, Schindelhauer D, Meitinger T, Ueffing M. Analysis of the human GDNF gene reveals an inducible promoter, three exons, a triplet repeat within the 3'-UTR and alternative splice products. Human molecular genetics 1998;7(12):1873–1886. [PubMed: 9811930]

160. Kinameri E, Matsuoka I. Autocrine action of BMP2 regulates expression of GDNF-mRNA in sciatic Schwann cells. Brain research Molecular brain research 2003;117(2):221–227. [PubMed: 14559157]

161. Verity AN, Wyatt TL, Hajos B, Eglen RM, Baecker PA, Johnson RM. Regulation of glial cell line-derived neurotrophic factor release from rat C6 glioblastoma cells. Journal of neurochemistry 1998;70(2):531–539. [PubMed: 9453547]

162. Marquardt LM, Sakiyama-Elbert SE. GDNF preconditioning can overcome Schwann cell phenotypic memory. Experimental neurology 2015;265:1–7. [PubMed: 25496841]

163. Jesuraj NJ, Marquardt LM, Kwasa JA, Sakiyama-Elbert SE. Glial cell line-derived neurotrophic factor promotes increased phenotypic marker expression in femoral sensory and motor-derived Schwann cell cultures. Experimental neurology 2014;257:10–18. [PubMed: 24731946]

164. Kokai LE, Bourbeau D, Weber D, McAtee J, Marra KG. Sustained growth factor delivery promotes axonal regeneration in long gap peripheral nerve repair. Tissue engineering Part A 2011;17(9–10):1263–1275. [PubMed: 21189072]

165. Gold BG, Storm-Dickerson T, Austin DR. The immunosuppressant FK506 increases functional recovery and nerve regeneration following peripheral nerve injury. Restorative neurology and neuroscience 1994;6(4):287–296. [PubMed: 21551759]

166. Gold BG, Katoh K, Storm-Dickerson T. The immunosuppressant FK506 increases the rate of axonal regeneration in rat sciatic nerve. The Journal of neuroscience : the official journal of the Society for Neuroscience 1995;15(11):7509–7516. [PubMed: 7472502]

167. Jost SC, Doolabh VB, Mackinnon SE, Lee M, Hunter D. Acceleration of peripheral nerve regeneration following FK506 administration. Restorative neurology and neuroscience 2000;17(1):39–44. [PubMed: 11490076]

168. Lee M, Doolabh VB, Mackinnon SE, Jost S. FK506 promotes functional recovery in crushed rat sciatic nerve. Muscle & nerve 2000;23(4):633–640. [PubMed: 10716776]

169. Yan Y, Sun HH, Hunter DA, Mackinnon SE, Johnson PJ. Efficacy of short-term FK506 administration on accelerating nerve regeneration. Neurorehabilitation and neural repair 2012;26(6):570–580. [PubMed: 22291040]

170. Yang RK, Lowe JB 3rd, Sobol JB, Sen SK, Hunter DA, Mackinnon SE. Dose-dependent effects of FK506 on neuroregeneration in a rat model. Plast Reconstr Surg 2003;112(7):1832–1840. [PubMed: 14663227]

171. Doolabh VB, Mackinnon SE. FK506 accelerates functional recovery following nerve grafting in a rat model. Plast Reconstr Surg 1999;103(7):1928–1936. [PubMed: 10359255]

172. Snyder AK, Fox IK, Nichols CM, Rickman SR, Hunter DA, Tung TH, Mackinnon SE. Neuroregenerative effects of preinjury FK-506 administration. Plast Reconstr Surg 2006;118(2):360–367. [PubMed: 16874203]

173. Tajdaran K, Chan K, Zhang J, Gordon T, Borschel GH. Local FK506 dose-dependent study using a novel three-dimensional organotypic assay. Biotechnol Bioeng 2019;116(2):405–414. [PubMed: 30345505]

174. Tajdaran K, Chan K, Shoichet MS, Gordon T, Borschel GH. Local delivery of FK506 to injured peripheral nerve enhances axon regeneration after surgical nerve repair in rats. Acta Biomater 2019.

175. Labroo P, Ho S, Sant H, Shea J, Gale BK, Agarwal J. Controlled Delivery of FK506 to Improve Nerve Regeneration. Shock 2016;46(3 Suppl 1):154–159. [PubMed: 27058050]

176. Davis B, Wojtalewicz S, Labroo P, Shea J, Sant H, Gale B, Agarwal J. Controlled release of FK506 from micropatterned PLGA films: potential for application in peripheral nerve repair. Neural regeneration research 2018;13(7):1247–1252. [PubMed: 30028334]

177. Labroo P, Shea J, Sant H, Gale B, Agarwal J. Effect Of combining FK506 and neurotrophins on neurite branching and elongation. Muscle & nerve 2017;55(4):570–581. [PubMed: 27503321]

178. Hobson MI, Green CJ, Terenghi G. VEGF enhances intraneural angiogenesis and improves nerve regeneration after axotomy. Journal of anatomy 2000;197 Pt 4:591–605. [PubMed: 11197533]

179. Hoben G, Yan Y, Iyer N, Newton P, Hunter DA, Moore AM, Sakiyama-Elbert SE, Wood MD, Mackinnon SE. Comparison of acellular nerve allograft modification with Schwann cells or VEGF. Hand 2015;10(3):396–402. [PubMed: 26330769]

180. Braga TT, Agudelo JS, Camara NO. Macrophages During the Fibrotic Process: M2 as Friend and Foe. Frontiers in immunology 2015;6:602. [PubMed: 26635814]

181. Klopfleisch R Macrophage reaction against biomaterials in the mouse model - Phenotypes, functions and markers. Acta Biomater 2016;43:3–13. [PubMed: 27395828]

182. Mokarram N, Merchant A, Mukhatyar V, Patel G, Bellamkonda RV. Effect of modulating macrophage phenotype on peripheral nerve repair. Biomaterials 2012;33(34):8793–8801. [PubMed: 22979988]

183. Mokarram N, Dymanus K, Srinivasan A, Lyon JG, Tipton J, Chu J, English AW, Bellamkonda RV. Immunoengineering nerve repair. Proc Natl Acad Sci U S A 2017;114(26):E5077–E5084. [PubMed: 28611218]

184. Lv D, Zhou L, Zheng X, Hu Y. Sustained release of collagen VI potentiates sciatic nerve regeneration by modulating macrophage phenotype. The European journal of neuroscience 2017;45(10):1258–1267. [PubMed: 28263445]

185. Stenberg L, Stossel M, Ronchi G, Geuna S, Yin Y, Mommert S, Martensson L, Metzen J, Grothe C, Dahlin LB, Haastert-Talini K. Regeneration of long-distance peripheral nerve defects after delayed reconstruction in healthy and diabetic rats is supported by immunomodulatory chitosan nerve guides. BMC Neurosci 2017;18(1):53. [PubMed: 28720074]

186. Neubrech F, Sauerbier M, Moll W, Seegmuller J, Heider S, Harhaus L, Bickert B, Kneser U, Kremer T. Enhancing the Outcome of Traumatic Sensory Nerve Lesions of the Hand by Additional Use of a Chitosan Nerve Tube in Primary Nerve Repair: A Randomized Controlled Bicentric Trial. Plast Reconstr Surg 2018;142(2):415–424. [PubMed: 30045179]

187. Pedrini FA, Boriani F, Bolognesi F, Fazio N, Marchetti C, Baldini N. Cell-Enhanced Acellular Nerve Allografts for Peripheral Nerve Reconstruction: A Systematic Review and a Meta-Analysis of the Literature. Neurosurgery 2018.

188. Jesuraj NJ, Santosa KB, Newton P, Liu Z, Hunter DA, Mackinnon SE, Sakiyama-Elbert SE, Johnson PJ. A systematic evaluation of Schwann cell injection into acellular cold-preserved nerve grafts. Journal of neuroscience methods 2011;197(2):209–215. [PubMed: 21354206]

189. Gambhir HS, Raharjo E, Forden J, Kumar R, Mishra C, Guo GF, Grochmal J, Shapira Y, Midha R. Improved method to track and precisely count Schwann cells post-transplantation in a peripheral nerve injury model. Journal of neuroscience methods 2016;273:86–95. [PubMed: 27546200]

190. Mathon NF, Malcolm DS, Harrisingh MC, Cheng L, Lloyd AC. Lack of replicative senescence in normal rodent glia. Science 2001;291(5505):872–875. [PubMed: 11157166]

191. Brenner MJ, Lowe JB 3rd, Fox IK, Mackinnon SE, Hunter DA, Darcy MD, Duncan JR, Wood P, Mohanakumar T. Effects of Schwann cells and donor antigen on long-nerve allograft regeneration. Microsurgery 2005;25(1):61–70. [PubMed: 15481042]
192. Ogden MA, Feng FY, Myckatyn TM, Jensen JN, Grand AG, Wood PW, Hunter DA, MacKinnon SE. Safe injection of cultured schwann cells into peripheral nerve allografts. Microsurgery 2000;20(7):314–323. [PubMed: 11119286]
193. Rbia N, Bulstra LF, Bishop AT, van Wijnen AJ, Shin AY. A Simple Dynamic Strategy to Deliver Stem Cells to Decellularized Nerve Allografts. Plast Reconstr Surg 2018;142(2):402–413. [PubMed: 29889737]
194. Rbia N, Bulstra LF, Thaler R, Hovius SER, van Wijnen AJ, Shin AY. In Vivo Survival of Mesenchymal Stromal Cell-Enhanced Decellularized Nerve Grafts for Segmental Peripheral Nerve Reconstruction. The Journal of hand surgery 2019;44(6):514 e511–514 e511. [PubMed: 30301645]
195. Szynkaruk M, Kemp SW, Wood MD, Gordon T, Borschel GH. Experimental and clinical evidence for use of decellularized nerve allografts in peripheral nerve gap reconstruction. Tissue engineering Part B, Reviews 2013;19(1):83–96. [PubMed: 22924762]
196. Fu SY, Gordon T. Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. The Journal of neuroscience : the official journal of the Society for Neuroscience 1995;15(5 Pt 2):3886–3895. [PubMed: 7751953]
197. Brushart TM. Neurotropism and neurotrophism. The Journal of hand surgery 1987;12(5 Pt 1):808–809.
198. Brushart TM. Preferential reinnervation of motor nerves by regenerating motor axons. The Journal of neuroscience : the official journal of the Society for Neuroscience 1988;8(3):1026–1031. [PubMed: 3346713]
199. 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. The Journal of neuroscience : the official journal of the Society for Neuroscience 2006;26(38):9646–9655. [PubMed: 16988035]
200. Madison RD, Robinson GA. Accuracy of regenerating motor neurons: influence of diffusion in denervated nerve. Neuroscience 2014;273:128–140. [PubMed: 24846614]
201. Kobayashi J, Mackinnon SE, Watanabe O, Ball DJ, Gu XM, Hunter DA, Kuzon WM Jr. The effect of duration of muscle denervation on functional recovery in the rat model. Muscle & nerve 1997;20(7):858–866. [PubMed: 9179158]
202. Saheb-Al-Zamani M, Yan Y, Farber SJ, Hunter DA, Newton P, Wood MD, Stewart SA, Johnson PJ, Mackinnon SE. Limited regeneration in long acellular nerve allografts is associated with increased Schwann cell senescence. Experimental neurology 2013;247:165–177. [PubMed: 23644284]
203. Poppler LH, Ee X, Schellhardt L, Hoben GM, Pan D, Hunter DA, Yan Y, Moore AM, Snyder-Warwick AK, Stewart SA, Mackinnon SE, Wood MD. Axonal Growth Arrests After an Increased Accumulation of Schwann Cells Expressing Senescence Markers and Stromal Cells in Acellular Nerve Allografts. Tissue engineering Part A 2016;22(13–14):949–961. [PubMed: 27297909]
204. Sahakyants T, Lee JY, Friedrich PF, Bishop AT, Shin AY. Return of motor function after repair of a 3-cm gap in a rabbit peroneal nerve: a comparison of autograft, collagen conduit, and conduit filled with collagen-GAG matrix. The Journal of bone and joint surgery American volume 2013;95(21):1952–1958. [PubMed: 24196465]
205. Vasudevan S, Huang J, Botterman B, Matloub HS, Keefer E, Cheng J. Detergent-free Decellularized Nerve Grafts for Long-gap Peripheral Nerve Reconstruction. Plastic and reconstructive surgery Global open 2014;2(8):e201. [PubMed: 25426384]
206. Pan D, Hunter DA, Schellhardt L, Jo S, Santosa KB, Larson EL, Fuchs AG, Snyder-Warwick AK, Mackinnon SE, Wood MD. The accumulation of T cells within acellular nerve allografts is length-dependent and critical for nerve regeneration. Experimental neurology 2019;318:216–231. [PubMed: 31085199]

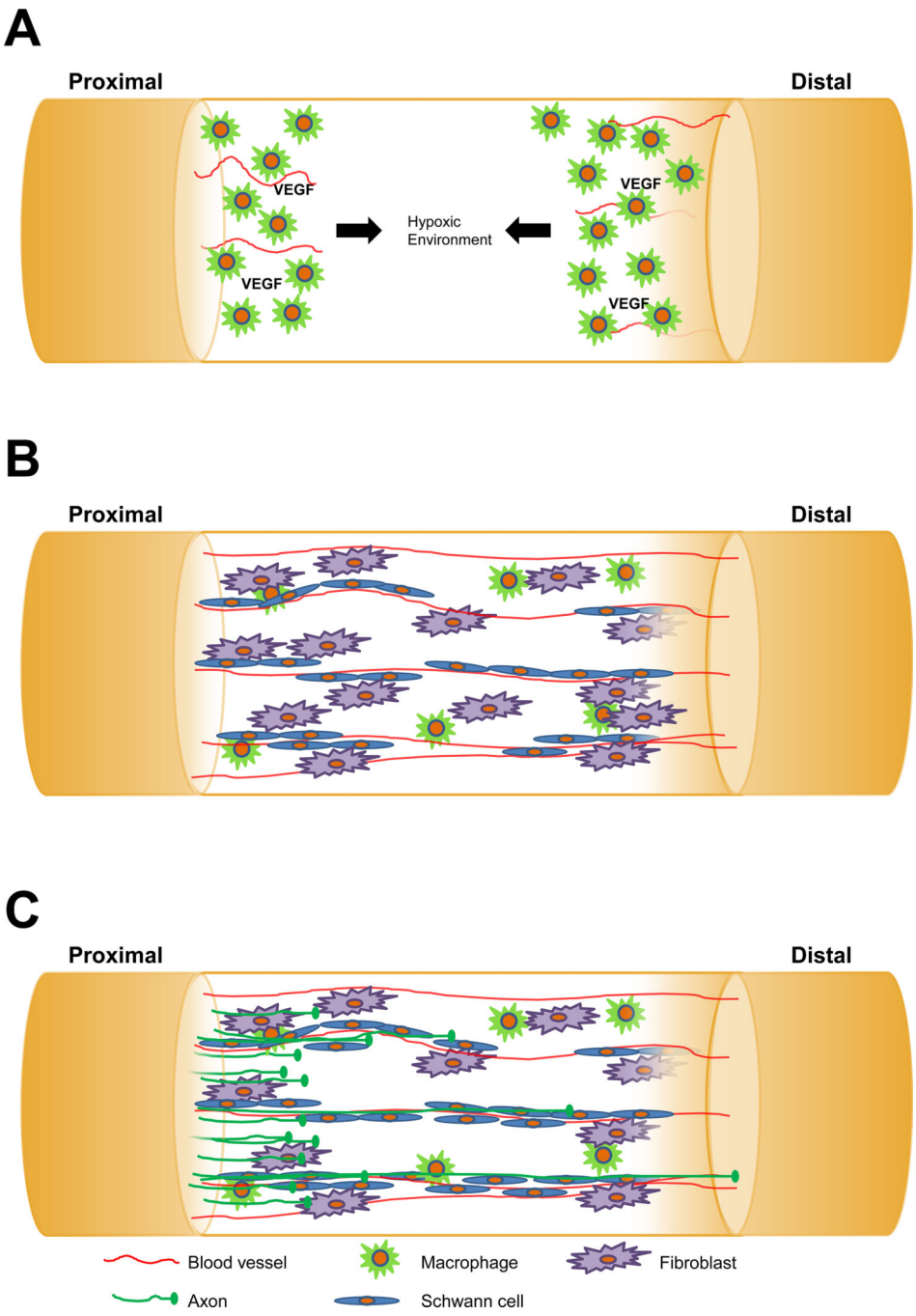

Figure 1.

Schematic of nerve regeneration across a short nerve defect. After formation of an endogenous matrix, the innate immune system infiltrates the defect. (A) Due to the hypoxic nature of this environment, macrophages produce factors to support angiogenesis, such as vascular endothelial growth factor (VEGF). The secretion of VEGF from macrophages recruits endothelial cells into the injured nerve bridge[56]. (B) As blood vessels form, Schwann cells migrate upon this network, and in turn fibroblasts interact with Schwann cells

forming cellular cords through EphrinA-EphrinB signaling[58]. (C) These processes enable axon growth across the nerve defect.

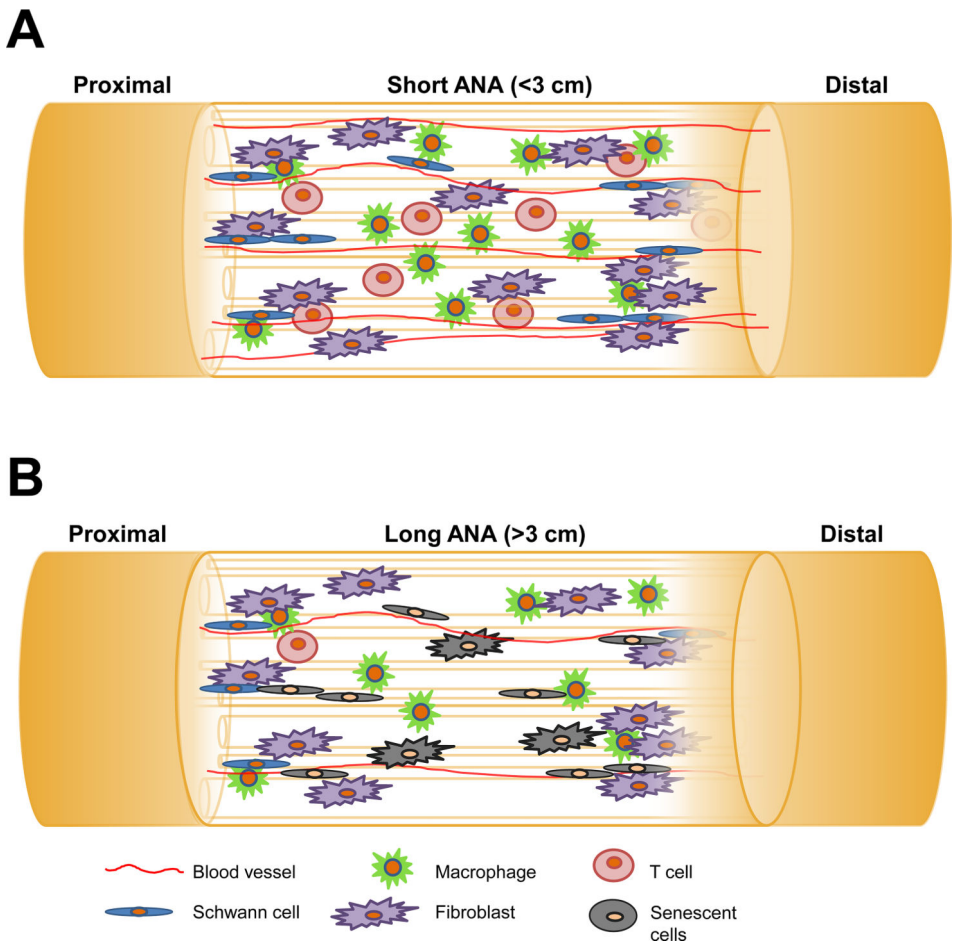

Figure 2.

Long nerve graft alternatives develop an environment limiting axon regeneration. Using acellular nerve allografts (ANAs) as a model, studies have compared the regenerative processes within short (non-critical length) vs long (critical-length) ANAs to discover factors contributing to successful or failed nerve regeneration across ANAs[202,203,206]. (A) Short ANAs are repopulated with cells similar to previously described in Figure 1. Conversely, (B) long ANAs are repopulated with an environmental imbalance consisting of altered populations of immune cells, cells expressing markers of senescence[203], and delayed angiogenesis compared to their shorter counterparts[72,70,71,206]. These cumulative changes to long ANAs limit axon regeneration and represent a “barrier” to axon regeneration[203,202].

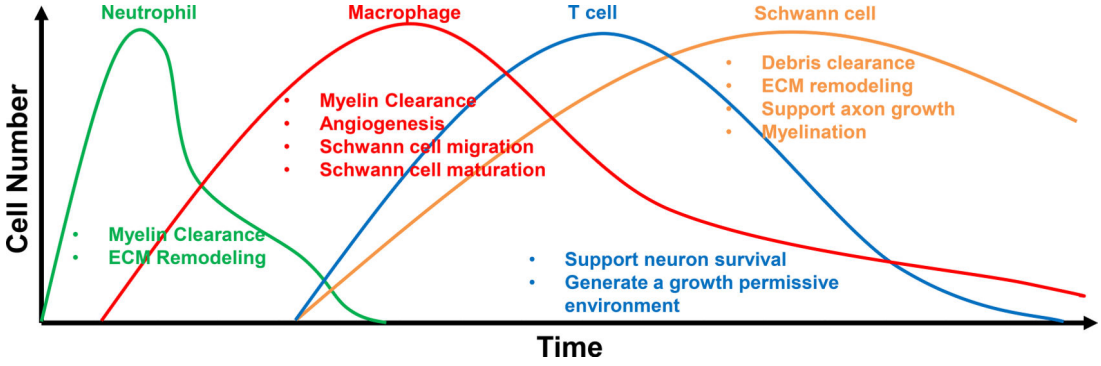

Figure 3.

Temporal relationship between immune system cells within nerve defects and regeneration. Each cell type migrates within a defect, where its accumulation peaks at different times during regeneration across nerve defects and precedes axon growth. Prior to substantial Schwann cell migration within a nerve defect, the innate immune system plays a pivotal role. Neutrophils are the first cells to infiltrate the defect, and have key processes involved in debris clearance, as well as yet unresolved roles in further cell recruitment. Macrophages shortly follow neutrophils and are responsible for the majority of blood vessel formation, as well as providing signaling for Schwann cell functions. Cells of the adaptive immune system, such as T cells, arrive at the same time as Schwann cells, and regulate axon myelination. The nature of how T cells regulate myelination is not yet resolved.

Table 1.

Medical Research Council (MRC) scale

Sensory Function[113]	S0	absence of sensibility in the autonomous area of the nerve
	S1	recovery of deep cutaneous pain and tactile sensibility
	S1+	recovery of superficial pain sensibility
	S2	recovery of some degree of superficial cutaneous pain and tactile sensibility
	S2+	as in S2, but with over response
	S3	return of pain and tactile sensibility with disappearance of over response, s2-PD: >15mm
	S3+	return of sensibility as in S3 with some recovery of 2-point discrimination, s2-PD: 7–15mm
	S4	complete recovery, s2-PD: 2–6mm
Motor Function[114]	M0	no contraction
	M1	flicker or trace of contraction
	M2	full range of active movement, with gravity eliminated
	M3	active movement against gravity
	M4	active movement against gravity and resistance
	M5	normal power

Abbreviations: S2-PD: static two-point discrimination test