INVITED REVIEW

An update–tissue engineered nerve grafts for the repair of peripheral nerve injuries

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Abstract

Peripheral nerve injuries (PNI) are caused by a range of etiologies and result in a broad spectrum of disability. While nerve autografts are the current gold standard for the reconstruction of extensive nerve damage, the limited supply of autologous nerve and complications associated with harvesting nerve from a second surgical site has driven groups from multiple disciplines, including biomedical engineering, neurosurgery, plastic surgery, and orthopedic surgery, to develop a suitable or superior alternative to autografting. Over the last couple of decades, various types of scaffolds, such as acellular nerve grafts (ANGs), nerve guidance conduits, and non-nervous tissues, have been filled with Schwann cells, stem cells, and/or neurotrophic factors to develop tissue engineered nerve grafts (TENGs). Although these have shown promising effects on peripheral nerve regeneration in experimental models, the autograft has remained the gold standard for large nerve gaps. This review provides a discussion of recent advances in the development of TENGs and their efficacy in experimental models. Specifically, TENGs have been enhanced *via* incorporation of genetically engineered cells, methods to improve stem cell survival and differentiation, optimized delivery of neurotrophic factors *via* drug delivery systems (DDS), co-administration of platelet-rich plasma (PRP), and pretreatment with chondroitinase ABC (Ch-ABC). Other notable advancements include conduits that have been bioengineered to mimic native nerve structure *via* cell-derived extracellular matrix (ECM) deposition, and the development of transplantable living nervous tissue constructs from rat and human dorsal root ganglia (DRG) neurons. Grafts composed of non-nervous tissues, such as vein, artery, and muscle, will be briefly discussed.

Key Words: *peripheral nerve injury; peripheral nerve repair; tissue engineered nerve graft; nerve conduit; stem cells; Schwann cells; dorsal root ganglia neurons; axon stretch-growth; autologous tissue graft*

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Introduction

Peripheral nerve injury (PNI) is a complex challenge due to its many etiologies and varying degrees of severity. Although the etiologies of PNI are vast, traumatic injuries, such as motor vehicle accidents and gunshot wounds, account for the majority of cases (Scholz et al., 2009). Approximately 360,000 people suffer a PNI in the United States annually, which amounts to an estimated annual expenditure of $150 billion healthcare dollars (Noble et al., 1998; Taylor et al., 2008). Moreover, patients are left with a spectrum of disability following PNI, ranging from mild sensorimotor dysfunction to the devastating loss of motor and/or sensory function.

The current approach to PNI repair depends on the degree of injury. If no gap exists in the transected nerve, a direct repair *via* an end-to-end neurorrhaphy can be performed by joining each perineurial defined fascicle (Siemionow and Brzezicki, 2009). In this technique, care must be taken to avoid tension, as this would diminish epineurial blood flow and risk tissue necrosis (Smith, 1966a, b; Lundborg and Rydevik, 1973). For gaps of 1 cm or less, either biological or synthetic nerve conduits have been used to approximate the nerve stumps and guide regeneration with good success (Meek and Coert, 2002; Battiston et al., 2009; Moore et al., 2009; Siemionow and Brzezicki, 2009). Although some authors have used synthetic nerve conduits for nerve gaps up to 2.5 cm, the complication rates have been high, including fistulization of the conduit requiring removal and tube extrusions (Chiriac et al., 2012; Safa and Buncke, 2016). Therefore, the current gold standard for repairing gaps greater than 1 cm is autologous nerve, which provides the native scaffolding of Schwann cells, extracellular matrix (ECM), and growth factors needed for optimal regeneration (Pfister et al., 2011).

The most common sources of autologous nerve include the sural nerve, medial antebrachial cutaneous nerve, and posterior interosseous nerves (Battiston et al., 2017). While harvesting sensory nerves results in the least of morbidity at the harvest site, using a sensory nerve autograft in a motor nerve or a mixed motor-sensory nerve injury can lead to poor functional outcomes (Rbia and Shin, 2017). In addition, despite the superior clinical efficacy of autografting, the supply of autologous nerve is limited and harvesting the nerve from an additional surgical site increases the potential for donor site morbidity, including painful neuroma formation, sensory loss, infection, and surgical scar (Liu et al., 2012).

One common alternative to nerve autografts are processed nerve allografts. In clinical practice, nerve allografts have been most commonly used to successfully repair nerve gap lengths up to 70 mm (Safa and Buncke, 2016). Although nerve allografts are a potential alternative for the repair of substantial gaps, the high immunogenicity of Schwann cells and myelin within allografts results in a high rate of rejection by the host, thereby necessitating concurrent immunosuppression (Berger et al., 2007; Rbia and Shin, 2017).

Due to the limitations of autografts and allografts, tissue engineering has been heavily utilized to find a suitable alternative for nerve repair. Specifically, tissue engineered nerve grafts (TENGs)

utilizing either decellularized allografts, also termed acellular nerve grafts (ANGs), or conduits composed of natural or synthetic material have been a central focus in finding a suitable alternative to autografting. In addition, some groups have looked beyond nerve tissue and experimented with grafts composed of non-nerve tissues, such as tendon membrane, skeletal muscle, vein grafts, and arterial grafts. While other types of biomedical engineering solutions, such as electrical stimulation, have shown success in the repair of peripheral nerve defects, this review will focus on recent advances in the development of TENGs utilizing acellular nerve grafts, bioengineered conduits, and non-nerve tissue grafts, as well as a discussion of their efficacy in experimental models.

Acellular Nerve Grafts

Decellularization of allogeneic nerve to create ANGs reduces immunogenicity by eliminating antigenic factors, particularly Schwann cells and myelin, while retaining the natural basement membrane and three-dimensional ECM to guide axonal regeneration (Hudson et al., 2004; Johnson et al., 2011). Many methods have been described on the preparation of ANGs ranging from the relatively quick preparation of nerve grafts with irradiation or a short cycle of freezing and thawing to more time intensive protocols requiring days of soaking in chemical detergents with intermittent agitation (Szynkaruk et al., 2013). In addition, a recent experiment used ANGs prepared with interspersed segments of cellular nerve grafts to determine if fragments of nerve grafts can provide a "stepping stone" to improve regeneration of myelinated axons across a peripheral nerve defect (Yan et al., 2018). Unfortunately, the stepping stone technique of preparing a hybrid ANG only showed to be effective in crossing short peripheral nerve gaps (< 3 cm) and yielded poor results with the regeneration of myelinated axons across long defects (> 6 cm) (Yan et al., 2018).

Regardless of the preparatory method, ANGs alone have not been as effective as grafting segments of nerve (Saheb-Al-Zamani et al., 2013). This is likely due to the pivotal role of Schwann cells in fostering the appropriate microenvironment for regeneration following PNI (Pfister et al., 2011). After nerve injury, the c-Jun transcription factor mediates de-differentiation of Schwann cells in the distal nerve stump into a pro-repair phenotype, resulting in the suppression of myelin genes and activation of trophic factors, such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and glial derived neurotrophic factor (GDNF), to help guide regenerating axons from the proximal nerve stump towards the distal end of the injury (Burnett and Zager, 2004; Scheib and Hoke, 2013; Thompson and Sakiyama-Elbert, 2018). Regenerated axons are then re-myelinated once neuron-derived neuregulin-1 (NRG1)-type III binds to receptors on repair Schwann cells (Thompson and Sakiyama-Elbert, 2018).

Instead of entering a pro-repair state, Schwann cells can also enter a state of cellular senescence, thereby leading to failure of the regeneration of axons in the ANGs. Although the mechanism by which Schwann cells enter a state of senescence is poorly understood, it is most likely felt to be related to long gap (> 3 cm) defects bridged with ANGs (Saheb-Al-Zamani et al., 2013; Poppler et al., 2016). In addition, ischemia of the ANGs and impairment of axonal regeneration is more likely to occur in ANGs bridging long defects versus short defects, since ANGs rely on diffusion of tissue fluids from each end of the graft to supply nutrients during the early stage (Zhu et al., 2017). Lastly, microvessel growth in ANGs occurs at a slower rate than it does in autografts, increasing the susceptibility of ANGs to ischemia (Zhu et al., 2017).

Several groups have investigated seeding ANGs with Schwann cells to imitate the natural microenvironment, however, this can result in additional morbidity since the Schwann cells must be host-derived to avoid immunoreactivity (Zheng et al., 2017). Given that stem cells have demonstrated the ability to differentiate into Schwann cell-like cells *in vitro*, many groups have seeded ANGs with stem cells from various sources, including nervous tissue, bone marrow, adipose tissue, and skin (Jiang et al., 2017). These approaches (summarized in **Additional Table 1**), as well as other novel approaches, such as nourishing ANGs with platelet-rich plasma (PRP), priming the ANG ECM with chondroitinase ABC (Ch-ABC) (**Additional Table 1**), and supplying additional neurotrophic factors (**Additional Table 2**) will be discussed.

Recellularizing acellular nerve grafts

Schwann cells

Jiang et al. (2016) created TENGs composed of rhesus monkey-derived ANGs injected with autologous Schwann cells. Five months after repairing 40 mm ulnar nerve defects in rhesus monkeys, the TENG and autograft groups displayed similar histological and electrophysiological outcomes (Jiang et al., 2016). In addition, the TENGs resulted in significantly superior repair versus empty ANGs (Jiang et al., 2016). While this study harvested Schwann cells from the common peroneal nerve, an earlier study isolated Schwann cells from either the motor or sensory branches of rat femoral nerve to determine if Schwann cell phenotype influences nerve repair (Jesuraj et al., 2014). Twelve weeks after the repair of 14 mm rat sciatic nerve defects, the TENGs, regardless of phenotype, resulted in similar nerve regeneration and functional recovery as the isograft group (Jesuraj et al., 2014).

A recent experiment showed that TENGs could be further improved by gene therapy (Wang et al., 2017). Krüppel-like factors (KLFs) are zinc finger DNA-binding proteins that are involved in several processes, including neuronal morphogenesis, and KLF7 is a transcriptional activator that has been shown to promote axonal regeneration in the CNS (McConnell and Yang, 2010; Blackmore et al., 2012). Accordingly, Wang et al. (2017) transfected Schwann cells with adeno-associated virus 2 (AAV2)-KLF7 *in vitro* to create Schwann cells that overexpress KLF7. When applied to 10 mm sciatic nerve defects in mice, TENGs seeded with KLF7-transfected Schwann cells promoted a greater level of axonal regeneration, myelination, and electrophysiological recovery compared with TENGs seeded with normal Schwann cells (Wang et al., 2017). Also, given that the TENGs consisting of KLF7-Schwann cells increased the expression of several downstream targets, including NGF, tyrosine receptor kinase A (TrkA), tyrosine receptor kinase B (TrkB), and growth associated protein 43 (GAP43), the authors posited that the KLF7-Schwann cells promoted improved nerve regeneration and functional recovery by upregulating the repair pathways that are triggered after PNI (Wang et al., 2017).

The methods of seeding Schwann cells into an acellular graft may also affect how well peripheral nerve regeneration occurs. Thompson et al. (2017) found that injection of Schwann cells into an acellular graft, instead of bathing in a cell-suspension, allows a large volume and more uniform distribution of supportive cells throughout the acellular nerve graft, thus potentially improving clinical outcomes. Likewise, in an experiment using acellular nerve grafts to repair rodent sciatic nerve defects, Isaacs et al. (2017) found that injection of Schwann cells into the grafts resulted in a larger number of viable Schwann cells at 21 days after

implantation when compared to bathing methods.

Neural stem cells (NSCs)

Recently, Xiang et al. (2017) constructed TENGs by culturing ANGs with NSCs derived from the embryonic ependyma of fetal rats. A subset of TENGs were cultured in a medium containing tetramethylpyrazine (TMP), a compound that has been shown to exert anti-inflammatory, anti-oxidant, and anti-apoptotic properties on ANGs (Xiang and Yang, 2015). Rat sciatic nerve defects of 15 mm were subsequently repaired, and twelve weeks after surgery, rats that received TENGs cultured in TMP displayed similar sciatic functional index (SFI), nerve conduction velocity, and gastrocnemius wet weight as the autograft group and significantly improved outcomes compared to the TENG alone and ANG groups (Xiang et al., 2017). Notably, the TENGs cultured in TMP resulted in significantly greater immunofluorescent cells positive for neural markers *versus* the TENGs cultured without TMP, which indicates the protective effect of TMP on the survival of NSCs within ANGs (Xiang et al., 2017).

Bone marrow stem cells (BMSCs)

Prior studies have reported the ability of BMSCs to differentiate into a Schwann cell phenotype and positively modulate Schwann cell behavior (Lin et al., 2008; Wang et al., 2009). As a result, several studies have created TENGs composed of ANGs seeded with BMSCs (Yin et al., 2013; Li et al., 2016; Zheng et al., 2016, 2017). A recent study showed that BMSCs injected into ANGs at multiple points resulted in outcomes similar to autografts and significantly improved outcomes compared to ANGs alone at 12 weeks after the repair of 15 mm rat sciatic nerve defects (Yin et al., 2013). Notably, the TENG group displayed superior motor endplate reconstruction and reinnervation of the distal target (Yin et al., 2013). A more recent study by Li et al. (2016) demonstrated that infusing BMSCs into ANGs to repair 30 mm sciatic nerve defects in rabbits resulted in similar electrophysiological function, morphological recovery, and tensile strength of the sciatic nerve as the autograft group, in addition to significantly improved outcomes compared to the ANG alone group.

The role of TrkA, the high affinity receptor for NGF, in promoting the survival and neural differentiation of BMSCs has been a recent focus of investigation to improve the effect of BMSCs on peripheral nerve regeneration (Zheng et al., 2016). Rat BMSCs infected with recombinant lentiviruses to create TrkA-overexpressing BMSCs resulted in superior survival and Schwann cell-like differentiation within ANGs derived from Sprague-Dawley rats; in addition, knockdown of TrkA accelerated cell death and reduced neural differentiation of BMSCs within ANGs, indicating the importance of TrkA on BMSC survival and differentiation (Zheng et al., 2016). The repair of 10 mm rat sciatic nerve defects with ANGs seeded with lentiviral vector mediated TrkA-overexpressing BMSCs resulted in significantly improved axonal growth and functional recovery at eight weeks post-surgery compared to ANGs loaded with control BMSCs, as well as similar outcomes as the autograft group (Zheng et al., 2017).

Adipose derived stem cells (ADSCs)

ADSCs can also differentiate into Schwann cell-like cells and thus have been widely utilized in the construction of TENGs (Kingham et al., 2007; Gao et al., 2014). Wang et al. (2012b) showed that ANGs seeded with BMSCs or ADSCs result in similar nerve regeneration and functional recovery when used to repair 15 mm sciatic nerve defects in rats; however, unlike BMSCs, harvesting ADSCs is easier, less invasive, and results in greater yield. Gao et al. (2014) harvested ADSCs from rat inguinal fat pad and induced differentiation into Schwann cell-like cells *in vitro*; the resulting cells were injected into ANGs to create TENGs. When applied to 10 mm sciatic nerve defects in Sprague-Dawley rats, the TENGs resulted in significantly improved outcomes over ANGs alone; furthermore, at six and twelve weeks post-surgery, the SFI, wet weight of gastrocnemius muscle, and density of regenerated myelinated nerve fibers were similar in the TENG and autograft groups (Gao et al., 2014).

While the study by Gao et al. (2014) showed the utility of differentiated ADSCs in promoting axonal regeneration, Luo et al. (2015) attempted to improve the distribution and viability of ADSCs within ANGs by creating the TENGs in a rotary cell culture system (RCCS). After repairing a 10 mm sciatic nerve defect in rats, TENGs constructed in the RCCS resulted in improved uniformity of ADSCs throughout the ANG, greater ADSC proliferation, improved neural differentiation, and ultimately greater nerve regenerative capacity compared to the TENGs constructed in static culture. Given these outcomes, this technique has the potential to improve the construction and efficacy of TENGs that are seeded with cells.

Skin derived precursors (SKPs)

The dermal layer of skin contains SKPs, a group of cells with several similarities to embryonic neural crest stem cells, which can be differentiated into Schwann cells *in vitro*, thereby providing an easier and less invasive alternative to harvesting autologous Schwann cells (Fernandes et al., 2004; Biernaskie et al., 2006). Walsh et al. (2009) experimented whether SKP-derived Schwann cells (SKP-SCs) injected into both ends of an ANG could provide comparable results to ANGs loaded with autologous Schwann cells. Four weeks after repairing 12 mm defects in rat sciatic nerves, ANGs injected with SKP-SCs resulted in similar axonal regeneration as the autograft and autologous Schwann cell groups. In addition, at eight weeks post-repair, the SKP-SC loaded ANG resulted in significantly higher compound muscle action potential versus the autologous Schwann cell loaded ANG, thereby indicating superior electrophysiological recovery (Walsh et al., 2009).

A more recent study by Wang et al. (2016a) showed that the efficacy of TENGs composed of ANGs injected with SKP-SCs can be improved by the co-injection of heregulin-1β, a multifunctional protein expressed by Schwann cells that is involved in Schwann cell proliferation and migration, as well as survival of SKPs after transplantation (Walsh et al., 2012; Wang et al., 2016a). After the repair of 15 mm sciatic nerve defects in rats, the group treated with ANGs injected with SKP-SCs and heregulin-1β displayed significantly improved functional and histological recovery compared to the autograft group. Of note, the SKP-SC and heregulin-1β group resulted in greater expression of collagen types I and III, reduced myelin damage, and increased nerve fiber size and density (Wang et al., 2016a).

Priming the ECM of ANGs for optimal axonal regeneration

Chondroitin sulfate proteoglycans (CSPGs) are components of the peripheral nerve ECM that are upregulated by Schwann cells following PNI, which results in inhibition of neurite growth (Zuo et al., 1998). The application of Ch-ABC, an enzyme that cleaves glycosaminoglycan (GAG) side chains from proteoglycans, reverses the inhibitory effects of CSPGs and promotes axonal regeneration following injury (Zuo et al., 2002). As a result,

several groups have investigated whether the efficacy of TENGs composed of ANGs loaded with stem cells can be improved by pretreating the ANG with Ch-ABC to prime the ECM structure for optimal axonal regeneration (Wang et al., 2012a, 2016b; Boyer et al., 2015; Jiang et al., 2015).

When used to repair 10 mm sciatic nerve defects in rats, ANGs that were treated with Ch-ABC and subsequently injected with BMSCs at four points along the graft resulted in significantly increased SFI, nerve conduction velocity, number of myelinated fibers, myelin sheath thickness, and axonal diameter compared to non-Ch-ABC treated ANGs loaded with BMSCs (Wang et al., 2012a). In addition, the number of BMSCs in the middle of the ANG was higher in the group treated with Ch-ABC, indicating the ability of Ch-ABC to promote the survival of BMSCs in ANGs (Wang et al., 2012a). In 2016, the group investigated the molecular mechanisms underlying the synergistic effects of BMSCs and Ch-ABC on nerve regeneration in a similar 10 mm rat sciatic nerve injury model (Wang et al., 2016b). While the previous study showed that Ch-ABC treated ANGs seeded with BMSCs do not significantly increase growth factor secretion *in vitro* compared to ANGs with BMSCs alone, the subsequent study showed significantly increased levels of NGF, vascular endothelial growth factor (VEGF), and BDNF after transplantation, indicating that the improved histological and functional recovery in the Ch-ABC group may be partly due to the ability of Ch-ABC to enhance *in vivo* growth factor secretion (Wang et al., 2016b).

Jiang et al. (2015) showed that treating ANGs with Ch-ABC and subsequently loading the grafts with differentiated ADSCs resulted in significantly higher compound muscle action potentials, nerve conduction velocity, and number of myelinated axons than the ANG + differentiated ADSC group when applied to 15 mm sciatic nerve defects in rats. While the autograft group resulted in the most superior outcomes, this study reiterates that the degradation of GAG branches by Ch-ABC promotes significantly improved nerve regeneration, particularly when the ANG is combined with differentiated ADSCs (Jiang et al., 2015).

Enriching ANGs with neurotrophic factors

Neurotrophic factors, including BDNF, GDNF, ciliary neurotrophic factor (CNTF), and NGF, play an important role in promoting axonal regeneration and functional recovery following PNI (Poppler et al., 2016). As such, several groups have investigated whether supplementing ANGs with neurotrophic factors improves the efficacy of ANGs in nerve repair. Recent strategies of neurotrophic factor delivery include virally transfected Schwann cells injected either directly into the ANG (Godinho et al., 2013; Santosa et al., 2013; Ee et al., 2017) or into the distal nerve stump (Marquardt et al., 2015), in addition to growth factor containing polymeric microspheres that are applied around ANGs (Yu et al., 2009; Zhang et al., 2014; Tajdaran et al., 2016).

Genetically modified Schwann cells overexpressing neurotrophic factors

Godinho et al. (2013) transduced Schwann cells with lentiviral vectors to express either BDNF, CNTF, or neurotrophin-3 (NT3) and subsequently transplanted these cells into ANGs derived from rat sciatic nerve to repair 10 mm peroneal nerve defects in rats. At ten weeks post-repair, each neurotrophic factor resulted in differential effects on axonal regeneration, myelination, and functional recovery. Notably, the NT3 grafts contained the most sensory axons versus other grafts, including autografts, whereas the CNTF grafts exhibited the lowest level of axonal regeneration of all grafts. TENGs containing Schwann cells expressing either BDNF or NT3 resulted in clearly apparent fascicles of axons; however, the fraction of myelinated axons was highest in BDNF grafts and lowest in NT3 grafts. Functional recovery as observed by gait analysis was concordant with the morphological findings; namely, the CNTF and NT3 groups displayed increased sensory sensitivity as evidenced by increased stance width and step size (Godinho et al., 2013). Given that each neurotrophic factor has a different effect on nerve regeneration after injury, the creation of a graft containing Schwann cells that overexpress all three neurotrophic factors is warranted to investigate if their complementary effects result in further improvement of outcomes.

GDNF is another neurotrophic factor that has been heavily studied. Following PNI, Schwann cells transiently upregulate the expression of GDNF in the distal nerve; however, this effect is only temporary and is often shorter than the time required for completion of axonal regeneration to the distal target (Hoke et al., 2000, 2002). While supplementation of exogenous GDNF after nerve injury should theoretically improve axonal regeneration, the repair of 14 mm rat sciatic nerve defects *via* ANGs seeded with Schwann cells that overexpress GDNF resulted in ineffective axonal growth into the distal stump due axon entrapment in the mid-graft; consequently, functional recovery was impaired as evidenced by significantly decreased extensor digitorum longus (EDL) force production compared to the isograft group (Santosa et al., 2013). Of note, repair of the nerve defect with TENGs containing wild-type Schwann cells resulted in comparable evoked force production in the EDL as the isograft group, as well as less muscle atrophy that the GDNF-overexpressing Schwann cells, thereby further suggesting that higher levels of local GDNF delivery may impede axonal regeneration. An earlier study described a similar phenomenon, called the "candy store effect," and while the exact mechanism is unclear, it appears that the constant supply of high levels of GDNF results in bundling of the regenerating axons within the graft, which thereby prevents axonal outgrowth and reinnervation of the distal target (Tannemaat et al., 2008).

To overcome the "candy store effect," Marquardt et al. transduced Schwann cells with a tetracycline-inducible GDNF expressing lentiviral vector, which allowed GDNF expression to be temporally controlled by doxycycline administration (Marquardt et al., 2015). These Schwann cells were injected into the distal nerve stump of a 30 mm rat sciatic nerve injury, and the defect was subsequently repaired with an ANG injected with fibrin containing a heparin-based delivery system (HBDS) and GDNF (Marquardt et al., 2015). The HBDS allowed for the controlled release of GDNF in the proximal nerve stump, whereas the transduced Schwann cells that were modified to overexpress GDNF when in the presence of doxycycline were utilized to promote axonal regeneration to the distal nerve (Wood et al., 2009; Shakhbazau et al., 2013). The study found that six weeks of doxycycline-induced GDNF overexpression resulted in axonal regeneration to and beyond the distal nerve stump, whereas four and eight weeks of doxycycline administration led to regeneration failure; in addition, six weeks of GDNF delivery resulted in significantly higher percent neural tissue and a lower amount of myelin debris, implying greater quality of nerve regeneration (Marquardt et al., 2015). Compared to the isograft group, TENGs supplying six weeks of GDNF expression resulted in higher axonal density and percent neural tissue in the midgraft, and similar axonal density and percent neural tissue in the distal nerve; furthermore, gastrocnemius and tibialis anterior muscle mass recovery was similar in both groups,

and although functional analysis was not performed, this suggests that the TENGs were able to achieve a similar level of end target reinnervation as the isograft group.

While the study by Marquardt et al. (2015) showed that the temporal and spatial control of GDNF release for six weeks promotes significantly improved axonal regeneration, a study by Ee et al. (2017) that also investigated temporal regulation showed disparate results. In this study, transgenic Schwann cells that conditionally overexpress GDNF in the presence of doxycycline were injected into ANGs, which were subsequently used to repair 14 mm sciatic nerve defects in rats. After six weeks, histomorphometric analysis of the distal nerve showed decreased number of myelinated axons and decreased percent neural tissue compared to the group that received ANGs loaded with normal Schwann cells. Electron microscopy of the nerve grafts that were seeded with the conditionally GDNF expressing Schwann cells revealed significant remodeling of the ECM with dense collagen bundles in areas lacking myelinated axons, and genetic analysis revealed that the transgenic Schwann cells caused significantly increased expression of certain collagen genes in fibroblasts. This suggests that the axonal entrapment associated with GDNF-overexpressing Schwann cells may be due to their effect on fibroblast gene expression (Ee et al., 2017). Of note, while Marquardt et al. (2015) injected transgenic Schwann cell directly into the distal sciatic nerve stump, Ee et al. (2017) injected the cells into the ANG, which has a higher number of stromal cells than native nerve, and thus could explain the disparate results between the two studies (Poppler et al., 2016).

Microsphere delivery systems

In 2016, Tajdaran et al. (2016) used 10 mm ANGs combined with a drug delivery system (DDS) composed of poly(lactic-co-glycolic acid) (PLGA) microspheres containing recombinant human GDNF embedded in fibrin gel to repair 5 mm common peroneal nerve defects in rats (Wood et al., 2013a, b; Tajdaran et al., 2016). The DDS was designed to release GDNF for either two or four weeks *in vitro*, after which it was implanted around the proximal and distal suture sites of the ANGs (Tajdaran et al., 2016). Of the three experimental groups, one group received a two-week release formulation at both sites, another group received a four-week release formulation at both sites, and the third group received a two-week formulation at the proximal suture site and a four-week formulation at the distal suture site (Tajdaran et al., 2016). Eight weeks after implantation, all experimental groups showed significantly improved regeneration of motor and sensory neurons as evidenced by retrograde labeling neurons compared to the controls that received either no DDS or fibrin gels with empty microspheres; most notably, motor and sensory neuron regeneration in the experimental groups was comparable to the isograft group (Tajdaran et al., 2016). Furthermore, histomorphometric analysis of the nerve showed similar number of myelinated axons, frequency of nerve fibers with larger diameter, and axonal density as the isograft group. Given the efficacy of the DDS, ease of administration, and lack of complications, this approach has great potential for clinical application; however, a study evaluating functional recovery and *in vivo* GDNF dose detection is warranted. Notably, the DDS did not result in the "candy store effect" that has been seen in studies utilizing transgenic Schwann cells to supply GDNF (Tannemaat et al., 2008; Santosa et al., 2013; Tajdaran et al., 2016).

CNTF has also been delivered to ANGs *via* a PLGA microsphere delivery system (Zhang et al., 2014). In this study, Zhang et al. (2014) repaired 10 mm sciatic nerve defects in rats with ANGs and applied the CNTF microspheres around the grafts *via* a micro-syringe. Eight weeks after repair, histological analysis revealed increased density of myelinated nerve fibers in the CNTF group versus the ANG alone group. Functional analysis showed significantly greater SFI in the CNTF group versus the ANG alone group, but the autograft group exhibited the highest SFI. Electrophysiological recovery was also highest in the autograft group, but the CNTF group had significantly improved outcomes compared to the ANG alone group (Zhang et al., 2014). While CNTF supplementation to an ANG does not compare to autografting, the study did show significantly improved outcomes compared to ANGs alone. Given that neurotrophic factors have differential effects on nerve repair, there is potential for CNTF to complement more potent neurotrophic factors, such as GDNF, in a drug delivery system.

Nourishing ANGs with PRP

Zheng et al. (2013) discovered that PRP significantly stimulates Schwann cell proliferation and migration and significantly increases NGF and GDNF expression. Accordingly, the group investigated whether nourishing ANGs with PRP improves the ability of ANGs to promote repair after PNI (Zheng et al., 2014). Autologous PRP was prepared into a gel that was subsequently wrapped around the surface of ANGs and sutured to the connective tissue. These grafts where then utilized to repair 15 mm rat sciatic nerve defects, and histological and functional recovery was analyzed twelve weeks after repair. Motor recovery, as evidenced by the SFI, was similar to the autograft group and significantly improved compared to the groups that received ANG with platelet-poor plasma (PPP) or ANG alone. In addition, electrophysiological assessment showed that functional reinnervation was greatest in the autograft group, but the PRP group was significantly improved compared to the PPP and ANG alone groups. Notably, histological analysis showed improved axonal regeneration from the proximal nerve stump to the distal nerve stump in the PRP and autograft groups.

Zheng et al. (2014) also determined gene expression of neurotrophic factors *via* quantitative real-time polymerase chain reaction (qRT-PCR) of mRNA levels. The PRP group achieved similar levels of GDNF and NGF as the autograft group and significantly increased levels compared to the PPP and ANG alone groups; this result was possibly due to the ability of PRP to cause Schwann cell proliferation and subsequent growth factor secretion, which is plausible given that the PRP group showed the highest level of S-100 positivity (Zheng et al., 2014). However, given that growth factors within PRP, such as TGF-1β and PDGF-BB, have also been implicated in Schwann cell proliferation and differentiation, it is possible that the release of these growth factors also contributed to the improved axonal regeneration in the PRP group (McLennan and Koishi, 2002; Jiang et al., 2013). PRP has also been employed in nerve guidance conduits and vein-muscle grafts with promising outcomes, and

While the study by Zheng et al. (2014) shows the potential clinical utility of wrapping PRP gel around ANGs, further studies with ANGs are needed to better define the exact parameters of growth factor release from PRP to optimize nerve regeneration. Despite this, PRP has been applied to nerve injuries clinically with success, notably in the repair of a 12 cm ulnar nerve gap in a patient who initially sustained the injury 3.25 years prior to the repair and was considering a complete upper extremity amputation due to excruciating neuropathic pain (Kuffler et al., 2011).

In this patient, autologous PRP was filled in a collagen tube and subsequently polymerized in fibrin after being secured to the nerve. Two years after surgery, the patient had some motor and sensory recovery with significant alleviation of his neuropathic pain, which thereby spared the patient of an amputation without requiring autologous sensory nerve for the repair (Kuffler et al., 2011). In another successful clinical report, a 5 cm long radial nerve gap at the elbow was repaired with sural nerve that was wrapped in collagen and subsequently filled with cryoprecipitate combined with thrombin (Reyes et al., 2007). At 1.5 years after surgery, the patient exhibited complete motor and sensory function without any adverse effects from the repair.

Bioengineered Conduits

In recent decades, a vast array of natural and synthetic biomaterials, including chitosan, collagen, alginate, poly(glycolic acid) (PGA), and poly(lactic-co-glycolic acid) (PLGA) have been utilized in an effort to develop nerve guidance conduits that are suitable alternatives to autologous nerve in peripheral nerve repair (Gu et al., 2014a). Despite this, empty conduits have shown inferior results versus empty ANGs and have limited utility for large nerve defects (Moore et al., 2009, 2011; Whitlock et al., 2009; Pfister et al., 2011). Recent advances in improving conduit efficacy center around replicating the structure of native nerve, including cellularizing conduits with Schwann cells or stem cells (**Additional Table 3**), enhancing conduits with ECM components or cell-derived ECM scaffolds to mimic nerve structure (**Additional Table 4**), and creating novel living nervous tissue constructs with DRG neurons (**Additional Table 5**).

Cellularized conduits

The incorporation of bone marrow mononuclear cells (BMMCs) and bone marrow mesenchymal stem cells (BMMSCs) within conduits to create TENGs has shown promising results in nerve repair. A 10 mm defect in the sciatic nerve of Sprague-Dawley rats was repaired using a chitosan/silk fibroin based conduit filled with BMMCs (Yao et al., 2016). At 12 weeks, large muscles distal to repair showed no significant weight difference between the autograft and TENG groups. More recently, a much larger gap of 30 mm in goat peroneal nerve was repaired using chitosan conduits filled with autologous BMMCs (Muheremu et al., 2017). Twelve months after nerve repair, functional recovery of muscles distal to the injury occurred with no significant differences seen in nerve conduction velocity between the autograft and TENG groups. An even larger defect of 50 mm in the median nerve of rhesus monkeys was bridged with TENGs composed of chitosan/PLGA conduits filled with autologous BMMSCs (Hu et al., 2013). Twelve months later, recovery of nerve function in the TENG group was superior to empty chitosan/PLGA conduits and morphological reconstruction resembled the autograft group.

ADSCs have also been used in conduits to repair peripheral nerve defects. A 10 mm defect in the sciatic nerve of Sprague-Dawley rats was repaired with a type I collagen conduit filled with autologous undifferentiated ADSCs immediately harvested from inguinal fat pads (Klein et al., 2016). Six months after repair, the cellularized conduit resulted in significantly greater motor and sensory nerve conduction velocities and improved quality of axonal regeneration versus empty conduits. In a different experiment, ADSCs obtained from adult rats were cultured *in vitro* for a minimum of two weeks to encourage differentiation into Schwann cell-like precursors and then loaded into a type I collagen matrix within NeuraWrap conduits to create TENGs (Georgiou et al., 2015). Eight weeks after the repair of 15 mm gaps in rat sciatic nerve, the TENG group resulted in 3.5 times more axons in the distal nerve stump versus empty conduits. An earlier study utilized TENGs composed of NeuraGen collagen conduits filled with fibrin-agarose hydrogels containing undifferentiated ADSCs to repair 10 mm sciatic nerve gaps in Wistar rats (Carriel et al., 2013). After 12 weeks, the TENG group displayed superior nerve regeneration, increased myelination, and improved axonal orientation within a more organized ECM versus saline filled control conduits.

Schwann cells alone and in combination with stem cells have successfully been used inside of conduits in peripheral nerve repair. For example, conduits composed of polyurethane and gelatin nanofibrils were loaded with autologous Schwann cells along with PRP and melatonin (MLT) to repair 10 mm rat sciatic nerve defects and resulted in superior outcomes versus empty conduits (Salehi et al., 2018). In a different experiment, hydrogel enriched chitosan conduits were loaded with either naïve Schwann cells or Schwann cells genetically modified to overexpress GDNF or fibroblast growth factor 2 (FGF-2) (Meyer et al., 2016). After repairing 15 mm rat sciatic nerve defects, Schwann cells overexpressing GDNF or FGF-2 demonstrated excellent sensory and motor regeneration at 17 weeks, although to a lesser extent than autografts. A previous experiment created engineered neural tissue *via* self-alignment of Schwann cells within a type I collagen matrix (Georgiou et al., 2013). The neural tissue was placed in NeuraWrap conduits and then applied to 15 mm rat sciatic nerve defects. Eight weeks after repair, histological analysis showed greater axonal regeneration to the distal nerve than the empty conduit, but less effective regeneration compared to the allograft group.

In an effort to more closely mimic the regenerative microenvironment following PNI, a recent study created TENGs composed of collagen/silk fibroin conduits loaded with a co-culture of Schwann cells and ADSCs (Xu et al., 2016). Twelve weeks after the repair of 10 mm sciatic nerve defects, morphological and electrophysiological outcomes were similar to the autograft group and superior to empty conduits; however, comparisons to individual Schwann cell and ADSC groups are needed to determine if the co-culture is superior to each cell type alone. Few studies have compared the efficacy of the various cells that are transplanted within conduits. A notable experiment used 10 mm rat sciatic nerve gaps bridged with fibrin conduits seeded with either Schwann cells, differentiated BMMSCs, or differentiated ADSCs (di Summa et al., 2011). Four months after repair, the Schwann cell group displayed significantly improved axon myelination and nerve fiber diameter, but the morphological and electrophysiological outcomes in the differentiated ADSC group were most similar to the autograft group.

Human dental pulp stem cells (hDPSCs) and olfactory ensheathing cells (OECs) have also been experimented within conduits. Recently, 15 mm rat sciatic nerve defects were repaired with TENGs composed of NeuraWrap conduits filled with engineered neural tissue that was derived from hDPSCs harvested from human patients undergoing routine wisdom tooth extractions (Sanen et al., 2017). Eight weeks later, the TENGs promoted comparable proximal nerve regeneration as the nerve allograft group but lower regeneration distally. In a different experiment, single walled carbon nanotube/PLA conduits were filled with OECs harvested from the olfactory bulb of adult donor rats to repair 8 mm rat sciatic nerve gaps (Kabiri et al., 2015). After nine weeks, conduits containing OECs showed better neu-

ral regeneration than empty conduits, with functional and histological results resembling the autograft group.

Conduits with extracellular matrix

Acellular nerve grafts promote greater nerve regeneration than silicone and type I collagen conduits, likely due to the intact three-dimensional extracellular matrix architecture to guide axonal regeneration (Whitlock et al., 2009; Johnson et al., 2011; Moore et al., 2011). Accordingly, some groups have tried to improve the efficacy of conduits either by incorporating individual ECM proteins, such as laminin and fibronectin (Gonzalez-Perez et al., 2017, 2018) or engineering ECM scaffolds *via* cell-based ECM deposition (Gu et al., 2014b, 2017).

Gonzalez-Perez et al. (2017) fabricated conduits by filling chitosan conduits with a type I collagen matrix, enriched with either laminin or fibronectin, in the form of either a simple hydrated hydrogel or as a stabilized and rolled scaffold for improved longitudinal orientation. Four months after repairing 15 mm sciatic nerve defects in rats, the conduit containing fibronectin in a stabilized and rolled collagen hydrogel resulted in the greatest growth of regenerating axons through the conduit and the highest number of myelinated fibers (Gonzalez-Perez et al., 2017). A subsequent study by the group expanded upon these constructs by adding either allogenic Schwann cells or BMMSCs (Gonzalez-Perez et al., 2018). Functional and histological analysis at four months after the repair of 15 mm rat sciatic nerve defects showed that conduits composed of self-aligned Schwann cells in either laminin or fibronectin enriched stabilized collagen scaffolds resulted in the greatest regeneration compared to the BMMSC conduits and the acellular ECM-enriched conduits, thereby indicating the ability of Schwann cell transplantation to synergistically improve ECM-based conduit efficacy (Gonzalez-Perez et al., 2018). When compared to the group's previous results of nerve autograft repair of a 15 mm rat sciatic nerve defect, the conduits containing fibronectin and Schwann cells achieved the closest level of myelinated nerve fibers at the middle of the construct and in the distal nerve stump (Gonzalez-Perez et al., 2015, 2018).

In an earlier study, Schwann cells were cultured with chitosan/silk fibroin conduits to stimulate ECM deposition within the conduits (Gu et al., 2014b). The scaffolds were subsequently decellularized and compared with plain chitosan/silk fibroin conduits and ANGs in the repair of 10 mm rat sciatic nerve gaps; twelve weeks after repair, morphological and electrophysiological results in the ECM-modified conduit group were significantly better than the plain conduit group but comparable to the ANG group (Gu et al., 2014b). More recently, the group utilized BMSCs for ECM deposition within chitosan/silk fibroin conduits and subsequently used these constructs to repair similar 10 mm rat sciatic nerve defects after decellularization (Gu et al., 2017). The BMSC-derived ECM-modified conduits facilitated significantly better regeneration than plain conduits, again displaying the utility of cell-derived ECM in improving conduit efficacy in nerve repair. However, while BMSCs are certainly more practical to harvest clinically, a study comparing these constructs to nerve autografts and Schwann cell-derived ECM constructs is needed to better understand their role in peripheral nerve repair.

Conduits with DRG neurons

Prior studies have shown that applying continuous mechanical tension on axons spanning two populations of neurons stimulates axonal stretch-growth at rates up to 10 mm/day (Smith et al., 2001; Pfister et al., 2004). Notably, the resulting nerve tracts consist of up to 1×10^6 axons and reach an incredible length of at least 10 cm while retaining normal morphology and function (Smith et al., 2001; Pfister et al., 2004). Huang et al. utilized this exciting mechanism of axonal growth to develop transplantable living nervous tissue constructs from both human and rat DRG neurons as a novel approach to peripheral nerve repair (Pfister et al., 2007; Huang et al., 2008, 2009; Wang et al., 2015; Dayawansa et al., 2016).

Huang et al. (2008) investigated whether human DRGs could be a clinically significant source of neurons for the development of transplantable nervous tissue constructs. Human DRG neurons harvested from live patients undergoing elective cervical ganglionectomy or from organ donors undergoing thoracic ganglionectomy survived in culture for at least three months under the optimal culture conditions; furthermore, axon fascicles spanning two populations of human DRG neurons were observed to undergo axonal stretch-growth when placed under continuous mechanical tension (Huang et al., 2008). The group achieved an axon stretch-growth rate of 1 mm/day, and the axon length increased significantly from an initial length of ~100 μm to 10 mm while maintaining normal morphology, thereby creating the first living human nervous tissue constructs *via* the process of stretch-growth (Huang et al., 2008).

A preliminary experimental study by Huang et al. (2009) used a 12–13 mm rat sciatic nerve injury model to demonstrate the utility of living nervous tissue constructs engineered from fetal DRG neurons in peripheral nerve repair. To develop these constructs, a motorized micro-stepper applied continuous mechanical tension on two apposed populations of fetal DRG neurons for seven days *in vitro*, which stimulated stretch-growth of the axons spanning the two sets of neurons to a desired length of 12–13 mm (Huang et al., 2009). The cultures were then embedded in collagen and inserted into PGA tubes, followed by implantation into the nerve defects. Four months after repair, histological analysis demonstrated long-term survival of the DRG neurons and the original axonal tracts within the construct despite the absence of immunosuppressive therapy, integration of the constructs into the host as evidenced by proximal and distal axonal outgrowth from the constructs into the host nerve, and regeneration of host axons through the constructs with intimate contact between host and transplanted neurites (Huang et al., 2009). Given these positive results, the authors postulated that the axons within the transplanted nervous tissue constructs provide a living axonal pathway to promote and guide axon-mediated axonal growth from the proximal nerve stump to the distal target (Huang et al., 2009).

A notable outcome of the study by Huang et al. (2009) was the *via*bility of the living nervous tissue constructs in the absence of immunosuppression despite being composed of allogeneic DRG neurons, especially considering that nerve allografts require concurrent immunosuppressive therapy due to the immunogenicity of Schwann cells and myelin (Berger et al., 2007; Huang et al., 2009). A subsequent study investigated this unexpected outcome by examining the immune response after the repair of 8 mm sciatic nerve defects in Sprague Dawley rats with NeuraGen® conduits seeded with either allogeneic DRG neurons or Schwann cells from Wistar rats (Liu et al., 2012). Compared to the DRG neuron group, the Schwann cell group exhibited significantly increased interferon-γ (IFN-γ) level and macrophage count at two weeks and four months post-repair, respectively (Liu et al., 2012). In addition, while major histocompatibility complex (MHC) I expression was increased in all groups compared to the

sham-operated group, the DRG neuron group displayed lower expression compared to the Schwann cell group (Liu et al., 2012). Histological analysis of the DRG neuron group at four months post-repair showed superior myelination at the proximal stump and healthier axons at the distal stump compared to the other groups, whereas the Schwann cell group exhibited fewer and less pronounced axons both proximally and distally (Liu et al., 2012). Given that the conduits containing allogeneic DRG neurons exhibited reduced immunogenicity without compromising histologic axonal regeneration, a subsequent study utilizing the same experimental groups and injury model was performed to assess functional recovery (Dayawansa et al., 2014). At four months post-repair, extensor postural thrust (EPT) motor evaluation showed no significant effect on motor recovery in all experimental groups; however, the DRG neuron group demonstrated superior sensory recovery *via* the Von Frey assay compared to the Schwann cell and empty conduit groups, as well as a lower frequency and severity of autotomy (Dayawansa et al., 2014).

Dayawansa et al. (2014) investigated the effect of their previously described living nervous tissue constructs on functional and electrophysiological recovery after the repair of 10 mm rat sciatic nerve defects (Huang et al., 2009; Dayawansa et al., 2016). Four months after repair, the angle board challenge test used to assess motor recovery showed significantly reduced recovery in the DRG group, autograft group, and unrepaired group compared to the sham operated group when performed at threshold (35°) level; however, at a subthreshold level of 30°, the DRG group performed similarly to the sham-operated group, whereas the autograft group had significantly reduced recovery compared to the sham-operated group (Dayawansa et al., 2016). Despite these disparate motor results, electrophysiological assessment showed intact and similar transmission of compound action potentials in all groups except the unrepaired group, and immunohistochemistry displayed close resemblance of the elongated DRG neurons to both the autograft and native bands of Büngner fibers (Dayawansa et al., 2016). Ultimately, elongated DRG neurons performed similarly to the autograft group, but further investigation of motor recovery following adequate healing, in addition to testing of sensory and autonomic recovery, is needed to verify these constructs as an alternative to autografting.

Tissue Engineered Grafts Using Non-Nerve Tissues

The following experiments within the last 15 years illustrate non-nervous tissue grafts composed of autologous tissues such as vein, muscle, artery, tendon, and even intestine, in the repair of peripheral nerve defects (**Additional Table 6**).

Vein grafts

Vein grafts loaded with various host derived materials have been employed experimentally. In 2016, femoral vein grafts filled with stromal vascular fraction (SVF) derived from host inguinal fat pad were used to repair 8 mm tibial nerve defects in rats (Özkan et al., 2016). Although myelin and axonal diameters were equivalent to nerve grafts, fewer myelinated axons were observed in the vein graft-SVF group. In an earlier study, vein grafts seeded with BMSCs derived from tibia and femur were used to bridge 8 mm defects in rat sciatic nerve and resulted in higher number of regenerated motor neurons when compared to autologous nerve (Fernandes et al., 2008).

Experiments combining vein grafts with PRP have shown promising results. In one study, 10 mm rat sciatic nerve defects were repaired with inside-out host jugular vein grafts, after which PRP collected *via* centrifugation of host whole blood was injected into the vein cavity (Kim et al., 2014). Results suggested the ability of PRP to promote neuroangiogenesis in the early period of axonal regeneration. In a more recent experiment, standard and inside-out jugular vein grafts were used to repair 10 mm rat sciatic nerve defects, followed by injection of autologous PRP into the vein cavity (Roque et al., 2017). Results showed that vein grafts filled with PRP, arranged in a standard or inside-out fashion, regenerated nervous tissues equivalently in most variables.

Muscle grafts

Muscle autografts have been used for peripheral nerve repair alone and in combination with vein tubes. Raimondo et al. proposed that the basal lamina of muscle is a migration pathway for Schwann cells as illustrated in their experiment involving freshly harvested rat biceps muscle that was placed inside an autologous femoral vein graft to repair 10 mm rat sciatic nerve defects (Raimondo et al., 2005). In a different experiment, rat tibialis anterior muscle was harvested for the repair of 15 mm rat sciatic nerve defects (Neto et al., 2004). At sixty days post-repair, the soleus muscle neuromuscular junctions distal to the repaired nerve were comparable to normal control muscle.

Denatured muscle has been incorporated within vein tubes for nerve repair. An 8 mm rat tibial nerve defect was repaired using an external jugular vein graft filled with muscle denaturized with liquid nitrogen (Fernandes et al., 2007). A retrograde neuronal marker visible under light microscopy revealed no significant difference in the number of cells regenerated between the vein filled muscle group and autologous nerve. A more recent study investigated whether denatured muscle alone or vein grafts filled with denatured muscle results in better nerve repair (Mohammadi et al., 2016). Superficial gluteal muscle was harvested from the contralateral side in a 10 mm rat sciatic nerve injury model, and after denaturation with liquid nitrogen and adequate thawing, the epimysium was sewn to epineurium of the proximal and distal nerve stumps. Vein grafts were constructed with external jugular vein filled with gluteal muscle fibers. Results showed that grafts consisting of denatured muscle and vein performed better than muscle alone.

Arterial grafts

In a recent experiment in rats, 10 mm sciatic nerve defects were repaired with arterial grafts composed of allogeneic aorta with and without ear cartilage (Firat et al., 2014). Axonal regeneration produced similar functional outcomes in the experimental groups, but autologous nerve produced the best results with the highest amount of myelination distal to the anastomosis. Another experiment utilized arterial grafts composed of donor abdominal aorta filled with femur BMSCs or a saline control to bridge 10 mm rat sciatic nerve defects (Mohammadi et al., 2014). Results indicated a much greater number of regenerated nerve fibers with increased diameter in the grafts filled with BMSCs versus saline.

Miscellaneous grafts

In the repair of 10 mm rat sciatic nerve defects, it was shown that autologous rat tendon membrane incubated with Schwann cells obtained *via* enzymatic digestion of the injury site produced nerve regeneration at a faster rate than the autograft group (Brandt et al., 2005). Although promising, the exact time period required to

allow for injured nerve to degenerate prior to harvesting Schwann cells is still under investigation. Of note, rat tail tendon and human plantaris muscle tendon share similar properties, including the ability to form collagenous membranes, thus suggesting an alternative non-nerve tissue approach for human PNI (Siemionow et al., 2010). While less practical, a recent experiment repaired a 10 mm rat sciatic nerve defect with a freshly harvested 15 mm segment of host ileum (Arda et al., 2017). Axonal regeneration and innervation of target muscles occurred, although to a much lesser extent than the autograft group. This approach may have a role in the trauma patient requiring a laparotomy, otherwise, this is a very invasive option for peripheral nerve repair.

Conclusions

TENGs have been extensively investigated in experimental studies, and many novel approaches utilizing acellular nerve grafts and bioengineered conduits have resulted in outcomes comparable to nerve autografts. ANGs seeded with Schwann cells or stem cells result in improved axonal regeneration compared to ANGs alone, and this can be further enhanced *via* gene therapy, supplemental neurotrophic factor delivery, PRP, and Ch-ABC. While conduits containing Schwann cells or stem cells usually don't attain the level of neural regeneration as their ANG counterparts, efforts to mimic the native nerve structure with cell-derived ECM has shown promising conduit efficacy. Moreover, the stretch-growth property of axons has been utilized to create transplantable conduits containing axons that span two populations of DRG neurons, thereby providing a living pathway for nerve regeneration.

While many of these tissue engineered approaches have shown positive histological and electrophysiological outcomes, more studies emphasizing functional outcomes are needed to further assess clinical utility. For example, utilizing monkey forelimb nerve defects would provide a more realistic injury model and could allow for evaluation of hand function. Furthermore, applying TENGs to various nerve defect sizes within the same study could help determine the maximum and optimal defect sizes that can be repaired with the particular TENG. While more experimental studies are needed, the continued research in the pathophysiology of PNI and the advances in tissue engineering techniques will one day result in a viable alternative to autografts for extensive nerve defects.

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Additional files:
Additional Table 1: Acellular nerve grafts seeded with cells.
Additional Table 2: Acellular nerve grafts supplemented with neurotrophic factors.
Additional Table 3: Conduits seeded with cells.
Additional Table 4: Conduits with extracellular matrix.
Additional Table 5: Conduits with DRG neurons.
Additional Table 6: Grafts constructed with non-nerve tissues.

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Additional Table 1 Acellular nerve grafts seeded with cells.

Author	Animal model	Nerve damaged	Defect size (mm)	Cell type	Cell source	Summary of outcome
Jiang et al. (2016)	Rhesus monkeys	Ulnar	40	Schwann cells	Host common peroneal nerve	At 5 months, CMAP, NCV, and neurofilament number were similar in TENG and autograft groups and significantly improved compared to empty ANGs
Jesuraj et al. (2014)	Lewis rats	Sciatic	14	Schwann cells	Host motor or sensory branch of femoral nerve	Similar number of regenerated nerve fibers and muscle force generation in the Schwann cell group compared to isograft at 6 and 12 weeks, respectively; Schwann cell phenotype had no significant effect on functional recovery
Wang et al. (2017)	C57BL/6 mice	Sciatic	10	KLF7-Schwann cells	Unknown	ANGs with KLF7 overexpressing Schwann cells resulted in increased CMAP, SFI, myelinated nerve regeneration, and survival of Schwann cells and spinal motoneurons compared to normal Schwann cells; upregulated signaling pathways also observed
Xiang et al. (2017)	SD rats	Sciatic	15	Neural stem cells	Fetal rat embryonic ependyma	At 3 months, the TENG + TMP group showed significantly higher SFI, NCV, gastrocnemius wet weight, HRP-positive cells, and myelinated nerve fibers compared to TENG without TMP and ANG groups
Zhang and Lv (2013)	Wistar rats	Sciatic	15	BMSCs	Rat femur and tibia	At 3 months, the TENG group showed similar NCV, tibialis anterior wet weight ratio, and hindlimb motor function as the autograft, all of which were improved compared to empty ANG. Immunohistochemistry of the TENG group revealed gastrocnemius motor endplate reconstruction
Li et al. (2016)	Japanese rabbits	Sciatic	30	BMSCs	Mouse BMSC cell line	At 6 months, there was no significant difference in CMAP, NCV, elastic limit load, stress-strain, and maximum load in the TENG and autograft groups, but both groups performed significantly better than empty ANGs
Zheng et al. (2017)	SD rats	Sciatic	10	TrkA-BMSCs	Rat femur and tibia	At 2 months, compared to control BMSCs, the TENG group with TrkA-overexpressing BMSCs resulted in increased MBP expression, myelinated fiber density and thickness, and axonal diameter. Electrophysiological recovery was also significantly improved, as evidenced by higher SFI, shorter onset latency, higher CMAP peak amplitude, and faster NCV
Wang et al. (2012b)	SD rats	Sciatic	15	Schwann cells, dBMSCs, or dADSCs	Rat sciatic nerve, femur and tibia, SQ tissue	At 2 weeks, TENGs consisting of Schwann cells, dBMSCs, or dADSCs showed similar axonal regeneration as autografts. At 3 months, von Frey test results, triceps surae muscular tension and weight, and regenerated nerve number were similar among all cellularized TENG groups with no significant difference compared to autograft; however, myelin sheath thickness was significantly higher in the autograft group compared to all other groups
Gao et al. (2014)	Fischer 344 rats	Sciatic	10	dADSCs	Rat inguinal fat pad	At 6 and 12 weeks, the TENG group showed significantly improved SFI, gastrocnemius wet weight, CMAP, NCV, myelin sheath thickness, and number of regenerated nerve fibers compared to empty ANG, with no significant difference compared to autograft
Luo et al. (2015)	SD rats	Sciatic	10	ADSCs	Rat SQ tissue	At 12 weeks, the group repaired with TENGs constructed with ADSCs in a RCCS showed more uniform distribution of ADSCs within the graft, improved differentiation and proliferation, and significantly higher number of regenerated axons and neuronal survival compared to TENGs made in static culture
Walsh et al. (2009)	Lewis rats	Sciatic	12	SKPs	Rat dermis	At 4 weeks, TENGs with SKPs resulted in similar axonal regeneration distal to the injury as the autograft and Schwann cell seeded ANG groups, with significantly improved regeneration compared to empty ANGs. At 2 months, the CMAP peak amplitude was significantly higher compared to the Schwann cell and empty ANG groups and similar to autograft; however, NCV showed no significant difference among all groups
Wang et al. (2016a)	SD rats	Sciatic	15	SKPs + heregulin-1β	Rat dermis	At 3 months, the TENG containing SKPs and heregulin-1β resulted in higher CMAP peak amplitude, NCV, recovery rate of gastrocnemius and triceps surae muscle wet weights, regenerated nerve fiber number, and myelin thickness compared to autograft, ANG, and TENG without heregulin-1β
Wang et al. (2012a)	Wistar rats	Sciatic	10	BMSCs + Ch-ABC	Rat femur and tibia	At 2 months, BMSC seeded ANGs resulted in improved axonal regeneration and higher secretion of neurotrophic factors compared to empty ANG. Ch-ABC treatment improved BMSC survival within the TENG and resulted in higher SFI, NCV, tibialis anterior wet weight restoration rate, myelin sheath thickness, and myelinated nerve number compared to BMSC seeded ANGs without Ch-ABC and empty ANGs
Wang et al. (2016b)	Wistar rats	Sciatic	10	BMSCs + Ch-ABC	Rat femur	At 2 months, the Ch-ABC + BMSC group resulted in significantly increased axonal regeneration, growth factor expression, motoneuron survival, NCV, and tibialis anterior muscle weight recovery compared to the Ch-ABC alone and BMSC alone groups
Jiang et al. (2015)	SD rats	Sciatic	15	dADSCs + Ch-ABC	Rat inguinal fat pad	At 3 months, NCV, CMAP, number of myelinated axons, and recovery rate of tricep surae muscle wet weight were significantly higher in the Ch-ABC + ADSC group compared to the ANG, Ch-ABC treated ANG, and ADSC seeded ANG groups; however, outcomes were inferior to the autograft group

ANG: Acellular nerve graft; TENG: tissue engineered nerve graft; SD: Sprague-Dawley; CMAP: compound muscle action potential; NCV: nerve conduction velocity; SFI: sciatic functional index; KLF7: kruppel like factor 7; BMSCs: bone marrow stem cells; dBMSCs: differentiated BMSCs; ADSCs: adipose derived stem cells; dADSCs: differentiated ADSCs; SKPs: skin derived precursors; Ch-ABC: chondroitinase ABC; TrkA: tropomyosin receptor kinase A; HRP: horseradish peroxidase; MBP: myelin basic protein; RCCS: rotary cell culture system; SQ: subcutaneous; TMP: tetramethylpyrazine.

Additional Table 2 Acellular nerve grafts supplemented with neurotrophic factors.

Author	Animal model	Nerve damaged	Defect size (mm)	Neurotrophic factor(s)	Delivery method	Summary of outcome
Godinho et al. (2013)	Fischer 344 rats	Peroneal	10	BDNF, CNTF, or NT3	Transgenic Schwann cells within ANG	At 10 weeks, NT3 grafts contained the most sensory axons and BDNF grafts showed the highest fraction of myelinated axons. CNTF grafts exhibited the lowest level of axonal regeneration. Functionally, the CNTF and NT3 groups displayed increased stance width and step size, indicating better sensory recovery
Santosa et al. (2013)	Lewis rats	Sciatic	14	GDNF	Transgenic Schwann cells within ANG	At 6 weeks, GDNF-Schwann cell grafts resulted in similar axonal regeneration at mid-graft as isograft, but significantly lower regeneration distally compared to all other groups, including grafts seeded with normal Schwann cells. At 12 weeks, the GDNF-Schwann cell group displayed lower maximum and specific force production. Electron microscopy revealed bundling of axons in the mid-graft in the GDNF-Schwann cell group, suggesting that GDNF overexpression traps axons and impairs functional recovery
Ee et al. (2017)	Lewis rats	Sciatic	14	GDNF	Tet-inducible transgenic Schwann cells within ANG	At 6 weeks, GDNF-Schwann cell grafts showed decreased myelinated axons and percent neural tissue compared to grafts seeded with normal Schwann cells. The GDNF group also displayed significant ECM remodeling and upregulation of fibroblast gene expression
Marquardt et al. (2015)	thy-1 gfp sd rats	Sciatic	30	GDNF	HBDS within ANG; tet-inducible transgenic Schwann cells injected into distal nerve	Six weeks of doxycycline treatment in the tet-inducible GDNF-Schwann cell group resulted in greater axonal regeneration through the distal stump, higher percent neural tissue, and increased gastrocnemius and tibialis anterior muscle weight compared to 4 and 8 weeks of treatment. Axonal density, axonal diameter, and muscle mass in the 6 week GDNF group were most similar to isograft
Zhang et al. (2014)	SD rats	Common peroneal	5	CNTF	PLGA microsphere delivery system around ANG	At 8 weeks, SFI, soleus MEP amplitude, triceps wet weight recovery rate, myelinated nerve fiber number, and myelin sheath thickness were highest in the autograft group but significantly improved in the CNTF group compared to empty ANG
Tajdaran et al. (2016)	SD rats	Sciatic	10	GDNF	PLGA microsphere delivery system at suture sites	At 8 weeks, motor and sensory axon regeneration, myelinated nerve fiber number, and nerve fiber diameter in the distal graft were similar in the GDNF and isograft groups

ANG: Acellular nerve graft; SD: Sprague-Dawley; MEP: motor evoked potential; SFI: sciatic functional index; BDNF: brain derived neurotrophic factor; CNTF: ciliary neurotrophic factor; NT3: neurotrophin-3; GDNF: glial derived neurotrophic factor; HBDS: heparin based delivery system; tet: tetracycline; PLGA: poly(lactic-co-glycolic acid); GFP: green fluorescent protein; ECM: extracellular matrix.

Additional Table 3 Conduits seeded with cells.

Author	Animal model	Nerve damaged	Defect size (mm)	Conduit type	Cell type	Cell source	Summary of outcome
Yao et al. (2016)	SD rats	Sciatic	10	Chitosan/silk fibroin	BMMCs	Rat femur and tibia	At 3 months, the TENG group displayed similar SFI, CMAP amplitude, NCV, regenerated nerve fiber number, and gastrocnemius and tibialis anterior wet weight ratio as autograft. Myelin sheath thickness and diameter of myelinated fibers were also similar in the distal nerve
Muheremu et al. (2017)	Goats	Peroneal	30	Chitosan	BMMCs	Goat femur	At 12 months, there was no significant difference in NCV, nerve fiber density, myelin sheath thickness, or axonal diameter between TENG and autograft groups
Hu et al. (2013)	Rhesus monkeys	Median	50	Chitosan/PLGA	BMMSCs	Monkey anterior iliac crest	At 12 months, there was no significant difference in NCV, motor or sensory neuron survival, and regenerated nerve fiber number between TENG and autograft groups; however, autograft resulted in significantly higher myelin sheath thickness and diameter of myelinated fibers
Klein et al. (2016)	SD rats	Sciatic	10	Type 1 collagen	ADSCs	Rat inguinal fat pad	At 6 months, motor and sensory NCV, regenerated nerve fiber number, and axonal organization were significantly improved in the TENG group compared to empty conduits
Georgiou et al. (2015)	SD rats	Sciatic	15	NeuraWrap + type 1 collagen matrix	dADSCs	Rat inguinal fat pad	At 2 months, the TENG promoted 3.5 times more axonal regeneration into the distal stump than empty conduit but significantly less than allograft. No significant differences in nerve fiber diameter or myelin thickness were noted
Carriel et al. (2013)	Wistar rats	Sciatic	10	NeuraGen + fibrin-agarose hydrogel	ADSCs	Rat inguinal fat pad	At 3 months, the ADSC seeded conduit resulted in significantly better sensory recovery, results of foot length and toe-spread motor testing, axonal sprouting, remyelination, and ECM organization compared to saline filled and hydrogel filled conduits
Salehi et al. (2017)	wistar rats	Sciatic	10	Polyurethane + gelatin nanofibrils	Schwann cells + PRP/MLT	Rat sciatic nerve	At 3 months, SFI, CMAP latency and amplitude, hot plate latency, gastrocnemius muscle wet weight loss percentage, and histological morphology were improved in the Schwann cell + PRP/MLT group compared to all other groups except autograft
Meyer et al. (2016)	wistar rats	Sciatic	15	Chitosan + hydrogel	Schwann cells, GDNF-Schwann cells, or FGF2-Schwann cells	Rat sciatic nerve	At 17 weeks, TENGs containing Schwann cells that overexpress either GDNF or FGF2 resulted in significant sensory and motor axonal regeneration, but autografts resulted in the most superior outcomes
Georgiou et al. (2013)	SD rats	Sciatic	15	NeuraWrap + type 1 collagen matrix	Schwann cells	Rat Schwann cell line	At 2 months, the TENG resulted in similar myelin and axonal diameters in mid-graft as the autograft group; axonal regeneration into the distal graft and nerve stump was significantly improved compared to empty conduits
Xu et al. (2016)	SD rats	Sciatic	10	Collagen/silk fibroin	Schwann cells + ADSCs	Schwann cells: rat sciatic nerve; ADSCs: rat inguinal fat pad	At 3 months, there was no significant difference in CMAP amplitude, mean NCV, or myelin shealth layer number and thickness between the TENG and autograft groups
di Summa et al. (2011)	SD rats	Sciatic	10	Fibrin	Schwann cells, dBMMSCs, or dADSCs	Schwann cells: rat sciatic nerve; BMMSCs: rat femur ADSCs: rat visceral fat	At 4 months, muscle atrophy was reduced in all groups compared to empty conduits; the dADSC seeded conduits resulted in axonal and fiber diameter regeneration, gastrocnemius evoked potentials, and motoneuron recovery most similar to the autograft group
Sanen et al. (2017)	SD rats	Sciatic	15	NeuraWrap	hDPSCs	Human third molars	At 2 months, axonal regeneration into the mid-distal graft was about 2.5 times greater in the nerve allograft group compared to the TENG and empty conduit groups; allograft and TENG groups displayed significantly greater number of blood vessels compared to empty conduits, but the allograft group had significantly more myelinated neurites compared to TENG and empty conduit groups
Kabiri et al. (2015)	SD rats	Sciatic	8	Carbon nanotube/PLA	OECs	Rat olfactory bulb	At 9 weeks, no significant difference in SFI or axonal diameter was observed in TENG *versus* autograft groups, the TENG group resulted in significantly higher nerve fiber diameter, myelin sheath thickness, and density of myelinated nerve fibers compared to empty conduits but significantly less than autograft

SD: Sprague-Dawley; TENG: tissue engineered nerve graft; CMAP: compound muscle action potential; NCV: nerve conduction velocity; SFI: sciatic functional index; BMMCs: bone marrow mononuclear cells; BMMSCs: bone marrow mesenchymal stem cells; dBMMSCs: differentiated BMMSCs; ADSCs: adipose derived stem cells; dADSCs: differentiated ADSCs; hDPSCs: human dental pulp stem cells; OECs: olfactory ensheathing cells; GDNF: glial derived neurotrophic factor; FGF2: fibroblast growth factor 2; PLA: poly(lactic acid); PLGA: poly(lactic-co-glycolic acid); ECM: extracellular matrix; PRP: platelet rich plasma; MLT: melatonin.

Additional Table 4 Conduits with extracellular matrix.

Author	Animal model	Nerve damaged	Defect size (mm)	Conduit type	ECM type	Summary of outcome
Gonzalez-Perez et al. (2017)	Wistar Hannover rats	Sciatic	15	Chitosan	Type I collagen matrix + laminin or fibronectin	At 4 months, the ECM scaffolds that were stabilized and rolled resulted in greater regeneration than fully hydrated scaffolds; enriching these stabilized scaffolds with fibronectin resulted in greater muscle reinnervation and myelinated fiber regeneration compared to laminin-enriched grafts
Gonzalez-Perez et al. (2018)	Wistar Hannover rats	Sciatic	15	Chitosan W/Schwann cells or BMMSCs	Type i collagen matrix + laminin or fibronectin	At 4 months, fibronectin enriched conduits seeded with Schwann cells resulted in better electrophysiological, histological, and functional recovery compared to laminin-enriched and BMMSC seeded groups.
Gu et al. (2014b)	SD rats	Sciatic	10	Chitosan/silk fibroin	Schwann cell-derived ECM deposition	At 3 months, there was no significant difference in regenerated nerve fiber density at the distal graft, CMAP amplitude, gastrocnemius and tibialis anterior wet weight, myelin sheath thickness, or diameter of myelinated fibers between the ECM-conduit and ANG groups; both groups performed significantly better than plain chitosan/silk fibroin conduits
Gu et al. (2017)	SD rats	Sciatic	10	Chitosan/silk fibroin	BMSC-derived ECM deposition	At 3 months, the ECM-conduit resulted in significantly greater regenerated nerve fiber density in the distal graft, CMAP amplitude, SFI, gastrocnemius and tibialis anterior weight ratio, myelin sheath thickness, and number of myelinated axons compared to plain chitosan/silk fibroin conduits

ECM: Extracellular matrix; ANG: acellular nerve graft; SD: Sprague-Dawley; CMAP: compound muscle action potential; SFI: sciatic functional index; BMSC: bone marrow stem cell; BMMSCs: bone marrow mesenchymal stem cells.

Additional Table 5 Conduits with DRG neurons.

Author	Animal model	Nerve damaged	Defect size (mm)	Conduit composition	Summary of outcome
Huang et al. (2009)	SD rats or R26-hPAP transgenic rats	Sciatic	12-13	DRG neuron derived nervous constructs in collagen within PGA tubes	At 4 months, DRG neurons and their axons were viable within the construct and facilitated axonal regeneration from the proximal nerve stump to the distal graft; axons within the construct also grew into the host nerve both proximally and distally
Liu et al. (2012)	SD rats	Sciatic	8	NeuraGen conduits seeded with DRG neurons or Schwann cells	At 4 months, there was no significant difference in axon density between DRG neuron and Schwann cell seeded conduits, both of which performed significantly better than empty conduit; however, myelination was superior in the DRG neuron group. Immunological assessment *via* IFN-ɣ level, macrophage count, and MHC I immunoreactivity showed reduced immunogenicity in the DRG neuron group compared to the Schwann cell group
Dayawansa et al. (2014)	SD rats	Sciatic	8	NeuraGen conduits seeded with DRG neurons or Schwann cells	At 4 months, extensor postural thrust motor testing showed no significant difference in the experimental groups; however, the DRG neuron group showed significantly improved sensory recovery compared to the Schwann cell group
Dayawansa et al. (2016)	SD rats	Sciatic	10	DRG neuron derived nervous constructs in collagen within PGA tubes	At 4 months, DRG nerve constructs were able to conduct compound action potentials, however, the angle board challenge at threshold level showed significantly inferior motor recovery compared to the sham group. At subthreshold angle of 30°, there was no significant difference between DRG and sham groups, whereas the autograft group performed significantly worse compared to the sham group

SD: Sprague-Dawley; DRG: dorsal root ganglia; hPAP: human placental alkaline phosphatase; PGA: poly(glycolic acid); CMAP: compound muscle action potential; NCV: nerve conduction velocity; SFI: sciatic functional index; IFN-ɣ: interferon-gamma; MHC: major histocompatibility complex.

Additional Table 6 Grafts constructed with non-nerve tissues.

Author	Animal model	Nerve damaged	Defect size (mm)	Description of graft	Summary of outcome
Neto et al. (2004)	Wistar rats	Sciatic	15	Autologous tibialis anterior muscle	At 60 days, the number of distal myelinated axons were the same as the control groups
Brandt et al. (2005)	Wistar rats	Sciatic	10	Autologous tail tendon, acutely dissociated Schwann cells from previously injured nerve	At 7 days, axonal outgrowth was longest. Nerve regeneration occurred at faster rate than standard autograft
Raimondo et al. (2005)	Wistar rats	Sciatic	10	Autologous femoral vein filled with fresh biceps muscle	At 2 weeks, vein grafts filled with muscle were filled with regenerating axons
Fernandes et al. (2007)	Wistar rats	Tibial	8	Autologous external jugular vein surrounding denatured muscle graft	No significant differences found between the vein covered muscle group versus autograft
Fernandes et al. (2008)	SHR rats	Sciatic	8	Autologous external jugular vein with or without BMMCs	BMMCs filled vein autografts had a higher number of regenerated motor neurons versus autograft
Firat et al. (2014)	Rats	Sciatic	10	Allogenic aorta graft with and without ear cartilage	Autograft outperformed allografts, but cartilage tissue showed positive effects on nerve healing
Kim et al. (2014)	SD rats	Sciatic	10	Autologous jugular vein grafted in an inside-out fashion with or without PRP	SFI improved faster and number of myelinated axons were greater in the vein filled with PRP group versus empty vein
Mohammadi et al. (2014)	Wistar rats	Sciatic	10	Donor abdominal aorta harvested and filled inside out with and without host BMSCs	Number and diameter of myelinated fibers higher in artery graft filled with BMSCs versus without
Mohammadi et al. (2016)	Wistar rats	Sciatic	10	Denatured autologous gluteus superficial muscle vs autologous external jugular vein filled with gluteus muscle	Veins filled with denatured muscle had increased gait functional recovery and axon number, diameter compared to muscle only
Özkan et al. (2016)	Wistar rats	Tibial	8	Autologous femoral vein with or without adipose derived SVF	SVF filled vein autografts had myelin and axonal diameters equivalent to nerve grafts
Arda et al. (2017)	Wistar rats	Sciatic	10	Cut ends of sciatic nerve were entubulated with segment of washed autologous ileum	Axonal regneration and innervation of targe muscles occurred with ileum group to a smaller degree versus autograft
Roque et al. (2017)	Wistar rats	Sciatic	10	Autologous left external jugular vein grafted in standard versus inside-out configuration with and without PRP	Veins filled with PRP in a standard or inside-out configuration showed equivalence in most variables and improvement of axonal regeneration versus empty vein graft

SD: Sprague-Dawley; SHR: spontaneously hypertensive rat; SFI: sciatic functional index; BMSCs: bone marrow stem cells; BMMCs: bone marrow mononuclear cells; PRP: platelet rich plasma; SVF: stromal vascular fraction.