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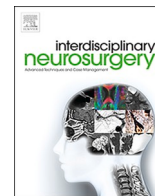
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Review Article

Application of electrical stimulation for peripheral nerve regeneration: Stimulation parameters and future horizons

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ABSTRACT

Peripheral nerve trauma impacts both social and occupational quality of life. Patients are typically young and subsequently suffer from lifelong disability. Unlike the central nervous system, the peripheral nervous system has the capacity to regenerate along previous or new connections. Yet, complete functional recovery has been an elusive clinical objective despite the development of advanced microsurgical techniques to repair nerves. In recent decades significant amount of work has expanded the focus towards establishing new facets of adjuvant treatment to improve nerve regeneration. One potential therapy is the application of electric stimulation of peripheral nerves immediately following microsurgical repair. Mounting pre-clinical and clinical evidence demonstrated the efficacy of electrical stimulation in improving nerve regeneration and functional recovery. In this paper, we review the potential therapeutic benefits of electrical stimulation and the current limitations of regeneration after nerve injury. We also summarize the proposed mechanisms of electrical stimulation in increasing the regenerative capacity of peripheral nerves, including evidence from human clinical trials. Finally, we discuss stimulation parameters and safety profiles with an eye towards future treatment strategies. Combining electrical stimulation with conductive scaffolds has the potential to improve successful nerve regeneration and may have profound clinical implications to nerve injury patients.

1. Introduction

Peripheral nerve injuries (PNI) are a significant source of disability for patients from various backgrounds. PNI affect 2.2% of patients with extremity trauma [1]. Greater than 80% of cases observed are males approximately 38 to 40 years of age [2,3]. Depending upon severity, majority of patients suffer from chronic pain, prolonged hospitalization and increased need for inpatient rehabilitation [4]. This subsequently leads to remarkable financial burden in addition to severely impacting health related quality of life [2,3]. Despite the tendency to be injured, peripheral nerves also have an inherent capacity to regenerate; however, regeneration does not always lead to full functional recovery [5]. Many factors influence the functional recovery following nerve injury – severity of nerve injury, time elapsed between injury and surgical repair, distance from the injury site to target muscle or organ, patient age, and appropriate guided axonal reinnervation [6,7].

Following PNI, functional recovery outcomes have been observed correlating with the grade of nerve injury [8]. Grade I neuropraxic

injuries only require medical management, Grade II to IV axonotmetic injuries are only repaired when meaningful regain of function is not seen within 3–6 months of electrodiagnostic testing. Severe Grade V neurotmetic injuries typically require surgical repair [8–10]. It is prudent to repair nerves soon after injury to avail the opportunity of increased regenerative capacity of freshly transected axons and Schwann cells. Early reinnervation of distal segment of transected nerve would protect both the nerve and end organs from undergoing atrophy [11]. However, early repair is not always feasible in the clinical settings; for example, after lesion in continuity is found significant time is given for spontaneous recovery before attempting surgical repair [12]. Still, even after timely repair axons may escape their normal regenerative pathways due to disruption of endoneurial tubes which further causes the delay of almost three to four weeks before axons are able to reinnervate the distal segment [13–15].

After facing staggering and crossing the barrier of scar tissue at injury site, axons are finally on path to the distal targets. Yet, axons have to traverse over long distances in proximal nerve injuries and due to their

Abbreviations: BDNF, Brain derived neurotrophic factor; ES, Electrical stimulation; trkB, Tyrosine receptor kinase B; PMR, Preferential motor reinnervation.

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sluggish regeneration rate of 1 mm per day; both the distal nerve and muscles undergo atrophy and be unable to support regeneration for meaningful functional recovery [16,17].

Experimental injuries have shown that the transected nerves may be salvageable if repaired within 3 months [18], and delaying repair for even 4 to 6 months can reduce the regenerative capacity to only 33% of normal [17]. Despite significant advances made in peripheral nerve repair in past decades, reasonable clinical outcomes have not achieved and majority of patients suffer from lifelong sensorimotor disability and chronic neuropathic pain [8,19]. Undoubtedly, there is a significant need of a therapeutic strategy that would overcome the limitations of peripheral nerve regeneration and improve the functional outcomes in patients.

One promising strategy to accelerate peripheral nerve regeneration is the application of electrical stimulation directly on the injured nerve. Immediately following nerve injury, electrical stimulation has been shown to enhance early regenerative stages, including neuronal survival and axonal sprout formation [20]. Evidence from rodent injury models have shown the potential of electrical stimulation to enhance regeneration in a variety of nerve injury types including crush [21,22], transection [23,24], and long distance injuries [25]. Electrical stimulation has been shown to increase intraneuronal cAMP in DRGs and nerve growth factor (NGF) in Schwann cells *in vitro* [26,27]. Sustained elevation in cAMP subsequently enhances regenerative capacity via increased expression of neurotrophins and cytoskeletal proteins [28,29].

Furthermore, effective electrical stimulation therapy requires determination of optimum stimulation parameters. Several parameters have been tested in animal models that led to the development of current standard protocol; a brief intraoperative electrical stimulation for one hour at 20 Hz immediately following nerve repair [14,30–34]. It has been hypothesized that additional electrical stimulation beyond the initial repair may have a dose dependent effect. Despite this, an optimum electrical stimulation paradigm beyond brief stimulation of one hour is yet to be determined. Recently, our lab has developed wireless nerve stimulators, which enables testing of various electrical stimulation paradigms beyond the initial repair [35].

Ultimately, the aim of this literature review is to elucidate the utility of electrical stimulation on improving axonal regeneration and appropriate stimulation parameters that may translate into improved functional outcome in the clinical settings.

2. Literature search methodology

PubMed and Google Scholar literature search was conducted using a combination of key words including “peripheral nerve regeneration”, “electrical stimulation”, “peripheral nerve injury”, “nerve repair”, “conductive scaffolds” and “delayed repair”. Only published articles up to 2020 were included. Abstracts were reviewed, studies were prioritized based on relevancy to our aim: electrical stimulation of peripheral nerves and optimum parameters. Further information was retrieved from via systematic snowballing in reference lists.

3. Historical perspective

The concept that electrical stimulation (ES) enhanced peripheral nerve regeneration arose from an observation of improved tissue healing after application of electrical field [36–38].

The effects of electrical fields on neurons has been known for more than a century (Verworn, 1889) [39] to be favorable in enhancing nerve growth and directing nerve fibers (Ingvar, 1920) [40]. In 1943, Hyden’s seminal work evaluated the effect of ES on axonal regeneration. The dorsal root ganglia were stimulated for 1 to 10 min with a sinusoidal alternating current of 1 to 2 mA. An increase of Nissl substance in the ganglion cells was observed following ES [41]. Less than a decade later, ES parameters to enhance axonal regeneration were proposed by Hoffman in 1952. ES at 50 to 100 Hz for 10 to 60 min were applied to the

sciatic nerve following partial transection of the L5 nerve root. Hoffman’s work demonstrated an increased rate of axonal sprouting following ES. He further postulated that ES led to a bombardment of antidromic signals to the neuronal cell body that accelerated axoplasm synthesis. Increased axoplasm resultantly raised the intracellular pressure leading to forward extrusion and hence enhanced axonal sprouting [41]. Nix and Hopf [42] further verified that ES enhanced axonal and functional recovery following a crush injury of the rabbit soleus nerve. Continuous ES was applied proximal to the crush site at 4 Hz for 4 weeks. The stimulated group recovered soleus muscle action potential and twitch force one week earlier than the controls. Similarly, Pocket and Gavin performed sciatic nerve crush lesions in rats, followed by ES at 1 Hz from 15 min to 1 h and observed the earlier recovery of toe spread reflex in the ES cohort [43]. Consistent findings of improved motor recovery were reported after ES at 0.5 Hz for 2 months following radial nerve crush lesioning in a canine model [21].

Ultimately, multiple pre-clinical studies have validated the efficacy of ES for proximal nerve stump axonal regeneration following injury [14,23,44,45] however, several questions remain: Does ES enhance the axonal regeneration by increasing the rate of regeneration? Does ES alter biochemical pathway selection within the regenerating axons? What is the most effective and clinically applicable stimulation protocol to aid axonal regeneration and functional recovery?

4. Biochemical effects of electrical stimulation

The exact cellular pathway by which ES enhance axonal regeneration remains largely unknown. Several *in vitro* studies have elucidated the temporal sequence by which ES exerts its effects at various phases of regeneration (Fig. 1A–D). Following nerve injury and repair brief ES may mimic natural wave of calcium influx that generates a retrograde signal. This retrograde signal might lead to subsequent activation of cell autonomous mechanisms initiating regeneration [33,46]. This hypothesis is supported by the evidence that ES accelerated the axons to enhance expression of genes associated with regeneration. Ultimately this increased translation of cytoskeletal proteins including actin, tubulin and growth associated protein-GAP-43 within 48 h of injury with contrast to without ES [14,24,47].

Neurotrophins like brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), and Neurotrophin 4/5 (NT 4/5) have integral role in development, maintenance, and regeneration of neurons [48–52]. After nerve injury and repair ES induced motoneuron regeneration was associated with increased mRNA expression of BDNF and its receptor tyrosine kinase b- trkB [11]. After ES of spinal cord neurons *in vitro*, confocal microscopy detected rise in intracellular Ca^{2+} that was associated with BDNF rise demonstrating role of ES mediated Ca^{2+} induced BDNF release [53,54]. BDNF acts in autocrine/paracrine pathways on regenerating motoneurons via its receptor trkB to enhance axonal growth [11]. Normally axons fail to grow through acellular allografts devoid of BDNF or NT-4/5, however ES resulted in regeneration in these grafts as it normally would through the wild type grafts. Conversely, ES treatment after nerve transection in NT-4/5 knockout mice failed to enhance axonal growth [55]. These findings support the evidence that ES indeed promoted regeneration via enhanced BDNF expression in motoneurons independent of glial trophic support. Moreover, occluding retrograde signal with tetrodotoxin, a sodium channel blocker, also eliminated the effects of ES [14,24].

Another key component by which ES exerts its effects downstream is cAMP. The role of cAMP in enhancing neurite outgrowth and axonal guidance has been demonstrated by several *in vitro* studies [56–58]. It was found that ES promoted DRG neurite outgrowth via increase in the intraneuronal cAMP [26]. Subsequently, ES induces cAMP which activates PKA (phosphokinase A) mediated phosphorylation of CREB (cAMP response element binding protein). This in turn activates downstream pathways increasing expression of BDNF and neurite outgrowth [29,59]. BDNF also prevents degradation of cAMP by inhibiting

phosphodiesterases ultimately maintaining sustained rise of cAMP [46,60].

Regenerating axons are encountered with inhibition from myelin debris, inhibitory CSPGs (chondroitin sulfate proteoglycans), and MAG (myelin associated glycoprotein) [61–63]. Recent studies have shown that ES may have a role in overcoming myelin inhibition and clearance to promote growth of regenerating axons. After treatment of axotomized motoneurons with Rolipram (a cAMP analog) similar effects were seen as after ES. The axonal growth promoted by increased cAMP likely overcome the inhibition from myelin and inhibitory chondroitin sulfate proteoglycans (CSPGs) [64,65]. Additionally, ES in DRG explants enhanced matrix metalloproteinases that degraded CSPGs overcoming the myelin inhibition [66]. ES also might have a role in clearing the myelin debris, as after nerve injury, *in vitro* experiments have shown an enhanced expression of M2 phenotype of macrophages in ES treated nerves potentiated myelin clearance, and therefore increased outgrowth [67].

ES also influence the behavior of glial supporting cells along with neurons. Schwann cells provide with guidance cues, trophic factors, and adhesion molecules that facilitate regeneration [68]. In an *in vitro* culture of Schwann cells, ES resulted in enhanced release of nerve growth factor-NGF [27]. After implanting Schwann cells that were pre-stimulated with ES at the site of injury, neurite outgrowth was significantly enhanced [69]. These findings suggest that ES may have a broader spectrum of activity than previously thought.

Recent studies have examined the role of ES in modulating other downstream signaling pathways. ES has been observed to reduce expression of a growth inhibitor PTEN [70]. PTEN an inhibitor of PI3-K/Akt cellular pathway which is critical in downstream signaling of neurotrophins to enhance regenerative pathways [71,72]. Inhibition of PTEN has been shown to facilitate peripheral nerve regeneration [73]. After nerve injury pharmacological blockade of PI3-K abolished the positive effects of ES. These findings suggest role of ES in promoting PI3-K/Akt pathway in increasing intrinsic regenerative capacity [70].

5. Electrical stimulation expedites the axonal crossing at site of coaptation

After nerve transection and repair, regenerating axons may sprout in a random, non-specific fashion, such that they stagger before entering the distal Schwann tubes. This staggering is responsible for a delay in distal stump reinnervation [13,74]. A delay in the reinnervation of end organs may ultimately lead to denervation of muscle and atrophy with a concomitant poor functional recovery [5]. In preclinical models, application of ES has shown to shrink the staggering time and accelerate the regeneration of motor neurons within 3 weeks, as compared to 8–10 weeks without ES [14]. Furthermore, ES effectively doubled the number of regenerating sensory axons [24] (Fig. 1A).

The growth of regenerating axons across a distal stump can be assessed by proximal labeling of axons, with the rate of new axonal crossing events determined per a specified time interval. In a rat femoral nerve injury model, ES facilitated both motor and sensory axonal crossing across the coaptation site 4 days earlier than without ES [23,24]. Axons typically regenerate at a speed of about 1–3 mm per day in rats [75]. To evaluate if ES increases the speed and distance of growth cone advancement, Brushart utilized a radioisotope that traveled anterogradely via fast axonal transport and labelled axons at the site of growth. By visualizing these axons, he found that ES did not actually increase the rate of regeneration or elongate the axon [23]. The early onset of regeneration is actually based upon enhancement of the cell body response stimulated by retrograde signaling. This early onset of regeneration shrinks the staggering time to increase the chance that more axons cross the site of coaptation, rather than increasing the true regeneration rate [14].

6. Electrical stimulation enhances guidance of regenerating axons

Regenerating axons from the proximal stump must select from a multitude of endoneurial tubes in the distal stump, while the reinnervation of accurate distal pathways is the key to functional recovery. As illustrated in Fig. 1C, on first pass, axons regenerate into muscle or cutaneous branches in a seemingly random fashion [76]. Motor axons that regenerate into cutaneous branches will remain permanently misguided and hinder the functional recovery after nerve injury [77]. However, upon closer inspection, regenerating motor axons demonstrate a preference to project into the motor pathways termed as preferential motor reinnervation (PMR) [76,78]. ES alleviated random reinnervation by accelerating PMR as shown in a recent rat study whereby more motoneurons regenerated into a target muscle branch when treated with ES [14]. The basis for promoting specificity of motor axon regeneration by ES might be due to increased expression of motor axon guidance cues. Polysialic acid (PSA), which has been linked to neural cell adhesion molecule (NCAM), and HNK-1 glycan are specifically expressed in motor axons. Certain femoral axons that demonstrate a preference to grow in motor pathways rather than sensory pathways showed increased expression of PSA and HNK-1 glycan [79–81]. Consequently, ES after nerve transection and repair was correlated with upregulation of both PSA and HNK-1 which showed improved preferential motor axon regeneration into motor targets [82,83] (Fig. 1C).

After nerve injury, end organs (such as muscles) provide trophic signals that attract regenerating motor neurons to efficiently regrow along their native pathways. Disconnection of the distal nerve stump from end organs resultantly removes these signal cues causing motor axons to no longer preferentially innervate motor pathways. ES was shown to increase trophic support signals in the distal nerve segment that enhanced motor axon regeneration selectivity even in the absence of end organ connection [84] as shown in Fig. 1D.

In mixed nerves, such as the femoral nerve, the dorsal root ganglion (DRG) neurons inherently project 2–3 times more in the sensory than the motor branches. After nerve transection and repair, the number of DRG neurons projecting into the motor branch abnormally equals those in sensory branch [76]. ES restored the original proportion of DRG neurons serving the sensory and motor branches. Moreover, ES increased the specificity of sensory axons to regenerate into their native pathways from 40% in non-stimulated to 75% in stimulated rats [31]. Therefore, ES may have a role in altering the pathway choices made by both motor and sensory axons in mixed nerves. Although, ES increased axonal regeneration along motor specific pathways, it also compromised the conformity of the original muscle targets. An axon projecting into a motor pathway does not mean that it would ultimately reconnect the original muscle it served before injury. In other words, enhanced regeneration may misdirect motor axons to inappropriate muscle reinnervation [85].

Nevertheless, this misdirection is secondary to the benefits observed through early reinnervation which promotes sensory feedback and the central plasticity of new neural connections [86]. Functional recovery ultimately requires reinnervation of the original targets but also the reorganization of newly regenerated axons within the central nervous system. Hyperreflexia and neuropathic pain may develop following nerve injury, due to aberrant connections (or interpretations) of peripheral axons with the spinal cord circuitry. ES has been reported to modulate plasticity at spinal cord level, via enhanced integration of sensory afferents with spinal cord circuitry. This preserved substance P levels in the sensory afferents of the dorsal horn which prevented the development of hyperreflexia and neuropathic pain [44].

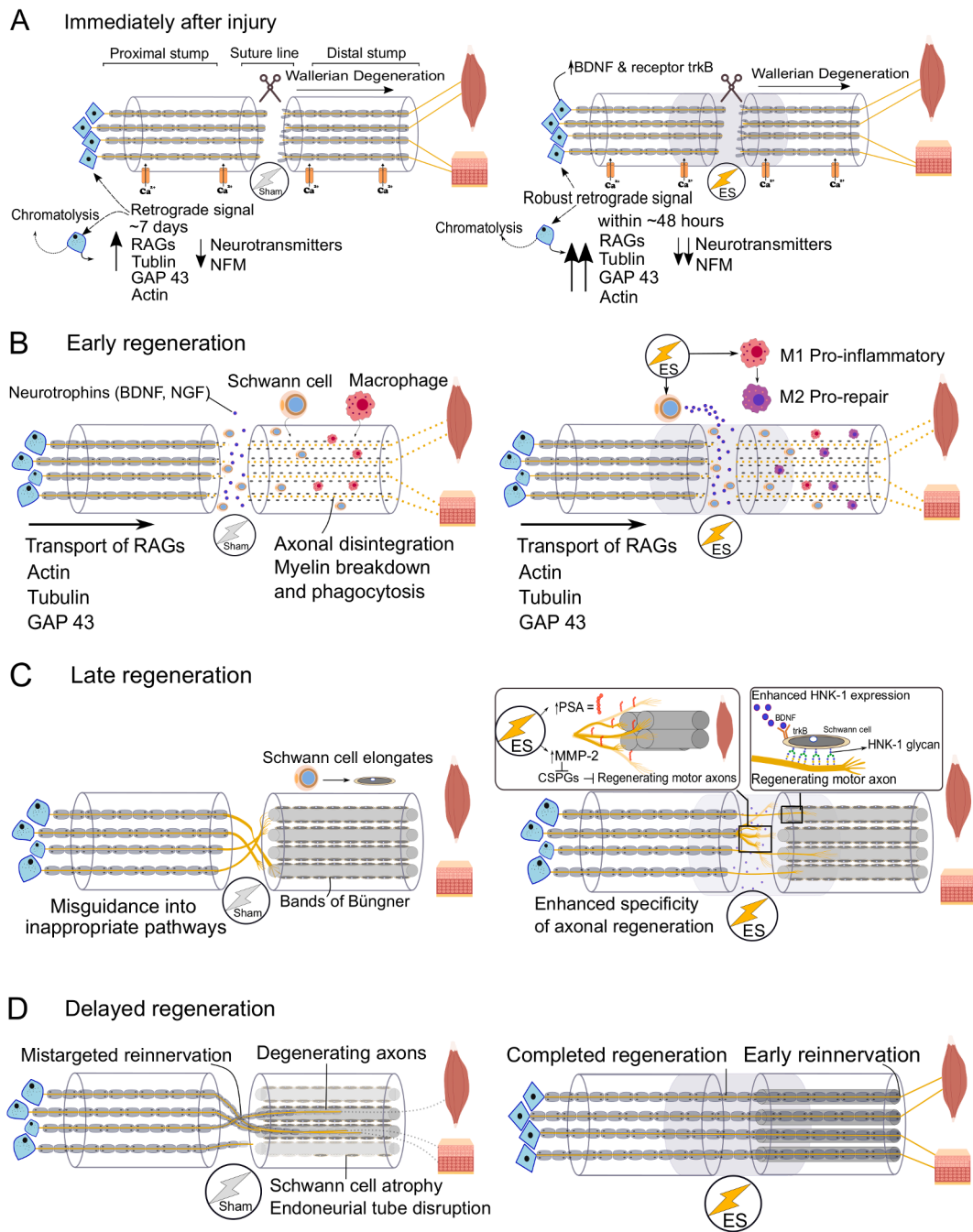


Fig. 1. Proposed Mechanism of Electrical Stimulation enhancing regeneration versus no treatment: A) Electrical stimulation enhance cell autonomous response after injury initiating regeneration. B) ES promotes neurotrophin release from axons and Schwann cells. C) ES limits staggering to ~ 3 weeks instead of 8 to 10 weeks. Enhanced PSA and HNK-1 expression promote PMR. M2 macrophages help clearing myelin. CSPGs are disintegrated via increased MMP-2 activity. D) ES reduces misguided reinnervation and protects end organs from undergoing atrophy.

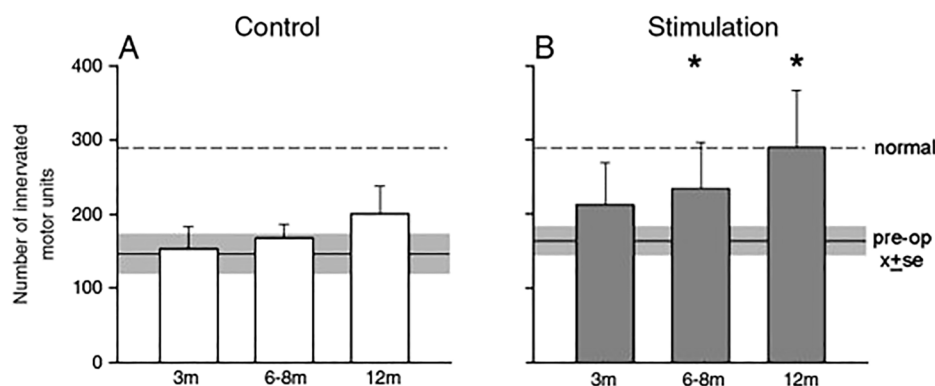


Fig. 2. After Carpal release surgery: A) No significant recovery in control group. B) Stimulated patients had reinnervation starting at 3 months, increasing at 6 to 8 months and completed reinnervation of motor units at 12 months. Figure adapted with permission from Elsevier from Gordon T, Amirjani N, Edwards DC, Chan KM. Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. *Exp Neurol.* 2010 May;223 (1):192–202.

7. Initial trials of clinical electrical stimulation

To our knowledge, only a few human clinical trials have been conducted to evaluate the potential of ES on peripheral nerve regeneration. To test the utility of ES on human peripheral nerve regeneration, Gordon *et al.* conducted a randomized clinical trial on patients with severe carpal tunnel syndrome [87]. Brief ES at 20 Hz was applied for an hour, shortly after carpal tunnel release surgery and patients were tracked over a 12 month period to measure the functional outcomes. Compound motor action potentials (CMAP) and surface recorded motor unit action potentials (S-MUAPs) were calculated at three time points during the post-operative period. The number of regenerated motor axons was calculated using motor unit number estimate (MUNE) derived from CMAP and the mean of S-MUAP. ES extensively enhanced MUNE in the hand within 3 months. The proportion of motor units innervating the thenar muscles was almost complete by 6–8 months in ES patients (Fig. 2). This was significant in contrast to the non-stimulated patients, who showed no sign of recovery even after 12 months of follow-up [87]. Cubital tunnel syndrome is the second most common compressive neuropathy after carpal tunnel syndrome [88]. In a recent clinical trial Power *et al.* tested the efficacy of ES on ulnar nerves after surgically relieving severe compression at the cubital tunnel. Following surgery, ES at 20 Hz was applied for an hour and patients were tracked longitudinally for three years. Results showed that electrically stimulated patients had significantly recovered grip and pinch strengths within a year. When motor units were estimated at three years of completed follow up, stimulated patients had twice the number of motor units innervating intrinsic hand muscles as compared to the control patients who failed to show any signs of recovery [89]. Entrapment neuropathies such as carpal and cubital tunnel syndromes are types of chronic nerve injuries as many patients have several years of symptoms before they ultimately seek care. The positive effects of ES in these severe entrapment neuropathy patients provide hope for the treatment of chronically injured neurons.

Digital nerves are the most commonly lacerated nerves due to trauma. Robust reinnervation after repair is essential for dexterity and preserving sensory function required for activities of daily living and employment [90]. Wong *et al.* studied the outcomes of supplementary treatment with ES at 20 Hz for 1 h after repair of a transected digital nerve in a randomized control clinical trial. Brief ES after the repair of a lacerated nerve led to a significantly early recovery of all sensory modalities within 6 months as compared to control patients [91].

As part of the surgical neck dissection for the oncological resection of head and neck cancers, the spinal accessory nerve (SAN) is skeletonized and manipulated. This manipulation sometimes causes a SAN injury that results in significant post-operative shoulder dysfunction. In a randomized clinical trial, Barber *et al.* treated SANs with brief ES after neck dissection. ES enhanced recovery and improved the preservation of shoulder function after surgery (Fig. 3). Therefore, the addition of brief ES along with other rehabilitative treatments can be considered an

adjunctive therapy to maintain shoulder function after oncological neck dissection [92].

Facial nerve injury after Bell's palsy cause significant morbidity. A recent randomized control clinical trial by Kim *et al.* demonstrated the benefit of supplemental ES along with steroids and acyclovir in improving facial nerve functional recovery. Subthreshold continuous daily stimulation at 20 Hz was applied via surface electrodes within 2 weeks of symptoms and continued for 2 months. Ultimately, ES treated patients had earlier return to function within 3 months of symptom onset and significant improvement in House-Brackman scores. Moreover, ES treatment also minimized synkinesis and hypertonia, the common sequelae to Bell's palsy [93].

While these five examples are encouraging (Table 1), the feasibility of implementing ES in the clinic still needs to be determined. For example, after extremity nerve injuries, the repair surgery is done under local anesthesia. Local anesthetics block sodium channels and thus limits the action potential generation. Since ES enhances regeneration through action potential generation retrogradely promoting a cell body response, the repair surgery with ES may require general anesthesia to prevent abolishing the effect of ES by local anesthetics. Using general anesthesia in these procedures would increase both time and complications attributed to anesthesia [91]. Perhaps application of ES by implantable and bioresorbable electrical stimulators after nerve repair would alleviate this issue and allow the ES treatment after surgery [35,94,95].

8. Clinically applicable paradigms of electrical stimulation

ES has significant effects on nerve regeneration, yet an appropriate stimulation paradigm and parameters to achieve optimal outcomes remain largely unknown. Al Majed *et al.* compared post-operative ES at 20 Hz only once for one hour to continuous daily ES over days immediately after nerve repair. Brief ES for one hour not only enhanced motor neurons [14] but also increased sensory neuron regeneration compared with longer ES times. It has been postulated that longer stimulation increases BDNF levels resulting in trkB receptor sensitization and decoupling, which negatively impacts regeneration [24]. Furthermore, 1-hour ES is comparably easy to apply in the clinic as a neuroregenerative therapy and has demonstrated benefit in clinical trials [87].

An ES paradigm must be safe and effective. The effects of ES are dependent on variables like electrical current, frequency, duration, site, electrode type, and nerve gap length. ES at different frequencies [96] and currents [97] have variable effects on neurons. For example, higher ES frequencies can induce neuronal injury and lead to degeneration [98,99]. Moreover, currents greater than 4 mA negatively affect regeneration, as excessive direct current is inhibitory to growing fibers. Ultimately, ES at a low frequency of 2 Hz and a current of 1 mA demonstrated the regeneration of a more mature nerve architecture. Regenerated axons had a smaller cross-sectional area, with higher myelination, density, and vascularity compared to controls [96,97].

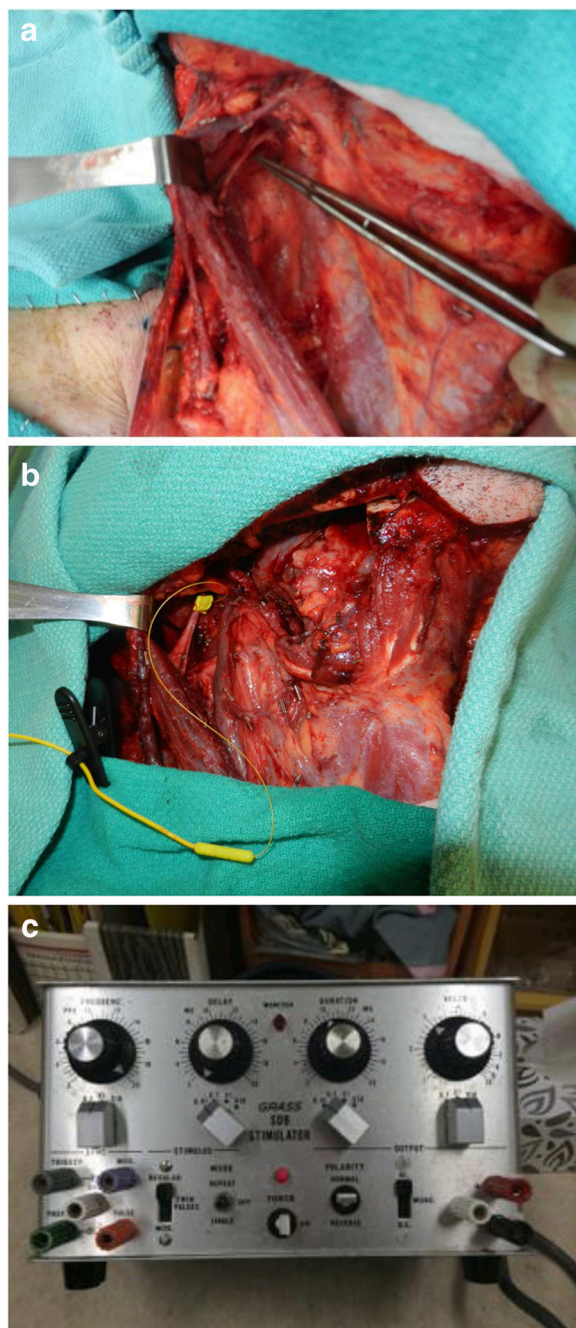


Fig. 3. A) Skeletonized spinal accessory nerve. B) Intraoperative brief stimulation with cuff electrode C) Electrical stimulator. Reprinted from Barber B, Seikaly H, Ming Chan K, Beaudry R, Rychlik S, Olson J, Curran M, Dziegielewski P, Biron V, Harris J, McNeely M, O'Connell D. Intraoperative Brief Electrical Stimulation of the Spinal Accessory Nerve (BEST SPIN) for prevention of shoulder dysfunction after oncologic neck dissection: a double-blinded, randomized controlled trial. *J Otolaryngol Head Neck Surg.* 2018 Jan 23;47(1):7.

Although 1-hour ES protocols exist and are effective, the limitation to using them clinically is that ES is applied via nerve hook or loop electrodes, that can only be used intraoperatively. Due to this limitation, the benefits of these ES protocols have not been established beyond 1-hour post-operative period. It is therefore presumed that application of ES beyond the intraoperative period with multiple daily ES could have additional therapeutic benefits if optimized. Previous studies have established that continuous chronic electrical stimulation is not beneficial, and instead prevents regeneration [14,24,100]. Indeed, prolonged

continuous ES may not improve regeneration; however, if nerves are stimulated intermittently with brief ES it could benefit regeneration and prevent neuronal injury due to excess current.

In the context of this need for long-term intermittent stimulation, our lab developed bioresorbable implantable wireless nerve stimulators with cuff electrodes to evaluate the benefits of additional electrical stimulation. Bioresorbable stimulator is clinically feasible as it permits ES beyond the intraoperative period. Moreover, it reduces operative time and the risk of infection, as there is freedom of stimulating nerves after surgery and no surgical extraction is needed. Interestingly, bioresorbable electrodes can also be customized to resorb at predetermined timeline (Fig. 4). This would allow testing various ES parameters and nerve injury models to determine the effective and safe ES protocols [35].

Ultimately, we developed a novel paradigm of repeated daily ES one hour per day for 6 days after repair of rodent nerve transection model. After completion of stimulation a significantly enhanced rate of regeneration with earlier return to electrophysiological baseline was observed. Both the muscle mass and twitch force were significantly higher when compared with one hour ES [35]. Recently validated by Ju and Park, 6 weeks of ES demonstrated faster functional recovery and superior axonal regeneration [101]. Similarly, intermittent ES of the pudendal nerve after injury twice weekly for 2 weeks resulted in recovery of urinary continence, which is in contrast to brief ES that failed to show any functional benefit [102].

Loop electrodes are merely wires looped around a nerve, in contrast to that cuff electrodes surround the nerve with close approximation with the epineurium. The electric field vector distribution in a 3D anatomical model demonstrated that cuff electrodes provide concentrated and local electrical fields parallel to the injured axons. Moreover, neurites are directed towards the electrical field. In contrast, transcutaneous electrodes provided an orthogonal and poorly localized electrical field [101]. Therefore, ES delivered to the injured nerves via a wireless stimulator with a cuff electrode provides a novel model that is clinically applicable and can be customized to establish an efficacious ES protocol.

Although ES may be safe and effective for one type of nerve, however that does not necessarily mean that same protocol may translate universally to all other nerves. As an example, ES applied after femoral nerve injury promoted significant regeneration, but a similar ES protocol failed to recover facial nerve function [103]. In this context, the development of an appropriate ES paradigm should be focused on individual types of nerves and nerve injuries. On a biophysiological level, ES depolarizes the cellular membrane and opens voltage gated calcium (Ca^{2+}) channels [53,54]. Adams *et al.* developed a computational model based upon intracellular Ca^{2+} elevation serving as a surrogate marker to assess the efficacy of ES paradigms. ES of various nerves demonstrated a differential response to alternative stimulation parameters. Based on the duration of pulse, frequency and current, ES paradigms can therefore be optimized for various neuron types or for an individual type of nerve injury [104].

We have summarized electrical stimulation paradigms tested in various preclinical nerve injury scenarios, that have a potential to be translated in the clinic in (Table 2).

To summarize, as illustrated in (Fig. 5) long term stimulation studies validating the efficacy of ES protocols with computational model testing should be further evaluated to develop an effective ES paradigm.

9. Conditioning of neurons with electrical stimulation

Crush lesions inflicted prior to an actual nerve injury condition the nerves. After subsequent injury and repair both the regeneration speed [105] and number of axons [106] increase. Conditioning lesion expedites regeneration before injury via upregulating genes associated with regeneration and transporting cytoskeleton proteins to the site of nerve repair [107]. However, clinical delivery of a conditioning crush lesion is not feasible. Although it seems counter-intuitive, ES of intact peripheral

Table 1
Summary of clinical trials of electrical stimulation in nerve injuries.

Clinical trials	Paradigm	Electrode type	Nerve injury	Outcomes	References
Chronic Compressive Neuropathy	ES – 1 h at 20 Hz after Carpal tunnel release	Wire electrode	Carpal tunnel syndrome	Increase MUNE with complete reinnervation of thenar muscles	[87]
	ES – 1 h at 20 Hz after Cubital tunnel release	Wire electrode	Cubital Tunnel Syndrome	Increase MUNE, grip and pinch strengths	[89]
Digital Nerve Transection	ES – 1 h at 20 Hz after Digital nerve repair	Wire electrode	Digital nerve transection	Earlier recovery of all sensory modalities	[91]
Bell's palsy	Continuous daily ES at 20 Hz until 2 months from onset	Cutaneous electrodes	Human facial nerve ES adjunct with prednisolone and acyclovir	Earlier regain of function and synkinesis prevention	[93]
Spinal accessory nerve damage	ES – 1 h at 20 Hz after neck dissection	Cuff electrode	Human spinal accessory nerve damage	Preserved shoulder function	[92]

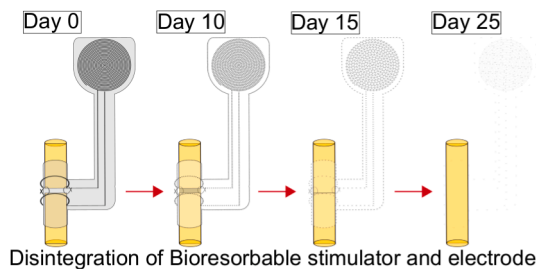


Fig 4. Predetermined timeline for resorption of bioresorbable cuff electrode.

nerves prior to nerve injury and repair can be used as a conditioning strategy [108] and has been shown to enhance neurite outgrowth *in vitro* [26].

Recently Senger *et al.* developed a novel protocol for conditioning the nerves without injury. ES was applied to the intact nerves for 1 h at 20 Hz a week prior to the injury. Following the nerve injury and repair, regenerated axons had increased in length. Compared to nerves that were crushed to enhance regeneration, conditioning with ES showed

superior sensory and locomotor recovery [109]. Alternatively, brief post-operative ES enhances early reinnervation by reducing staggering. However, the major limitation is that it does not increase the speed of regeneration nor does it elongate axons [23]. In contrast, conditioning with ES increases the speed of regeneration and axonal elongation which is critical for early distal reinnervation (Fig. 6) [109].

These effects of conditioning with ES will mostly benefit situations requiring nerve autografts, where robust regeneration is required; as axons have to traverse two coaptation sites. Conditioning of nerves with ES prior to autograft implantation demonstrated faster regeneration of axons through the graft and enhanced functional recovery [110]. When the creation of a nerve gap is anticipated, such as after oncological resections or severe soft tissue trauma, electrical conditioning of nerve prior to repair could benefit patients by expedited regeneration. Furthermore, it can be applied before distal nerve transfer surgery to yield a better functional outcome. Clinical trials on humans would further elucidate the benefits of conditioning ES paradigm.

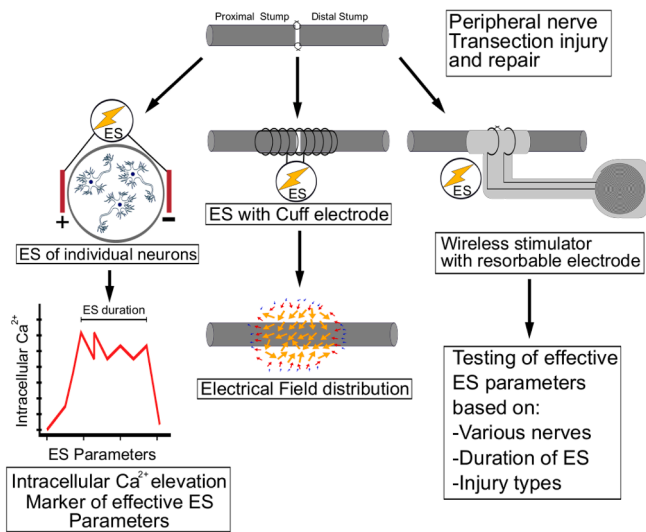
10. Electrical stimulation after repair of chronic nerve injuries

ES is capable of increasing the regenerative capacity after repair of

Table 2
Electrical stimulation potential in different clinical scenarios (Pre-Clinical Studies).

Clinical translation	Paradigm	Electrode type	Nerve injury model	Outcomes	References
Immediately after nerve cut repair	Brief ES after repair 1 h 20 Hz	Wire electrode	Rat Femoral nerve transection	Earlier reinnervation	[14]
Non-invasive stimulation after operation	Intermittent ES after repair 1 h at 20 Hz daily for 6 days	Cuff electrode Bioresorbable and wireless stimulator	Rat sciatic nerve transection	Preserved muscle mass and enhanced functional recovery	[35]
Chronic compressive neuropathy, nerve transfer for chronic nerve injury	ES after repair – 1 h at 20 Hz after 3 months of injury	Wire electrode	Rat common peroneal and tibial nerve transection; repair delayed –3 months	Accelerated axon outgrowth Comparable to immediate repair	[116,114]
	ES 20 min at 20 Hz after delayed repair	Wire electrode	Rat sciatic nerve transected; repair delayed until 2, 4, 12 and 24 weeks	Improved functional recovery that progressively declined from 2 to 24 weeks delay	
	ES – 1 h at 20 Hz a week before repair (Conditioning)	Wire electrode	Rat tibial nerve conditioned with ES a week prior to transection	Increased speed of regeneration & axonal length	[109]
Anticipated nerve gap & distal nerve transfers	ES 1 h at 20 Hz after repair	Wire electrode	Rat femoral nerve transection repaired with autograft	Improved motor functional recovery	[119,110]
	ES 1 h at 20 Hz a week before repair (Conditioning)	Wire electrode	Rat tibial nerve conditioned with ES a week prior to autograft repair of 5 mm gap	Enhanced functional recovery and longer axonal length	
Nerve gap injuries	ES 1 h at 20 Hz every 2nd day –7 times	Wire electrode on conductive NGC	Rat sciatic nerve section repaired with 10 mm PPY/SF* NGC‡	Enhanced axonal growth, recovery similar to autograft	[124]
	ES 1 h at 100 mV on 1, 3, 5, & 7 days	Wire loop electrode on conductive NGC	Rat sciatic nerve section repaired with 15 mm PPY/PLCL† NGC	Enhanced axonal myelination, recovery similar to autograft	[126]
Nerve injury in diabetics	Intermittent ES 15 min at 2 Hz for 2 weeks after 1–15 days of repair	Needle electrodes	Rat sciatic nerve transection in induced diabetes	Enhanced functional recovery, vascularity and macrophage recruitment at injury site	[137]
Stress urinary incontinence	Intermittent ES 1 h at 20 Hz 2X/week for 2 weeks	Needle electrodes	Rat pudendal nerve crush injury	Continence recovery and increased BDNF expression	[102]

*PPy/SF -polypyrrole/silk fibroin †PPy/PLCL -polypyrrole/poly (lactic acid-co-caprolactone) ‡NGC -Nerve guidance conduit.



Advances in exploring effective ES paradigms

Fig. 5. Determining appropriate electrical stimulation paradigm.

chronic nerve lesions. Of particular importance, early nerve repair is not possible in situations where severe soft tissue trauma occurs with wound contamination. Moreover, after a majority of closed nerve injuries, surgeons often delay operative repair for 3–6 months to evaluate for

spontaneous regeneration on clinical evaluation [111,112].

Various ES paradigms have been proposed for promoting functional recovery in chronic nerve injuries [113]. Huang *et al.* demonstrated that ES for 20 min at 20 Hz can improve functional recovery after delays up to 24 weeks [114]. Interestingly, chronically denervated Schwann cells in the distal stump have been shown to retain regenerative capacity *in vitro* [115].

With this context, the repair of various chronic nerve injury scenarios was simulated in a rat cross suture paradigm by Elzinga *et al.* The nerve repair was delayed for 3 months after transection injury. Firstly, a freshly cut common peroneal nerve was sutured to a chronically injured tibial nerve (mimicking chronic denervation of Schwann cells and muscles). Secondly, the chronically injured common peroneal nerve (mimicking chronic axotomy) was sutured to the freshly cut tibial nerve. Finally, the chronically injured common peroneal nerve was cut and sutured to the chronically injured tibial nerve (simulating the delayed nerve repair). After repair of chronic injuries in all scenarios, ES for 1 h at 20 Hz was applied. ES enhanced regeneration after both chronic axotomy and chronic denervation of Schwann cells. The functional recovery was comparable to freshly injured and repaired nerves [116]. However, Huang *et al.* demonstrated that efficacy of ES after repair of chronic nerve injuries progressively declined with delays ranging from 2 to 24 weeks [114].

Nevertheless, these studies set the foundation that ES has a potential to enhance regeneration after repair of chronic nerve injuries that were previously believed impossible to treat [116]. Moreover, ES could be beneficial in nerve transfer surgeries where chronically injured nerves

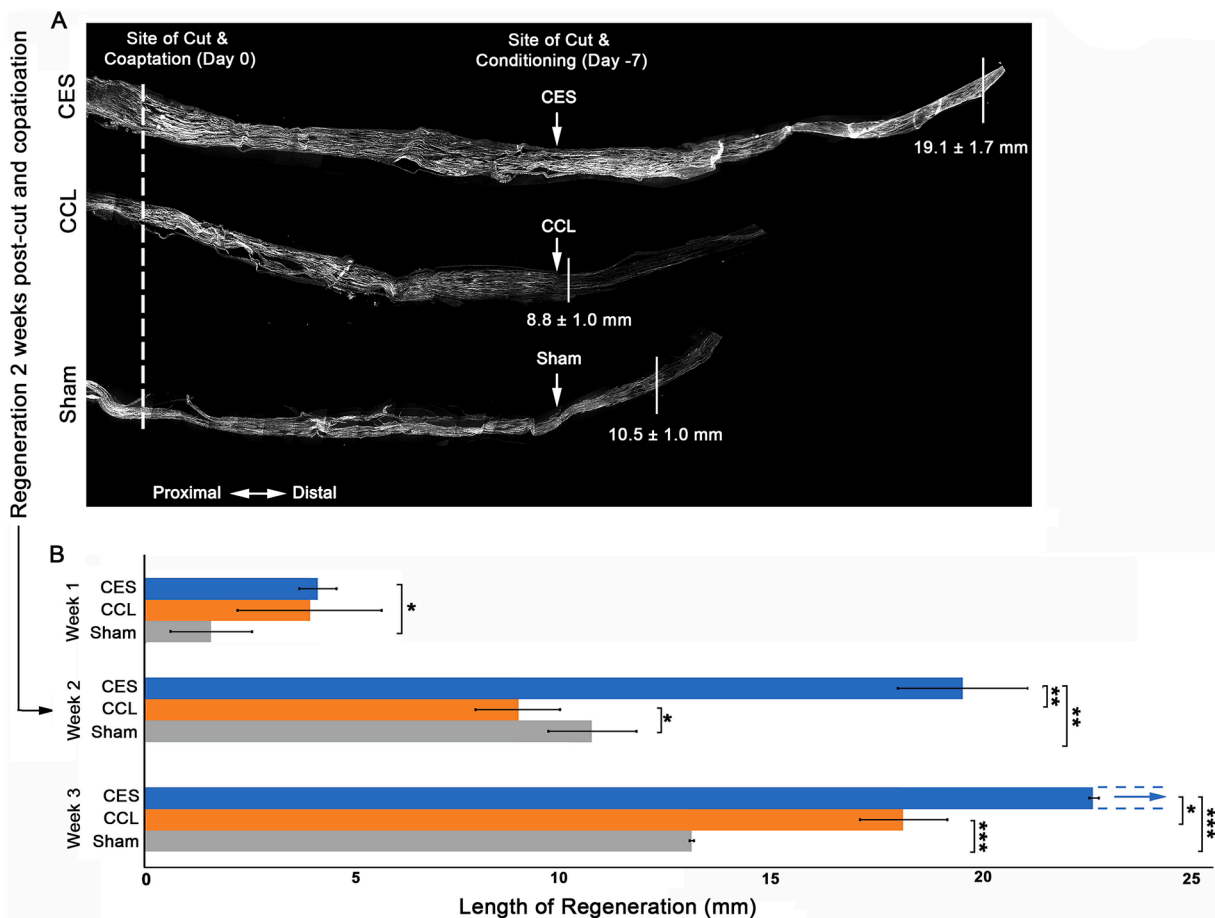


Fig. 6. Conditioning with electrical stimulation enhances regeneration and elongates axon significantly as compared to conditioning crush lesion: Longitudinal sections of regenerated tibial nerves labelled with NF200 antibodies. Conditioned with either ES (CES), conditioning crush lesion (CCL), sham one week prior to transection and repair. Figure reprinted with permission from Elsevier from Senger JL, Chan KM, Macandili H, Chan AWM, Verge VMK, Jones KE, Webber CA. Conditioning electrical stimulation promotes functional nerve regeneration. *Exp Neurol.* 2019 May;315:60–71.

are coated with freshly transected donor nerves to reinnervate the distal targets [117,118].

11. Electrical stimulation after reconstruction of discontinuous nerve injuries

After severe nerve trauma where a significant gap exists, nerve reconstruction with an autograft is standard of care. ES of autografts has been shown to improve regenerative capacity, nevertheless, donor site morbidity and limited availability warrants alternative options [110,119,120]. Nerve scaffolds have been used in the repair of gap injuries clinically for more than a decade [121]. These scaffolds have been shown to successfully repair a median nerve gap of up to 30 mm in humans [122].

Nerve guidance scaffolds are bioengineered to mimic the extracellular matrix architecture of a nerve graft. Engineering these scaffolds with techniques, like electrospinning and 3D printing, mimics the topographical cues and microchannel guidance of autografts [123]. Recently, conductive nerve guidance scaffolds have been developed using materials such as polypyrrole [124]. ES facilitates the guidance of axons along an electrical field, and the supporting matrix of the nerve guidance scaffold, further enhances this regeneration.

Multiple *in vitro* and *in vivo* studies have examined the efficacy of conductive nerve guidance scaffolds. In an *in vitro* study, ES of a conductive scaffold promoted Schwann cell migration, adhesion, and division. Further ES promoted the release of neurotrophins by Schwann cells within the scaffold matrix [125]. This Schwann cell support within the scaffold make for an optimum environment for incoming axons. In rat models of nerve gap injuries ranging from 10 to 15 mm, ES with a conductive nerve guidance scaffolds have shown functional recovery comparable to autografts [124,126].

Various materials have been utilized to develop conductive and bioresorbable nerve guidance scaffolds, thus increasing their usefulness in clinical practice. Discussing these materials in detail is beyond the scope of this review. The evidence provided by *in vitro* and *in vivo* studies forms the basis of using ES with conductive scaffolds for nerve gap repair clinically.

12. Novel synergistic approaches and future directions

Peripheral nerve regeneration is an intricate process – from axonal extension, guidance, and entry into the distal segment with the modulation of trophic support and remyelination – each step has its own significance. While ES increases the regenerative capacity, but in order to achieve successful regeneration, a multifaceted approach that targets the different aspects of regeneration is essential to achieve successful results.

Exercise and activity dependent therapies are neurorehabilitative strategies that help maintain muscle properties during denervation and promote functional recovery. During reinnervation period enhanced motor activity and sensory inputs influence neuromuscular functional outcomes. Enhancing neuronal cell body response after injury promotes cell autonomous mechanism to accelerate regeneration [127]. Electrical stimulation is an artificial way to mimic activity without movement. Combining ES with exercise has been shown to improve sensory input, reducing hyperreflexia and neuropathic pain. Therefore, supplementing ES with exercise may have a synergistic role in regeneration [128–131]. Recently optogenetics have been applied in this perspective. Activation of nerves to target neuronal cell body via light stimulation has been proposed as a novel approach in activity based therapies [132]. Optical stimulation of sciatic nerves after transection showed greater motor and sensory regeneration with reformation of neuromuscular junctions [133–135]. Androgens have long been known to have neuroprotective properties in both central and peripheral nervous system [136]. Supplementing androgens with ES after nerve injury accelerated greater nerve regeneration with completed functional recovery. Androgens have

been thought to sustain nerve regeneration in the later stages of reinnervation. Addition of testosterone with ES may have an additive effect in reducing the time required to obtain complete functional recovery [32,45]. Future work should design treatment paradigms including activity based therapies and androgens in synergism to elucidate potential in treatment of peripheral nerve injuries.

Supplementing ES with activity dependent therapies, and/or gonadal steroids has boundless therapeutic potential and may be offered as standard of care in future.

13. Conclusions

Addressing peripheral nerve injuries has been a significant challenge. In this review we have summarized various aspects of electrical stimulation therapies for peripheral nerve injury. Based upon the evidence provided by pre-clinical and a few clinical studies, ES remains in its infancy and has a prospective role in conjunction with surgery. The optimum and safe parameters of ES application to individual nerves needs to be further established. Elucidating the mechanisms and limitations of peripheral nerve regeneration has also opened the avenue to explore multiple novel therapeutic approaches. Ultimately, targeting regeneration from multiple angles and combining these with electrical stimulation has the opportunity to be translated into clinical practice and improve patient outcomes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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