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A shock to the (nervous) system: Bioelectricity within peripheral nerve tissue engineering

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A shock to the (nervous) system: Bioelectricity within peripheral nerve tissue engineering

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Impact Statement

Tissue engineering is becoming increasingly complex, with multiple therapeutic modalities often included within the final tissue engineered construct. Electrical stimulation is emerging as a viable therapeutic intervention to be included within peripheral nerve tissue engineering strategies, however to date, there have been no review articles which collates the information regarding the effects of electrical stimulation on key cell within peripheral nerve injury. This review article aims to inform the field on the different therapeutic effects that may be achieved by using electrical stimulation and how they may become incorporated into existing strategies.

Abstract

The peripheral nervous system has the remarkable ability to regenerate in response to injury. However, this is only successful over shorter nerve gaps and often provides poor outcomes for patients. Currently, the gold standard of treatment is the surgical intervention of an autograft, whereby patient tissue is harvested and transplanted to bridge the nerve gap. Despite being the gold standard, over half of patients have dissatisfactory functional recovery after an autograft. Peripheral nerve tissue engineering aims to create biomaterials that can therapeutically surpass the autograft. Current tissue engineered constructs are designed to deliver a combination of therapeutic benefits to the regenerating nerve, such as supportive cells, alignment, extracellular matrix, soluble factors, immunosuppressants and other therapies. An emerging therapeutic opportunity in nerve tissue engineering is the use of electrical stimulation (ES) to modify and enhance cell function. ES has been shown to positively affect four key cell types, neurons, endothelial cells, macrophages, and Schwann cells, involved in peripheral nerve repair. Changes elicited include faster neurite extension, cellular alignment, and changes in cell phenotype associated with improved regeneration and functional recovery. This review considers the relevant modes of administration and cellular responses that could underpin incorporation of ES into nerve tissue engineering strategies.

Introduction

The peripheral nervous system is responsible for skeletal muscle movement, sensory feedback from the environment, and numerous autonomic functions[1]. Injuries to the peripheral nervous system are common, occurring in 2.8% of trauma patients and often affect young and otherwise healthy individuals[2, 3]. Peripheral nerve injuries often have significant long term detrimental effects on quality of life[4], with the people affected being predominately young males[3]. Peripheral nerve injuries and the resulting disabilities have a high associated cost. A recent study highlighted that traumatic injuries to the brachial plexus for example are estimated to incur over $1.1 million in indirect costs per patient[5].

The peripheral nervous system, in contrast to the central nervous system, has the capability to regenerate in response to injury[6]. Despite this ability, in many cases of severe trauma to the peripheral nervous system, surgical intervention is required to reconstruct large injury sites[7]. The current gold standard of treatment is utilization of an autograft, which is the act of excising a section of the patient's own non-essential peripheral nerve tissue, often the sural nerve, in order to provide an autologous section of tissue which can be grafted across a nerve gap to act as a bridge to aid regeneration[7]. Despite being the gold standard for peripheral nerve repair, the autograft has serious limitations in terms of donor site morbidity and tissue availability, and has been found to provide dissatisfactory recovery in more than half of patients[8]. There is obvious merit to creating a non-immunogenic construct that facilitates similar mechanical properties[9] and guidance/adhesion cues[10], and tissue engineering strategies often attempt to mimic key aspects of endogenous nerve tissue using biomaterials. Research in this area has led to a range of conduits, decellularized allografts and engineered tissues that aim to match the performance of the nerve autograft. However, since the autograft itself is often unsatisfactory in terms of functional recovery, there is much interest in the incorporation of additional factors in future nerve repair technology to create a therapeutic solution that can surpass the performance of the gold standard. This can be achieved by utilizing the understanding gained of peripheral nerve regeneration through primary neurobiological studies, and harnessing advances in biomaterials science, to elicit stimuli that

induce cellular and regenerative changes. Electrical stimulation (ES) is a forerunner therapeutic option which is being applied in a variety of different tissue engineering contexts[11], as it has both the ability to positively influence the pro-regenerative phenotype of cells[12-14] and provide directional cues[15, 16] via the orientation of an electrical field applied to the injury site. This brief review highlights how electrically conductive biomaterials can be incorporated into tissue engineering strategies for treating peripheral nerve injuries and identifies the benefits that can be realized using this therapeutic stimulus.

Naturally Occurring Bioelectricity Within Human Physiology

Bioelectricity refers to electrical potentials and currents that occur within, or are produced by, living organisms. These electrical potentials and currents play a role in spatiotemporal tissue patterning in early stage embryogenesis[17-19], wound healing[20, 21] and signalling throughout the body[20]. Possibly the first thought when discussing bioelectricity is nerve conduction, whereby a potential difference across the membrane of a single neuron leads to a cascade of events, voltage gated channels opening and closing, extracellular vesicles released at synapses and electrical impulses traveling the length of the human body to deliver crucial signals[20].

There is mounting evidence that altering endogenous bioelectrical signalling can have a profound therapeutic effect on different disease pathologies. Metastatic tumours are found to have abnormal expression levels of voltage gated channels associated with cell migration, and a recent experimental report investigated this effect in a metastatic tumour X. *laevis* model. By hyperpolarizing related but distant voltage gated channels from the primary tumour site, tumour metastasis was suppressed[22, 23]. Primary T-cell activation can be greatly suppressed by electrical fields, which could be relevant in the treatment of autoimmune diseases[24]. Furthermore, stimulation of specific nerve bundles responsible for communication with target organs provides a possible future treatment for chronic inflammatory conditions and this type of application is currently at preclinical stages[25]. Deep brain stimulation is currently a clinically approved option for patients with Parkinson's disease where pharmacological interventions are insufficient[26].

Harnessing the therapeutic potential of bioelectricity

Investigations surrounding ES for peripheral nerve regeneration have been ongoing for around two decades, due to the ability of ES to influence a myriad of cellular behaviours[15]. Much ES research in peripheral nerve repair has utilized a single dose of ES prior to surgical repair of nerve tissue, with 3V, 20 Hz being the most investigated parameter [27-29]. An important early study in this area was published in 2000, by Al-Majed *et al*. Their experiments were performed in a 20 mm rat femoral nerve gap bridged via a silastic nerve cuff, and electrical stimulation was provided by wires wrapped around the proximal stump[30]. This manner of stimulation at the proximal stump has been shown to improve regeneration and elicit earlier, and more sustained upregulation of regeneration associated genes in animal models of peripheral nerve regeneration[28, 30-32]. Furthermore, single dose ES has been investigated within clinical trial protocols for improving peripheral nerve regeneration outcomes in carpel tunnel syndrome within patients[29], and is currently under several clinical investigations (**Table 1**)

Advances within materials sciences and research into softer, more biocompatible organic semiconductors as opposed to traditional electronics, means that ES may also be administered directly to the injury site and throughout the nerve regeneration process. A recent review provides a comprehensive overview of recent progress in the development of conducting

polymer biomaterial, to which the reader is referred to for more details about this aspect which is beyond the scope of this review[11]. This has the potential for the application of multiple, repeat stimulations that can create positive effects on regeneration associated genes at different time points following nerve repair, rather than just a one-time stimulation, which appears to elicit similar benefits to a conditioning lesion when investigated *in vivo* using a 5mm autograft rat model[33].

In 2018, J. Koo *et al*. published work on a wireless bioresorbable electronic system that can enable future developments in nonpharmacological neuroregenerative therapy[34]. The wireless electronic system was wrapped around the sciatic nerve in rats, immediately after nerve crush and nerve transection injuries, and a stimulus of 20 Hz was applied for 1 h per day for 1, 3 or 6 days, post operatively. Multiple administrations of electrical stimulation showed increased functional nerve recovery, which was measured as increased electrophysiological response, muscle mass, and force generation in downstream muscles.

The Bao group at Stanford University have conducted research into skin-inspired, biocompatible electronics[35-37] which yields materials that may be leveraged towards tissue engineering. The materials are stretchable, can be micropatterned, biodegradable and possess Young's modulus values within the kilopascal range, similar to that of human nerve tissue, and can retain their electronic properties through bodily movement[35]. There have been experimental reports of the effects bioelectronic devices can have on the surrounding tissue. One such report investigated H_2O_2 formation because of the redox reactions that occur within bioelectronic devices, and how to avoid these types of reactive metabolites through chemical manipulation of materials[38], which will aid the understanding and translation to medical devices. While this review focuses specifically on biomaterials for electrical stimulation of nerves, for a more complete perspective of recent advances in the field of conducting biomaterials used more generally in regenerative medicine, the reader is directed to a recent review[11].

In the context of tissue engineering, biocompatible conductive polymers may act as the starting point for scaffold design and can incorporate a wide range of functionality, such as conjugation of peptide or small molecule therapeutics[39], functional groups which can encourage supramolecular self-assembly into pro-regenerative scaffolds and also ES-mediated therapeutic factor release[40]. Organic semiconductor based components are well suited to be directly incorporated into biomaterial strategies, such as electrospinning[41], hydrogel fillings[42] or micro/nano-patterning[43]. An electrospun conduit comprised of polypyrrole coated poly (l-lactic acid-co-ε-caprolactone (PPy-PLCL), combined with repeated ES, was tested in a 15 mm gap rat peripheral nerve model[44]. The construct was stimulated for 1 h per day, 1, 3, 5 and 7 days post implantation, showing similar performance to the autograft, and significantly better performance than the non-stimulated implanted materials (**Figure 1**). More recently, an injectable, conductive self-assembling peptide-carbon nanotube (pCNT) material was shown to promote axon regeneration and Schwann cell migration in dorsal root ganglion cultures when electrically stimulated over 7 days, and over 30 days (**Figure 2**). The hybrid hydrogel was composed of a self-assembling peptide, RADA-16, with HGF and IKVAK peptides on either end of the primary RADA-16 peptide[42].

This review attempts to evaluate the experimental evidence surrounding electrical stimulation and peripheral nerve cells and discuss the extent to which further development of biocompatible electronics for peripheral nerve regeneration is a worthy pursuit.

Key cell types involved in peripheral nerve regeneration

Peripheral nerves have the capability to regenerate following trauma[45]. **Figure 3** shows a brief overview of the highly orchestrated cellular response to damage within the peripheral nervous system, and the key cell types that are involved. Important cell types involved within this response are Schwann cells[46], endothelial cells[47], macrophages[48] and neurons[49]. The process of regeneration can be considered in three main parts: The initial injury response[50], Wallerian degeneration[51] and axonal guidance by Schwann cell alignment[52].

Often cited as a key molecular feature of successful peripheral nerve regeneration is the alignment and reprogramming of Schwann cells to a regeneration-supportive phenotype, which is induced by the local cellular environment including signalling factors secreted by macrophages that become activated due to inflammation and tissue damage following injury[46, 53, 54]. Macrophage responses to hypoxic conditions because of injury, cause changes in endothelial cell activity, resulting in a new blood vessel front, which Schwann cells migrate along within nerve injury gaps[48, 55, 56]. Repair phenotype Schwann cells align, forming cellular guidance tracks termed the Bands of Büngner which can orientate and direct regenerating axons[57]. This is a rather simplified description of the mechanisms at play during peripheral nerve regeneration, but hopefully gives the reader an idea of the multicellular and orchestrated nature of the regeneration process.

In vitro studies on Bioelectricity

Neurons

Before discussing the effects that electrical fields have on neuronal cells, it is important to distinguish this topic of using these fields to influence regeneration outcomes, from that of using electrical fields to elicit action potentials within nerves. Much of the work regarding electrical stimulation has been conducted on the latter[58, 59], however there is increasing evidence that small electrical currents, with and without a directionality bias, can provide a cue for nerves to grow faster, and towards the negative pole within an electrical field. C. E. Schmidt pioneered work involving neurite outgrowth using electrically conducting polymers and in 1997 used oxidized polypyrrole (PP) as a material for stimulating PC-12 nerve cell growth *in vitro* & *in vivo*. Schmidt *et al*. found that using low strength electrical fields increased neurite outgrowth, and hypothesized that the presence of charge within the growth environment encouraged adsorption and adhesion of the cells whilst growing in culture[60]. This hypothesis was then demonstrated in 2001, with PC-12 cells subjected to ES increasing the adsorption of serum proteins, specifically the extracellular matrix protein fibronectin, which then increased neurite extension[61].

Experimental work by A. M. Rajnicek *et al*. in 2018 using a variety of transparent conductive substrate materials found that electrical fields could control the direction of *X. laevis* spinal neuron outgrowth[62]. Interestingly, this was not conserved across all materials, despite the same electrical field strength. This highlights that material properties and conductivity play an important role and that the effective field strength is dependent on the materials

'delivering' the stimulus to cells. Overall, it appears that directionality of neurite outgrowth can be influenced by external fields, which may find potential use within tissue engineering constructs. Many current tissue engineering approaches, such as electrospun constructs[63], self-assembling peptide hydrogels[64, 65] and aligned collagen gels seeded with cells[66, 67] have exhibited positive effects due to alignment and improved guidance of neurons. Incorporating conductive materials into these fabrication and design techniques may provide further improvement to direct regenerating neurons. Alternatively, incorporating conductive materials into tissue engineering strategies that do not provide topographical directionality cues may then provide that beneficial guidance.

Schwann Cells

Increased myelination markers in DRG neurons/Schwann cells co-culture following ES compared to a control has been demonstrated *in vitro*, however the mechanism was not discussed[68]. The main therapeutic outcome from an ES (8-hour duration) within primary rat Schwann cells appears to be release of NGF[69]. From this experiment, it suggests that ES alters the Schwann cell growth factor secretion, which in turn has a positive growth effect on neurites *in vitro* when Schwann cells are cocultured with neurons. This highlights how the interaction between different types of cells within the regenerating peripheral nerve can be influenced in different manners under ES. Applying ES to influence Schwann cell paracrine activity has the potential to be utilized in tissue engineering, exploiting their endogenous regenerative capacity and perhaps providing on demand release of exosomes and neurotrophic growth factors. This approach may gain increasing traction, as evidence is emerging for the pro-regenerative role of exosomes and the microRNAs contained within them, secreted from Schwann cells in peripheral nerve regeneration[70-72].

Further experiments have characterized the secretions of Schwann cells under ES, showing that ES can induce increased Brain Derived Neurotrophic Factor (BDNF)[73] and neurotrophic growth factor (NGF)[74] secretion in a calcium dependant manner. Interestingly, this increase in BDNF was not detected in the previous mentioned study[69]. Electrical fields have also been shown to alter Schwann cell morphology and alignment. In 2013, H. T. Nguyen *et al* applied electrical fields to Schwann cells in a variety of different substrates; culture media, matrigel or collagen I, on an indium tin oxide coated glass slide[75]. When ES was applied to Schwann cells without any extracellular matrix features present, the cells aligned perpendicularly to the field provided and elongated. Schwann cells cultured on extracellular matrix-like substrates, did not exhibit any difference in alignment or elongation, suggesting that the relative influence of ES on alignment and elongation was significantly lower than other directionality cues present in those groups. The study also highlighted that ES influences extracellular matrix structure, as ES caused reorganization of collagen I fibres and alterations in Matrigel macroscopic structure. The research group led by C. E. Schmidt that led initial work into the effects of ES on PC-12 neuronal cells used polypyrrole substrates and isolated neonatal rat sciatic nerves to show that Schwann cell migration directionality could be influenced by ES, identifying an optimal voltage of ES above which effects on migration diminished[76].

Endothelial Cells

Angiogenesis has a fundamental role within development, wound healing, tumour formation[77] and axonal regeneration[78]. Blood vessels have been shown to precede Schwann cell migration and axonal elongation following nerve injury[46, 54]. In 2015, L. Cattin *et al*. performed a detailed set of experiments which aimed to dissect the role macrophages and

hypoxia had on the regenerating peripheral nerve[55]. They administered a peripheral nerve lesion in a rat, then misdirected the newly formed blood vessel front away from the nerve gap, showing that both Schwann cells and the regenerating axons followed the redirected blood vessel front[55].

Electrical fields of low strength have been shown to alter the migration speed and directionality of newly forming blood vessels and can also induce pre-angiogenic responses in vascular endothelial cells by VEGF signalling[79, 80]. In 2004, M. Zhao *et al*. showed that a field strength of 75-100 mV/mm for 72 h significantly altered the elongation and migration of cultured human umbilical vein endothelial cells (HUVECs), causing the endothelial cells to align perpendicularly to the field applied[80]. Interestingly, when an electrical field and VEGFR inhibitor was applied to the HUVECs, the orientation and morphology was significantly different (**Figure 4**). In a further report investigating direct current (DC) ES and its effects on endothelial cells in culture, VEGF expression along with IL-8, an angiogenic cytokine were upregulated in response to ES[81]

Electrical stimulation has been shown to upregulate chemokine receptors CXCR4 and CXCR2 within endothelial cells, as well as influencing migration speed and directionality (**Figure 5**) [79], which may find potential within peripheral nerve regeneration, with experimental work highlighting the importance of endothelial cell migration[82]. This behaviour could be exploited in neural tissue engineering; providing ES directly after construct implantation could encourage initial endothelial cell migration into the construct from the proximal nerve stump, followed closely by pro-regenerative Schwann cells and regenerating neurons [66]. This could address the challenge of encouraging cells across the proximal stump/ construct interface associated with some tissue engineering approaches [83].

Macrophages

Schwann cell responses following nerve injury cause secretion of signalling molecules which can polarize macrophages[46], activating them and inducing switching to a pro-regenerative / M2 phenotype[84]. Macrophages can further release pro-regenerative factors, influencing Schwann cell reprogramming and endothelial cell recruitment[48], leading to Schwann cell alignment and blood vessel formation, respectively[82]. The impact of delayed (5 days after initial injury) brief ES on macrophages was investigated in a rat model of focal tibial nerve demyelination and found to induce a shift to a pro-repair M2 phenotype [13]. Earlier work using the same process of delayed ES showed axon-protective neurofilament phosphorylation, accelerated immune cell clearance and remyelination *in vivo*[85]. Both studies involved a brief ES of 1 hour at 20 Hz. If ES promotes immune cell clearance via macrophage phenotype switching within the early stages of peripheral nerve injury, this may be responsible for some benefits seen for ES-surgical interventions in peripheral nerve injury, as often the nerve repair will occur after a significant delay to the initial injury[29].

In 2016, J. I. Hoare investigated the effects electrical fields can have on macrophage functions. This study showed for the first time that by applying electrical fields to macrophages, macrophage phagocytic uptake was significantly enhanced against a variety of targets, including pathogen *candida albicans*[86]. Li, C., Levin, M. & Kaplan, D investigated bioelectric modulation of macrophage polarization via potassium sensitive ATP channels (K_{ATP}), using two experimentally treated groups, subjected to K_{ATP} channel blocker and K_{ATP} channel opener. It was shown it is possible to exert control over macrophage phenotype by pharmacologically altering the potential difference across the cell membrane, and this may

translate further, harnessing ES to control macrophage phenotype directly[87]. Further investigation is required to fully elucidate the level of influence ES can have on macrophage phenotype in the peripheral nerve, and explore whether there is an opportunity for temporal control to aid transition through the distinct stages of peripheral nerve regeneration[51, 53]. There is now an emerging body of work implicating macrophages as key targets in peripheral nerve regeneration, as they can help to maintain the regeneration-supportive status of distal tissue. Mouse models have been used to show that macrophage-derived VEGF-A is integral to neuromuscular junction reinnervation after nerve injury[88], and there is evidence mounting for a dynamic interplay between Schwann cells and macrophages in the distal stump[89].

Electrical Stimulation of Stem Cells in Tissue Engineering Applications

Stem cells are frequently used as cell sources for tissue engineering, partly because they can differentiate into the cells required for regeneration, a process which can be controlled by factors in their local environment[90-93]. ES has been shown to significantly increase the proliferation and differentiation of foetal neural stem cells into neuronal cells[94, 95]. In 2011, electrospun conducting polymer nanofibers were used to administer ES to nerve stem cells, which resulted in extended neurite outgrowth compared to nerve stem cells grown on the non-stimulated scaffold[96]. More recently, ES was applied to neural crest stem cells (NCSCs) derived from human embryonic stem cells[97]. NCSCs were incorporated into a nerve guidance conduit, and implanted into athymic nude rats, with controls for NCSCs and ES established. Upon surgical implantation of the conduit, ES was applied for 1h (20 Hz, 3V). The results showed that the conduit containing NCSCs + ES showed improved regeneration in comparison to just stem cells alone, or ES alone. This experimental design utilized ES in the manner of a conditioning lesion, whereby a single 'dose' was administered to the stem cells. It is possible there could be benefits to applying multiple doses of ES over time following implantation, rather than a single dose during surgery. Combining stem cell therapy with materials that can deliver ES multiple times throughout regeneration may hold promise within the field of nerve repair, and wider applications within regenerative medicine.

In cell culture studies, ES (20 Hz, 100 us, 3 V) administered to peripheral blood stem cells taken from Sprague Dawley rats caused the cells to differentiate into Schwann cells[98]. ES has also been used to differentiate mesenchymal stem cells into Schwann-cell-like phenotypes using flexible graphene circuits[99]. Using softer, organic electronics provides the benefit of being able to match the mechanical properties of the implanted materials to those of the host nerve tissue at the injury site. One of the issues in peripheral nerve regeneration is the diminishing population of pro-regenerative cells in cases of long gap nerve injuries[46]. There is therefore a case to consider for using stem cell-seeded conductive scaffolds to repair nerves, with ES used to promote their differentiation at specific times to provide pro-regenerative cells.

Outlook for Future Incorporation of ES into Tissue Engineering Solutions

Electrically conductive biocompatible scaffolds have a wide range of potential applications in regenerative medicine including interfacing electronics with nerve tissue following injury and repair. What remains to be investigated is how different ES 'dosage' regimes might be harnessed to alter cellular phenotype, migration patterns and induce release of pro-regenerative factors from cells. ES parameters of 20Hz, a voltage of 50 mV/mm, and a current within the region of 100 µA appears to be a sensible starting point for preclinical investigations. The current is not often stated within experimental investigations of ES, this value of around 100 µA taken from the neural interface field and is used without causing

cellular destruction[100, 101]. This field strength appears to provide therapeutic benefits to each of the cell types within the regenerating peripheral nerve (Table 2). These stated parameters also have the benefit of not causing damage to the cells. They are well established within the clinic and are an alternating current (AC). Translation of DC currents from *in vitro* holds many challenges, as setting up a DC electrical field *in vivo* whilst avoiding toxic metabolite build up at the poles of the field is challenging. *In vitro,* electrodes are not in direct contact with the cells as agar bridges are used. AC ES avoids this issue, as there is no significant net migration of charged metabolites, whilst still having the potential to influence cell phenotype.

ES has the potential to improve nerve repair conduits if applications of ES *in vivo* can elicit some of the positive experimental results shown *in vitro*. One of the biggest limitations of innate peripheral nerve regeneration is that Schwann cells distal to the injury site cannot uphold their pro-regeneration phenotype over long periods of time[46]. If ES, for example administered at multiple time points, could maintain and promote Schwann cell pro-regenerative phenotype, this would potentially improve long-range peripheral nerve regeneration. There is experimental evidence that c-Jun upregulation may rescue the regenerative Schwann cell phenotype after chronic denervation in mice[102] and, since c-Jun has been shown to be upregulated in cultured DRG-derived Schwann cells upon application of ES [42], this is just one of several potentially useful therapeutic targets for ES in improving peripheral nerve repair beyond the lesion site. A key piece to translating ES into a clinically relevant tissue engineering solution for peripheral nerve regeneration is understanding the relationship between ES parameters, the material employed, and peripheral nerve regeneration. Therapeutic performance will likely be affected by bioelectronic material design and the mechanism of delivery of ES, either through inductive coupling, direct stimulation or stimulation through the scaffold [11].

Clinical Translation of ES in Peripheral Nerve Engineering

Nerve tissue engineering as a field is progressing towards increasingly sophisticated construct design, drawing on therapeutic stem cells[103, 104], gene therapy[105], precisely fabricated scaffolds[106] constructed from either synthetic or natural materials[107], and controlled release of therapeutic molecules[108, 109]. Experimental constructs often combine multiple features[110], which reflects the need to address simultaneous diverse and dynamic biological processes but can create additional hurdles for commercial and clinical translation. ES may be perceived as adding yet another level of complexity, but the potential benefit to tissue engineers of being able to continue to modulate cells within regenerating nerve tissue long after surgical repair is a compelling possibility. A hypothetical ES-NGCs might be comprised of a conductive scaffold within an insulating outer tube, combined with a method of delivering ES.

There are examples of interfacing tissue with electronics within medicine. Pacemakers, first used within the 1960s, are implantable devices that interface directly with the heart to treat conditions of cardiac arrhythmias[111]. Approximately 1,000,000 new pacemakers implanted each year[112], and the technology is still evolving to date[111, 113]. Deep brain stimulation (DBS) is a therapeutic option for patients with advanced Parkinsons Disease (PD) who cannot control their symptoms using pharmacological interventions, such as L-Dopa administration. In the late 1980s, the first deep brain electrodes were implanted to treat parkinsonian tremors[114], which has since evolved for worldwide application of DBS[115]. Interfacing electronics with human physiology is not entirely novel, and the application of electronics to

regenerative medicine is certainly feasible by leveraging the experience of pre-existing technologies.

Understanding how different peripheral nerve cell types respond to ES is the first step towards developing an effective strategy. This information can then be used to determine the most appropriate timing, location and stimulation parameters to achieve targeted modulation of specific cell populations. For example, neurons could be stimulated to regenerate faster, macrophages and Schwann cells could be encouraged to adopt and sustain pro-regenerative phenotypes, endothelial cell behaviour could be guided and improved, and the migration and orientation of cells and tissues could be enhanced. These sorts of positive interventions could be applied not only to the proximal stump (Table 2) but also to the distal nerve stump to improve and prolong the pro-regenerative environment, and to the cells within a nerve conduit itself, e.g. to stimulate differentiation, migration and alignment. Being able to target distinct cell populations at specific times to create an optimal regeneration environment is a tantalising prospect that holds great promise for the future of the field.

Conclusions

There are different distinct categories of ES for peripheral nerve regeneration. Currently, providing ES to the peripheral nerve stump during intervention is being explored in several randomized clinical trials (**Table 1**). However, due to materials advances, it is becoming possible to interface electronics directly with the regenerating nerve throughout the regeneration process, which may be key to providing therapeutic benefits post-surgery, especially as the pro-regenerative Schwann cell phenotype fades which impairs long gap regeneration[46, 102]. *In vitro* experiments over the past decade have provided evidence that there is a myriad of benefits to be realized by subjecting cells involved in peripheral nerve regeneration to low strength ES over significant durations of >1 hour. Low strength ES can provide directionality cues, phenotypical changes, and alterations in neurotrophic factor secretion for cells present within the regenerative peripheral nerve niche, however the evidence is less clear for the effect of ES on macrophages (Figure 6).

Bioelectronics has the possibility to provide these positive therapeutic effects, on demand, *in vivo,* by providing the cell-electronics interface. A key consideration in the development of this promising technology will be to determine the influence of electrical fields, relative to other cues present in the regenerating tissue.

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Author Contributions

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Table 1: Current clinical trials being explored of ES for peripheral nerve regeneration. Data collected on the 19th of October, 2021

Study Title	ClinicalTrials.gov Identifier	Sponsors	Study Summary
Electrical Stimulation to Enhance Peripheral Nerve Regeneration	NCT02403661	University of Alberta	Primary goal is to quantify the functional defects caused by injuries to the bracial plexus and peripheral nerves within the arm. Secondary goal is to test the possible benefit of ES, and if ES will improve functional outcomes
Electrical Stimulation to Improve Recovery after Peripheral Nerve Injury	NCT03996525	The Hospital for Sick Children, Canada	The study aims to assess if ES accelerates motor axon regeneration and improves muscle recovery in patients undergoing two-staged facial reanimation for facial palsy.
The Effect of Pre-operative Electrical Stimulation on Peripheral Nerve Regeneration	NCT03205124	Ming Chan, University of Alberta	Study attempts to assess if pre- and post-operative ES is providing an addictive benefit on sensory nerve axonal regeneration using a 3-arm clinical trial.
Feasibility Study of a Temporary Peripheral Nerve Stimulator	NCT04732936	Epineuron Technologies Inc.	A novel temporary peripheral nerve stimulator will be evaluated for safety, usability and preliminary efficacy
Extracorporal Shock Wave Treatment to Improve Nerve Regeneration	NCT03147313	Ludwig Boltzmann Gesellschaft	*"This study evaluates the impact of extracorporeal shock wave treatment after microsurgical coaptation of finger nerves. Participants will be randomized into two treatment groups with different settings and a sham group. The participants will thereafter followed-up in a prospective, double-blind study design"*

Table 2; Summary of experimental studies using ES on cells involved in peripheral nerve regeneration, with brief experimental and outcome descriptions. Studies were included based on stimulation parameters that were long duration (>1h), low field strength and that have translational potential through post-operative stimulation of implanted tissue engineered constructs

Cell type Effect studied	Material/Method	Model/Cell Type	ES Parameters	Experimental Groups	Outcomes Description	Reference
Neurons						
Neurite outgrowth using polypyrrole	Polypyrrole films	PC-12	100 mV for 2h	Stimulated; Not Stimulated; Solution Control; Tissue Culture Polystyrene	Neurites longer on PP compared to controls; ES of PP resulted in greater median neurite length	[60]
Fibronectin adsorption on polypyrrole	Polypyrrole films	PC-12	10 µA for 2h	Immediate ES; Delayed ES 2h after protein adsorption; Control	Increased neurite outgrowth is attributed to increased fibronectin adsorption from the ES	[61]
Schwann Cells						
ES effect on Schwann cell exosomes	Polyester membrane	DRG & Co-culture with RSC96	100 mv/mm; 200 mv/mm	3 groups for each parameter, 0.5 h, 1 h, 2 h; controls	Conditioned media from ES treated Schwann cells improved neuronal activity	[12]
ES induced BDNF release from Schwann cells	Indium Tin Oxide (ITO) slides	Primary Schwann Cells (SD rat)	3Hz and 6V, different V and Hz, 30 mins	3Hz (1 V, 4 V, 6 V, 10 V, 16V); 6V (1Hz, 3Hz, 10Hz, 100Hz, 300Hz); controls	ES promotes BDNF release from SCs through increased calcium flux through voltage gated calcium channels	[73]
ES induced calcium dependant release of NGF	Indium Tin Oxide slides	Primary Schwann cells (SD rat)	1-10 V/cm and frequencies 1-100 Hz, 3 h	1Hz (1, 2, 5, 10 mV/mm); 5 mV/mm (1, 5, 10, 100 Hz); Controls	ES causes calcium influx over plasma membrane and release from internal stores - Results in NGF release	[74]
Schwann Cell orientation	poly-l-lysine coated glass	Primary Schwann cells (SD rat)	0-200 mV/mm for 8 h	0, 10, 25, 50, 75, 100, 150, 200 mV/mm	Schwann cells showed changes in orientation and at higher mV/mm showed abnormal clustering.	[69]
Macrophages						
Brief electrical nerve stimulation encourages pro-repair phenotype in demyelinated nerves	surgical re exposure of regenerating nerve & stainless-steel wires	Sprague Dawley rat	20 Hz, 3 V, 1 h	Control, LPC (5d, 8d) and LPC + ES (5d, 8d)	Delayed ES showed decreased M1 phenotype markers and increased M2 phenotype markers. Results were greater when ES was applied after a greater delay.	[13]
Delayed ES promotes immune cell clearance	surgical re exposure of regenerating nerve & stainless-steel wires	Wistar rats	20 Hz, 3 V, 1 h	Control, LPC (5d, 8d) and LPC + ES (5d, 8d)	Brief ES increases myelin basic protein expression, accelerated Node of Ranvier reorganization and enhanced macrophage clearance	[85]
Macrophage	glass slides	RAW264.7	50 mV/mm, 4 h	Control, LPS, LPS +TGF-B1	No major changes in phenotype were observed in	[90]

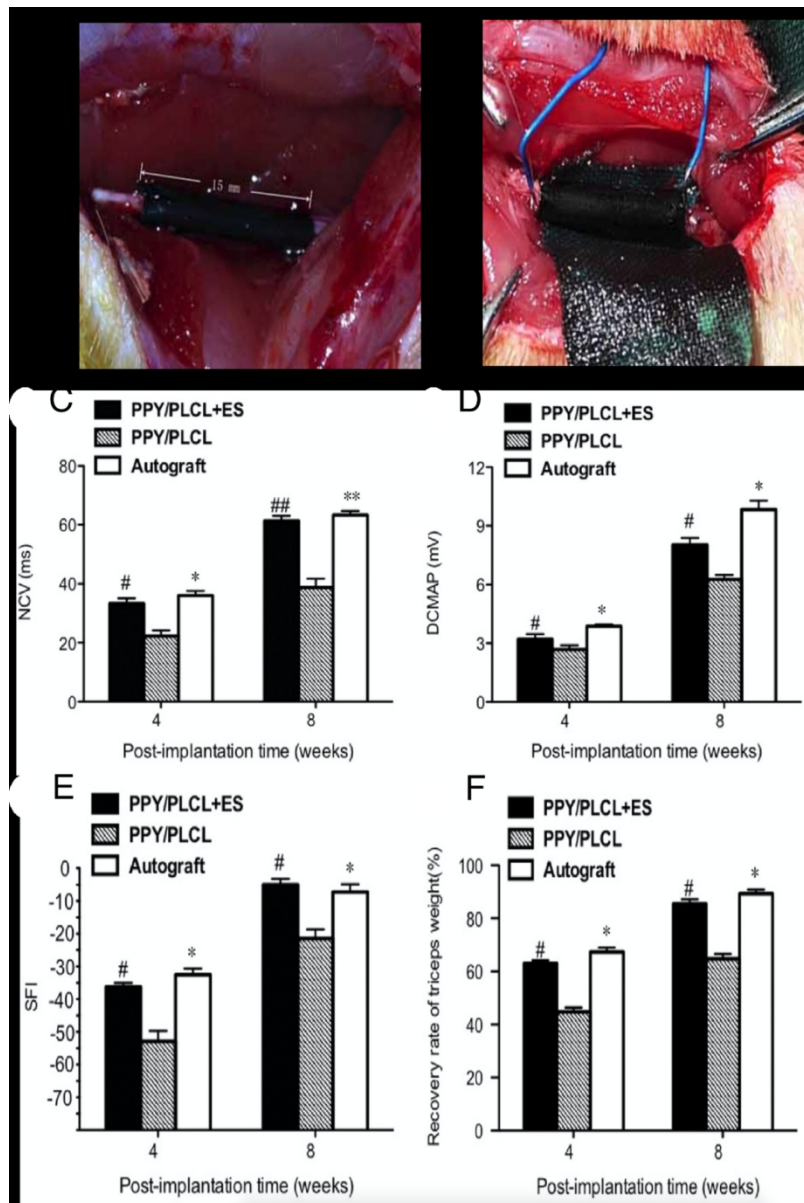|						
					response to low strength ES	
Endothelial Cells						
ES directs migration, orients cell division, and upregulates chemokine receptors	glass	HUVECs & HMEC	50-300 mV/mm 3 h	Control; 50, 100, 150, 175, 200, 300 mV/mm	Migration of endothelial cells is faster towards the cathode under stimulation and chemokine receptors CXCR4 and CXCR2 were upregulated	[79]
Endothelial cell migration and orientation	poly-l-lysine coated glass	m.Bend.3 (microvascular endothelial cell line	50 mV/mm, 8 h	Compared vs 50 mV/mm Schwann cells	No morphological changes or changes in cell number were observed compared to Schwann cells, which elongated and migrated	[69]
Pre-angiogenesis responses induced by ES	glass	HUVEC	100-300 mV/mm	Multiple timepoint and duration, over 24 hours	ES caused VEGF release from endothelial cells. Endothelial cells orientated and migrated towards the anode	[80]
Pre-angiogenesis responses induced by ES	glass	HUVEC	200mV/mm	Multiple timepoint and duration, over 24 hours	PCR and ELISA showed ES upregulating mRNA of angiogenic proteins, VEGF165, VEGF121 and IL-8	[81]

Figure 1 A) Immediately after implantation of the 15 mm electrospun PPy/PLCL conduit B) 1/4 circle electrode implantation C-F) Electrophysiology and functional evaluation C) Nerve conduction velocity (NCV) D) Distal compound motor action potential (DCMAP) E) Sciatic function index (SFI) E) Recovery rate of triceps weight (C-F (n = 5, #p < 0.05, ##p < 0.01 the PPY/PLCL + ES group vs. PPY/PLCL group; *p < 0.05, **p < 0.01 the autograft group vs. PPY/PLCL group). Panel recreated with permission from J. Song, B. Sun, S. Liu et al[44]
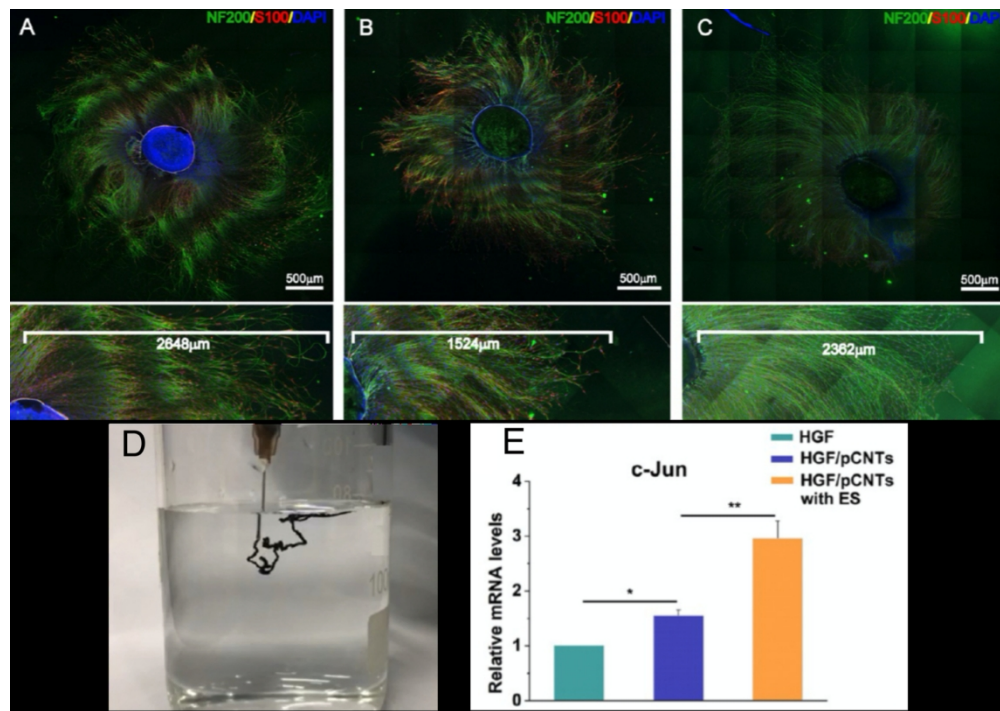
Figure 2 Confocal images of Dorsal Root Ganglion (DRG) neurons (Green, NF200+) and Schwann Cells (red, S100+) cultured over 7 days. Cell nuclei stained with DAPI (blue) A) HGF/pCNTs + ES B) HGF/pCNTs without ES C) HGF hydrogel alone D) Immediate formation of a cylindrical hydrogel upon the injection of HGF/pCNTs into PBS through a 26 gauge needle E) Relative mRNA expression after culturing DRG neurons for 30 days. Fold changes are reflected on the vertical axis compared to the control group (HGF), which has been normalized to 1. *p < 0.05, **p < 0.01, and ***p < 0.001. Figure reproduced with permission from L. He, Q. Xiao, Y. Zhao et al.[42]

Figure 3 Schematic representation of key cell types and neuron response to injury in the peripheral nervous system. Figure created using Biorender.com

Figure 4 HUVECs grown in various conditions. a) No electrical field, b) electrical field of 100 mV/mm applied for 72h, c) 100 mV/mm electrical field applied for 72h with addition of VEGFR inhibitor. Panel reproduced with permission from [80]

Figure 5, Electrical stimulation of human microvascular endothelial cells (HMECs) shows changes in endothelial cell migration speed and directionality (a, b). (c, d) shows the effect an EF has on directionality of human umbilical vein endothelial cells (HUVECs) growth on different substrates, collagen, fibronectin. (e, f) shows the relationship between EF and cell division for HUVEC cells (e) and HMEC cells (f). In all cases, electrical stimulation was provided for 3h. Panel reproduced with permission from [79]

Figure 6 Summary of the beneficial effects that can be achieved through the application of ES cells involved with peripheral nerve regeneration. Figure created using Biorender.com

Figure 1 A) Immediately after implantation of the 15 mm electrospun PPy/PLCL conduit B) 1/4 circle electrode implantation C-F) Electrophysiology and functional evaluation C) Nerve conduction velocity (NCV) D) Distal compound motor action potential (DCMAP) E) Sciatic function index (SFI) E) Recovery rate of triceps weight (C-F (n = 5, #p < 0.05, ##p < 0.01 the PPY/PLCL + ES group vs. PPY/PLCL group; *p < 0.05, **p < 0.01 the autograft group vs. PPY/PLCL group). Panel recreated with permission from J. Song, B. Sun, S. Liu et al[44]

101x150mm (300 x 300 DPI)

Figure 2 Confocal images of Dorsal Root Ganglion (DRG) neurons (Green, NF200+) and Schwann Cells (red, S100+) cultured over 7 days. Cell nuclei stained with DAPI (blue) A) HGF/pCNTs + ES B) HGF/pCNTs without ES C) HGF hydrogel alone D) Immediate formation of a cylindrical hydrogel upon the injection of HGF/pCNTs into PBS through a 26 gauge needle E) Relative mRNA expression after culturing DRG neurons for 30 days. Fold changes are reflected on the vertical axis compared to the control group (HGF), which has been normalized to 1. *p < 0.05, **p < 0.01, and ***p < 0.001. Figure reproduced with permission from L. He, Q. Xiao, Y. Zhao et al.[42]

159x112mm (300 x 300 DPI)

Figure 3 Schematic representation of key cell types and neuron response to injury in the peripheral nervous system. Figure created using Biorender.com
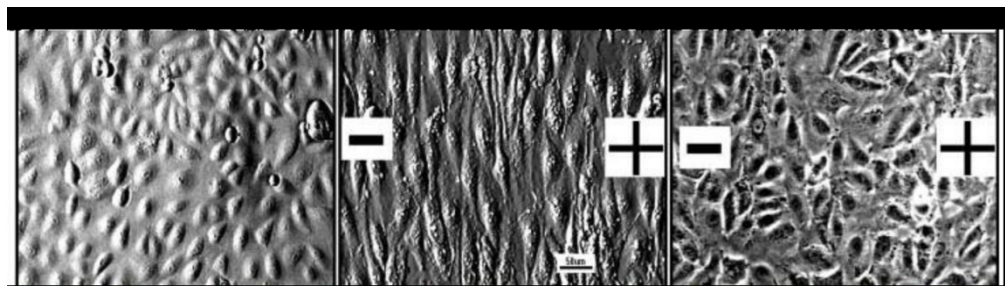
159x74mm (220 x 220 DPI)

Figure 4 HUVECs grown in various conditions. a) No electrical field, b) electrical field of 100 mV/mm applied for 72h, c) 100 mV/mm electrical field applied for 72h with addition of VEGFR inhibitor. Panel reproduced with permission from [80]
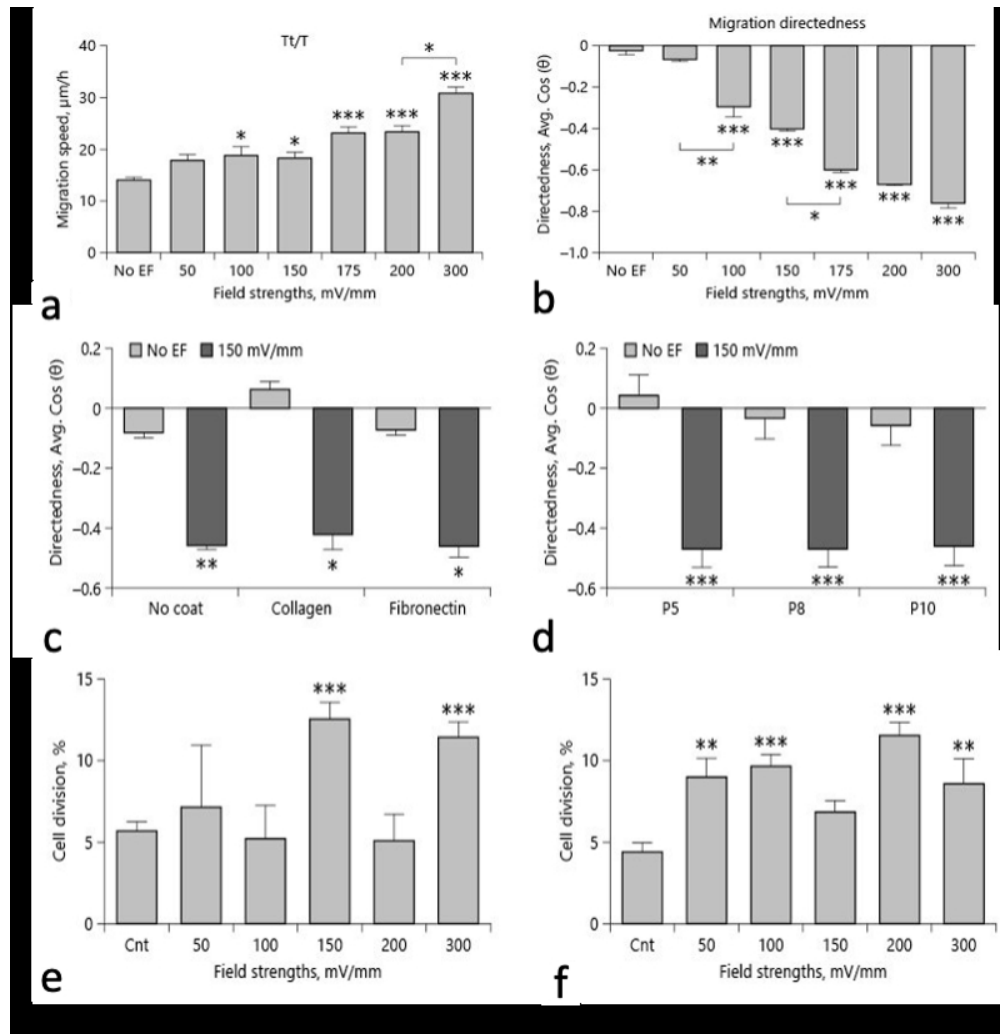
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Figure 5, Electrical stimulation of human microvascular endothelial cells (HMECs) shows changes in endothelial cell migration speed and directionality (a, b). (c, d) shows the effect an EF has on directionality of human umbilical vein endothelial cells (HUVECs) growth on different substrates, collagen, fibronectin. (e, f) shows the relationship between EF and cell division for HUVEC cells (e) and HMEC cells (f). In all cases, electrical stimulation was provided for 3h. Panel reproduced with permission from [79]
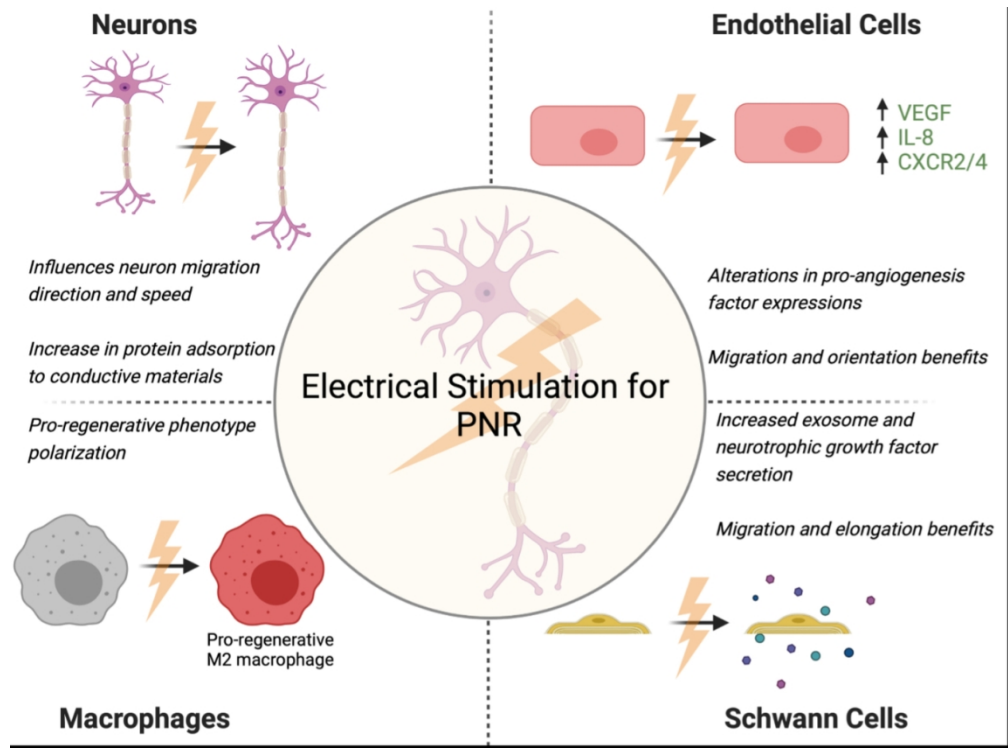
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Figure 6 Summary of the beneficial effects that can be achieved through the application of ES cells involved with peripheral nerve regeneration. Figure created using Biorender.com

118x87mm (300 x 300 DPI)