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Therapeutic Application of Electric Fields in the Injured Nervous System

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Abbreviations and Acronyms

AC = alternating current
BDNF = brain-derived neurotrophic factor
Cdc42 = cell division cycle 42
CNS = central nervous system
DC = direct current
EF = electric field
OFS = oscillating field stimulator
PNS = peripheral nervous system
SCI = spinal cord injury
TCPD = transcorneal potential difference
TES = transcorneal electrical stimulation

Significance: Nervous system injuries, both in the peripheral nervous system (PNS) and central nervous system are a major cause for pain, loss-of-function, and impairment of daily life. As nervous system injuries commonly heal slowly or incompletely, new therapeutic approaches may be required.

Recent Advances: The observation that cultured neurons are able to respond to exogenous electric fields (EFs) by sprouting more neurites and directing growth along the field, along with the presence of endogenous EFs in the developing vertebrate nervous system have led to the suggestion of the use of EFs in a regenerative therapeutic setting. This review discusses the effects of EFs on nervous cells, and their use in the treatment of nervous injuries in the eye, limb nerves, and the spinal cord. Exogenous EFs have been shown to be neuroprotective in various injury models of the eye, including traumatic injury, congenital degenerative retinopathy, and glaucoma. In the PNS, EFs are able to stimulate regrowth and functional recovery in damaged limb nerves. In the spinal cord, axonal regeneration and improved quality of life may be achieved using EF stimulation.

Critical Issues: The optimal paradigm for electrical stimulation has not been determined, and the mechanisms behind the effect of EF are still largely unknown.

Future Directions: Although the therapeutic use of EFs in the nervous system is still in its infancy, it is a promising therapeutic avenue for otherwise hard to treat injuries. The cellular/molecular mechanisms of such regulation need to be fully investigated, and the efficiency of applied EFs during wound healing needs to be optimized in a systematic approach in both animal models and future clinical trials.

SCOPE AND SIGNIFICANCE

Nervous system injuries can be divided into two categories: central nervous system (CNS) insult and peripheral nervous system (PNS) injury. CNS and PNS injuries commonly have poor clinical outcome, and cause heavy financial burdens to the society, as well as emotional and physical challenges to the patients and their families. In the United States alone ~450,000 people have sustained traumatic spinal cord injuries (SCIs), with more than 10,000 new cases emerging every year. In China, the incidence of SCI is ~60,000 per year. The average annual medical cost ranges from $15,000 to $30,000 per year per SCI patient, and the estimated lifetime cost ranges between $500,000 and more than $3 million, depending on injury severity.[1] Despite various efforts in treatment of nervous system injuries; the clinical outcomes were not satisfactory largely due to the limited regeneration capacity of the nervous system. Many new

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therapeutic approaches, including chemical, physical, and electrical means have been suggested. This work discusses the application of exogenous electric fields (EFs) in the treatment of CNS and PNS injuries, including in the eye, limb nerves, and spinal cord. It does not address the clinical use of exogenous EFs for pain control.

TRANSLATIONAL RELEVANCE

Previous studies have demonstrated endogenous EFs are formed immediately at wound formation.[2] EFs regulate the wound healing[3] and promote the nervous reinnervation during wound healing.[4] Much preclinical work in animal models suggests exogenous EFs are beneficial in nervous system injury. The stimulation paradigms and surgical procedures used in animal studies would be potentially suitable for clinical trials, as proved by previous pre-clinical studies.

CLINICAL RELEVANCE

Nervous system injuries heal slowly and often incompletely. Several trials using electric stimulation have been performed in both PNS and CNS injury repairs (see details below). Although the clinical outcome has been encouraging in some cases, there is an apparent lack of systematic characterization and optimization of using EFs in such treatments, including the EF pattern and voltage, as well as potential interaction and combined application with other guidance factors (*e.g.*, chemical gradients, physical cues, extracellular matrix, and others). Nevertheless, EFs still hold great potential and may represent a new therapeutic avenue in treating nervous system injuries.

Clinical problems addressed

The sensitive nature and limited regenerative capacity of the nervous system makes it exquisitely sensitive to injury. Traumatic injury can cause severe damage to nerve tracts throughout the PNS or CNS. Neuropathy of the PNS is nontraumatic damage caused by various factors among prolonged diabetes mellitus, infection, inflammation, compression, or congenital disorders. What is common in these assaults to the nervous system is that regeneration can be limited, either due to the inherent limits of the cells involved or due to local inhibitory factors generated from cellular debris or inflammation.

In both the PNS and CNS, after injury, axons severed from the cell body will undergo Wallerian degeneration and fragment and disintegrate over the course of several days.[5] After injury, immune cell infiltration leads to clearing of the debris. In the PNS, the ensheathing Schwann cells dedifferentiate and express appropriate chemoattractants and repellents to stimulate and guide axonal regrowth and reinnervation (Fig. 1).[6] However, for large-scale injuries, or those with large amounts of cellular debris and inflammation, these mechanisms are inadequate, and consequently regeneration is incomplete.

In contrast, such a mechanism does not operate in the CNS. The equivalent to Schwann cells—the oligodendrocytes—do not stimulate neural regrowth, and in combination with the hypertrophy of supporting astrocytes and immune cells, the wound environment remains un-supportive for

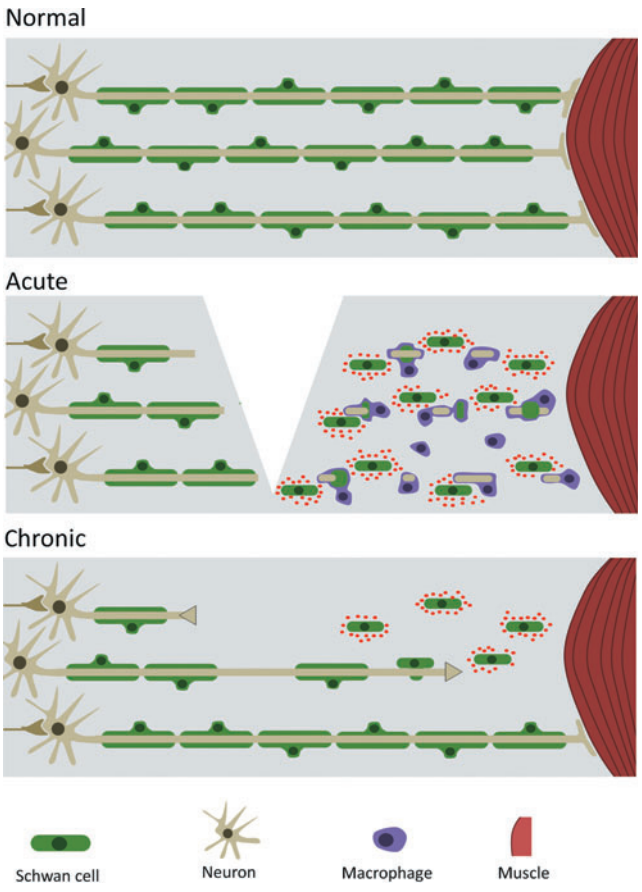

Figure 1. Effects of injury in the peripheral nervous system. In the normal situation, peripheral axons are enveloped by supportive Schwann cells. After an injury, damaged axons retract from the site of injury, and infiltrating macrophages clear axonal and cellular debris. Intact Schwann cells dedifferentiate and start expressing chemoattractants and neurotrophic factors. After the initial inflammatory phase, axons that have a short distance to cross will be attracted by the Schwann cells, and eventually reinnervate their muscle target and will once again be enveloped by differentiated Schwann cells. However, when the distance is too large, no reinnervation will take place, and the axonal stump will retract and the neuron will likely undergo apoptosis. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

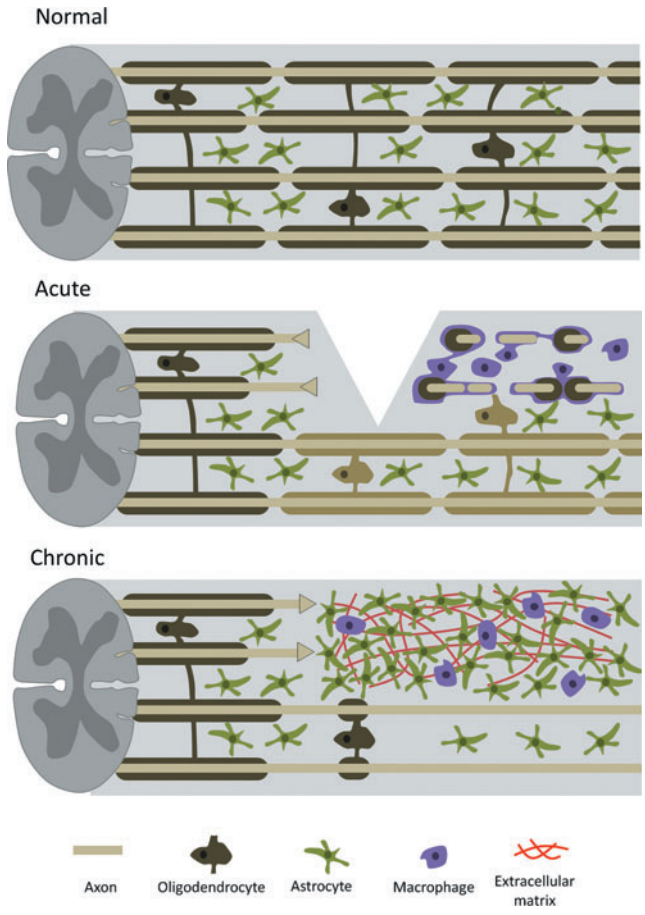

Figure 2. Cellular response in spinal cord injury. In the healthy spinal cord, intact nerve bundles are surrounded by oligodendrocytes and supported by astrocytes. In the acute phase of injury, severed axons retract toward the soma, while their distal stumps and remaining myelin debris are phagocytosed by microglia and infiltrating macrophages. Damaged oligodendrocytes lead to demyelination of nearby intact axons. In the chronic phase, when the acute inflammatory response has been resolved, reactive astrocytes have proliferated and formed a dense glial scar, which includes trapped immune cells and dense networks of extracellular matrix, which is inhibitory to axons attempting reinnervation to the distal end of the injury. Remyelination may take place to once again envelop naked axons. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

regrowth. For an overview of the cellular response to spinal cord damage, see Fig. 2. In these situations where regrowth and reinnervation is absent or limited, the use of exogenous EFs may offer a new therapeutic option.

RELEVANT BASIC SCIENCE CONTEXT

Endogenous and exogenous EFs in the nervous system

EFs in the nervous system have been used clinically in several settings. Their use was pioneered in the field of pain control. Chronic pain may be controlled by EF stimulation of the affected area, the spinal cord, or brain. This is based on the "gating theory" of pain, where the exogenous EF signals override the pain signals coming from the periphery. Direct deep brain stimulation has also been beneficial in improving quality of life in Parkinson's disease patients, by ameliorating symptoms through stimulation of basal ganglia.[7] The activation of output signals and the consequent pattern changes in neuronal activity throughout the basal ganglia motor circuit is the mechanism responsible for improvement of symptoms. However, one of the major disadvantages is the diminished response over time to deep brain stimulation, limiting its long-term usefulness. From a regenerative point of view, EFs are still a largely untried therapeutic avenue.

A number of effects of exogenous EFs on nervous cells *in vitro* and *in vivo* have been described in the past decades. Initial studies in cultured *Xenopus* embryonic neurons showed that applied EFs induced neurite sprouting and promoted the turning of the growth cones of extending neurons (Fig. 3A).[8] The presence of an exogenous EF is able to increase the number of filopodia on the growth cones, even when these were first collapsed pharmacologically,[9] promoting growth cone motility.

The effects of EFs on nerve growth have also been noted during normal physiological development. In the early embryonic development, endogenous EFs exist during the neural tube formation in *Xenopus*[10] and chicken.[11] Blocking the electric current or reversing its polarity in developing *Xenopus* embryos hinders closure of the neural tube,[12] and when the field is disrupted following closure of the neural tube internal tissues disaggregate.[13] The role of the endogenous EF in patterning the neural tube is illustrated by the complex three-dimensional pattern of the field, with both rostral-to-caudal and left-to-right gradients (Fig. 3B, C).[14] The response of neuronal outgrowth to EF has been shown to be dependent on dynamic microtubules and small GTPases Cdc42 and Rac.[15] The effects of EFs can be further modulated by neurotransmitters or neurotrophic factors.[16,17] For an overview of these mechanisms, see Fig. 4. The observed effects of EFs both *in vivo* and *in vitro* have led to the suggestions of their use in a number of pathological situations affecting the PNS or CNS. The application of exogenous EFs has been used in various injury models, including in the eye, PNS, and in the spinal cord.

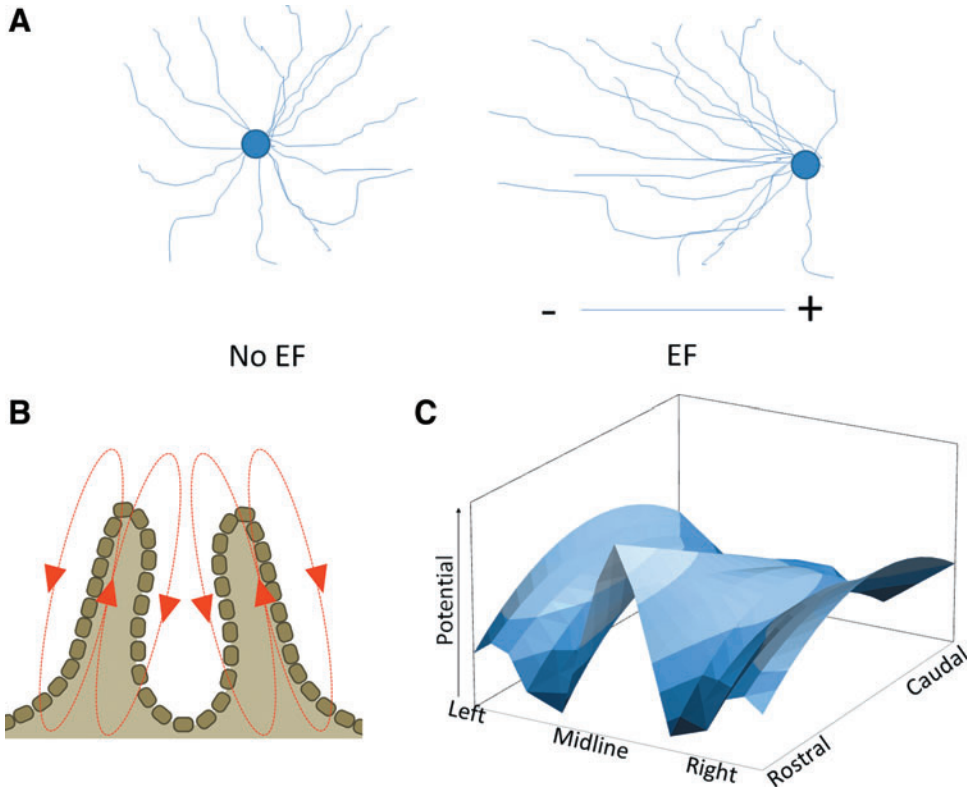

Figure 3. Electric fields (EFs) during neural development. **(A)** Effects of applied EFs on cultured neurons. In the absence of EF, neurite outgrowth has no directionality, whereas in an applied field, outgrowth is strongly directed toward the anode. **(B)** Direction and location of the EF before neural tube closure. **(C)** During vertebrate development, EF strength along the neural tube varies by anatomical location, with both rostal-caudal and lateral-midline gradients. Plot represents the fields in a stage 15 axolotl embryo. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

DISCUSSION OF FINDINGS AND RELEVANT LITERATURE

EFs in the eye

Damage to a number of components of the eye has been shown to respond favorably to the presence of both endogenous and exogenous EFs. The cornea normally maintains a transcorneal potential difference (TCPD) of approximately +40 mV, positive internally, by active pumping of Cl^- outward and Na^+/K^+ inward.[18–20] In the event of an injury, the TCPD collapses. However, at ~1 mm from the injury site, normal TCPD is maintained, leading to the establishment of an EF parallel to the cornea, between injured and uninjured epithelium, of at least 40 mV/mm (Fig. 5A).[21] This field has been observed to stimulate wound closure by promoting cell division and migration in corneal epithelial cells.[4,21,22] However, apart from the effect on epithelial cells, the endogenous EFs also promote nervous repair in the injured cornea. In the damaged cornea, new nerve sprouting occurs rapidly, perpendicular to the wound edge (Fig. 5B).[23] By manipulating the endogenous EF in *ex vivo* wounded corneas, Song *et al.* showed EFs are important guiding factors for directionality of nerve regrowth.[4] The perpendicular direction of new sprouting is enhanced by pharmacologically enhancing EFs, and directionality is lost when EFs are diminished. The effect of the EF is strong enough to induce turning in newly sprouted nerve that did not have an initially perpendicular direction. Apart from determining nerve sprouting direction, the presence of EFs also increases the number of newly sprouting nerves. In the stronger field close to the wound edge, sprouting rate is higher, and this can be further modified by pharmacological manipulation of the field.

Further into the eye, retinal damage is a prime cause for loss of vision. In a number of different injury situations, the beneficial effect of exogenous EFs on retinal damage has been described. In these studies, transcorneal electrical stimulation (TES) was used, with two concentric ring electrodes are placed on the surface of the eye to deliver current. In an experimental setup modeling glaucoma, ischemic damage to the retina was caused by raising intraocular pressure. EF application via TES had a

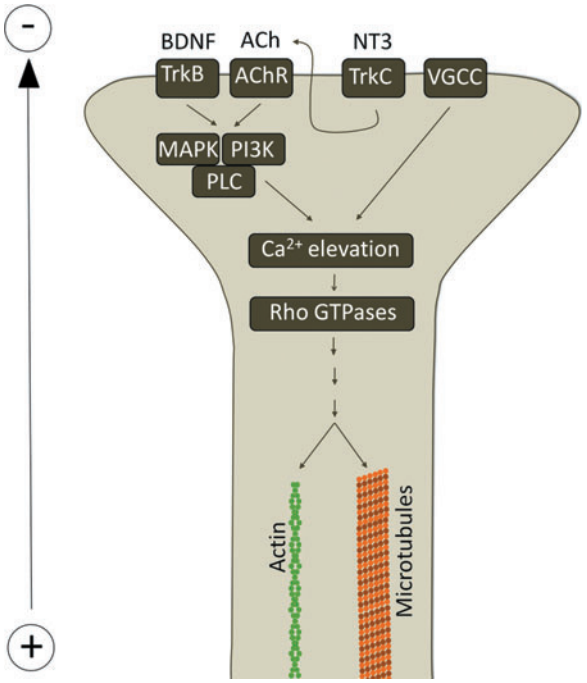

Figure 4. Mechanisms of electric growth cone guidance. Electrotaxis of growth cones is at least partially mediated by elevation of intracellular calcium though activation of voltage gated calcium channels (VGCC). The effect is increased in the presence of neutotrophins, such as BDNF or NT3 and neurotransmitters, such as acetylcholine (ACh), via activation of PLC through MAPK or PI3K. The elevated calcium levels activate a number of Rho GTPases, leading to actin and microtubule modification and growth cone mobility. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

neuroprotective effect in the model, preserving both the morphology and electrophysiological properties of the retinal ganglion cells.[24] TES also has a neuroprotective effect following optic nerve transsection or crush, rescuing de-innervated ganglion cells from apoptosis, possibly in an insulin-like growth factor 1 dependant mechanism.[25,26] Retinal degeneration following exposure to intense light is also ameliorated by both pre- and post-exposure TES, which is accompanied by increased expression of growth factors ciliary neurotrophic factor and brain-derived neurotrophic factor (BDNF) and of the anti-apoptotic factor B-cell lymphoma 2.[27] In a rat model of degenerative retinitis pigmentosa, TES protected photoreceptor cells from degeneration.[28] Although EF treatment of the eye has so far not been used in a clinical setting, the ease of application means this will likely translate to the clinic soon.

EFs in the PNS

PNS injuries are common as a result of trauma and other conditions, such as diabetic neuropathy. Although in contrast to the CNS, extensive regeneration of the PNS is possible, it is often incomplete. PNS damage leads to loss of function, often accompanied by atrophy in the affected muscles, and either loss of sensation or persistent neuropathic pain. While chronic neuropathic pain has been controlled using EFs, stimulation may prove to be useful in regenerative medicine too. In general, a positive effect of EF stimulation on regrowth

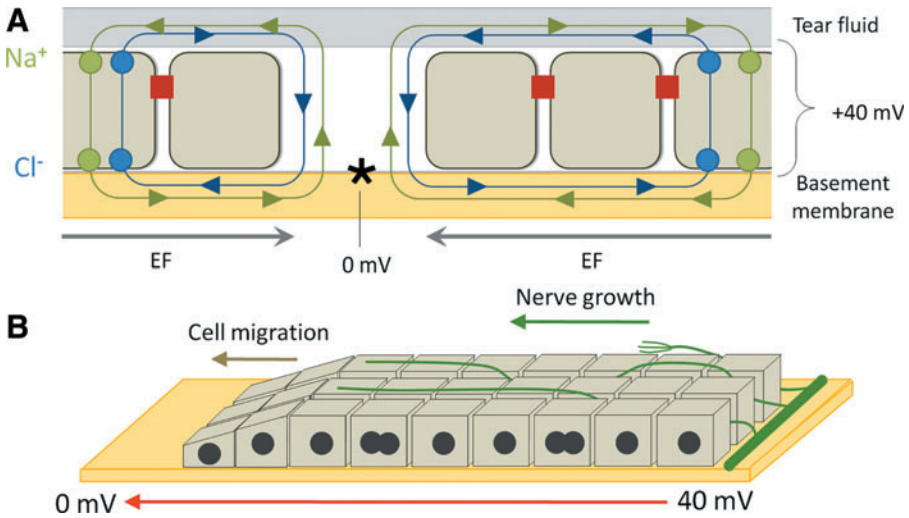

Figure 5. The EF in the damaged cornea. **(A)** The transcorneal potential is normally maintained by the inward flow of sodium and the outward flow of chloride, with tight junctions between epithelial cells prohibiting free return of ions. At a wound (asterisk), this ionic barrier is broken, leading to short-circuit of the potential and the establishing of a lateral EF between intact epithelium and wound site. **(B)** The transcorneal potential is disrupted at the site of injury, leading to a net EF, along which cell division, cell migration, and nerve growth takes place. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

is seen in the PNS, although some studies show contradicting results.

The most direct method to stimulate nerve regrowth is to implant electrodes directly over the injured nerve. Early work using short-term or long-term alternating current (AC) stimulation following a femoral nerve transsection or sciatic nerve crush significantly sped up reinnervation.[29,30] Application of a direct current (DC) EF following a sciatic nerve crush injury resulted in a functional improvement, along with an increased vascularisation and higher nerve fiber density.[31] The alternative approach to direct electrode implantation is to implant an electrically conductive nerve conduit across both ends of the damaged nerve. After implantation, an AC was delivered through the nerve conduit, which led to improvement in both motor and sensory function, along with increased axonal regeneration, myelination, and increased expression of BNDF.[32] Human trials have been limited, but in patients with degenerated median nerves due to severe carpal tunnel syndrome, high-frequency AC EF stimulation through implanted electrodes promoted axonal regrowth and led to improved electrophysiological parameters but did not significantly improve functional measurements.[33]

Apart from stimulation on the damaged nerve, several groups have studied the effects of using EFs on target muscles deinnervated due to nerve damage. Using long-duration (28 day) transcutaneous AC EFs on the tibialis anterior muscles following a sciatic nerve crush injury was found to not to have an effect on reinnervation of the muscle, and increased muscle atrophy during the stimulation period.[34] In a resection of the sciatic nerve, AC EF stimulation through intramuscular needle electrodes resulted in improved electrophysiological parameters and increased vascularisation, but only at moderate currents (≤ 2 mA) whereas high currents (4 mA) had a detrimental effect. This effect was independent of nerve fiber number or size, as these parameters remained unchanged.[35] Effects of stimulation on the muscle is inconclusive, due to the limited data available. For an overview of the stimulation methods used, see Fig. 6.

It has been suggested that the beneficial effect of AC EFs on regrowth in the PNS is growth factor mediated. EF stimulation has been shown to increase BDNF expression in injured nervous tissue. The EF driven BDNF expression may then increase the expression the human natural killer-1 carbohydrate motif, which is contained in a number of cell surface adhesion molecules, such as neural cell adhesion molecule.[36] Based on the literature, it appears a short stimulation period (< 1 day) is sufficient to stimulate significant regrowth, making it likely that a growth factor mediated mechanism is involved in the effect.

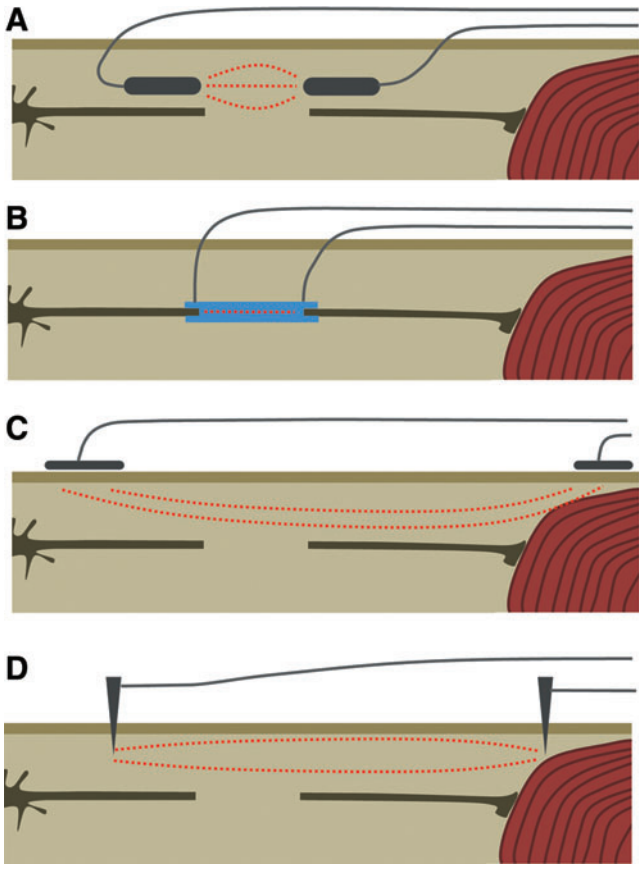

Figure 6. The different approaches for electric stimulation in peripheral nerve damage. **(A)** Implantation of electrodes over the severed end of the axons. **(B)** Implantation of an electrically conductive nerve conduit over the nerve stumps. **(C)** Noninvasive stimulation by skin patch electrodes over the target muscle and the proximal end of the damage nerve. **(D)** Needle electrodes on target muscle and proximal end of the damaged nerve. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

EFs in the SCI

Traumatic SCI is one of the main reasons for lower body paralysis. In humans, functional recovery after SCI is limited, and treatment options are few. The only currently accepted therapeutic option is the early administration of large doses of steroids to limit tissue damage due to acute inflammatory responses. However, this is not without controversy. The main limiting factor in functional recovery is the inability of severed or damaged axons to reconnect with their corre-

sponding targets on the other side of the lesion. The axons of damaged spinal neurons will retract and cell bodies degenerate if not reinnervated swiftly. However, local inflammatory responses and glial hypertrophy form a glial scar which is not conductive to the passage of growing axons. The implantation of electrodes over the injury site has been suggested as a means to promote and guide regrowth of the axons. Early work using a hemisection of the spinal cord of the guinea pig with DC stimulation over 4 weeks or more showed enhanced axonal growth, although not directly though the lesion,[37] and showed functional recovery in some of the treated animals.[37,38] In a rat spinal compression injury model, functional improvement following DC stimulation was seen, although no noticeable histological improvement was observed.[39]

It has been noted that the direction of the DC field is crucial for the beneficial effect, in a crush spinal injury, with the cathode caudal of the injury site, improvement was seen in histological, electrophysiological, and functional parameters, while no such enhancement was seen with the opposite polarity.[40] As a mono-polar field would stimulate axonal growth in one direction, but repress growth in the opposite, a steady field would only promote either efferent motor neurons or afferent sensory neurons, but not both. To circumvent this problem, an oscillating field has been used, which changes direction every 15 min, which was hypothesized to be sufficient time to promote growth, but not to induce regression in the opposite direction. This oscillating field stimulator (OFS) has been tested in two studies in dogs with sub-acute spinal injuries, with beneficial outcomes.[41,42] These studies led to a phase 1 trial with an OFS in humans with acute traumatic SCI.[43] The stimulator (Fig. 7) was implanted within 3 weeks after injury, and remained in place for 15 weeks. The stimulation provided significant improvement in two measures of sensory sensitivity and in seven out of nine patients improved motor scores were observed, compared to historical data for untreated patients. Although an erratum was later published concerning some discrepancies in functional scoring,[44] the fundamental conclusions of the trial are still compelling.

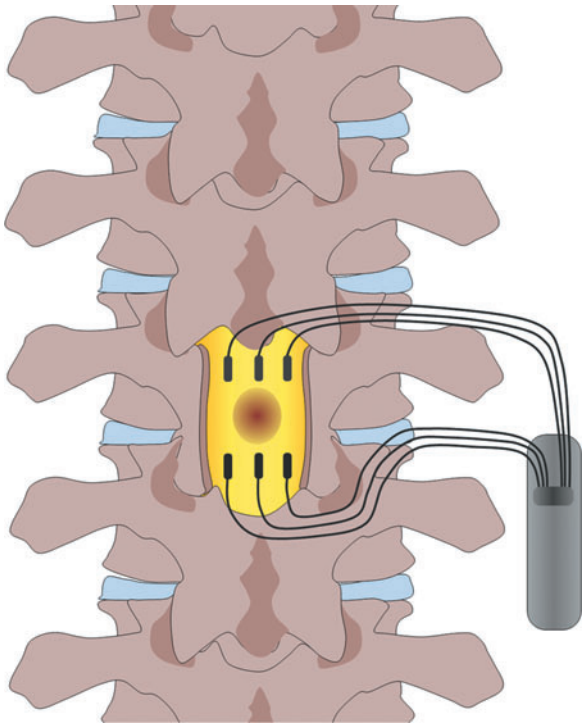

Figure 7. The design of oscillating field stimulators as used in a human phase 1 trial. The injury site is flanked by two sets of three electrodes, with the polarity of the field changing every 15 min. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

As reactive astrocytes are an important component of the inhibitory scar formed at the site of injury, the effect of EFs on the glial scar have also been investigated. An OFS stimulation on rats spinal cords with a puncture wound showed that the number of reactive astrocytes were reduced and astrocytes outside the injury site rearranged to align to an angle close to the EF.[45] Interestingly, the other major component of the inhibitory glial scar, macrophages, do not seem to be affected by exogenous EFs.[46]

Apart from enhancing endogenous repair mechanisms, EFs may also play a role in cell transplantation therapies. In recent years, neural stem cell transplantation therapy has been investigated for its potential in the treatment of CNS damage. The mechanisms of the beneficial effects of neural stem cell implantation are under active debate. They may be due to functional reconnection by the neurons differentiated from the implanted cells, or alternatively due to neuroprotection via neurotrophic factors released by implanted cells. Regardless of the actual mechanism, the positive supporting role of stem cells replacement has been acknowledged.[47] EFs may be used to enhance the effectiveness of stem cell implantation. Interestingly, Cao and colleagues showed in a recent study that endogenous electric signals could regulate the directional migration of neuroblast cells toward the rostral migratory stream *in vivo*.[48] This suggests exogenous EFs may modulate neural stem cell

behavior. Indeed, we and other labs proved that neural stem cells can be controlled to recruit directionally in an applied physiological level of EF,[49,50] and our lab has shown is it possible to regulate such cellular response in a three-dimensional organotypic spinal cord slice culture model with electric stimulation,[50] which suggests great potential for the use of exogenous EFs after grafting *in vivo*, as an innovative approach to optimize the stem cell based therapy in treating nervous system injuries.

TAKE-HOME MESSAGES

- Endogenous EFs play an important role in the development of the nervous system.
- Applied EFs stimulate neuronal outgrowth.
- In the eye, EFs are neuroprotective and may stimulate regrowth of axons into damaged areas.
- In peripheral nerve injuries, applying an EF over the site of nerve damage may promote regrowth and reinnervation, although the exact stimulation protocol is important, as it may also be detrimental to healing when not applied correctly.
- In the spinal cord, EFs can stimulate axonal growth over the injury site, and cause functional recovery. A human trial of EF stimulation in SCI has shown promising results.
- The sensitivity of neurons and neural stem cells EFs can potentially be used in clinical practice.

CONCLUDING REMARKS

Although the use of EFs for nervous system injuries in a therapeutic setting is still limited, pre-clinical work suggests a potential role for otherwise hard to treat injuries. It is unlikely that the use of EFs alone will turn out to be the silver bullet for nervous system injuries, but it may certainly be a useful addition to the clinic. The exact mechanisms by which EFs promote nervous regrowth are still unclear, and may be dependent on the choice of stimulation; AC or DC. It is likely that these mediate beneficial effects through different mechanisms. In AC stimulation, the likely effect is the stimulation of release and/or production of various neurotrophic factors, providing a nonspecific supportive environment for the regeneration of nervous cells. In contrast, DC is able to provide directional attractive cues for regeneration, potentially speeding up reinnervation, likely in addition to altering gene expression. Further fundamental research to elucidate the exact mechanisms of the beneficial effects of various stimulation paradigms will be required to validate their use in the clinic. The observations that the effect of EFs may be enhanced by certain neurotransmitters or neurotrophic factors opens up an avenue for treatment by combining these factors with EF to perhaps synergistically act on regrowing axons. Important future research direction will be to optimize the stimulation protocol for any given application, and to gain a more fundamental understanding of the mechanisms of EF-mediated cell behavior. Although the field of therapeutic EFs is still in its infancy, it is nonetheless a promising avenue for both PNS and CNS injuries.

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