Review

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Regenerative Medicine

Cell therapy for spinal cord injury informed by electromagnetic waves

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Spinal cord injury devastates the CNS, besetting patients with symptoms including but not limited to: paralysis, autonomic nervous dysfunction, pain disorders and depression. Despite the identification of several molecular and genetic factors, a reliable regenerative therapy has yet to be produced for this terminal disease. Perhaps the missing piece of this puzzle will be discovered within endogenous electrotactic cellular behaviors. Neurons and stem cells both show mediated responses (growth rate, migration, differentiation) to electromagnetic waves, including direct current electric fields. This review analyzes the pathophysiology of spinal cord injury, the rationale for regenerative cell therapy and the evidence for directing cell therapy via electromagnetic waves shown by *in vitro* experiments.

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Physiopathology of spinal cord injury

Spinal cord injury (SCI) is a trauma-induced disease state occurring in the CNS. SCI causes variant degrees of destruction and functional loss to the axons of the spine and their downstream targets. The initial injury in SCI is often a contusion rather than a full nerve transection. The secondary injury begins as hemorrhaging fills compartments of the epidural, subdural, subarachnoid and intramedullary spaces. Once the integrity of the blood–spinal cord barrier is lost, the spinal cord is vulnerable to inflammatory molecules. Chronic, multiphasic inflammation has been analyzed via flow cytometry to show continuing disturbances within 180-day postinjury [1]. During the secondary injury, axonal dieback worsens nerve function beyond the initial lesion. This is caused partially by the autoimmune actions of macrophages crossing the blood–spinal cord barrier. Macrophages are 'classically activated' by a wound response and release a handful of neurotoxic effectors including nitric oxide and inflammatory cytokines. In the damaged spinal cord, classically activated (M1) macrophages vastly outnumber M2 macrophages (which are associated with neuroprotection and angiogenesis) [2–4].

A pathological hallmark of SCI is the formation of glial scars within 5–14-day postinjury. This is effected when astrocytes intertwine with other cells to form a mesh-like barrier. Formation of the glial scar exacerbates neural loss. Neural loss results from the lack of glial support, as well as actual stretching and mechanical damage due to glial cells, reinforce an area of cavitation around the wound epicenter [5]. Astrocytes of the glial scar will further disrupt the wound environment by expressing surface proteins, such as CSPGs, potent inhibitors of axonal growth and full neural recovery [6]. Axons (even those spared from trauma and dieback) were once believed to undergo chronic demyelin-

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ation; the current understanding is that axons undergo acute demyelination and partial remyelination [7]. After injury, the microenvironment is so fundamentally changed that abnormal synapse reformation could result in worsened motor outcomes [8].

Growth cones, the receptor tips rich of migrating axons, are present in the developing and injured CNS. These finely tuned cellular mechanisms obtain remarkable precision during development. Unfortunately, they falter after CNS injury. The lesion site, which separates the growth cone from natural targets, is rich in inhibitory proteins, such as SEMA, NOGO, MAG and OMPG [9,10]. Although many molecules and receptors are implicated in the stumbling and successful guidance of the growth cones, a successful pharmaceutical solution to modulate them has yet to be presented [11].

Existing treatment options for SCI & challenges

Acute management

Guidelines for acute management of SCI strongly recommend stabilization of the injury and surgical decompression to reduce damage and inflammation [12].

Methylprednisolone is an anti-inflammatory agent, which may offer neuroprotective benefits if administered in the early acute phase. Expert opinions disagree whether or not methylprednisolone should be considered a standard of care. Low intravenous dosage of methylprednisolone is currently recommended by AOSpine, if administration begins less than 8 h from injury [13]. However, the American Association of Neurological Surgeons does not currently recommend routine administration of methylprednisolone [14]. A comprehensive study of trials from 1948 to 2011 has suggested that high doses could be responsible for improving America Spinal Injury Association impairment scores by up to four points [15]. Higher dosages are possibly linked to increase mortality and complications, such as sepsis, pneumonia and gastrointestinal bleeding. A recent investigation of randomized trials failed to find significantly improved motor outcomes in methylprednisolone recipients after accounting for the level of initial injury as well as baseline neurological function [16]. Though methylprednisolone is by far the most administered and studied (if not most contested) neuroprotective agent for SCI, several other compounds are currently undergoing clinical trials [13,17].

Palliative innovations

Because chronic SCI is associated with a broad spectrum of symptoms, there are numerous theoretical and active therapies available, focusing specifically on managing SCI spasticity, pain, bladder control, diaphragm pacing as well as other symptoms [18–23]. Phenomenal advances in technology have given us possible solutions to SCI's hallmark symptom, paralysis. A small cohort of patients with tetraplegia was able to control a robotic arm attached to their motor neurons via a microelectrode array. Comfort and improvement of neural-interfacing prostheses (and neural prostheses themselves) await advances in computing and engineering, potentially offering drastic improvement in quality of life for some tetraplegic patients [24–28]. None of these innovations are cures for the disease; but the many symptoms associated with destruction of CNS structures necessitate varied and creative medical solutions.

Growth factor manipulation

Targeting various neurotrophins with the aim of neural regeneration has been met with mixed results. In one such example, rats were treated with hyperexpression of BDNF after hemisection at the C5 segment. Axonal regeneration was observed but neuroexcitatory effects interfered with (and actually worsened) motor outcomes [29]. Axons may be coaxed to elongate, but adult CNS axons often behave dysfunctionally as they are not genetically primed for development or regeneration. However, tight spatiotemporal control over competing growth factors offers many insights into neural regeneration and synaptic remodeling [30].

Downstream intracellular pathways have been studied to better develop successful therapies. Interest has been shown in the PI3K–Akt–mTOR pathway, which is implicated in the activation of growth cones and the development of the dendrites and the spines. The mTOR pathway is linked to neural protein production as well as cell soma size; as may be expected, it is also linked to cancer development [31].

Rehabilitation & endogenous sprouting

Physical rehabilitation is the current standard for ensuring the best prognosis after SCI. Numerous studies support findings that acute exercise and training can significantly increase locomotor function by preserving functional synapses and allowing modest endogenous regeneration. Locomotor training was shown to beneficially modulate the expression of NGF by the mRNA output of adult rats after T10 contusion [32]. Physical training in rats has also been associated with a beneficial inhibition of allodynia-related c-fibers after C5 contusion [33].

Studies have shown that results of physical therapy could be enhanced by combinatorial therapies with technological approaches. Epidural electric stimulation in combination with growth factor cocktails enabled rats with dual opposite hemisections (T7 and T10) to regain plasticity and form functional relays after training [34].

Vasudeva *et al.* reported that chronic human patients with complete motor lesion regained function after epidural electric stimulation, and eventually advanced to stand and step training [35]. Regaining function is a great advancement, begging for a better understanding of cellular regeneration and endogenous electric cues. We believe that the study of these two fields may be key to developing a strong regenerative therapy.

Cell therapy for SCI

In the past two decades, undifferentiated precursor cells, or stem cells, have been proposed as a therapy for SCI. Purported benefits include replacement of neural and glial cells, modification of wound environment, aid for surviving cells, promotion of angiogenesis and construction of neural scaffolding [36]. Initial rodent studies have given some credence to these claims. Rat-derived multipotent adult progenitor cells have been shown to combat inflammation and support neurite outgrowth in rat-derived cultures of dorsal root ganglia (DRG) [3]. Human-derived induced (nonembryonic-derived) neural-class stem cells have been reported to differentiate, integrate and ultimately improve motor outcomes (shown by Basso Mouse Score, rotarod test, digigait and testing for motor-evoked potentials) in murine models after T10 contusion. Implanted cells developed independently into all three neural classes: neuron, astrocyte and oligodendrocyte. Furthermore, an abundance of GABAergic neurons was reported. GABAergic repopulation may be a key strategy in SCI pain and spasticity management [37]. Various robust human-derived primitive neural-class cells were shown to differentiate into adult neurons (especially serotonergic neurons). These neurons integrated and extended axons rostral and caudal within the spinal cords of adult rats after lesion after complete transection at C4 [38].

Intrinsic properties of neural stem cells may trump the necessity of extensive pharmaceutical remodeling in the hostile wound environment. Stem cells showed little adverse response to the post-SCI inhibitory milieu. Various cell classes (rat embryonic-derived neural stem cells as well as human embryonic-derived HUES7 and 566RSC neural stem cells from NeuralStem Inc. [MD, USA]) survived implantation to fibrin matrices at sites of complete T3 lesion. Differentiated axons extend in all directions (including through adult white matter) with distances up to 25 mm (566RSC). These axons form functional synapses, which improve motor outcomes (all cell classes) [39]. The presence of neural precursors at the wound site tames inflammatory phagocytes by altering gene expression. Implantation of mouse-derived BV-2 microglial precursors at subacute and early chronic stages after T12 contusion in mice has shown significant modulatory action upon several inflammatory genes, revealed by mRNA markers. Genes for astrogliosis show upregulation. Furthermore, undifferentiated neural precursor cells (NPCs) form junctions with macrophages, which lead to reduced M1 (neurotoxic) activation [40].

Neural precursors also exhibit the ability to repair dysfunctional myelin segments. One major goal of stem cell transplantation in SCI is to achieve the axonal myelination necessary for normal axonal functions. With remyelination, axons can also re-acquire a normal molecular arrangement [41,42]. Transplantation in shiverer mice resulted in adult NPCs (aNPCs, mouse subventricular zone [SVZ]-derived) differentiating to oligodendrocytes. Post-transplantation, mice exhibited improved ion profiles, conduction velocity and activation thresholds [43]. Therefore, some studies are now focusing on direct transplantation of cultured aNPCs to the injured area to improve remyelination [41–42,44]. Remyelination research probes a variety of cell types in addition to aNPCs, including embryonic stem cells, mesenchymal cells and nonstem cells including olfactory ensheathing cells and Schwann cells [45,46]. Another cell class exhibiting promise for SCI-based therapy is the Asterias Oligodendrocyte Precursor. Injections of these cells have reduced cavitation at the injury site, improved myelination and supported neural outgrowth. Asterias Oligodendrocyte Precursor cells have recently been approved for human clinical trials after preclinical studies, which have shown very low risk of tumorigenesis, unwanted migration or hypersensitivity [47].

A handful of clinical studies are underway to assess the safety and efficiency of cell therapy for SCI (Table 1). Stem cell therapy remains unapproved in several countries with advanced medical systems due to serious concerns regarding safety and efficacy. Many unknowns will have to be addressed before a viable therapy can be implemented.

Steps needed to advance stem cell therapy

Several unknowns remain before the therapeutic benefits from stem cells can be realized. NPCs respond with great variance due to location of injection, growth factor cocktails and time postinjury [48]. Because the post-SCI microenvironment is dynamic, cell therapy protocols will differ between chronic and acute periods, as well as distinct stages within the acute period. Such factors play a pivot role in not only cell survival but also the fate of cell differentiation and integration [49].

Implantation and successful integration of a living cell population demands careful controls for several factors. Many growth and inhibitory factors are known to control cell growth, but their precise application would be difficult due to the nature of chemi-

Table 1. Neural precursor cell, clinical trials for spinal cord injury†.

Primary sponsor (contact location)	Status	Study type	Last verified	Ref.
Federal Research Clinical Center of Federal Medical and Biological Agency (Moscow)	Recruiting	Phase I and II	October 2015	[63]
StemCells, Inc. (Calgary, Toronto, Zurich)	Completed (no results posted)	Phase I and II	June 2015	[64]
StemCells, Inc. (Downey, Miami, Chicago, Baltimore, Ann Arbor, New York City, Philadelphia, Pittsburgh, Houston, Salt Lake City, Milwaukee)	Terminated (no safety concerns cited)	Phase II	May 2016	[65]
StemCells, Inc. (Zurich)	Terminated (no safety concerns cited)	Long-term follow-up observation	May 2016	[66]
Neuralstem, Inc. (San Diego)	Active (not recruiting)	Phase I	August 2015	[67]
Asterias Biotherapeutics, Inc. (Los Angeles, Stanford, Atlanta, Chicago, Indianapolis, Milwaukee)	Recruiting	Phase I/II	August 2016	[68]
Chinese Academy of Sciences (Tianjin)	Recruiting	Phase I/II	February2016	[69]

Listing of clinical trials for spinal cord injury therapy with neural-class stem cells registered with clinicaltrials.gov.
†Information accessed from clinicaltrials.gov.

cal diffusion. Clinicians are concerned with cells reaching target areas (and extending through hostile environments such as glial scars). Clinicians are also concerned with methods to manipulate migration in order to keep cells within the target area. For example, neural precursors may affect unintentional targets and form dysfunctional or harmful synapses [50]. Wandering cells present the dangers of malformed synapses and tumor genesis. Cell therapy will not be safely regulated and implemented until an efficacious method of controlling cell growth and migration is presented [51].

Current knowledge is limited by *in vivo* tracking of stem cells. MRI efforts have shown the ability to track cells labeled with superparamagnetic iron oxide for up to 3 months. Unfortunately, this method cannot yet distinguish between living and deceased cells [52]. A combinatorial approach using both nuclear medicine imaging and optical imaging might assess cell fate with greater precision and accuracy [53]. Unless a novel imaging strategy presents itself, stem cell researchers will have difficulty determining the migration and integrational fate of stem cells *in vivo*.

Bioelectricity in the neuronal tissue & its application

Biological tissues maintain their normal homeostatic functions by utilizing electric fields. Cells maintain integrity via membranes that are interspersed with selective ion channels and transporters. Various configurations of ion channels and transporters endow each cell with a characteristic bioelectric state, even in nonexcitable cells. This bioelectric state can be expressed *en masse* by a tissue (such as the transepithelial potentials generated by the skin) or singularly as the resting potential of a cell. In addition, some neuronal cells also communicate via 'ephaptic interactions' – neuronal firing in a specific neuron, or a neuron group, that can alter the external electric field and in turn, further affect the membrane potential of neighboring neuronal or glial cells.

Bioelectric properties are known to be associated with cell migration, growth, differentiation and apoptosis [54]. Long distance electrotactic migration has been observed in the healthy adult mammalian brain. An endogenous voltage gradient exists between the subventricular zone and the olfactory bulb, which plays significant roles in guided migration of adult neuroblasts [55].

Therefore, a deliberately applied electric field could control the fate of cells in certain physiological and pathological simulations. As early as 1920, neurites were found to align along direct current (DC) vectors when subjected to constant electric current [56]. Later studies in the 1980s further illustrated that neurite orientation, neurite outgrowth and cell body migration could be controlled by electric fields [57,58]. Here, we review several fields of study in which the effects of electromagnetic fields have manifested critical cellular reactions.

Development

Endogenous fields have been identified as instructive cues during morphological development. Perturbation of these endogenous fields leads to malformation of the embryo [59]. In the developing CNS, distinct voltage gradients are formed ventrally and dorsally early in the blastomere stage (*Xenopus laevis*). These gradients maintain significant control over early neural tissue differentiation. Hyperpolarization of ventral blastomeres will increase cell proliferation in the embryonic spinal cord. In contrast, hyperpolarization of dorsal blastomeres will result in significant upregulation of apoptosis-related activated caspase-3 within the developing spinal cord [60]. Ion channels, which maintain and adapt to cell voltage, are necessary for cell-to-cell communication in nonexcitable cells. Removal of individual ion channels that regulate the membrane potential will result in the loss of function or other developmental malformation [61]. Manipulation of transmembrane potential is sufficient to induce whole-scale organogenesis (well-formed eyes outside of the neural field) in *Xenopus* embryos (Table 2) [62].

Wound regeneration

Endogenous electric fields not only play significant roles in development but also in regeneration. When the skin of an animal is perturbed, natural transepithelial potentials are perturbed. The wound edge builds up a voltage of approximately 50 mV. This leads to a healing response in which epidermal epithelial cells proliferate and migrate toward the wound edge [73,74]. The transepithelial potential at a wounds edge will also stimulate peripheral nerve sprouting toward the wound edge [75]. With respect to SCI models, there is evidence that electric signaling modifies wound response in deeper vascular tissues as well. Following aortic injury in a rabbit model, low-level DC current was significantly associated with reduced collagen expression [76]. We were unable to find any existing research concerning electrotactic signaling associated with trauma to nervous tissues.

Spinal cord injury

Despite unclear mechanisms, guinea pig models of thoracic hemisection subjected to electric current therapy have yielded functional recovery [77]. A recent study with a rat model (chronic contusion at T10) confirms that electromagnetic stimulation significantly improved functional outcomes. Electromagnetic stimulation prior to exercise training and growth factor application improves functional outcomes, measured by histology, electrophysiology and behavioral testing [78].

Signal transduction in electric stimulation: mechanisms of action

Although publications describing the movement of cells under the influence of an externally applied electric field can be retrieved from the 1920s [79], the underlying mechanisms of electrotaxis in its many cell classes and pathways are not yet fully understood. Regarding cellular migration, many leading theories purport Ca^{2+} signaling as crucial. Under DC electric fields, Ca^{2+} will move to the anodal side of the cell, eliciting myosin contraction and advancing of the rear. The relative paucity of Ca^{2+} at the cathodal end promotes actin stability and polymerization along the leading edge [80]. Variances in kinematics among the cells are reasoned by differing expressions of voltage-gated ion channels, cotransporters and exchangers, which result in dynamic intracellular Ca^{2+} waves [81].

Beyond Ca^{2+}, cell migration could also be altered by factors presented in the cellular structures: EGFR, ion channels and actin proteins. EGFR and actin proteins will aggregate along the cathodal edge of a cell under an electric field [82,83]. Ion channel localization appears to be necessary for the cell's directional sensing [84]. $K_v1.1$,

Table 2. Electrotactic kinematics of human stem cells.

Study	Year	Model	0 mV	50 mV	100 mV	200 mV	300 mV	Ref.
Zhang *et al.*	2011	Induced Pluripotent Stem Cell (3D Matrigel)	3.6 (0.05)	4.8* (-0.045)**	8.4** (-0.8)**	18.6** (-0.85)**		[70]
Zhao *et al.*	2011	Bone Marrow Derived Mesenchymal Stem Cell (2D DMEM)	24 (0)	24 (0.2)*	26 (0.24)*	28* (0.52)*		[71]
Feng *et al.*	2012	Embryonic Derived Neural Stem Cells (2D Laminin)	58 (0)	62 (0.45)*	65 (0.55)**	–	75** (0.7)**	[72]

Speed is shown as micrometer per hour. Directedness (shown in parentheses) as Cosine θ, where θ is the angle between electric field vector and a line drawn over total cell displacement. Directedness was shown to be the greatest for induced pluripotent cells in 3D matrigel; whereas speed was the greatest for embryonic-derived neural cells on 2D laminin.
*$p < 0.05$.
**$p < 0.01$ statistical analysis corresponds to Student's *t*-test compared against a 0 mV baseline for studies from Zhang and Feng.

$K_v1.3$, $K_v3.1$, TRPC1 and dozens of other ion channels affect cellular response [85]. These responses vary from ion-directed migration to voltage-controlled expressions of apoptosis/proliferation/differentiation [86]. Ionic- and voltage-based signaling is also transduced and mediated in a cell-to-cell fashion through gap junctions and serotonergic signaling. Cells utilize bioelectric communication to coordinate not just organogenesis but also the innervation of nerve targets [87].

DC electric field signals are transduced by intracellular mechanisms toward cohesive cellular response such as concerted migration or epigenetic change in cell state. Activity of GC and PI3K is observed in regulating intracellular Ca^{2+} and actin activity, respectively. In a simple protista, simultaneous inhibition of these processes will initiate a reverse toward the anode, while control protista migrate toward the cathode under the electric field [88]. Electrotactic movement within corneal and epithelial cells is regulated by the ERK family of MAPK downstream of EGFR [83]. Corneal cells are driven to orient by EF cues as well as mechanical cues such as contact guidance. When presented with opposing directional cues, GTPase activity is responsible for processing the signals. Inhibition of GTPase Cdc42 abolished electrotactic response, whereas inhibiting GTPase-Rho selected against contact guidance [89]. Within the spinal growth cone electrotaxis is observed. However, it is abolished when CRIB domains (within Cdc42 and Rac GTPases) are chemically blocked, evidenced as no filopodial or lamelipodial orientation occurs [90]. Rho-associated protein kinases are also implicated in the electrotaxis of human-induced pluripotent stem cells. Inhibition of Rho-associated protein kinases will decrease the directedness of an EF response but increase cell track speed [91].

Control neural precursors with electric field: *in vitro* evidence

Stem cell migration under electric field

There is a blooming body of study concerned with the electrotactic responses of NPCs, an ideal candidate for stem cell therapy in SCI. Adult mammalian NPCs migrate with the aid of electric fields in the CNS – a selective gradient is maintained along the rostral pathway from the subventricular zone to the olfactory bulb. In mice, these cells respond to an electric field as low as 3 mV/mm, and electric field maintained by ATPase activity. Calcium-modulating purinergic receptor P2Y1 was found necessary for maintaining directedness in this process [55].

In vitro studies have reported that cells differentiated from aNPCs (mouse derived from the subependyma) will remain static within a DC EF while undifferentiated counterparts exhibit electrotaxis. *In vitro* studies have also been repeated with special chambers where cells are perfused with a constant fluid current against the direction of DC EF. These studies reveal that migration is due to electrotaxis alone, rather than the formation of extracellular chemotactic gradients [92]. *Ex vivo* studies performed with mouse-derived aNPCs and mouse spinal cord slices suggest that the electrotactic response of the neural progenitor cells still exhibited strong cathodal migration in 3D [93].

Electrotaxis in both adult and embryonic rat-derived neural stem cells requires EGF and FGF. Greater DC electric field shows positive correlation with migration speed and directedness values. Intracellular cathodal migration of EGFR, PIP3 and actin, were observed within 1 min of DC EF application. An increase in EF strength was correlated with upregulation of PIP3 and AKT phosphorylation. Inhibition of PIP3 was found to decrease electrotactic migration in NPCs [94].

Stem cell differentiation under electric field

The abilities of stem cells to differentiate into the desired cell types, migrate into the target area and functionally integrate with the existing tissue are the ultimate goals for stem cell therapy. In addition to the intensive studies on stem cell migration, electric fields have also be shown to be capable of guiding various stem cells into neuronal lineage.

Properties of the electric field seem to play a significant role for the differentiation. For example, an intermittent and systematic DC electric stimuli could guide human mesenchymal stem cells toward neural-like cells [95]. By contrast, an alternating electric current (AC) [96], or a pulsed electric field in combination with an optimized biochemical microenvironment) [97], introduced osteogenic differentiation of human mesenchymal stem cells. Another example, when monophasic- and biphasic-pulsed electric fields were applied to the human cardiac progenitor cells, they induced early differentiation of the cells toward a cardiac phenotype. Interestingly, only the biphasic fields showed effectiveness in the upregulation of cardiac transcription factors [98]. Frequency of the field is also a major parameter in inducing cell differentiation. Osteogenic differentiation of human adipose-derived stem cells depended on the frequency of the applied electromagnetic field [99].

Electric field has also shown potential in promoting neural stem cell differentiation toward neurons, and enhance their maturation. For example, short duration electrical pulses at physiological intensities (0.53 or 1.83 V/m) were effective in enhancing neurite outgrowth and maturation of adult rat neural stem progenitor cells [100]. Rat NPCs treated with a 437 V/m DC electric field had a greater tendency to differentiate

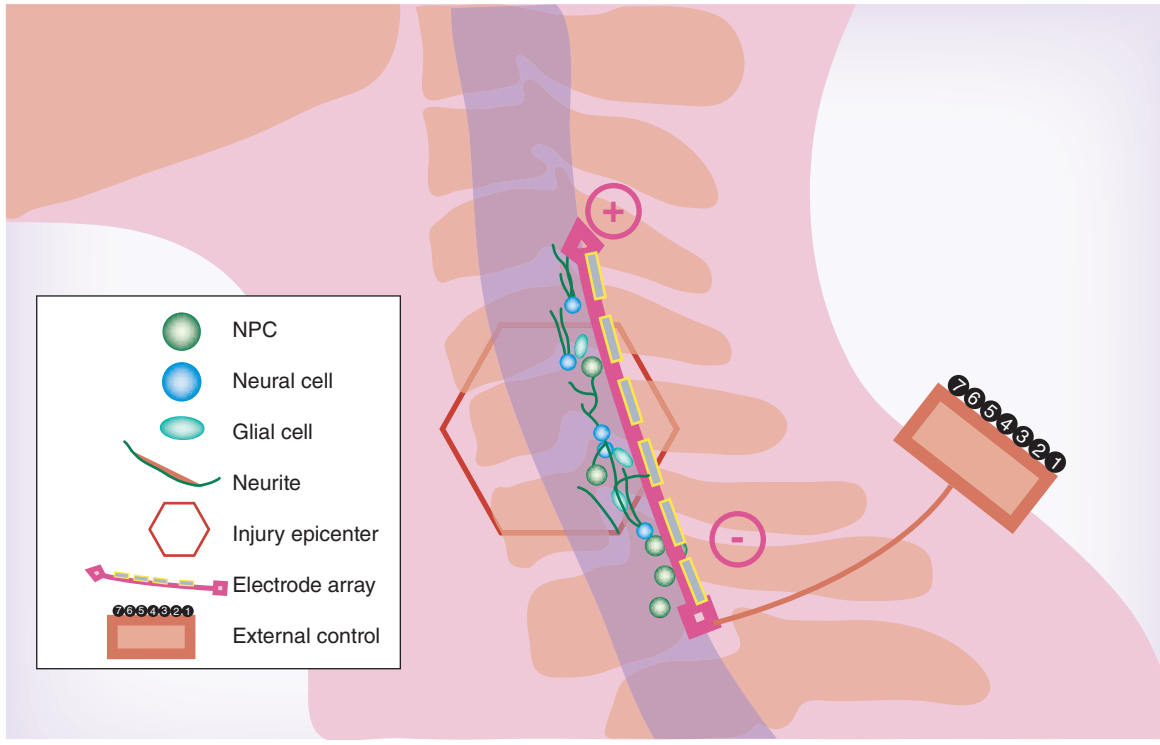

Figure 1. Direct current electrode. Representation of the electrode that is placed dorsal longitudinal to enhance cell therapy in spinal cord injury. Numbers along external control represent individual voltage controls for each electrode band. Here, a rostral current is shown along the dorsal axis of the spine.
NPC: Neural precursor cell.

into neurons, rather than oligodendrocytes or astrocytes [101]. A single electric field that can both control stem cell migration and induce desired differentiation would hold significant potential for stem cell therapy. Recently, we found that a DC field (115 mV) could both induce migration in mouse NPCs and encourage their differentiation into neurons [102].

These discrete phenomena support the idea that regeneration of the CNS could be enacted by NPCs and safely guided by the clinician's control with electric fields. As the kinetics of NPC electrotaxis are not fully resolved, the best method of electric field modulation for cell therapy has yet to be established. A few methods have been proposed.

Technology for application

Electric control of transplanted NPCs in SCI

Researchers exploring electric control of biological tissue could benefit from the existing body of research concerning functional electric stimulation. Several tools and guides are available concerning electric application for epidural stimulation of spinal sensorimotor circuits [103]. A Phase I clinical trial has approved DC stimulation for SCI by direct surgical implantation of electrodes. Such devices are currently used for functional electric stimulation. Gross voltaic manipulation of the wound area may also stimulate neurotrophic cues associated with the developmental voltaic state. *Xenopus* embryos show that modulation of EF is sufficient to switch between apoptotic and proliferative states in spinal cord development [60]. If the electric field extends to the area of local astrocyte populations, then they could align and create a naturally favorable substrate for neurite growth (experimentally shown *in vitro* with rat astrocyte and DRG cultures) [104]. Under electric stimulation, rat-derived Schwann cells exhibited a 30% increase in neurite outgrowth and an 11-fold increase in NGF secretion [105].

The devices could be adapted to synergistically aid in cell therapy, to enhance stem cell migration, differentiation and interaction with the local neural circuits (Figure 1). Current practice involves placing electrodes adjacent to the vertebral canal to protect cells from excess heat, including both the cells of the spinal cord and the transplanted cells. Possible disadvantages of this approach include the damage to superficial tissues, limited strength of EF due to the resistivity of deeper tissue and limited precision due to distance from the target [106]. Recently, murine-derived NSCs were grafted in a healthy mouse brain that was subjected to transcranial DC stimulation. Cells increased in general migration, but no difference in directedness was found in comparison with the control group. It is unclear whether control of directedness persists in a

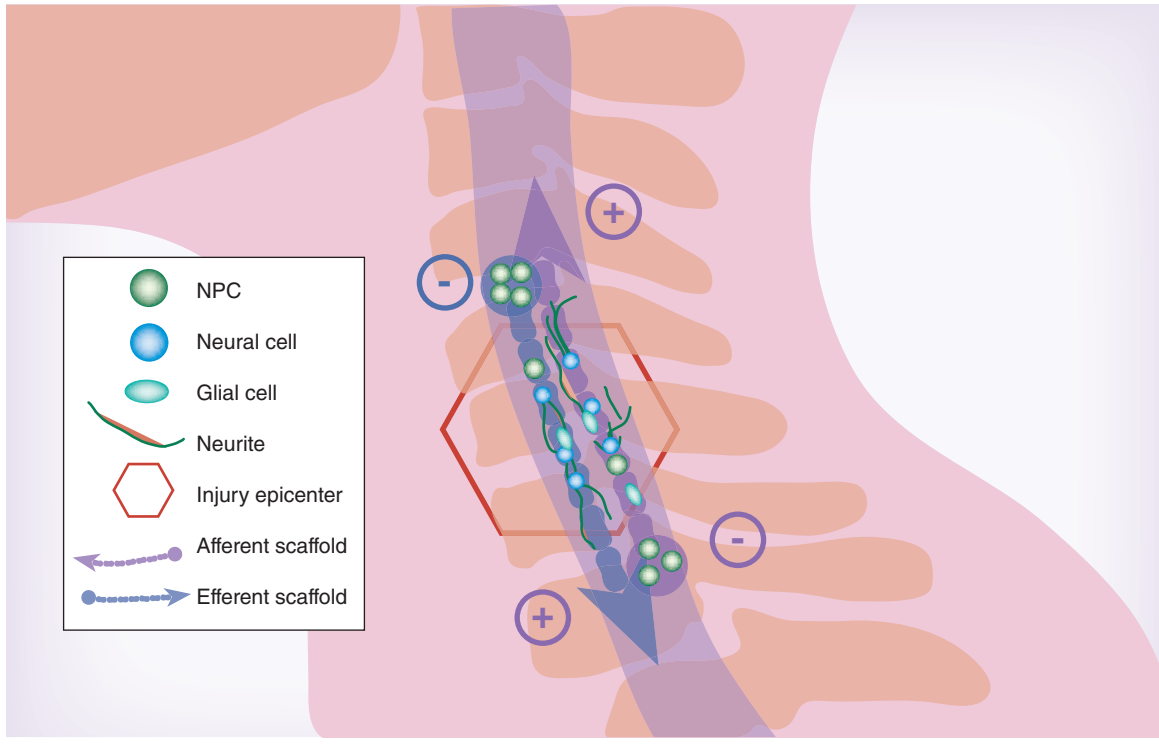

Figure 2. Conductive scaffolds. Two conductive scaffolds bridging glial scar for both afferent and efferent axonal tracts. A piezoelectric material would be self-powered and surgically immersed. Alternatively, an external power supply could be utilized to afford control of voltage.
NPC: Neural precursor cell.

fundamentally different environment such as the spinal cord (healthy or damaged), or whether the outcome may be changed by varying cell class, growth factors, grafting technique or EF application [107].

Conductive scaffolds for the transplanted NPCs in SCI

To enhance the outcomes of electric stimulation in cell therapy, electrically conductive scaffolds could be introduced and combined with the stimulation protocol (Figure 2). Several bioengineering labotoratories have developed these scaffolds for neural regeneration under the electric stimulation. These scaffolds have been purported to support damaged axons, nerve transplants and the transplanted stem cells. An advantage of scaffolds over the direct electrode approach would be the ability to manifest two parallel and opposite voltaic gradients to guide the simultaneous formation of both afferent and efferent neurons. Materials are carefully developed for neural adhesion, biocompatibility and conductivity. Materials under consideration include polypyrrole, polyaniline, polythiophene derivatives and carbon nanotubules [108].

A variation of the conductive scaffold is the piezoelectric scaffold, a material that autonomously generates electric charge as a result of mechanical deformation. Such a variation has the advantage of freedom from an external power source. However, it lacks the specificity and precision of bioelectric control afforded by conductive scaffolds and is more difficult to produce [Gascon MNR. Neuroplasticity of spinal cord neurons based on piezoelectric stimulation and electrophysiological analysis after stem cell-derived progenitor transplant (2011) unpublished data].

Mechanical confinement, provided by a conduit or scaffold, may enhance the effects of galvanotactic migration. Neural scaffolds have received significant attention over the years. However, the relatively new combination of scaffold and electric stimulus has been shown to outperform existing nonconductive scaffolds in neurite proliferation [109]. Huang *et al.* reported increased velocity, persistency and directedness associated with the confined cells in the electric field [110]. Conductive scaffolds offer several advantages in developing cell therapies for SCI, such as the ability to chemically dope for adhesion factors and growth factors, the ability to control axonal trajectory over relatively large distances and the ability to confine cell proliferation. Challenges moving forward include improving cell adhesion and testing for biocompatibility and cytotoxicity beyond the acute period.

Magnetic control of transplanted NPCs in SCI

An alternate noninvasive strategy that could potentially replace electric stimulus, is the time-varying magnetic

field, which induces electric current inside the tissue (Figure 3). Transcranial magnetic stimulation (TMS) is a technology that was originally developed to stimulate the deep brain for the treatment of depression [111], seizures [112,113] and Parkinson's disease [114]. This technology can induce peripheral motor response and is used in eliciting MEPs downstream of damaged neural circuity, enabling possible identification of residual spinal connectivity [115]. It is worth noting that TMS technique has the precision to stimulate discrete structures within the rat CNS [116]. Because magnetic fields experience minimal attenuation as they travel through tissues, the induced current thereof can travel deeper than current generated by external electrodes [117]. This technique would bypass the necessity of any surgically invasive implants, reducing risk of discomfort and infection. Under low frequency time-varying magnetic fields (including frequencies for TMS), a cell experiences negligible surface pressure and translational force. Surface charges on the cell membrane are induced, however, such charges are significantly smaller than intrinsic charges on cell proteins under physiologic conditions [118]. These observations suggest that no adverse cellular trauma would results from magnetic fields. Instead, cells depolarize under the magnetic field stimulation [119], which could play significant roles in orchestrating stem cell differentiation. TMS offers a method to induce electric current within a tissue with readily controllable location and intensity, and low risk to the subject (Figure 3).

Optogenetic control of transplanted NPCs in SCI

Rhodopsin family proteins (such as ChR2 and Arch) form light-sensitive ion channels. Selective genetic engineering can, in turn, induce these proteins to depolarize or hyperpolarize individual cells in response to LED light (Figure 4). LED-activated (stem cell-derived) motor neurons were shown to fire specific motor groups. Attenuation to specific light wavelengths allows graded control of response via LED frequency [120]. Recent evidence has shown that this technology can control stem cell differentiation, migration and integration with host tissue. For example, mouse-derived DRG that expressed ChR2 were induced to directional nerve growth by optically controlled depolarization in culture. Optical depolarization stimulated release of NGF and BDNF, which in turn, directed responses among neighboring cells [121]. Arch-based H^+ pumps can directly hyperpolarize cells, revealing a bioelectrically controlled regenerative state within *Xenopus* embryos [122]. The light-sensitive and pH-neutral

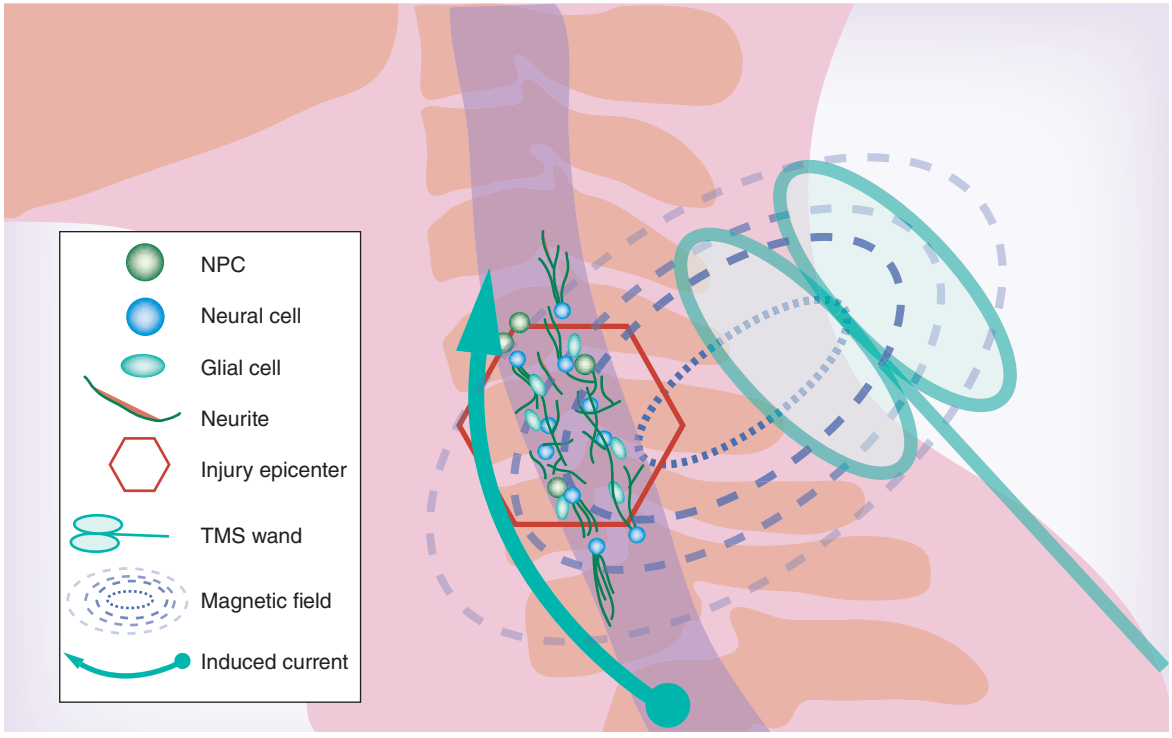

Figure 3. Transcranial magnetic stimulation/neural precursor cell therapy. Apparatus for transcranial magnetic stimulation applied to spine. An external magnetic field generator placed near the patient emits a magnetic field, which can safely travel into deep tissues. A direct current field is induced in the plane perpendicular to the magnetic field, producing a positive electrotactic effect.
NPC: Neural precursor cell; TMS: Transcranial magnetic stimulation.

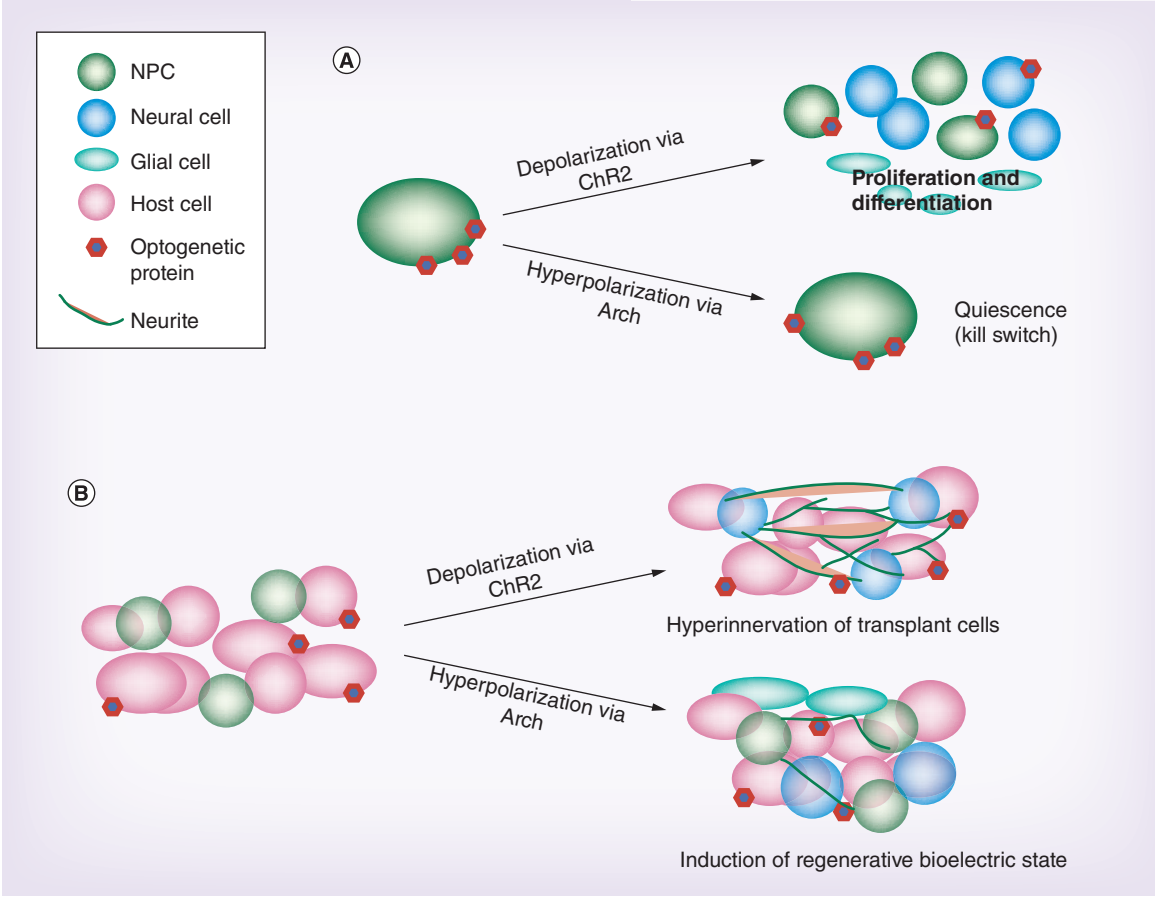

Figure 4. Infographic of optogenetic controls. Theoretical applications of optogenetic technology for cell therapy in spinal cord injury. **(A)** Opsin proteins engineered into NPCs enable cellular depolarization (resulting in cellular proliferation and differentiation into neural and glial cell classes) or hyperpolarization (resulting in quiescence). **(B)** Opsin proteins engineered into a host or 'scaffold' cell allows control of cellular resting potentials via manipulation of endogenous ion pumps. Depolarization of host tissue via ChR2 was shown to result in hyperinnervation of transplanted CNS structures [87]. Hyperpolarization of host cells (via Arch) in an embryonic amphibian model was shown to rescue a regenerative proliferative state, manipulating resting membrane potential by approximately 20 mV and upregulating expression of Notch1 and Msx1 [122].
NPC: Neural precursor cell.

H^+ pumps expressed on NPCs could control cell cycle via manipulation of membrane potential. Theories suggest that voltage-gated channel activity is sufficient to switch between proliferation and quiescence for many cell types [86,123–124]. We speculate that a direct modulation of membrane potential could be employed to guide stem cells by surgical implantation of an LED microarray to aid cell therapy in SCI.

Alternatively, a support cell population could be selected and optogenetically controlled as a biological scaffold in cell therapy in SCI. This supporting-bioscaffold could be populated mesenchymal stem cells or a glial-restricted cell line. Expression of light-sensitive and pH neutral H^+ pumps by a large group of cells could create an endogenous electric field. When endogenous voltage gradients are perturbed, *Xenopus* embryos lose their natural regenerative functions. Hyperpolarization of mesenchymal stem cells by Arch-activated ion pumps can rescue the regenerative state in the perturbed embryos, which is regulated by the activation of downstream effectors Notch1 and Msx1 [122]. Other *Xenopus* studies also show that bioelectric manipulation can induce hyperinnervation of transplanted CNS structures (the primordial eye). Depolarization of host cells initiates movement of 5-HT through gap junctions into the relatively negative transplant cells and assists the innervation the transplanted cells into the existing tissue [87]. Thus, light signals could possibly be transduced to DC or ionic cues for a regenerative NPC population.

It is worth noting that optogenetic manipulation could be conducted with a novel imaging strategy that assesses stem cell integration and functionality *in vivo*. Human-induced pluripotent stem cells expressing opsins were injected into the rats, and neurons descended from this line were selectively activated

by LED. High-field functional MRI of transplanted cells during stimulation was performed to visualize the cells. This allowed the successful detection of local and remote neural activity, enabling the assessment of the global graft-host neural circuit functions [125].

All hypothetical optogenetic therapies would require trans-species gene therapy, which has yet to be tested for medical safety. This may change in the near future. A Phase I/II clinical trial is currently recruiting for administration of ChR2 into the retina (Retrosense Therapeutics, clinicaltrials.gov ID: NCT02556736).

Cautions

Tumorigenesis

The formation of cancers is a reasonable concern for any cell therapy. The concern is heightened for any theoretical therapy, which may use donor cells subjected to gene therapy. Given that electric signaling informs development throughout many organisms, the same consideration for oncogene activation should be considered. Experiments have shown depolarization can induce cancer-like behavior in amphibian melanocytes [126]. Furthermore, studies implicate electric signaling with metastasis of both rat (Walker Carcinosarcoma 256) and human (SCP2) cancer cells [127,128]. Some researchers believe that parsing out the relationship between oncogenes and bioelectrics will improve our methods of screening cancers. However, until reliable pathways are deduced or disproven, researchers are advised to proceed with caution [129].

Procedural considerations

The same risks for any invasive surgical implantation are present for these hypothetical therapies. Obvious risks include risk of infection as well as risks of rejection for hardware (scaffolds and electrodes) and cell populations. Tissue damage could also be a factor after prolonged exposure to heat/electrotoxicity from implanted devices [106].

Furthermore, if the proposed techniques are attempted before a thorough study of electrotactic parameters for various cell classes, the technique would be just as dangerous as an unmeasured application of neurotrophins. The subject would be at risk for malformed synapses, the interruption of functional circuits and other possible neurological deficits.

Conclusion

Few illnesses can rival the tragic prognosis of a severe SCI. The body of research and data for SCI is enormous and ever-growing, but treatment options remain scant. Numerous and contradictory chemical signals in the postinjury environment obscure pharmaceutical solutions. Stem cell therapy has shown promise,

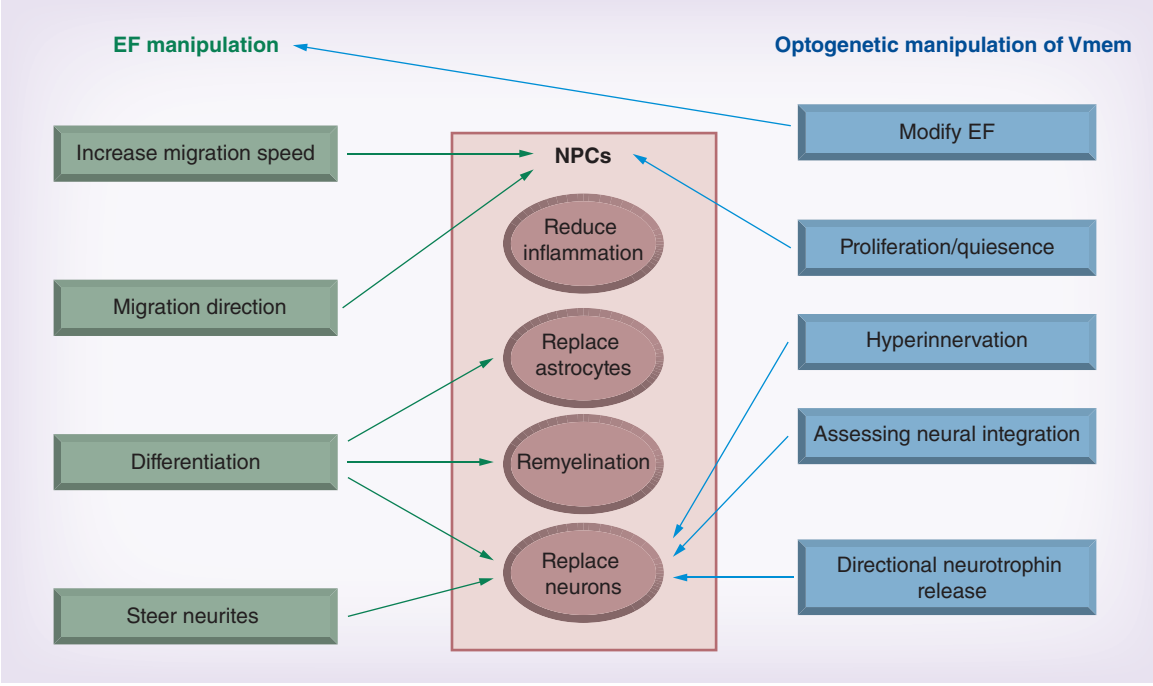

Figure 5. Summary of proposed electrotactic controls for neural precursor cell therapy. It summarizes proposed benefits of NPC therapy as well as highlighting modes of electrotactic technology for guiding cell therapy. In green, modes are shown by electrode manipulation of direct current EF. In blue, modes are shown by optogenetic control of Vmem.
EF: Electric Field; NPC: Neural precursor cell.

but greater scientific understanding is needed before clinical application is possible. Bioelectric cues have shown to be instructive in regard to development, regeneration and specific behaviors of NPCs. Stem cell therapy combined with electromagnetic stimulation would be a novel strategy for SCI and may become possible with a greater understanding of the biophysical cues that inform developing cells and regenerating systems.

Future perspective

Theoretically, directing NPC therapy with electric cues could provide several advantages. Safety of cellular therapy would be improved if electric variables are identified in the migration speed, migration directedness, differentiation and apoptosis. Perhaps cells could be engineered with an optogenetic kill switch in the event of tumorigenesis. Cell behavior could be controlled precisely in real-time, rather than relying upon the diffusion of pharmaceutical neuromodulatory agents throughout the complicated post-SCI environment. Transplant efficiency and the survival of expensive donor cells would be improved with more reliable migration, differentiation and integration. A clinician might guide migration and differentiation of cell populations to best suit the varying needs of the individual patient. The most ambitious application would occur as optogenetic- or microelectrode-guided regeneration of the individual synapse.

These possible benefits are currently unsubstantiated. But if the phenomena that occur *in vitro* and *ex vivo* were to be observed and consistently replicated with *in vivo* models, electrical modulation may prove to be an invaluable tool for the neurosurgeon.

The future of this therapy waits upon further research. Specifically, it requires detailed documentation of electrotactic thresholds, kinetics, side effects and voltage-transduced epigenetic effects. In addition, transitioning to clinical applications will require many years of effort. Clinicians will need technologies for electrotactic control, novel imaging strategies and above all a greater understanding of electrotactic physiology.

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Executive summary

Spinal cord injury

- Spinal cord injury (SCI) is a trauma-induced neuroregenerative disorder with a complicated pathophysiology that often results in life-long debilitation.

Existing treatment options

- Beyond acute stabilization, there is little more than palliative care afforded to SCI patients.

Cell therapy for SCI

- Stem cell therapy shows benchside promise for inducing neural regeneration, glial repopulation and normalization of the wound site.

Steps needed to advance cell therapy

- Stem cell therapy in the CNS is limited by a paucity of clinical knowledge, which is in part due to clinician's inability to see and control stem cells *in vivo*.

Electrotaxis

- Cells of the body respond to electric stimulation.

Signal transduction

- Intracellular ionic movement, ionic channels, chemical messengers and epigenetic changes are associated with various electrotactic behaviors.

Modulating neural precursors with electromagnetic waves

- Neural precursors exhibit strong and significant responses to electric fields, such as increase in migration speed, control for directedness in 2D and 3D, and control over proliferation and differentiation.

Technology for application

- The combination of cell therapy and electrotaxis could be realized as a therapy using technologies such as direct electrode implantation, electromagnetic current induction, conductive scaffolds for tissue engineering or optogenetic manipulation of tissue. Each method presents advantages as well as technical challenges.

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