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The regenerative effects of electromagnetic field on spinal cord injury

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\section*{ABSTRACT}

Traumatic spinal cord injury (SCI) is typically the result of direct mechanical impact to the spine, leading to fracture and/or dislocation of the vertebrae along with damage to the surrounding soft tissues. Injury to the spinal cord results in disruption of axonal transmission of signals. This primary trauma causes secondary injuries that produce immunological responses such as neuroinflammation, which perpetuates neurodegeneration and cytotoxicity within the injured spinal cord. To date there is no FDA-approved pharmacological agent to prevent the development of secondary SCI and induce regenerative processes aimed at healing the spinal cord and restoring neurological function. An alternative method to electrically activate spinal circuits is the application of a noninvasive electromagnetic field (EMF) over intact vertebrae. The EMF method of modulating molecular signaling of inflammatory cells emitted in the extra-low frequency range of <100 Hz, and field strengths of <5 mT, has been reported to decrease inflammatory markers in macrophages, and increase endogenous mesenchymal stem cell (MSC) proliferation and differentiation rates. EMF has been reported to promote osteogenesis by improving the effects of osteogenic media, and increasing the proliferation of osteoblasts, while inhibiting osteoclast formation and increasing bone matrix in vitro. EMF has also been shown to increase chondrogenic markers and collagen and induce neural differentiation, while increasing cell viability by over 50%. As advances are made in stem cell technologies, stabilizing the cell line after differentiation is crucial to SCI repair. Once cell-seeded scaffolds are implanted, EMF may be applied outside the wound for potential continued adjunct treatment during recovery.

\section*{Introduction}

The annual incidence of spinal cord injury (SCI) in the United States is approximately 12,500 new cases each year with a current estimate of 853 per million (SCI-Database, 2014). Primary traumatic SCI results from mechanical impact to the spine, leading to an acute compression of the spinal canal from displaced bone or intervertebral disk, or acute kinking of the spinal cord (SC) at the time of the accident (Levecchi, 2011). After the initial injury, damage to the SC continues in the hours following trauma, leading to secondary SCI. This secondary damage results from the presence of increased free radicals, cell membrane dysfunction, cell death and neuroinflammation, not only within the injured area, but also from intact neighboring SC, leading to neurodegeneration (Austin et al., 2012; Bareyre and Schwab, 2003; Bethea and Dietrich, 2002; Jones et al., 2005; Olson, 2010; Patel, 2010). Injured spinal cord rarely, if ever, heals properly. Pro-inflammatory macrophages potentiate a prolonged inflammatory phase and remodeling is not properly initiated, whereby macrophage activation is a likely contributor (Donnelly and Popovich, 2008). Microglia phagocytose damaged material within the first 24 h after SCI (Greenhalgh and David, 2014). When peripheral macrophages from the circulation influx into the injured spinal cord 2–3 days later, they immediately begin to phagocytose damaged axons and other debris; and by day 7, when they reach maximum numbers, they far outplace microglia in phagocytosing damaged tissue (Greenhalgh and David, 2014). Indefinite presence of both macrophages and microglia can contribute to the secondary effects of SCI, whereby neuroinflammation leads to scarring (Yuan and He, 2013) and nerve damage (Silver, 2004b).

To date there is not a Food and Drug Administration (FDA)-approved pharmacological agent that prevents the development of secondary SCI and induces regenerative processes aimed at healing the spinal cord and restoring neurological function (Courtine et al., 2011; Kuffler, 2012). Beneficial effects of therapies such as electric fields (EFs) applied epidural or directly to lesioned spinal cord are well...
documented (Petragna et al., 2015); however, a disadvantage to this technique is the need for surgery and an implanted electrode, resulting in risk of adverse effects. An alternative method to electrically activate spinal circuits is the application of a noninvasive electromagnetic field (EMF) over intact vertebrae. Pulses of EMF can deliver electrical and magnetic stimuli through intact tissue deep into underlying structures, and have been used in studies on patients with complete motor and sensory SCI (Shapiro et al., 2005). In noninjured humans (Gerasimenko et al., 2010) and decerebrated cats (Avleve et al., 2009), EMF at the thoracic level was found to induce locomotor-like movements in legs. In SCI-induced rodents (Ahmed and Wieraszko, 2008) and ganglions neurite outgrowth from spinal neurons and dorsal root system (CNS). PEMF has been reported to enhance has examined the effects of EMF on the central nervous system (CNS). PEMF has been reported to reduce neuroinflammation by regulating the balance of pro-inflammatory and anti-inflammatory cytokines (Gensel, 2015b). While pro-inflammatory cytokines such as interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) are of the M1 macrophage phenotype, M2 macrophage phenotypes include the anti-inflammatory cytokines interleukin-4 (IL-4), IL-13 and IL-10, as well as transforming growth factor-beta (TGF-β). PEMF has been reported to downregulate TNF-α, as well as its transcription factor nuclear factor kappa B (NFkB) (Ross and Harrison, 2013). NFkB is the protein complex that controls transcription of DNA, cytokine production and cell survival. Other pro-inflammatory markers involved in SCI include IFN-γ, where PEMF has been shown to significantly decrease its levels, as well as dramatically increase anti-inflammatory cytokines IL-4 and IL-10 (Luo et al., 2012). In studies by Sandyk et al., the therapeutic effects of PEMF have been reported to show significant effects in the treatment of neurological disorders in frequency ranges as low as 4–5 Hz, using a 7.5 picoTesla (pT) field strength for the treatment of multiple sclerosis (MS), showing improvements in balance, locomotion and gait, bladder function and fatigue (Sandyk and Iacono, 1993;}

**Effect of PEMF on SCI in vitro studies**

Treatment for SCI requires both the downregulation of inflammation along with nerve regeneration. Once inflammation is contained, the regeneration of nerves can begin.

**PEMF modulates neuroinflammation**

Two major barriers to SCI repair have been identified and include the local inflammatory response, known for its neurotoxic potential, and the creation of glial scarring which impairs regeneration (Block et al., 2007; Popovich et al., 1999; Silver, 2004a). A pivotal role for recovery has been attributed to monocytes in the circulatory system that infiltrate the damaged CNS (Rolls et al., 2008; Rolls et al., 2009). Monocyte-derived cells such as macrophages acquire matrix-degrading properties enabling their resolution of the gliarial scar. They have been demonstrated to promote the removal of tissue debris, secrete neurotrophic factors, support axonal regeneration (Kigerl et al., 2009; Ma et al., 2002; Schechter et al., 2011), and terminate local microglial response (Gensel, 2015a; Zhou et al., 2014), as well as produce the anti-inflammatory cytokine interleukin 10 (IL-10) (Didangelos et al., 2014). Microglia reside in spinal parenchyma and survey the microenvironment through the signals of injury or infection. Macrophages are derived from monocytes recruited to injured sites from the peripheral circulation. Activated resident microglia and monocyte-derived macrophages induce and magnify immune and inflammatory responses not only by means of their secretory molecules and phagocytosis, but also through their influence on astrocytes, oligodendro-cytes, and demyelination (Donnelly and Popovich, 2008). Macrophages play a central role in response to SCI, especially in the removal of debris; however, their indefinite persistence at the site of injury inhibits regeneration (David et al., 2015). Both macrophages and microglia are very plastic cells that can change their phenotype drastically in response to both in vitro and in vivo conditions. They can change from pro-inflammatory, cytotoxic cells to anti-inflammatory, pro-repair phenotypes (David et al., 2015). M1 macrophages facilitate innate immunity to remove foreign microbes and wound debris from the injury site, while M2 macrophages exhibit tissue repair properties and show attenuated production of pro-inflammatory cytokines (Gensel, 2015b).
Sandyk R, 1994; Sandyk, 1995) and ischemia (Capone et al., 2014; Smith et al., 2004).

PEMF stimulates nerve regeneration and mesenchymal stem cell differentiation

Recovery following SCI is limited due to axonal damage (Kurnellas et al., 2005), demyelination and scar formation (McDonald and Belegu, 2006). Hemorrhagic lesions can also form, lacking normal neurons and glia, and reducing oligodendrocytes and astrocytes by almost 50% within 24 h after surgery (Rosenberg and Wrathall, 1997). Both Szpara et al. and Vogelezang et al. propose that these neuronal processes have the ability to regenerate when damaged, and the direction in which these processes regenerate are strongly influenced by local environmental signals (Szpara et al., 2007; Vogelezang et al., 2007). Goldberg and Park suggest that one of the unique properties of neurons that enables them to perform their required functions is their ability to relay electrical signals by adjusting their concentrations of positive and negative ions on the interior and exterior membranes of axons or dendrites (Goldberg, 2004; Park and Lee and McLeod, 2007). These electrical neuronal activities are known to be primarily mediated by ions or affected chemicals that modulate their receptors (Kopell and Ermentrout, 2004; Smith and Pereda, 2003). McCaig et al. suggest endogenous electrical signals are co-regulators of developmental and regenerative cell growth and guidance, especially in nerve regeneration (2009). Endogenous electrical signals existing in the CNS evoke voltage-and time-dependent responses, and McCaig et al. envision these endogenous electrical signals can be modulated by exogenous guidance cues such as EMF. PC12 cells are a cell line derived from a pheochromocytoma of the rat adrenal medulla that have an embryonic origin from the neural crest, which has a mixture of neuroblastic cells and eosinophilic cells. This cell line is often used to measure neurite outgrowth after treatment and is a basic model for studying neuronal differentiation. Zhang et al. studied the effect of PEMF on PC12 cells to determine percentages of neurite-bearing cells, average length of neurites and directivity of neurite outgrowth using 50 Hz, 1.36 mT field. They found that pulse duty cycle had a significant effect on neurite outgrowth. Low (10%) pulse significantly inhibited the percentage of neurite-bearing cells, but at the same time increased the length of the neurites, while 100% on-time DC fields had the exact opposite effect (Zhang et al., 2005). They also discovered that neurites were prone to extend along the direction of PEMF with 10% pulse.

Clinical applications of grafted cells into neural cells have produced some beneficial results; however, these cells are limited in supply, and can create the need for immunosuppressant drugs due to rejection. The use of the patient’s adult stem cells for treating SCI has become a focus of regenerative medicine (Brundin et al., 2003; Lindvall and Kokaia, 2006; Martino and Pluchino, 2006). Differentiation of adult stem cells such as mesenchymal stem/stromal cells (MSCs) to a specific phenotype is achieved by culturing them in appropriate culture media under precise conditions; however, precautions need to be considered for using actively proliferating cells in vivo, so that implanted cells and cell scaffolds remain controlled by molecular signals and avoid the development of malignancy (Otto and Sarraf, 2012). Such signals are produced endogenously during human development, and MSC differentiation can be heavily influenced by molecular and biophysical regulating factors present within their environment.

Exogenous EMF has been reported to be effective in the enhancement of osteogenesis (Fu et al., 2014; Yong et al., 2014), chondrogenesis (Mayer-Wagner et al., 2011) and neurogenesis (Kim et al., 2013; Park et al., 2013), with no documented negative effects. Transplanting MSCs into injured lesions has been investigated as a therapeutic approach for SCI. The effects of PEMF on injured rat spinal cord were studied using bone-marrow MSCs (BM-MSCs), with histological analysis revealing significant differences in treatment of injured site compared with control. Results reported that human BM-MSCs incorporated with magnetic nanoparticles can be guided by PEMF to the SCI lesions. At 8 h/day over a 4-week period, treatment promoted behavioral recovery in SCI rats (Cho et al., 2013). EMF was also reported to stimulate neurogenic differentiation of human MSCs. Neurogenesis is the process by which neurons are generated from neural stem/progenitor cells (NSCs). Most active during prenatal development, neurogenesis is responsible for populating the growing brain with neurons (Fuchs and Flügge, 2014). Neurons can be regenerated by the elongation of neuronal processes and the reconnection with other neurons via synapse formation (Horner and Gage, 2000). The formation of neuronal processes has been observed to be influenced by several factors, such as nerve growth factor (Lakshmi and Joshi, 2006; Liu et al., 2007), insulin-like growth factor I (Shiraishi et al., 2006), retinoic acid (So et al., 2006), basic fibroblast growth factor (Zamburlin et al., 2006) and vascular endothelial growth factor (VEGF), in cerebral cortical neurons (Jin et al., 2006).

Bai et al. used a Helmholtz coil to produce a 50 Hz, 5 mT EMF to stimulate BM-MSCs to differentiate into
neural cells when exposed for 60 min/day for 12 days (2013). Results indicated that EMF exposure facilitates MSC differentiation to neural cells expressing neuronal-specific markers and genes. Cells formed synaptic junctions and pulsed post-synaptic currents. They further add that mechanisms promoting MSCs to differentiate to neurons included modulations in the $Ca^{2+}$ ion channel, along with activation of protein kinase C (PKC) signaling pathways. Piacentini et al. also suggest that PEMF promotes the differentiation of MSCs to NSCs via voltage-gated calcium channels (VGCCs) (Piacentini et al., 2008b).

**Effects of EMF on SCI in animal models**

The biological effects of EMF on motor function following SCI have been reported in well-designed studies, using animal models (Crowe et al., 2003b; Das et al., 2012; Hannemann et al., 2014). Investigation of the effect of EMF on axon regeneration, prevention of scar tissue formation and preservation of axon function is essential, as is the downregulation of inflammation. Unlike neurons in the CNS, peripheral nerves have a greater potential to regenerate. Peripheral nerve injury has time-dependent changes which are based on the integrity and function of the target tissue or organ (Walker et al., 2007). The longer it takes for nerve regeneration to occur, the greater the chance of permanent paralysis in the limbs. Also the time it takes for a peripheral nerve to regenerate depends on the severity of the injury (e.g. crush vs. transection). EMF was reported to improve increased regeneration in a crush injury in as little as 6 days with a 2 Hz, 0.3 mT PEMF (Sisken, 1989a). Using this same dosimetry, O’Brien et al. applied EMF to crushed nerve lesions which stimulated the regeneration of rat sciatic nerve by 22% (Sisken, 1989b), and enhanced functional locomotion by the same amount (Walker, 1994a). In these studies the whole rat was exposed to the fields.

In crush injury experiments, EMF wave functions and times of exposure vary. A 2 Hz, 0.3 mT field was shown to enhance functional recovery in 43 days (Walker, 1994b), and enhance regeneration rates using a sinusoidal wave function (Rusovan and Kanje, 1991). Along with 2 Hz, 25 and 50 Hz treatments have been reported to show beneficial effects. Studies to determine whether sinusoidal electromagnetic fields (SEMFs) were as effective as single-pulse, 50 Hz, 0.4 mT fields were found to be comparable (20%) to PEMF using the same crush nerve model (Rusovan and Kanje, 1991). Pretreating the rats with SEMF did not result in a heightened regenerative response found with a 2 Hz, 0.4 mT PEMF signal (Blank, 1995). Times of exposure necessary for beneficial outcomes are dependent on the strength of a rat’s immune system, along with external stress factors such as housing space, lighting that effects circadian rhythms, access to, or competition for food and water, etc. (Health, 2011).

Crowe et al. used a crush injury model to study the effect of PEMF on cats in order to determine whether PEMF results in improvement of motor function after SCI (2003a). Eight adult cats underwent laminectomy at T5/T6, exposing the dorsal surface of the dura mater. The spinal cord was contused using a weight drop apparatus. Animals were randomly assigned to two groups: (1) treatment group using a PEMF of 10 microsecond ($\mu$s) pulse width and 25 Hz frequency. PEMF was delivered via a two-coil device designed to non-invasively expose an area of PEMF stimulation to the dorsal midline of the subjects. The coils were wound together in a parallel figure eight fashion. The device was secured to the dorsal midline of SC-injured subjects with the coils positioned directly above the level of injury (T5/T6). Treatment began the first day after SCI, and was applied for 4 h/day for 12 weeks, or terminated if motor function returned to preinjury levels. Somatosensory-evoked potentials (SEPs) were recorded before SCI, immediately after SCI, 10 min after SCI, and before euthanasia at 12 weeks. Motor functions were tested and recorded weekly by a blinded investigator using an open-field walking score called the Tarlov Scale, developed for testing the functional evaluation of SCI in animal models. Animals receiving PEMF stimulation attained higher Tarlov scores than non-stimulated animals. The mean value of the scores was $4.25 \pm 0.37$ for the stimulated group, and $2.1 \pm 0.2$ for the non-stimulated group. Morphological assessment of lesion volumes was measured where the PEMF-stimulated group presented an overall increased sparing of white matter and smaller lesion volume than the non-stimulated group.

Following mild crush injuries, regenerating axons are often able to spontaneously regenerate without surgical intervention; however, for severe large-gap nerve transection (>4 cm), nerve autografts are implanted to bridge the gap and create a more supportive environment for axonal extension (Portincasa et al., 2007; Reyes et al., 2005). To restore function following injury, regenerating axons must navigate and bridge the injury site to reconnect with appropriate distal targets and peripheral glia (Schwann cells) to remyelinate the regenerating axons (Koppes et al., 2011). A substrate called chitosan (CHIT) has been used to bridge this gap. CHIT is a polysaccharide composite made by treating shrimp and other crustacean shells with alkali sodium hydroxide. CHIT is hypoallergenic and has natural...
antibacterial properties (Oyarzun-Ampuero et al., 2015). In one group of 15 rat subjects, CHIT was used to bridge the defects, while in the treated group both CHIT and PEMF (2 Hz, 0.3 mT) were applied using Helmholtz coils. Exposure times were 4 h/day for 1–5 days. In the control group sciatic nerve was dis-sected and manipulated without PEMF treatment. Nerve fibers in each group were studied at 4, 8 and 12 weeks post-op. Behavioral, functional, electrophysio-logical, biomechanical, gastrocnemius muscle mass findings as well as morphometric indices confirmed faster recovery of regenerated axons in CHIT/PEMF group than in CHIT group alone (p < 0.05) (Mohammadi et al., 2014).

Recovery after transection injury is much slower than after crush injury due to axonal and neural tube architecture disruption. EMF has demonstrated increased functional recovery in transection models, whereby rat sciatic nerve transection showed significantly better functional recovery at 165 days when exposed to 2 Hz, 0.3 mT field, as measured by the sciatic function index (Zienowicz et al., 1991). An example of this type of injury model was performed by Das et al. (2012), who investigated the effect of 50 Hz, 17.96 µT EMF on rat sensory and locomotor function after hemisection of T13 spinal cord (hSCI). Rats were divided into groups which included treatment and sham, then exposed to the EMF for 2 h/day for 6 weeks. Outcomes were measured using Basso, Beattie and Brennham for locomotor function; threshold of tail flick in response to noxious stimuli; simple vocaliza-tion; and neuronal excitability by H-reflex. Results showed a statistically significant improvement in treat-ment group compared with controls, promoting recovery of sensorimotor behavior, including locomotion and bladder control.

An example of how EMF stimulation affects action potentials was conducted by Hunanyan et al. (2012), who reported that EMF stimulation evoked action potentials through the spared fibers over intact T2 vertebrae by activating synaptic inputs to lumbar motor-neurons thereby enhancing the plasticity of spinal neural circuits after mid thoracic lateral hemi-section (HX) in rats. They also discovered that EMF activated synaptic inputs to lumbar motor-neurons and motor-evoked potentials in hind-limb muscles of adult anesthetized rats. For EMF stimulation the investigators used figure eight coils with 50 mm diameter, with each coil centered over intact T2 vertebrae. Stimulation was applied at 100 µs pulse duration and 0.2 Hz frequency. Repetitive EMF stimulation for 50 min at this frequency facilitated the amplitudes of lateral white matter of dorsal corticospinal tracts in HX rats. Interestingly, this response was sustained 1.5 h after termination of EMF stimulation. Also of note the injection of N-methyl-D-aspartate (NMDA) receptor blocker MK-801 did not alter this response, but blocked the EMF-induced response, suggesting that the activation of NMDA receptors is required to initiate an EMF-evoked increase. The authors report that repetitive EMF stimulus over intact vertebrae could be used as a therapeutic approach to evoke synaptic plasticity after incomplete mid-thoracic injuries.

**EMF treatment of SCI in clinical trials**

Studies have been conducted showing electric and EMF stimulation can facilitate locomotion and motor function after SCI in humans. Sayenko et al. used epidural stimulation (ES) of the lumbosacral spinal cord to facilitate standing and voluntary movement after clinically motor-complete SCI. Electric field intensity from 0.5 to 10 V at a frequency of 2 Hz was used to treat three paralyzed patients. Recruitment curves of evoked potentials in knee and ankle muscles were collected at three localized and two wide-field stimulation configurations. Epidural electrical stimulation of rostral and caudal areas of lumbar spinal cord resulted in selective movement in both proximal and distal leg muscles (Sayenko et al., 2014). EMF treatment of long-term para- and quadriplegics for pain control also reported patients with significantly increased motor function and sensory awareness (Ellis, 1987).

Shapiro et al. used a custom-built human oscillating field stimulator (OFS) that delivered a field of 500–600 µV/mm and a current density of 42.4 µA/mm² for each electrode in a Phase 1 trial to study 10 humans with complete motor and sensory SCI (2005). Entry criteria included complete SCI between C-5 and T-10 in patients 18–65 years of age, with no transection as shown on MRI. All subjects received the National Acute Spinal Cord Injury Study (NASCIS) III methylprednisolone protocol. Cord compression and/or vertebral instability was treated before study entry. After implantation of OFS patients underwent evaluation every 2 weeks, and OFS was explanted at 15 weeks. Independent neurologi-cal status was assessed based on the American Spinal Injury Association (ASIA) score, visual analog scale pain score and SEPs at 6 weeks, 6 months and 1 year. The mean overall gain in light-touch points at year 1 was 25.5 for p < 0.001. The mean overall gain in motor function points at year 1 was 6.3 for p < 0.01. While this research is promising, implanted devices require surgery and may induce adverse effects. Some reports indicate epidural hematoma and severe compression of the spinal cord following implantation of epidural
electrodes (Dam-Hieu et al., 2010; Franzini et al., 2005). Compared with the outcomes obtained in compliant National Acute Spinal Cord Injury Study III plegiac patients, the results of this study indicate efficacy, and the FDA has given permission for enrollment of 10 additional patients.

Heterotopic ossification (HO) is a complication of SCI, whereby bone tissue forms outside the skeleton. Durović et al. used a PEMF as prophylaxis of HO in patients with traumatic SCI (2009). Twenty-nine patients with traumatic SCI were divided into treatment group (n = 14) and control (n = 15) and treated with exercise and range of motion therapy. The treatment group was exposed to a 25 Hz, 10 mT PEMF for 30 min/day starting the 7th week after injury, and treatment continued for 4 weeks. Functional capabilities and motor impairment was measured using Functional Independent Measure (FIM), Barthel Index and ASIA standards. Outcomes showed the control group had HO, while the treatment group improved from ASIA-A class to ASIA-B class, indicating that PEMF could provide prophylaxis of HO in patients with SCI.

While these clinical studies use different parameters of EMF to treat different aspects of SCI, there are baseline mechanisms of action that apply to the down-regulation of inflammation and upregulation of neurite outgrowth. PEMF is known to increase blood flow to areas experiencing pain or inflammation, bringing more oxygen to the area and removing toxic sub-stances, which would apply to all these systems (i.e. epidural stimulation, blood flow, ion flux modulation) (Bragin et al., 2014; Hernández-Labrado et al., 2011; Smith et al., 2004; Walleczek, 1992).

**Side effects of EMF**

Ten studies investigated the effects of EMF stimulation by showing treatment response and testing blood serum, liver and kidney function along with levels of serum calcium and phosphorous in humans with osteoporosis (Chen et al., 2003; Gao, 2004a, Gao, 2004b; Gao and Zhang, 2006; Tabrah et al., 1990; Weng et al., 2003; Xiong and Zhao, 2007; Yang et al., 2004; Zhao and Chen, 2005; Zhou, 2006). No study reported side effects using EMF. Thermal effects from EMF can affect normal physiological processes; however, the low-frequency, low-intensity fields (<100 Hz, <5 mT) used did not increase ambient temperature exogenously or endogenously in vitro or in animals (Walker et al., 2007).

**Discussion**

The treatment of SCI includes strategies for containing initial damage, preserving residual motor function and improving functional recovery after SCI. These include improving local blood supply, regulating ion flow, suppressing free radical information and inflammation, promoting axon remyelination, while enhancing axonal transport, and inducing axon regeneration. In vitro studies have reported that low-frequency EMF down-regulates pro-inflammatory proteins such as NFkB, TNF-α, IL-6 and INF-γ, while upregulating anti-inflammatory cytokines such as IL-4, IL-10 and IL-13. Growth factor levels can also be affected (Longo et al., 1999), as can axonal transport and neurite outgrowth, all shown to benefit from treatment with EMF (Koppes et al., 2011; Lekhraj et al., 2014). PEMF therapy in the extra-low frequency ranges of <100 Hz, and field strengths of <5 mT, has been reported to increase MSC proliferation and differentiation rates, as well as promote osteogenesis by improving the effects of osteogenic media, and increasing the number of osteoblasts while inhibiting osteoclast formation, and increasing bone matrix (Ross et al., 2015). A 15 Hz, 5 mT EMF has been reported to increase chondrogenic markers and collagen, induce neural differentiation, while increasing cell viability by over 50% (Sun et al., 2009). As advances are being made in stem cell technologies, stabilizing the cell line after differentiation is crucial to SCI repair. Once cells and cell scaffolds are implanted, EMF can be applied outside the wound for continued treatment during recovery. The need for more research in the areas of anti-inflammatory treatments and stem cell therapies is essential to understanding the regenerative effects of EMF on SCI.

In animal models, both 2 Hz, 0.3 mT and 50 Hz, 1 mT fields have reported beneficial results in promoting the recovery of sensorimotor behavior, including locomotion and bladder control, decreased neuroinflammatory rates, and significant increases in neural differentiation rates. Studies have reported that the rate of regrowth of damaged nerve processes can be accelerated (Kanje et al., 1993; Mohammadi et al., 2014; Orgel et al., 1984; Raji and Bowden, 1983; Sisken, 1984; Sisken, 1989a). Clinical trials demonstrated that PEMF increases bone mineral density (Garland et al., 1999), provides prophylaxis of HO (Durović et al., 2009) and improves respiratory function (Estenne et al., 2000). Comparisons of in vitro, in vivo and human studies show a combination of neuroinflammatory and HO decrease, as well as increases in neural differentiation, sensory function, locomotion and bladder control (Table 1).
<table>
<thead>
<tr>
<th>Subject</th>
<th>Author(s)</th>
<th>Injury</th>
<th>Field type</th>
<th>Frequency (Hz)</th>
<th>Intensity (Tesla)</th>
<th>Time of exposure</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>Piacentin, et al. (2008)</td>
<td>Ca\textsubscript{v}1 channel blocker</td>
<td>ELF-EMF</td>
<td>50</td>
<td>1 mT</td>
<td>Continuous for 12 days</td>
<td>Decreased neuroinflammatory rates. Ca\textsubscript{v}1 increases in CREB phosphorylation</td>
</tr>
<tr>
<td>Neuronal SH-SY5Y and PC12 cells</td>
<td>Kim et al. (2008)</td>
<td>Forskolin or retinoic acid</td>
<td>Static</td>
<td>N/A</td>
<td>1 mT</td>
<td>Continuous for 0–36 h</td>
<td>Modulates the orientation and direction of neurite formation</td>
</tr>
<tr>
<td>Mesenchymal stem cells (MSCs)</td>
<td>Kim et al. (2013)</td>
<td>Undifferentiated bone-marrow derived</td>
<td>ELF-EMF</td>
<td>50</td>
<td>1 mT</td>
<td>Continuous for 2, 6 and 12 days</td>
<td>Significantly increased neural differentiation levels compared with controls</td>
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<td>Animal</td>
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<tr>
<td>Rat</td>
<td>Das et al. (2012)</td>
<td>T13 Hemisection</td>
<td>ELF-EMF</td>
<td>50</td>
<td>17.96 µT</td>
<td>2 h/day for 6 weeks</td>
<td>Promotes recovery of sensorimotor behavior including locomotion and bladder control</td>
</tr>
<tr>
<td>Rat</td>
<td>Mohammadi et al. (2014)</td>
<td>Sciatic nerve transection</td>
<td>Pulsed EMF</td>
<td>2</td>
<td>0.3 mT</td>
<td>4 h/day 1–5 days</td>
<td>Faster recovery of regenerated axons as compared with controls</td>
</tr>
<tr>
<td>Rat</td>
<td>Hunanyan et al. (2012)</td>
<td>T2 Hemisection</td>
<td>Pulsed EMF 100 µs</td>
<td>0.2</td>
<td>Max 2.8 T</td>
<td>50 min</td>
<td>Increased plasticity of LWM and dCST</td>
</tr>
<tr>
<td>Cat</td>
<td>Crowe et al. (2003)</td>
<td>Laminectomy at T5/T6</td>
<td>PEMF</td>
<td>25</td>
<td>6.7 mV/cm</td>
<td>4 h/day for 12 weeks</td>
<td>Overall increased sparing of white matter and smaller lesion volume than controls; higher Tarlov scores</td>
</tr>
<tr>
<td>Cat</td>
<td>Avelèv et al. (2009)</td>
<td>Decerebration</td>
<td>Pulsed magnetic</td>
<td>1</td>
<td>0.5 T</td>
<td>Continuous until results</td>
<td>Single magnetic stimulation of lumbar enlargement elicited reflex responses in proximal and distal hind limb muscles</td>
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<tr>
<td>Human</td>
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<tr>
<td>Long-term paraplegic</td>
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<td>Prophylaxis of heterotopic ossification (HO)</td>
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<td>Tetraplegic</td>
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<td>Fracture–dislocation of the cervical spine between the fourth and seventh cervical vertebra</td>
<td>EMF</td>
<td>30 and 100</td>
<td>10 mT</td>
<td>Immediately upon application</td>
<td>EMF stimulation of the abdominal muscles elicits increases in intrathoracic pressure therefore, may improve the clearing of airway secretions</td>
</tr>
</tbody>
</table>
Possible mechanisms of action for EMF on nervous system dysfunction target immunomodulatory effects on inflammation, allowing tissue regeneration to take place. Inflammatory responses are a major component of secondary injury and play a central role in mediating the pathogenesis of acute and chronic SCI. SCI induces the expression of pro-inflammatory cytokine TNF-α in the spinal cord and via SCI-activated monocytes isolated in the peripheral circulation (Bethea et al., 2009). The transcription factor NFκB is necessary for the transcriptional activation of a variety of genes regulating inflammatory, proliferative and cell death responses (Bethea et al., 1998). TNF-α activates NFκB during inflammatory response (O’Donnell and Ting, 2010). Low-frequency PEMF (5 Hz) has been reported to downregulate both TNF-α and NFκB in a macrophage cell line (Ross and Harrison, 2013).

PEMF has been used to stimulate the growth of neurons and neurites both in vivo and in vitro (Macias et al., 2000b). In vitro, nerve regeneration is studied by measuring neurite outgrowth. Through computational analysis of PEMF treatment, it is known that stimulation of neurons by PEMF occurs mainly through depolarization, followed by action potential initiation (Pashut et al., 2011). In the regeneration of neurons, electrical currents can generate magnetic force in the perpendicular direction (via Faraday’s Law), and these forces have been reported to interact with the electrical activity of neurons (Peridou et al., 2006; Schnabel and Struijk, 1999). Both PEMF (Macias et al., 2000a) and static magnetic fields (MFs) (Kim, 2008) have been reported to directly affect neurite properties, and PEMF stimulates a rise and fall in ion fluxes such that the current through them is varied (Barnett and Larkman, 2007). This variation of flux, like all electrical messages of the nervous system, creates action potentials, which cause changes in the membrane potential by the flow of ions through channels in the membrane (Hille, 1992) (Figure 1).

Freeman et al. suggest the PEMF could be modulating calcium (Ca²⁺) ion flux in neuronal growth cones causing enhancement (Freeman et al., 1985). ELF-EMF has been used as a clinically therapeutic option for treating neurodegenerative diseases (Kim HJ, 2013 Aug 1), based largely on studies showing that exposure to extremely low frequency (ELF)-EMF (0.3–100 Hz) increases the expression and function of VGCCs, where Ca²⁺ influx through CaV1 channels plays a key role in promoting the neuronal differentiation of NSCs (Park et al., 2013). ELF-EMF exposure has been reported to effect the neuronal differentiation of NSCs isolated from the brain cortices of newborn mice by modulating CaV1-channel function. In cultures of differentiating NSCs exposed to ELF-EMFs (50 Hz, 1 mT), the percentage of cells displaying immunoreactivity for neuronal markers (beta-III-tubulin, microtubule-associated protein 2 [MAP2]) and for CaV1.2 and CaV1.3 channels was markedly increased (Piacentini et al., 2008a). These same NSC-differentiated neurons in EMF-exposed cultures exhibited significant increases in spontaneous firing, in the percentage of cells exhibiting Ca²⁺ transients in response to potassium chloride (KCl) stimulation in the amplitude of these transients and of Ca²⁺ currents generated by the activation of CaV1 channels. When the CaV1-channel blocker nifedipine was added to the culture medium, the neuronal yield of NSC differentiation dropped significantly, and EMF exposure no longer produced significant increases in beta-III-tubulin- and
MAP2-immunoreactivity rates. In contrast, the effects of ELF-EMF were preserved when NSCs were cultured in the presence of either glutamate receptor antagonists or Ca\textsubscript{v}2.1- and Ca\textsubscript{v}2.2-channel blockers. EMF stimulation during the first 24 h of differentiation caused Ca\textsubscript{v}1-dependent increases in the number of cells displaying cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) phosphorylation. Piacentini et al. also suggest that EMF exposure promotes neuronal differentiation of NSCs by upregulating Ca\textsubscript{v}1-channel expression and function (Piacentini R1, 2008).

Shapiro et al., along with other investigators, have reported naturally occurring voltage gradients in the range of 500–1000 mV/mm in the wall of early neural tube invertebrates which are required for guiding cranial-to-caudal nervous system development (Borgens and Shi, 1995; Hotary and Robinson, 1991; Shapiro, 2005; Shi and Borgens, 1994; Shi and Borgens, 1995). It has been documented that an applied voltage gradient of 10–500 mV/mm instantly directs the growth of neurites projection from a frog neuroblast toward the cathode (negative pole) (Hinkle et al., 1981; Jaffe and Poo, 1979; Patel and Poo, 1982b). When aligned with the cathode (negative) source of the applied field, neurite growth increased two- to threefold in fibers turning toward the cathode. This corresponded with an increase in growth cone filopodia and cytoplasmic spines, the specialized neuronal structures that integrate multiple signaling events by which neurons communicate with each other. The growth rate was shown to be dependent on the magnitude of the field exposure, where there was also a window of opportunity between 70 and 140 mV/mm that marked the most significant response. McCaig et al. have reported that mammalian neurons in culture respond similarly, where neurite regrowth was demonstrated to be dependent of the polarity of an applied EF in vitro (1986, McCaig, 1987; Rajnicek et al., 1998). Neurites are programmed to respond to electrical guidance cues during development, and this response is accessed by axons to regenerate after injury. Research has shown that this electrical effect is mediated by plasma membrane receptors and second messengers such as adenylate cyclase and neuropeptides, and is linked to other guidance cues in physiological substrates (McCaig, 1990; McCaig et al., 2000; Patel and Poo, 1982a; Stewart et al., 1995).

**Conclusion**

In order to treat SCI, several factors must be taken into consideration. Reparative and regenerative mechanisms include the ability to release anti-inflammatory soluble factors and immunomodulatory properties, along with the capacity to regenerate tissue at the site of injury. Both whole body and site-specific exposure to EMF has been shown to improve functional recovery and morphometric indices of transected sciatic nerve, crushed nerve and neurite outgrowth. Suggested mechanisms of action for EMF on SCI include increased blood flow, as well as changes in ionic currents associated with the electric fields that are induced by the EMF, which in turn interact at the plasma membrane and stimulate signal transduction processes and ion transport. Studies suggest that in order for the EMF frequency to be effective, it must be in the range of 1–100 Hz, which is close to the body’s normal functioning activity frequency (Lee and McLeod, 2000; Muehsam and Pilla, 1999). Although the therapeutic use of EMF on 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 EMF dosimetry needs to be optimized in a systematic approach in both animal models and future clinical trials.

**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

**References**


synapses perform complementary roles in the
outgrowth is significantly increased by the simultaneous
presentation of Schwann cells and moderate exogenous
Plasma membrane calcium ATPase deficiency causes
neuronal pathology in the spinal cord: A potential mechanism
for neurodegeneration in multiple sclerosis and spinal cord
phospholipase C/mitogen-activated protein kinase and
inducin of neurite expression by ATP, independent of
Lee, J., McLeod, K. J. (2000). Morphologic responses of
osteoblast-like cells in monolayer culture to ELF electro-
Pulsed electromagnetic fields potentiate neurite outgrowth
92:761–771.
(Minneap. Minn), 17:568–583.
factor-mediated neurite outgrowth via regulation of Rab5.
Electromagnetic fields influence NGF activity and levels
steep pulse (ECSP) treatment suppresses tumor growth in
rats implanted with Walker 256 carcinosarcoma cells
through apoptosis and an antitumor immune response.
Ma, M., Wei, T., Boring, L., et al. 2002 Monocyte
recruitment and myelin removal are delayed following
spinal cord injury in mice with CCR2 chemokine receptor
Directed and enhanced neurite growth and pulsed mag-
Directed and enhanced neurite growth with pulsed mag-
Effects of low frequency electromagnetic fields on the
chondrogenic differentiation of human mesenchymal stem
growth and the effects of an applied electric field. J.
Physiol. 375:55–69.


Zhang, Y., Ding, J., Duan, W., Fan, W. (2005). Influence of pulsed electromagnetic field with different pulse duty