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Prospects on Clinical Applications of Electrical Stimulation for Nerve Regeneration

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Abstract Regenerative capability is limited in higher vertebrates but present in organ systems such as skin, liver, bone, and to some extent, the nervous system. Peripheral nerves in particular have a relatively high potential for regeneration following injury. However, delay in regrowth or growth, blockage, or misdirection at the injury site, and growth to inappropriate end organs may compromise successful regeneration, leading to poor clinical results. Recent studies indicate that low-intensity electrical stimulation is equivalent to various growth factors, offering avenues to improve these outcomes. We present a review of studies using electric and electromagnetic fields that provide evidence for the enhancement of regeneration following nerve injury.

Electric and electromagnetic fields (EMFs) have been used to heal fracture non-unions. This technology emerged as a consequence of basic studies [Yasuda, 1953; Fukada and Yasuda, 1957] demonstrating the piezoelectric properties of (dry) bone. The principle for using electrical stimulation for bone healing originated from the work of Bassett and Becker [1962], who described asymmetric voltage waveforms from mechanically deformed live bone. These changes were presumed to occur in bone during normal physical activity as a result of mechanical forces, and it was postulated that these forces were linked to modifications in bone structure. Endogenous currents present in normal tissue and those that occur after injury were proposed to modify bone structure [Bassett, 1989]. These investigators proposed that tissue integrity and function could be restored by applying electrical and/or mechanical energy to the area of injury. They successfully applied electrical currents to nonhealing fractures (using surgically implanted electrodes or pulsed currents using surface electrodes) to aid endogenous currents in the healing process.

A considerable technological improvement was made with the noninvasive application of EMFs [Bassett et al., 1974] to accelerate fracture repair. This newer technique allowed the treatment of hard tissues without the complications of invasive electrode insertion. In addition, soft tissue injuries were now accessible for treatment by electromagnetic fields.

In this article, we will first define the basic problems encountered in nerve injury and regeneration, and then review both in vitro and in vivo studies on the use of electric and electromagnetic fields to stimulate the healing process.

Key words: nerve regeneration, electromagnetic fields, electric fields, trophic factors, nerve therapy, stimulation of healing

OVERVIEW OF PERIPHERAL NERVE REGENERATION

Basic Science

Successful regeneration of peripheral nerves is dependent upon a number of variables, the extent of injury being the most critical. Regeneration after a crush injury is faster and more precise because (1) the parent neurons in the dorsal root ganglia and ventral motor area of the spinal cord do not degenerate and are not severely impaired, and (2) individual endoneurial tubes remain intact so that axonal regeneration proceeds from the injury distally to the end-organs with little chance of inappropriate (synaptic) connections. Transection injuries, however, are more severe with a potential loss of sensory and motor parent neurons, and destruction of axonal and endoneurial continuity between proximal and distal stumps. Therefore, there is a high probability of inappropriate synaptic connections and an increased incidence of target organ (skin, muscle, tendon) atrophy prior to regeneration.

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The processes of nerve regeneration are complex, involving a series of steps pertaining to the nerve cell, its axonal fiber, and the milieu through which it grows. Within the first few hours after nerve injury, sprouting of nerve fibers occurs while Wallerian degeneration is underway. As the newly formed fibers grow to and beyond the injury site, the surviving neuronal cell bodies recover from the trauma. The nerve cell receives retrograde signals and reacts by synthesizing new building materials for regeneration. Large amounts of new proteins and membrane components are synthesized to participate in the growth of axons and the formation of new synaptic contacts. At the injury site, circulating macrophages enter and engulf cell debris and are at least one source of mitogens and growth factors such as nerve growth factor (NGF), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF). In the distal segment, Schwann cells (which have lost contact with axoplasm) are activated to divide, participate in macrophagic activity, and, with other non-neuronal cells, produce trophic (growth-stimulating) factors. A substrate containing elements of basal lamina and Schwann cells permissive for facilitating axon regrowth is formed. The neuronal cell body transports material to its distal portions via two systems—fast axonal transport (~ 400 mm/day) of membrane and membrane-bound materials, and slow axonal transport (0.1–30 mm/day) for cytoskeletal components (such as actin, tubulin, and neurofilaments). The rate of regeneration is correlated with the rate of slow axonal transport [Maier and McQuarrie, 1990]. Appropriate growth to and synaptic contact with the target tissue occur after a period of axon elongation that is dependent upon the distance of the injury from the spinal cord. After a prolonged time period, some of the Schwann cells assume their normal role of myelinating newly formed axonal fibers.

Basic Problems in Nerve Regeneration

Injury to peripheral nerves may result in long-term disability despite the regenerative capacity of these structures. In extensive trauma the distal nerve segments are deprived of trophic factors from the parent nerve cell bodies, resulting in extensive degeneration of these segments; also, as noted, some of the neuronal cells die. The cut ends of both proximal and distal segments can be reapproximated surgically, but there is always misalignment of the fibers due to extrinsic scar and endoneurial tube collapse with time. In addition, the closer the injury to the spinal cord, the further distance the nerves must regenerate through the tubular sheaths so that the target organs are denervated for an extensive period of time and may undergo atrophy. Therefore, the surgical treatment of peripheral nerve injury is limited to careful microscopic approximation of proximal and distal stumps. Enhancement of further regenerative potential must try to manipulate cellular and other (extracellular) factors.

Influence of Electric Fields on Nerve Regeneration

In vitro. Culture models are useful for screening the influence of various chemicals (hormones, growth factors, ions) and electric and electromagnetic fields on regenerative processes. Moreover, they are often mandatory for determining underlying mechanisms of action. Such studies relating to nerve regeneration in vitro can be found in Table I. Direct current-induced (DC) electric fields ranging from low levels (nV/cm) to high levels (V/cm) evoke neurite outgrowth that is significantly greater than that observed in control cultures. Moreover, this growth is consistently oriented to the cathode. Patel et al. [1985] applied electric pulses (pA/μV) focally near growth cones of *Xenopus* neurons and noted directional growth toward the negative (sink) electrode. Both steady and pulsatile fields have been found to be effective in promoting directed neurite outgrowth despite the endogenous occurrence of pulsatile fields.

Studies on pulsed electromagnetic fields (PEMF) are fewer in number, but are also effective in stimulating growth and upregulating neurotransmitter release. Since it appears that the neurons respond vigorously to the electric fields specifically, it is likely that the currents induced by PEMF, rather than the magnetic fields themselves, are responsible for growth and functional changes [Sisken and Mullins, 1991].

In vivo. Both electric and electromagnetic fields have been tested in vertebrate peripheral nerve models. Tables II and III list the electromagnetic fields used and the extent of the response obtained.

Direct current studies. Electrodes implanted to deliver direct current to lesioned peripheral nerves have enhanced or caused no effects on regeneration. Axonal regeneration was

TABLE I. Nerve Regeneration In Vitro

Fields	Induced electric	Response	Authors
DC low	nV/cm	Increased neurite outgrowth to cathode	Sisken and Smith [1975]
DC high	mV/cm-V/cm	Increased neurite outgrowth to cathode	Marsh and Beams [1946] Jaffe and Poo [1979] McCaig [1986] Freeman et al. [1985] Bedlack et al. [1992]
Pulsed DC	0.1 V/cm	Neurite outgrowth focally to cathode	Patel et al. [1985]
PEMF	mV/cm	Increased neurotransmitter release; increased neurite outgrowth; increased neurite outgrowth directionally	Dixey and Rein [1982] Sisken et al. [1984] Sisken and Mullins [1991] Subramanian et al. [1991]

enhanced by implanting bimetallic electrodes into the distal segment of transected sciatic nerves [Winter et al., 1981]. Regeneration was measured by analyzing the area of the compound action potential and significant changes were found only when the current delivered was 50–100 nA and when the cathode was placed distal to the lesion. Kerns et al. [1991] used silver–silver chloride wick electrodes to deliver 0.6 μA current with the cathode 5 mm distal to a transection lesion. Seven days later, current density was measured along the nerve with a vibrating probe. An increase of 69% in the regenerated distance of the axons was found. However, when Kerns and Lucchinetti used this electrode system in a crush preparation [1992], significant changes were noted in twitch tension recovery of motor function only during the middle time period (days 12–21); no differences were noted at the early or late stages of recovery. McGiness [1989] found no differences in the extent of regeneration between DC-treated and untreated sciatic nerve preparations when assessing numbers of myelinated and unmyelinated axons and determining the time of return of the toe-spread reflex. Quite different methods of delivery of current to the nerve were employed in these studies.

TABLE II. Studies Using Electromagnetic Fields on Peripheral Nerve Regeneration

EMF	Rep Rate (Hz)	Peak Magnetic (mT)	Pos. dB/dT (T/sec)
Clinical, pulse train PEMF [O'Brien et al., 1984; Orgel et al., 1984]	15	1.9	9
Clinical, single pulse PEMF [Ito and Bassett, 1983; O'Brien et al., 1984; Orgel et al., 1984]	72	3.5	9
PEMF, single pulse PEMF [Sisken et al., 1989; Sisken et al., 1990; Zienowicz et al., 1991; Kanje et al., 1992]	2	0.3	0.6 *dB/dT* (T/sec)
Sinusoidal AC [McLeod and Rubin, 1992]	15	2.5	0.236
Sinusoidal AC [Rusovan and Kanje, 1991]	50	0.5	0.157

The use of direct current to accelerate human nerve healing would require invasive surgery to implant electrodes. Electrodes may produce scarring in surrounding tissue even when they are constructed of agar or wick; metal electrodes should be avoided because of electrolysis byproducts. Thus, this technique may have limited clinical application.

Electromagnetic (EMF) and Pulsed Electromagnetic Fields (PEMF). The influence of electromagnetic fields on nerve regeneration was first reported in a comprehensive study by Raji and Bowden [1983], who applied a 27-MHz signal (Diapulse, 10 mW/cm^2) to the transsected common peroneal nerve in a rat model. This PEMF was administered 15 min daily for various time periods and was found to accelerate the recovery of function (toe-spread reflex), increase the maturation of myelinated axons, reduce scar tissue, and increase the size of intraneural blood vessels.

PEMF signals used clinically for healing fracture non-unions have also demonstrated effects on nerve regeneration. When whole rats were

TABLE III. Factors Used to Stimulate Nerve Regeneration Rate

Treatment	Model	Authors	Increase
PEMF-2 Hz	Crush	Sisken et al. [1989]	22%
SEMF-50 Hz	Crush	Rusovan and Kanje [1991]	21%
Conditioning lesion	Transection	McQuarrie and Grafstein [1973]	20%
Testosterone	Crush	Kujawa et al. [1991]	25%
Org. 2766 (ACTH/MEL)	Crush	DeKoning et al. [1986]	20–40%
Triiodothyronine	Crush	Berenberg et al. [1977]	22%
Forskolin	Crush	Kilmer and Carlsen [1987]	18%

exposed to 72 Hz PEMF (ElectroBiology Inc., Parsippany, NJ) after sciatic nerve transection, an earlier return of motor function (plantarflexion) was reported [Ito and Bassett, 1983]. In a different model, the common peroneal nerve of a cat was transected and the limb exposed to PEMF for 5 days [Orgel et al., 1984] with either a 15-Hz pulse burst signal or a single 72-Hz repetitive pulse (ElectroBiology Inc., Parsippany, NJ). To test for integrity of the regenerated unit, the retrograde transport of horseradish peroxidase from muscle to spinal cord motor neurons was used. The 15-Hz pulse burst signal induced labeling of significantly more neurons, indicating the increased survival of ventral motor neurons. This finding was correlated with functional recovery [O'Brien et al., 1984]. No significant improvement was obtained with the 72-Hz signal.

Zienowicz et al. [1991] transected and surgically repaired (immediately or after a 5 day interval) the sciatic nerve of adult rats and the whole animals were exposed to PEMF (2-Hz, 3 Gauss, Bietic Research Inc., Lyndhurst, NJ). Assessment of functional return by the sciatic function index (calculated from footprint measurements) indicated significant recovery of gait (by the PEMF-treated, surgery-delay group) at the 140th day; this recovery continued to increase to the end of the experiment (165 days).

In our laboratory we have tested the same 2-Hz PEMF signal on in vitro preparations of regenerating dorsal root ganglia explants and in an in vivo crushed sciatic nerve model [Sisken et al., 1989; Sisken et al., 1990]. The ganglionic explant cultures, in the presence of serum with no added growth factors, demonstrated significantly increased neurite outgrowth with PEMF relative to unexposed controls. In the crush model experiments we found the rate of regeneration to be enhanced significantly (by 22%) over that of nontreated animals. Animals were restrained and placed between Helmholtz coils for 4 hr/day, for 3, 4, or 6 days. The regeneration distance was determined at these different times and extrapolated to zero. A plot of this outgrowth distance as a function of time estimates an initial delay period of 1–2 days due to Wallerian degeneration (die-back) after injury. This degeneration period was not affected by PEMF.

The enhancement of the regeneration rate by PEMF is equal to that obtained by others using conditioning lesions [McQuarrie and Grafstein, 1973], growth factors, hormones, etc., on nerve regeneration models [Fawcett and Keynes, 1990]. This enhancement is also similar to the increase in area reported by McLeod and Rubin [1992] using sinusoidal electromagnetic fields (SEMF) on bone growth in a turkey ulna model.

We have shown that treating a noninjured animal with PEMF before nerve crush injury without further treatment also resulted in a significant increase in the nerve regeneration rate [Sisken et al., 1990]. These studies have been extended by Kanje et al. [1992], who found that pretreatment for 4 hr/day, followed by an interval of up to 14 days after the end of the PEMF stimulation, still maintained the regeneration rate at an elevated level. However, when the amplitude was reduced to 0.6 Gauss from 3 Gauss, the pretreatment effect was lost.

This pretreatment effect with PEMF is similar to the stimulation of regeneration obtained after a "conditioning lesion" (two lesions made on the same nerve with an appropriate interval between them [McQuarrie and Grafstein, 1973]). The conditioning lesions, and perhaps the PEMF pretreatment, "prime" the nerve cell-axonal system to respond maximally. This would be manifest by upregulation of RNA and protein synthesis, the latter of which has been reported in transected nerves [Sisken et al., 1990] and in cellular systems [Goodman and Henderson, 1988] as a consequence of PEMF treatment. In addition, Maier and McQuarrie

[1990] have also reported an increase in slow axonal transport after a conditioning lesion.

Sinusoidal fields have been tested for influences on the regeneration rate. Rusovan and Kanje [1991] exposed rats after a sciatic nerve crush to 1 Gauss sinusoidal fields at frequencies of 50–2,000 Hz. Significant increases in regeneration rates were obtained at 250, 500, and 1,000 Hz with no effect at 50 or 2,000 Hz. The maximal response was obtained at a frequency of 1,000 Hz with the regeneration rate increased by 24%. Earlier, they had reported an increased regeneration rate of 21% when rats with similar lesions had been exposed to 4 Gauss, 50-Hz sinusoidal fields. Thus, it appears that various electrical techniques can be used to promote faster healing after nerve injury. The questions to be addressed now are, which set of electrical parameters (frequency, amplitude, etc.) provides the optimal combination of effectiveness and practical adaptation for clinical use.

Basic Mechanisms

Thus far, no single unifying mechanism has been accepted to explain electric field bioeffects. A number of laboratories are exploring how electric and EMF influence signal transduction mechanisms. One of the more obvious candidates is the calcium ion; it is involved in growth cone formation and cathodal orientation of neurites in vitro as a result of electric field-evoked calcium influx [Freeman et al., 1985; Bedlack et al., 1992]. Changes in calcium concentration may influence the interaction with calmodulin, activate protein kinase C, or act directly on the activity of intracellular enzymes. Other signal transductive candidates are membrane receptors whose interactions with G-proteins are modified by electrical stimulation [Luben, 1991] or ligand-gated channels that are acted upon by the fields. Furthermore, whether it is the electric rather than the magnetic field that is responsible for the bioeffects has yet to resolved. Downstream to these membrane-located events in the nerve injury model are remodeling phases that may also be influenced by electric and electromagnetic fields, such as mitosis of Schwann cells, increased macrophage activity, upregulation of trophic factor(s) production, increased axonal transport, and basal laminin and cytoskeletal unit production.

CONCLUSIONS AND FUTURE DIRECTIONS

The use of electric and electromagnetic field therapy for the enhancement of nerve regeneration in humans has been used sporadically and with anecdotal results. The experimental evidence presented justifies the use of these modalities in well-controlled clinical trials. The development of a local (rather than total body) electromagnetic delivery system is needed. However, successful experimental results may have been due to systemic rather than local effects. This local treatment should also be studied in the laboratory animal. Future directions should not only address the development of new technologies in this area, but should increase our understanding of the underlying mechanisms.

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