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The Role of Electrical Stimulation in Bone Repair

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Electric and electromagnetic fields regulate the expression of genes for extracellular matrix proteins in connective tissue cells. In vitro, this process has been observed in limb rudiments, osteoblasts, chondrocytes, and osteoprogenitor cells, among other systems [1]. Enhancement of both proliferation and differentiation has been demonstrated with a variety of exposure techniques, including direct current (DC), capacitive coupling (CC), and inductive coupling (IC) or pulsed electromagnetic fields (PEMF). In vivo models, specifically healing fractures and osteotomies, the increase in extracellular matrix is observed as enhanced repair with accelerated synthesis of fracture callus and maturation of physical parameters (eg, stiffness and strength). Detailed studies have demonstrated that exposure to PEMF upregulates gene expression for the extra-cellular matrix constituents proteoglycan and collagen, and accelerates endochondral bone formation [2].

In this review, the authors consider evidence from numerous preclinical and clinical investigations of accelerated bone repair by electric and electromagnetic fields. This review also examines studies on signal transduction at the membrane level and on stimulation of growth factor synthesis, which may be an intermediary mechanism of action, and possibly a mechanism of amplification, of electric and electromagnetic fields.

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Preclinical studies

Numerous in vitro and in vivo studies have shown that appropriately configured electric and electromagnetic energy stimulates the synthesis of extra-cellular matrix proteins. This increased synthesis is reflected in healing fractures and nonunions as enhanced bone repair.

In vitro studies

The results of in vitro studies have been previously reviewed [1,3]. These studies demonstrated that cells involved in bone formation, particularly endochondral bone formation, can be stimulated by appropriately configured electric and electromagnetic fields at several phases in their cell cycle. Cell responses depend upon the predominant activity of the cell population (eg, proliferation in pre-confluent cultures or matrix synthesis in post-confluent cultures).

Osteoprogenitor cells of bone marrow or fracture callus origin respond to electric and electromagnetic stimulation by increasing their synthesis of extracellular matrix molecules. Bone marrow cells in diffusion chambers have been stimulated to synthesize cartilage and undergo endochondral calcification by demineralized bone matrix or DC stimulation [4]. A significantly greater number of electrically stimulated cultures exhibited chondrogenesis and calcification than did controls. Fracture callus cells harvested from healing closed rat tibial fractures significantly increased ^{3}H thymidine incorporation during proliferation in response to DC electric stimulation [5]. This effect was not seen in either confluent or low-density cultures. In a different model, bone marrow osteoprogenitor cells exposed to PEMF stimulation exhibited an increase in collagen synthesis [6]. Collagen synthesis was significantly increased in stimulated post-confluent cultures, and enhanced collagen synthesis was not observed until confluence was reached and proliferation ceased. No effect was observed in either DNA content or the incorporation of ^{3}H thymidine.

Experimental osteotomies

The effects of electric and electromagnetic field exposure have been studied in several animal models. Studies have examined repair of bone defects, fresh fractures and osteotomies, and fracture nonunions (Table 1). Experimental models of bone repair exhibited enhanced cell proliferation, calcification, and gain of mechanical strength when stimulated with DC fields [7,8]. CC stimulation has been reported to improve mechanical strength of experimental fracture repair and healing osteotomies [9,10]. Several studies using PEMF stimulation have demonstrated increased calcification and enhanced radiographic and mechanical strength in healing bone [11,12]. Exposure to PEMF has been shown to enhance callus formation and mechanical parameters of healing in osteotomies [13]. The

Table 1
Stimulation of osteogenesis in animal long bone models

Study	Model	Technique	Stimulation
Petersson et al [8]	Rabbit fibula delayed union	DC	Accelerated union
Brighton et al [9]	Rabbit fibula osteotomy	CC	Accelerated union
Bassett et al [11]	Dog radius osteotomy	IC	Accelerated union
Fredericks et al [14]	Rabbit tibia osteotomy	IC	Accelerated union
Fredericks et al [15]	Rabbit tibia osteotomy	IC	Accelerated union
Inoue et al [13]	Dog tibia osteotomy	IC	Accelerated union

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volume of periosteal callus, new bone formation, and the normalized maximum torque and torsional stiffness were significantly greater at 6 weeks in PEMF-treated osteotomies compared with untreated control osteotomies. In a study focusing on the dosimetry of PEMF stimulation in experimental osteotomies, dose was expressed as daily exposure duration [14]. Osteotomies treated with PEMF for 60 minutes/d achieved intact torsional strength by 14 days after osteotomy, compared with 21 days for osteotomies treated for 30 minutes/d, and 28 days in the untreated control group. Other dosimetry studies, examining daily exposure of several electric and electromagnetic fields, have shown a linear effect of daily exposure over 0.5, 3, or 6 hours per day, with a 6-hour stimulation being most effective [15].

Clinical studies

Bone repair

The efficacy of electric and electromagnetic stimulation on bone repair has been studied in a formal meta-analysis [16]. Twenty randomized controlled trials were identified, with most using PEMF. Although 15 trials supported the effectiveness of electric stimulation, 5 did not, possibly because of the inclusion of patients with Perthes' disease and fresh fractures, and other patients who were undergoing bone grafts and chemotherapy. After application of quality assessment and tests for the appropriateness of data pooling, data were combined and analyzed from 12 controlled trials. Pooling data from all the studies could not be done because of heterogeneity of study design and outcome measurements. Results from pooled trials of 765 cases of stimulated versus non-stimulated cases revealed a difference of 0.26, which supported the effectiveness of electric and electromagnetic energy in the stimulation of bone repair in a number of diverse clinical conditions. However, because of the inability to pool data from all the studies, the conclusions regarding efficacy of electric and electromagnetic stimulation in bone repair cannot be considered definitive [3].

Table 2
Clinical stimulation of osteogenesis

Study	Model	Technique	Stimulation
Borsalino et al [17]	Femoral osteotomy	IC	Accelerated union
Mammi et al [18]	Tibial osteotomy	IC	Accelerated union
Traina et al [19]	Tibial osteotomy	IC	Accelerated union

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Electric and electromagnetic stimulation with DC, CC, or PEMF techniques has been used clinically to treat fresh fractures, osteotomies, and spine fusions (Table 2). Several randomized, placebo-controlled studies from Italian centers have described the role of PEMF techniques in enhancing union of femoral and tibial osteotomies [17–19]. An evaluation of 31 femoral intertrochanteric osteotomies, randomized to active PEMF or placebo devices and treated for 3 months, demonstrated greater bone density and trabecular bridging in the treated group compared with the untreated group [17]. A similar study evaluated the results of PEMF treatment in 40 high tibial osteotomies [18]. Blinded radiographic assessment demonstrated advanced healing in 72% of patients treated with PEMF compared with 26% of those treated with the placebo device.

Delayed union and nonunion

Four controlled studies of electric or electromagnetic stimulation have been reported: two compared results to placebo treatment and two compared results to bone graft (Table 3). Two studies compared PEMF to bone graft and demonstrated equivalence of the techniques in a total of 60 delayed unions or nonunions [20,21]. One study described the efficacy of PEMF stimulation

Table 3
Stimulation of delayed and nonunions

Study	Technique	Controls
Brighton et al [27]	DC	Observational
Paterson et al [26]	DC	Observational
Bassett et al [28]	IC	Observational
Heckman et al [29]	IC	Observational
Brighton and Pollack [30]	CC	Observational
Sharrard [22]	IC	Placebo
Scott and King [23]	CC	Placebo
DeHaas et al [20]	IC	Graft
Dunn and Rush [21]	IC	Graft

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compared with placebo in 45 patients with delayed union of severe tibial diaphyseal fractures [22]. Fractures were at risk for delayed union because of displacement, angulation, comminution, or soft tissue injury. Forty-five percent of patients treated with the active device demonstrated union compared with 12% with the placebo device after 12 weeks of treatment. In a study of CC treatment of 23 tibial nonunions, 60% of patients treated with the active device healed in a mean of 21 weeks compared with 0% with the placebo device [23].

Using a logistic regression analysis, investigators examined the effects of DC, CC, and bone graft in tibial nonunions [24]. Seven risk factors were identified for delayed healing. When no risk factors were present, no significant differences were observed among the three treatment methods. However, both electric techniques were more effective than bone graft in nonunions with previous bone graft failures.

Many observational studies have suggested the efficacy of DC, CC, or PEMF techniques in stimulating healing of delayed unions and nonunions. Investigators using invasive DC techniques have reported union rates of 70% to 90% [25]. In a large multi-center study, DC techniques were used in 84 patients who had 47 delayed and 37 un-united tibial fractures. Fracture healing, measured clinically and radiographically, was achieved in 72 of 84 patients (86%) [26]. A similar success rate of 78% of 258 patients treated with DC stimulation has been reported [27]. Several studies have reported improved bone repair with PEMF stimulation techniques. One study of 127 delayed unions and nonunions of the tibial diaphysis reported an 87% union rate with a median healing time of 5.2 months [28]. Another study demonstrated a success rate of 64% of 149 patients with most healing in 3 to 6 months after treatment [29]. In a study using CC techniques, 77% of 22 nonunions healed; the mean healing time was 23 weeks [30].

The effectiveness of PEMF compared with surgery in promoting healing of delayed nonunions has been the subject of an extensive review [31]. This work undertook a MeSH search of the English language literature, but a formal meta-analysis was not performed. Twenty-eight studies of un-united tibial fractures treated with PEMF were compared with 14 studies of similar fractures treated with bone graft with or without internal fixation. The overall success rate for the surgical treatment of 569 un-united tibial fractures was 82% (range = 70%–100%). By comparison, the overall success rate of PEMF treatment of 1718 un-united tibial fractures was 81% (range = 13%–100%).

Surgical bone grafting and electric stimulation have each been reported to fail to heal 10% to 15% of nonunions. Several reports of multiple bone grafting procedures for failed previous grafting of nonunions reveal progressively diminishing success rates as low as 33% to 50% for the third to fourth grafting procedure [31]. Electric and electromagnetic stimulation may be especially useful in these multiple grafted nonunions. In 44 patients with tibial nonunions after one or more surgical procedures, 36 (82%) achieved successful union with electric stimulation [26]. In this series, 13 of 15 infected fractures (87%) also healed. Results of PEMF stimulation together with autogenous graft have been

reported in 83 patients with complicated, persistent nonunions; the mean interval from initial fracture to treatment was 1.5 years [32]. These patients had undergone an average of 2.4 prior surgical procedures for treatment of their nonunions, and one third of the patients also had a history of infection. In one group of 38 patients previously treated with bone graft and PEMF stimulation, healing of the nonunion was achieved in 87% of the patients. Another group consisted of 45 patients with nonunions resistant to prior treatment by PEMF alone. A combination of PEMF stimulation and bone graft healed 42 of these 45 nonunions (93%). Healing was achieved in 12 of 14 patients (86%) with actively infected nonunions and in all 10 patients with a prior history of infection.

Electric and electromagnetic fields as an adjunct to foot and ankle surgery

Electric fields with DC-implantable electrodes and externally applied PEMF have been used as adjuncts to reconstructive surgery of the hindfoot and in other foot and ankle applications (Table 4). A prospective, randomized, blinded study compared the use of PEMF to non-stimulated hindfoot arthrodeses. In subtalar arthrodeses, no significant difference was observed in the time to radiographic union, although four nonunions in the control group and none in the PEMF-treated group were observed. The PEMF-treated group consisted of 22 primary and 5 revision subtalar arthrodeses, compared with 33 primary arthrodeses and no revisions in the control group. In talonavicular arthrodeses, the average time to fusion in the control group was 17.6 weeks compared with 12.2 weeks in the PEMF-treated group ($P = 0.003$). In calcaneocuboid arthrodeses, the average time to fusion in the control group was 17.7 weeks compared with 13.1 weeks in the PEMF-treated group ($P = 0.01$) [33].

Implantable DC electric stimulation has been used in high-risk hindfoot fusions [34]. Thirteen patients who were identified as high risk for nonunion because of smoking, previous nonunion, osteonecrosis, infection, or comorbidities underwent hindfoot arthrodesis with adjunctive DC stimulation. Successful arthrodesis was achieved in 12 of 13 (92%) patients. The study was uncontrolled and the results were compared with historical controls that reported much higher rates of nonunion in high-risk hindfoot fractures. DC electric stimulation has

Table 4
Electric and electromagnetic stimulation of the foot and ankle

Study	Model	Technique	Fusion rate
Midis and Conti [35]	Revision ankle fusion	DC	100%
Reynolds [37]	Fusion	DC	100%
Donley and Ward [34]	Hindfoot at risk	DC	92%
Holmes [36]	Metatarsal delayed and nonunion	PEMF	100%
Dhawan et al [33]	Hindfoot fusions	PEMF	100%

also been used as an adjunct in revision ankle arthrodesis for aseptic nonunions [35]. In this group of 10 patients, each patient had an average of 2.5 previous surgical procedures before the original fusion attempt. Arthrodesis occurred in all 10 patients at an average of 12.8 weeks after revision surgery. Two other studies that focused on the use of electric and electromagnetic fields as adjuncts to foot and ankle surgery have been presented. One study suggested that implantable DC electric stimulation is a useful adjunct in hindfoot arthrodesis, and the other used PEMF stimulation for the treatment of metatarsal fractures with radiographic signs of delayed union and nonunion. All fractures healed, on average, in 4 months [36,37].

Many preclinical in vitro and in vivo studies suggest a role for electric and electromagnetic stimulation of bone repair in delayed unions and nonunions of fractures by demonstrating that exposure to electric and electromagnetic energy stimulates the synthesis of extra-cellular matrix molecules. Similar results have been observed in a variety of clinical conditions in which electric and electromagnetic energy has been used to stimulate bone formation, including fractures and osteotomies, spine fusions, arthrodeses, and augmentation of bone grafts. Evidence for the efficacy of electric and electromagnetic stimulation techniques in delayed union and nonunion of long bone fractures comes largely from a small number of controlled studies and self-controlled observational studies in which spontaneous healing occurs in only a small number of nonunions. Meta-analyses and compendia of clinical studies support the effectiveness of these stimulation techniques primarily in osteotomies, delayed unions and nonunions of fractures, and spine fusions.

Stimulation of growth factor synthesis

The initial reports of an enhancement of growth factor synthesis in response to electric and electromagnetic fields demonstrated an increase in insulin-like growth factor II (IGF-II) mRNA and protein and suggested that IGF-II may in part mediate proliferation of osteoblast-like cells [38]. Additional studies with combined magnetic field exposure of both human osteoblast-like and rat fracture callus cells demonstrated increases in IGF-II levels after 30 minutes of exposure [39]. These results are similar to those observed in response to mechanical strain, and the stability of the signaling pathways suggests that growth factor synthesis serves to amplify electric and electromagnetic signaling [40].

PEMF exposure also stimulates mRNA expression of several bone morphogenic proteins (BMPs; Table 5). Exposure of developing chick embryos to PEMF enhances bone formation in calvaria. In this model, BMP-2 mRNA levels were increased 2.7-fold on day 15 and 1.6-fold on day 17 of incubation. A similar increase in BMP-4 for mRNA expression was observed: 1.6-fold on day 15 and 1.5-fold on day 17. These results suggest that upregulation of BMP-2 and BMP-4 mRNA mediates the bone-inductive effect of the PEMF [41]. Similar

Table 5
Regulation of TGF-β/BMPs

Study	Technique	Model	Result
Zhuang et al [45]	CC	MC3T3	↑ proliferation, ↑ $TGF\beta_1$ mRNA
Bodamyali et al [43]	IC	osteoblasts	↑ proliferation, ↑ BMP-2, -4 mRNA
Nagai and Ota [41]	IC	osteoblasts	↑ BMP-2, -4 mRNA
Yajima et al [42]	IC	osteoblasts	↑ BMP-4, -5, -7 mRNA
Aaron et al [49]	IC	E.O. in vivo	↑ differentiation, ↑ $TGF\beta_1$ mRNA + protein
Lohmann et al [47]	IC	MG63 osteoblasts	↑ differentiation, ↑ $TGF\beta_1$
Guerkov et al [46]	IC	human nonunion cells	↑ $TGF\beta_1$
Aaron et al [50]	IC	E.O. in vivo	↑ differentiation, ↑ $TGF\beta_1$
Lohmann et al [48]	IC	MLO-Y4 osteocytelike cells	↑ $TGF\beta_1$, PGE_2

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studies were performed in SV-HFO human osteoblasts. Unstimulated cells express significant amounts of mRNA for BMP-1, -2, -4, -5, and -7. PEMF exposure markedly increased the levels of mRNA for BMP-4, -5, and -7 in a time-dependent manner, with a maximal increase observed after 24 hours of exposure [42]. Upregulation of BMP-2 and BMP-4 mRNA expression in rat calvarial osteoblasts in vitro has also been reported coincident with bone induction [43].

Electric and electromagnetic fields have been shown to upregulate transforming growth factor beta (TGF-β) mRNA in a number of experimental models (Table 5) [44]. CC field exposure induced proliferation and increased DNA content of MC3T3 osteoblast-like cells [45]. TGF-β content was increased by 39% compared with unstimulated cultures. Whether TGF-β is related to the proliferation of the osteoblast-like cells was unclear in this study. In a series of studies examining the response of mesenchymal cells to PEMF, stimulation affected both TGF-β1 and prostaglandin E2 (PGE_2) levels, with the magnitude and time course of the effect being dependent on the maturation state of the cell in the osteoblast lineage [46–48]. Confluent cultures of cells at various stages in the osteoblast lineage were exposed to PEMF. Human MG63 osteoblast-like cells were studied as a model of a relatively undifferentiated osteogenic cell [47]. PEMF produced a differentiating effect, with a reduction in cell proliferation and an increase in alkaline phosphatase-specific activity, collagen synthesis and osteocalcin levels. At the early time points, TGF-β1 levels in cultures exposed to PEMF were elevated over those seen in the control cultures. Terminally differentiated MLO-Y4 osteocyte-like cells did not show an effect of PEMF on cell number or osteocalcin levels, but both TGF-β1 and PGE_2 levels were increased [48]. These studies also demonstrated that the effect of PEMF on TGF-β1 levels was through a prostaglandin-dependent mechanism. PEMF exposure of cells

isolated from hypertrophic and atrophic nonunions resulted in a time-dependent increase in TGF-β1 levels of the hypertrophic nonunion cells by day 2 and of the atrophic nonunion cells by day 4, although cell number, alkaline phosphatase, and levels of osteocalcin and PGE_2 were not affected [46]. These observations support the central role of TGF-β1 in the cascade of regulatory events initiated by PEMF. PEMF-dependent changes in mRNA levels of TGF-β1, as well as BMP-2, -4, and -7 and their receptors, demonstrate considerable variability in response among samples.

PEMF upregulated TGF-β1 protein synthesis and mRNA expression coincident with increases in extra-cellular matrix protein synthesis and gene expression in an in vivo model of endochondral bone formation [49,50]. Regulation of protein synthesis occurred in a dose-dependent manner in terms of both amplitude and duration of exposure. In response to PEMF consistent with certain environmental exposures, TGF-β1 mRNA levels increased 68%, the active protein 25%, and number of immunopositive cells 119% compared with control tissues [49]. Detailed studies of endochondral bone formation demonstrated that the therapeutic use of PEMF for bone healing upregulated chondroprogenitor and osteoprogenitor cell differentiation, extracellular matrix synthesis, and TGF-β1 expression. The pattern of TGF-β1 expression was preserved throughout the developmental sequence, suggesting that PEMF treatment enhances chondrogenesis, endochondral calcification, and the normal physiologic expression pattern of TGF-β1 [50] without disrupting these processes. These studies demonstrated no difference in DNA content between control and PEMF treatment during endochondral bone formation, which indicates an absence of a proliferative response to field exposure.

Chondrogenesis, however, was markedly stimulated, exhibiting a twofold increase in sulfate incorporation and a 64% increase in glycosaminoglycan content compared with controls. The glycosaminoglycan content expressed per unit of tissue, or per chondrocyte, and the chondrocyte:matrix ratios were not different in control and PEMF-treated tissues, which indicates the differentiation of additional chondrocytes in PEMF-treated tissue rather than an increase in matrix synthesis per chondrocyte [2]. The total cell number was unchanged because of PEMF treatment, which suggests that the increased number of chondrocytes made up a greater fraction of the total cell content in PEMF-treated ossicles compared with control ossicles. PEMF exposure stimulated earlier and quantitatively greater levels of aggrecan and type II collagen mRNA and the earlier appearance and deposition of cartilage-specific proteoglycans. A significant sustained increase in TGF-β1 protein was observed in PEMF-stimulated tissues compared with control tissues throughout the developmental sequence (Fig. 1). Overall, PEMF exposure resulted in a 32% increase in TGF-β1 protein and a 2.5-fold increase in mRNA levels compared with unstimulated tissues. Immunohistochemical studies demonstrated that TGF-β1 was synthesized by chondrocytes rather than by mesenchymal cells. These studies demonstrate that the transcription of TGF-β1 mRNA as well as the accumulation of active TGF-β1 protein is upregulated by PEMF coincident with accelerated chondrogenesis,

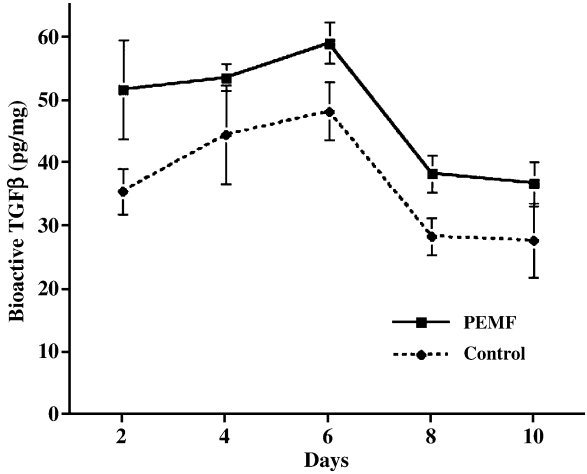

Fig. 1. Increase in bioactive TGFβ_1 protein with PEMF stimulation. Significant increases are seen at days 2, 6, and 8 ($P < 0.05$). TGFβ_1 protein is increased in pre-chondrogenic and early chondrogenic tissue (days 2–6) with a decrease on day 8 just before matrix calcification. The constitutive pattern of TGF-β1 expression in endochondral bone formation is increased but not disorganized. (*From* Aaron RK, Wang S, Ciombor DM. Upregulation of basal TGFβ_1 levels by EMF coincident with chondrogenesis: implications of skeletal repair and tissue engineering. J Orthop Res 2002;20:233–40. Later reprinted in Aaron RK, Boyan BD, Ciombor DM, et al. Stimulation of growth factor synthesis by electric and electromagnetic fields. Clin Orthop Relat Res 2004;419:30–7, with permission.)

which suggests that the PEMF-mediated effects on bone healing are mediated through TGF-β1 [44].

Interactions at the cell membrane

With current electric and electromagnetic devices, the induced electric fields are considerably weaker than the levels required to depolarize cell membranes and, therefore, the biological activity of these fields most likely depends on amplification mechanisms that occur during transmembrane coupling. Probable sites of amplification are transmembrane receptors (Table 6). In fact, it was demonstrated years ago that the effects of electric and electromagnetic fields were mediated at the cell membrane either by interference with hormone receptor interactions or by blocking of receptor–adenyl cyclase coupling [51].

The first demonstration of receptor-mediated signal transduction described the interactions of PEMF and parathyroid hormone (PTH) receptors [51]. Normally, PTH increases cyclic adenosine monophosphate activity in bone cells. However, in the presence of PEMF, this effect was abolished. The field blocked the inhibitory effects on collagen synthesis by PTH but not by 1,25 dihydroxy vitamin D_3, supporting the hypothesis that PEMF acts through membrane receptors. Further studies suggested that the effects of PEMF on PTH signaling

Table 6
Receptor modulation

Study	Technique	Model	Receptor
Luben et al [51]	IC	osteoblasts	PTH
Cain et al [52]	IC	osteoblasts	PTH
Hiraki et al [53]	IC	chondrocytes	PTH
Brighton and McCluskey [54]	CC	osteoblasts	PTH
Bourguignon et al [55]	DC	fibroblasts	Insulin
Cossarizza et al [56]	IC	lymphocytes	IL-2
Cho et al [57]	AC	fibrosarcoma	Transferrin, LDL
Fitzsimmons et al [58]	IC	TE-85 osteoblasts	IGF-2
Shankar et al [59]	IC	osteoclasts	Calcitonin
Varani et al [60]	IC	neutrophils	Adenosine A_{2A}

Abbreviations: IL-2, Interleukin 2; LDL, Low density lipoprotein; PTH, parathyroid hormone.
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were mediated through conformational changes in the transmembrane portion of the PTH receptor [52]. In chondrocytes, by contrast, PEMF enhanced the cAMP response to PTH [53]. In an osteoblast culture model, a CC field decreased the cAMP response to PTH and desensitized osteoblasts to PTH [54]. Studies with human fibroblasts have demonstrated an increase in calcium translocation and the number of insulin receptors in response to an electric field [55]. These studies suggest that electric fields trigger the opening of voltage-sensitive calcium channels followed by an increase in intracellular calcium. Inductively coupled fields stimulate lymphocyte proliferation by enhancing the use of IL-2 and the expression of the IL-2 receptor [56]. Electric fields may in some cells reorganize cytoskeletal and plasma membrane structures, providing pathways for cell surface receptors to migrate. Studies with large AC electric fields have demonstrated that cell surface molecules redistribute in response to these fields in a frequency-dependent manner [57]. Other electric field exposures increased mitogenic activity and the number of IGF-II receptors in a dose-dependent manner with dose being expressed as both frequency and duration of exposure [58]. Studies of osteoblast/osteoclast co-cultures have demonstrated that PEMF disrupts the interaction between calcitonin and its receptor systems and renders the osteoclasts insensitive to calcitonin [59]. These observations collectively demonstrate interactions of electric and electromagnetic fields with a wide variety of membrane receptors.

Detailed studies have recently been reported on the effects of PEMF on adenosine A_{2A} receptors [60]. Adenosine interacts with at least four cell membrane receptor subtypes—A_1, A_{2A}, A_{2B}, and A_3—that are coupled to G-proteins. A_{2A} receptors are present on neutrophils, monocytes, macrophages, lymphocytes, and platelets, and their activation appears to be associated with an inhibition of TNF-α, IL-6, and IL-8. Several anti-inflammatory drugs are mediated via adenosine receptors, including aspirin and methotrexate. In a series of Italian studies, detailed pharmacological assessment of the effects of PEMF on the

adenosine A_{2A} receptor has been performed [61–63]. Saturation-binding experiments revealed a significant increase in the adenosine A_{2A} receptor density in neutrophils treated with PEMF. The effect of these fields was specific to this receptor population and was dose dependent. The data suggest that the increase in adenosine receptor density is probably because of translocation of this receptor to the membrane surface rather than to synthesis of new receptors.

These studies demonstrated that electric and electromagnetic fields can affect ligand binding and alterations in the distribution and activity of receptor populations, thereby modulating transmembrane signaling [61,63,64].

Summary

Electric and electromagnetic fields signal connective tissue cells about the biophysical demands of their physical environment and the adequacy of the extracellular matrix to meet these demands. Muscle, ligament, bone, and cartilage all respond to electric and electromagnetic fields, and these biophysical agents can be applied in therapeutic contexts. Many laboratories have observed that electric and electromagnetic fields upregulate growth factor mRNA levels and protein synthesis, enhancing the synthesis of extra-cellular matrix proteins and accelerating tissue repair. Electric and electromagnetic fields produce sustained increases in growth factor concentrations at local sites of repair, making them useful for multiple applications in clinical repair and tissue engineering. Over the past 15 years, investigations have begun to clarify how cells respond to biophysical stimuli by means of transmembrane signaling and gene expression for structural and signaling proteins. Different cell types and cell cycle positions, as well the configuration and dose of electric or electromagnetic input, may determine which transmembrane signaling mechanisms are activated. Several studies have implicated factitious receptor activation or blockade as key mechanisms [44]. Subsequent studies will need to address the relationship of receptor interactions to changes in phenotypic expression of relevant cells, especially as regards extracellular matrix synthesis, in repair.

References

[1] Aaron RK, Ciombor DM. Electrical stimulation of bone induction and grafting. In: Habal M, Reddi AH, editors. Bone grafts and bone substitutes. Philadelphia: WB Saunders; 1992. p. 192–205.

[2] Ciombor DM, Lester G, Aaron RK, et al. Low frequency EMF regulates chondrocyte differentiation and expression of matrix proteins. J Orthop Res 2002;20:40–50.

[3] Aaron RK, Ciombor DM, Simon BJ. Treatment of nonunions with electric and electromagnetic fields. Clin Orthop Relat Res 2004;419:21–9.

[4] Friedenberg ZB, Brighton CT, Michelson JD, et al. The effects of demineralized bone matrix and direct current on an "*in vivo*" culture of bone marrow cells. J Orthop Res 1989;7(1):22–7.

[5] Aro H, Eerola E, Aho AJ, et al. Electrostimulation of rat callus cells and human lymphocytes *in vitro*. J Orthop Res 1984;2:23–31.
[6] Farndale R, Murray JC. Pulsed electromagnetic fields promote collagen production in bone marrow fibroblasts via athermal mechanisms. Calcif Tissue Int 1985;37:178–82.
[7] Connolly JF, Ortiz J, Price RR, et al. The effect of electrical stimulation on the biophysical properties of fracture healing. Ann N Y Acad Sci 1974;238:519–29.
[8] Petersson C, Holmer N, Johnell O. Electrical stimulation of osteogenesis: studies of the cathode effect on rabbit femur. Acta Orthop Scand 1982;53:727–32.
[9] Brighton CT, Hozack WJ, Brager MD, et al. Fracture healing in the rabbit fibula when subjected to various capacitively coupled electrical fields. J Orthop Res 1985;3:331–40.
[10] Brighton CT, Pollack SR, Windsor RE. Stimulation of fracture healing by a capacitively coupled electric field in the rabbit fibula. Trans Orthop Res Soc 1981;6:93.
[11] Bassett CAL, Valdes MG, Hernandez E. Modification of fracture repair with selected pulsing electromagnetic fields. J Bone Joint Surg 1982;64:888–95.
[12] Bassett CAL, Choski HR, Hernandez E, et al. The effect of pulsing electromagnetic fields on cellular calcium and calcification of nonunions. In: Brighton CT, Black J, Pollack S, editors. Electrical properties of bone and cartilage. New York: Grune and Stratton; 1979. p. 427–42.
[13] Inoue N, Ohnishi I, Chen D, et al. Effect of pulsed electromagnetic fields (PEMF) on late-phase osteotomy gap healing in a canine tibial model. J Orthop Res 2002;20:1106–14.
[14] Fredericks DC, Nepola JV, Baker JT. Effects of pulsed electromagnetic fields on bone healing in a rabbit tibial osteotomy model. J Orthop Trauma 2000;14:93–100.
[15] Fredericks DC, Piehl DJ, Baker JT, et al. Effects of pulsed electromagnetic field stimulation on distraction osteogenesis in the rabbit tibial leg lengthening model. J Pediatr Orthop 2003;23: 478–83.
[16] Akai M, Hayashi K. Effect of electrical stimulation on musculoskeletal systems: a meta-analysis of controlled clinical trials. Bioelectromagnetics 2002;23:132–43.
[17] Borsalino G, Bagnacani M, Bettati E, et al. Electrical stimulation of human femoral intertrochanteric osteotomies. Clin Orthop Relat Res 1988;237:256–63.
[18] Mammi GI, Rocchi R, Cadossi R. The electrical stimulation of tibial osteotomies. Double-blind study. Clin Orthop Relat Res 1993;288:246–53.
[19] Traina GC, Sollazzo V, Massari L. Electrical stimulation of tibial osteotomies; a double blind study. In: Bersani F, editor. Electricity and magnetism in biology and medicine. New York: Kluwer Academic/Plenum; 1999. p. 137–8.
[20] DeHaas WG, Watson J, Morrison DM. Noninvasive treatment of ununited fractures of the tibia using electrical stimulation. J Bone Joint Surg 1980;62B:465–70.
[21] Dunn AW, Rush GA. Electrical stimulation in treatment of delayed union and nonunion of fractures and osteotomies. South Med J 1984;77:1530–4.
[22] Sharrard WJ. A double blind trial of pulsed electromagnetic fields for delayed union of tibial fractures. J Bone Joint Surg 1990;72B:347–55.
[23] Scott G, King JB. A prospective double blind trial of electrical capacitive coupling in the treatment of nonunion of long bones. J Bone Joint Surg 1994;76A:820–6.
[24] Brighton CT, Shaman P, Heppenstall RB, et al. Tibial nonunion treated with direct current, capacitive coupling, or bone graft. Clin Orthop Relat Res 1995;321:223–34.
[25] Black J. Electrical stimulation: its role in growth, repair and remodeling of the musculoskeletal system. New York: Praeger; 1987.
[26] Paterson DC, Lewis GN, Cass CA. Treatment of delayed union and nonunion with an implanted direct current stimulator. Clin Orthop Relat Res 1980;148:117–28.
[27] Brighton CT, Black J, Friedenberg ZB, et al. A multicenter study of the treatment of nonunion with constant direct current. J Bone Joint Surg 1981;63A:2–13.
[28] Bassett CA, Mitchell SN, Gaston SR. Treatment of ununited tibial diaphyseal fractures with pulsing electromagnetic fields. J Bone Joint Surg 1981;63:511–23.
[29] Heckman JD, Ingram AJ, Loyd RD, et al. Nonunion treatment with pulsed electromagnetic fields. Clin Orthop Relat Res 1981;161:58–66.

[30] Brighton CT, Pollack SR. Treatment of recalcitrant nonunions with a capacitively coupled electrical field. J Bone Joint Surg 1985;67:577–85.
[31] Gossling HR, Bernstein RA, Abbott J. Treatment of ununited tibial fractures: a comparison of surgery and pulsed electromagnetic fields (PEMF). Orthopedics 1992;15(6):711–9.
[32] Bassett CA, Mitchell SN, Schink MM. Treatment of therapeutically resistant nonunions with bone grafts and pulsing electromagnetic fields. J Bone Joint Surg 1982;64:1214–24.
[33] Dhawan SK, Conti SF, Towers J, et al. The effect of pulsed electromagnetic fields on hindfoot arthrodesis: a prospective study. J Foot Ankle Surg 2004;43:93–6.
[34] Donley BG, Ward DM. Implantable electrical stimulation in high-risk hindfoot fusions. Foot Ankle Int 2002;23:13–8.
[35] Midis N, Conti SF. Revision ankle arthrodesis. Foot Ankle Int 2002;23:243–7.
[36] Holmes Jr GB. Treatment of delayed unions and nonunions of the proximal fifth metatarsal with pulsed electromagnetic fields. Foot Ankle Int 1994;15:552–6.
[37] Reynolds JC. The use of implantable direct current stimulation in bone grafted foot and ankle arthrodeses: a retrospective review. Presented at the 15th Annual Meeting of the Mid-America Orthopedic Association. Hilton Head Island, SC, April 23–27, 1997.
[38] Fitzsimmons RJ, Strong DD, Mohan S, et al. Low-amplitude, low-frequency electric field-stimulated bone cell proliferation may in part be mediated by increased IGF-II release. J Cell Physiol 1992;150:84–9.
[39] Ryaby J, Fitzsimmons RG, Khin NA, et al. The role of insulin-like growth factor II in magnetic field regulation of bone formation. Bioelectrochem Bioenerg 1994;35:87–91.
[40] Fitzsimmons RJ, Ryaby JT, Mohan S, et al. Combined magnetic fields increase insulin-like growth factor-II in TE-85 human osteosarcoma bone cell cultures. Endocrinology 1995;136: 3100–6.
[41] Nagai M, Ota M. Pulsating electromagnetic field stimulates mRNA expression of bone morphogenetic protein -2 and -4. J Dent Res 1994;73:1601–5.
[42] Yajima A, Ochi M, Hirose Y. Effects of pulsing electromagnetic fields on gene expression of bone morphogenetic proteins in human osteoblastic cell line *in vitro*. J Bone Min Res 1996;11(Suppl.1):381.
[43] Bodamyali T, Bhatt B, Hughes F, et al. Pulsed electromagnetic fields simultaneously induce osteogenesis and upregulate transcription of bone morphogenetic proteins 2 and 4 in rat osteoblasts *in vitro*. Biophys Biochem Res Comm 1998;250:458–61.
[44] Aaron RK, Boyan BD, Ciombor DM, et al. Stimulation of growth factor synthesis by electric and electromagnetic fields. Clin Orthop Relat Res 2004;419:30–7.
[45] Zhuang H, Wang W, Seldes R, et al. Electrical stimulation induces the level of TGF-beta1 mRNA in osteoblastic cells by a mechanism involving calcium/calmodulin pathway. Biochem Biophys Res Commun 1997;237:225–9.
[46] Guerkov HH, Lohmann CH, Liu Y, et al. Pulsed electromagnetic fields increase growth factor release by nonunion cells. Clin Orthop Relat Res 2001;384:265–79.
[47] Lohmann CH, Schwartz Z, Liu Y, et al. Pulsed electromagnetic field stimulation of MG63 osteoblast-like cells affects differentiation and local factor production. J Orthop Res 2000;18: 637–46.
[48] Lohmann CH, Schwartz Z, Liu Y, et al. Pulsed electromagnetic fields affect phenotype and connexin 43 protein expression in MLO-Y4 osteocyte-like cells and ROS 17/2.8 osteoblast-like cells. J Orthop Res 2003;21:326–34.
[49] Aaron RK, Ciombor DM, Keeping HS. Power frequency fields promote cell differentiation coincident with an increase in transforming TGFβ expression. Bioelectromagnetics 1999;10: 453–8.
[50] Aaron RK, Wang S, Ciombor DM. Upregulation of basal TGFβ$_1$ levels by EMF coincident with chondrogenesis: implications for skeletal repair and tissue engineering. J Orthop Res 2002;20:233–40.
[51] Luben RA, Cain CD, Chen MC-Y, et al. Effects of electromagnetic stimuli on bone and bone cells *in vitro:* inhibition of responses to parathyroid hormone by low-energy, low-frequency fields. Proc Natl Acad Sci USA 1982;79:4180–4.

[52] Cain CD, Adey WR, Luben RA. Evidence that pulsed electromagnetic fields inhibit coupling of adenylate cyclase by parathyroid hormone in bone cells. J Bone Min Res 1987;2:437–41.
[53] Hiraki Y, Endo N, Takigawa M, et al. Enhanced responsiveness to parathyroid hormone and induction of functional differentiation of cultured rabbit costal chondrocytes by a pulsed electromagnetic field. Biochim Biophys Acta 1987;931:94–100.
[54] Brighton CT, McCluskey W. Response of cultured bone cells to a capacitively coupled electric field: inhibition of cAMP response to parathyroid hormone. J Orthop Res 1988;6:567–71.
[55] Bourguignon GJ, Jy W, Bourguignon LY. Electric stimulation of human fibroblasts causes an increase in Ca2+ influx and the exposure of additional insulin receptors. J Cell Physiol 1989;140:379–85.
[56] Cossarizza A, Monti D, Bersani F, et al. Extremely low frequency pulsed electromagnetic fields increase interleukin-2 (IL-2) utilization and IL-2 receptor expression in mitogen-stimulated human lymphocytes from old subjects. FEBS Lett 1989;248:141–4.
[57] Cho MR, Thatte HS, Lee RC, et al. Induced redistribution of cell surface receptors by alternating current electric fields. FASEB J 1994;8:771–6.
[58] Fitzsimmons RG, Ryaby JT, Magee FP, et al. IGF-II receptor number is increased in TE-85 cells by low-amplitude, low frequency electromagnetic field (EMF) exposure. J Bone Min Res 1995;10:812–9.
[59] Shankar VS, Simon BJ, Bax CM, et al. Effects of electromagnetic stimulation on the functional responsiveness of isolated rat osteoclasts. J Cell Physiol 1998;176:537–44.
[60] Varani K, Gessi S, Merighi S, et al. Effect of low frequency electromagnetic fields on A2A adenosine receptors in human neutrophils. Br J Pharma 2002;136:57–66.
[61] Bersani F, Marinelli F, Ognibene A, et al. Intramembrane protein distribution in cell cultures is affected by 50 Hz pulsed magnetic fields. Bioelectromagnetics 1997;18:463–9.
[62] Cadossi R, Bersani F, Cossarizza A, et al. Lymphocytes and low-frequency electromagnetic fields. FASEB J 1992;6:2667–74.
[63] Chiabrera A, Bianco B, Moggia E, et al. Zeeman-Stark modeling of the RF EMF interaction with ligand binding. Bioelectromagnetics 2000;21:312–24.
[64] Massot O, Grimaldi B, Bailly JM, et al. Magnetic field desensitizes 5-HT(1B) receptor in brain: pharmacological and functional studies. Brain Res 2000;858:143–50.