BIOELECTRICITY
Volume 1, Number 4, 2019

DOI: 10.1089/bioe.2019.0026

The Use of Pulsed Electromagnetic Field to Modulate Inflammation and Improve Tissue Regeneration: A Review

Christina L. Ross, PhD,[1] Yu Zhou, PhD,[2] Charles E. McCall, MD,[3]
Shay Soker, PhD,[2] and Tracy L. Criswell, PhD[2]

Abstract

Pulsed electromagnetic field (PEMF) is emerging as innovative treatment for regulation of inflammation, which could have significant effects on tissue regeneration. PEMF modulates inflammatory processes through the regulation of pro- and anti-inflammatory cytokine secretion during different stages of inflammatory response. Consistent outcomes in studies involving animal and human tissue have shown promise for the use of PEMF as an alternative or complementary treatment to pharmaceutical therapies. Thus, PEMF treatment could provide a novel nonpharmaceutical means of modulating inflammation in injured tissues resulting in enhanced functional recovery. This review examines the effect of PEMF on immunomodulatory cells (e.g., mesenchymal stem/stromal cells [MSCs] and macrophages [M_Φ]) to better understand the potential for PEMF therapy to modulate inflammatory signaling pathways and improve tissue regeneration. This review cites published data that support the use of PEMF to improve tissue regeneration. Our studies included herein confirm anti-inflammatory effects of PEMF on MSCs and M_Φ.

Keywords: pulsed electromagnetic field (PEMF), inflammation, regeneration, mesenchymal stem cells (MSCs), macrophages (THP-1s), muscle cells (C2C12).

Introduction

The immune system plays an essential role in tissue regeneration following tissue damage as well as during cell signaling homeostasis. The immune response to tissue injury is crucial in determining the efficacy and rate of the healing process, including the extent of scarring and the restoration of organ function.[1] To integrate the immune system into regenerative strategies, one of the first challenges is to modulate the precise functions of the different immune components during the tissue healing process. The regulatory interactions of the immune system with tissue regeneration are not unidirectional, and stem cells, as key players in regeneration, can modulate the immune system in several ways to facilitate regeneration.[1,2]

However, the immune system does not always perform a complementary role in regeneration, and several reports have suggested that increased inflammation can inhibit the regeneration process. An argument can be made that there are immune-mediated mechanisms of regeneration and repair that can be modulated by pulsed electromagnetic field (PEMF) therapy to improve the ability of tissue to regenerate. Until recently, allopathic medicine rejected the possibility that an electromagnetic field (EMF) could affect biochemical mechanisms with weak electrical fields. Biochemistry, however, is based on an understanding of the flow of energy that drives chemical reactions.[3]

Physical properties of molecules can be combined to express internal energy and thermodynamic potentials, which are necessary for equilibrium and homeostasis in spontaneous processes.[4] New models of biophysics emphasize cooperative electrical activity of highly ordered elements at all levels of physiology: cells, tissues, organs, organ systems, as well as the entire human organism. Research has shown that effects caused by low-frequency or weak EMF therapies can induce changes in cell proliferation, alterations in membrane structure and function, changes in nucleic acids, protein phosphorylation and adenosine triphosphate (ATP) synthesis, as well as entrainment of brain rhythms and conditioned brain response *in vitro* and *in vivo*.[5–7] Parameters of these EMFs include frequency, intensity (field strength), waveform, and time

[1]Center for Integrative Medicine, Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina.
[2]Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina.
[3]Department of Microbiology and Immunology, Wake Forest School of Medicine, Winston-Salem, North Carolina.

of exposure. Recognition of physiological sensitivities to *exogenous* EMF came from the observation of *endogenous* internal electrical processes.[4] For example, the piezoelectric properties of bone use electromechanical control to determine either osteoblastic or osteoclastic phenotype of cells.[8] However, biophysical properties of cell function have mostly been ignored when choosing treatments for inflammation/immune modulation and regenerative medicine therapies.

Using PEMF to regulate cell signaling mechanisms involved in the inflammatory/immune response pathways of different cell types has become an innovative alternative treatment in the pursuit of regenerative therapies.[9,10] Several studies have reported that PEMF can modulate both cell surface receptor expression/activation and downstream signal transduction pathways, thereby restoring homeostatic cell functions such as viability, proliferation, differentiation, communication with neighboring cells, and interaction with components of the extracellular matrix (ECM).[11–18] PEMF can activate multiple intracellular pathways, including numerous processes and biochemical mechanisms within both the immune system and tissue regenerative processes, such as the musculoskeletal system[7,19,20] and the nervous system.[21–23] PEMFs are physical stimuli that affect biological systems through the production of coherent or interfering fields that modify fundamental electromagnetic frequencies generated by living organisms.[7,24] These endogenous frequencies are ubiquitous in tissue, for instance, frequencies from 5 to 30 Hz have been found during postural muscle activity (quiet standing) and 10 Hz during walking.[25] Successful regeneration requires a balanced immune cell response, with the recruitment of accurately polarized immune cells in an appropriate quantity.[2] Here is where PEMF could have an influential role in the inflammatory process and thereby support tissue regeneration.

Immune Function and Inflammation

Following injury, the immune system is responsible for maintaining a delicate balance between proinflammatory and pro-regenerative immune cells. A successful response to acute inflammation eliminates immune activation, followed by a resolution of inflammation and tissue repair by numerous anti-inflammatory cytokines as well as lipid mediators.[26–28] However, chronic inflammation can occur when the inflammatory triggers persist and shift the homeostatic set points, and cellular effectors fail to control the triggers activating inflammation, causing persistent tissue damage, and the inability to restore homeostasis.[29] Multipotent mesenchymal stem/stromal cells (MSCs) home to damaged tissues and contribute to their repair by secretion of cytokines, chemokines, and ECM proteins.[30] MSCs are capable of modulating the immune response.[31] M1 macrophages (M_Φ) promote cell recruitment and proliferation, whereas M2 macrophages promote differentiation and tissue regeneration. The polarization switch from M1 to M2 macrophage phenotype is important for promoting functional tissue regeneration. Both cell phenotypes are critical; however, the presence of more M1 than M2 macrophages has been shown to inhibit tissue repair.[32] Plasticity is a main feature of these cells, resulting in extreme heterogeneity in normal and pathological conditions.[33,34] Neither MSCs nor M_Φ are homogenous populations of cells but can be generally categorized into two broad, but distinct subsets: (1) classically activated type 1 proinflammatory MSC1/M1, which present a proinflammatory phenotype, or (2) alternatively activated MSC2/M2 type 2, an anti-inflammatory phenotype.[35] This phenomenon, termed polarization, results from cell/cell or cell/molecule interactions governing MSC/M_Φ function within the host tissue. During normal regulation of inflammation, healthy immune cells return the activated system back to homeostasis by downregulating proinflammatory cytokines, stabilizing the immune system by upregulating anti-inflammatory cytokines, and regenerating damaged tissue.[36] Specifically, MSCs have been shown to induce anti-inflammatory cytokine interleukin (IL)-10 secreting macrophages in both *in vitro* and *in vivo* studies. In particular, lipopolysaccharide (LPS)-stimulated macrophages produced more IL-10 when cultured with bone-marrow MSCs; however, this effect was eliminated if the bone-marrow MSCs lacked the genes encoding TLR-4, myeloid differentiation primary response gene-88, tumor necrosis factor-α (TNF-α), or Cox-2,[37] and in diabetic rats, MSC treatment upregulated anti-inflammatory cytokine IL-10.[38] MSCs appear to play a dual role whereby they promote the M1 differentiation of naive M_Φ, yet in the presence of M1, MSCs promote the M1 to M2 conversion in MSC-M1 cocultures.[39] The differentiation of specific MSC/M_Φ cell phenotypes is closely related to the surrounding environment that acts as an orchestrator of immune activity. The return to homeostasis of the immune function after acute activation is the sign of a healthy immune system.[40] Based on previous studies, the introduction of PEMF could provide beneficial outcomes for modulating inflammation and promoting tissue regeneration.

Inflammation and Tissue Regeneration

Successful mammalian regeneration requires precise coordination of multiple processes, including scavenging cellular debris, proliferation and activation of progenitor cells, immune modulation, and angiogenesis and innervation of the newly forming tissue.[41] MSCs have differentiation and immunomodulatory properties that are influenced by the inflammatory microenvironment at the sites of injury; however, they are quiescent until activated by inflammatory mediators.[42] The specific molecular and cellular mechanisms involved in the immunoregulatory activity of MSCs are still being investigated, but there is evidence that these cells can be influenced to regulate immune response at the molecular level. Toll-like receptor (TLR) activation triggers intracellular signaling pathways that lead to the induction of inflammatory cytokines and upregulation of co-stimulatory molecules resulting in activation of the adaptive immune response. TLRs polarize MSCs toward proinflammatory and antigen presenting-like phenotypes, leading to a release of proinflammatory cytokines and chemokines enhancing the recruitment of inflammatory immune cells.[42,43] Polarization occurs in the immune response driven by M1 pro- and M2 anti-inflammatory M_Φ.[44] Both arms of the inflammatory response are required for repair in many systems, such as heart, skeletal muscle, and the central nervous system.[32,41,45,46] Unregulated proinflammatory signals result in premature initiation of the anti-inflammatory cell signals, which can disrupt effective tissue healing.[41] For example, skeletal muscle regeneration is impaired when M_Φ are prematurely activated by

treatment with the anti-inflammatory cytokine IL-10.[47] In both skeletal muscle regeneration and nerve remyelination, M1 recruit and stimulate progenitor cell proliferation, whereas M2 regulate differentiation, demonstrating the necessity of both responses.[41] Many soluble factors secreted by M1 and M2 are important to skeletal muscle regeneration, including M1 activation and stimulation of proliferation of the local stem cell pool (i.e., satellite cell proliferation through production of IL-1β, IL-6, and TNF-α), whereas the anti-inflammatory cytokines IL-3, IL-4, and IL-10 are secreted by M2.[48] The same argument can be made for MSC1 (proinflammatory) and MSC2 (anti-inflammatory) phenotypes, where MSC1-based therapies attenuate tumor growth and MSC2-based therapies promote tumor growth and metastasis.[49] The importance of inflammatory homeostasis cannot be overstated; therefore, a therapeutic regulator is necessary for restoring immune function and tissue homeostasis. Our hypothesis is that low-frequency PEMF can modulate inflammatory response in activated MSC and M_Φ production and also affect the transcription factor NF-κB in C2C12 cells. Hence, we decided to test this hypothesis and perform a feasibility study to investigate the therapeutic potential of PEMF to influence inflammatory mediators in both immune and muscle cells.

Anti-inflammatory Effects of PEMF on MSCs, M_Φ, and Satellite Cells

PEMF therapy is based on Faraday's law, a basic law of electromagnetism that predicts how a magnetic field will interact with an electric circuit to produce an electromotive force known as electromagnetic induction.[50] To deliver PEMF dosimetry, it is necessary to include three important parameters: frequency, intensity, and duration/time of exposure.[24] PEMF intensity is dependent on wave intensity/field strength measured in units of Tesla (T) or Gauss (10,000 T). To test the effect of PEMF on cytokine signaling, a Helmholtz Coil (Micro Magnetics) was used to expose the cells to the fields. Frequency was measured with an oscilloscope and field strength measured with a Gauss/Tesla meter. Activated MSCs (human bone marrow), THP-1 (human macrophages), and C2C12 (mouse muscle) cells were exposed to dosimetry based on previous studies shown to therapeutically effect cytokine signaling.[19,51] Both bone-marrow-derived human MSCs and THP-1 M_Φ were activated by treatment with LPS and/or polyinosinic:polycytidylic acid [Poly(I:C)] and then monitored for changes in inflammatory cell signaling pathways using fluorescent immunocytochemical (ICC) analyses (R & D Systems, Minneapolis, MN). Quantitative analysis of the ICC staining was determined using GraphPad Prism 5.[52]

TLR priming protocol

Mesenchymal stem/stromal cells. Incubation with LPS (2 μg/mL) or Poly (I:C) (2 μg/mL) was added as the MSC agonists for TLR-4 and TLR-3, respectively, for no longer than 1 h (per Waterman et al. protocol[44]) before being exposed to PEMF. Since half-life time periods are short (15–20 min) for anti-inflammatory cytokines IL-3 and IL-4, samples were taken immediately to histology and fixed onto slides. Table 1 shows which ILs were tested for which cell type and their respective inflammatory phenotype.

THP-1 cells. Incubation with LPS (2 μg/mL) per Yoon et al. protocol[53] was added as the THP-1 agonist for TLR-4 for exactly 4 h before being exposed to PEMF. Since half-life time periods are short (15–20 min) for anti-inflammatory cytokines, samples were taken immediately to histology and fixed onto slides.

PEMF exposure

Once activated, both the MSC and THP-1 cells were exposed to PEMF for 5 min using a sine wave frequency of 5.1 Hz, magnetic field intensity of 0.04 mT, and induced electric field (EF) intensity of 0.07 mV/cm. TLR-3-activated [2 μL/mg Poly (I:C)] MSCs increased secretion of anti-inflammatory cytokines IL-3, IL-4, and IL-10, and TLR-4-activated (2 μL/mg LPS) MSCs increased the production of proinflammatory cytokines (IL-1b, IL-6, and IL-17A) molecules (Figs. 1 and 2). Results showed that the production of anti-inflammatory cytokine secretion (IL-3, IL-4, and IL-10) was stabilized, whereas PEMF significantly decreased the production of proinflammatory signaling in IL-1b, IL-6, and IL-17A cytokines (Figs. 1 and 2) in the MSCs. The TLR-4 receptor was activated (2 μL/mg LPS) in the THP-1 M_Φ to stimulate the production of proinflammatory cytokines IL-1b, IL-6, and TNF-α, and anti-inflammatory cytokine IL-10. After exposure to PEMF, outcomes show decreases in the proinflammatory cytokines secretion (IL-1b, IL-6, and TNF-α) and increase/stabilization of IL-10 in THP-1s (Figs. 3 and 4).

The C2C12 murine myoblast cell line, expressing an NF-κB-responsive fluorescent mKATE reporter, was used to examine PEMF effects on inflammatory pathways in skeletal muscle cells (Fig. 5). Cells were treated with 10 ng/mL TNF-α for 20 min, followed by PEMF treatment for 15 min at 15 Hz and 2 mT sine wave. Growth and differentiation of

Table 1. Mesenchymal Stem/Stromal Cells Were Activated with Lipopolysaccharide to Stimulate TLR-4 Proinflammatory Cytokines IL-1b, IL-6, and IL-17A

Activated cell type/ biomarker	*Proinflammatory cytokine (LPS)*	*Proinflammatory cytokine (LPS)*	*Proinflammatory cytokine (LPS)*	*Anti-inflammatory cytokine [Poly(I:C)]*	*Anti-inflammatory cytokine [Poly(I:C)]*	*Anti-inflammatory cytokine [Poly(I:C)]*
MSC	IL-1b	IL-6	IL-17A	IL-3	IL-4	IL-10
THP-1	IL-1b	IL-6	TNF-α	IL-10[a]		

Poly (I:C) stimulated TLR-3 anti-inflammatory cytokines IL-3, IL-4, and IL-10. THP-1 cells were activated with LPS to stimulate TLR-4 proinflammatory cytokines IL-1b, IL-6, and TNF-α.

[a]LPS was used to activate the TLR-4 anti-inflammatory cytokine IL-10.

IL, interleukin; LPS, lipopolysaccharide; MSC, mesenchymal stem/stromal cells; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-α.

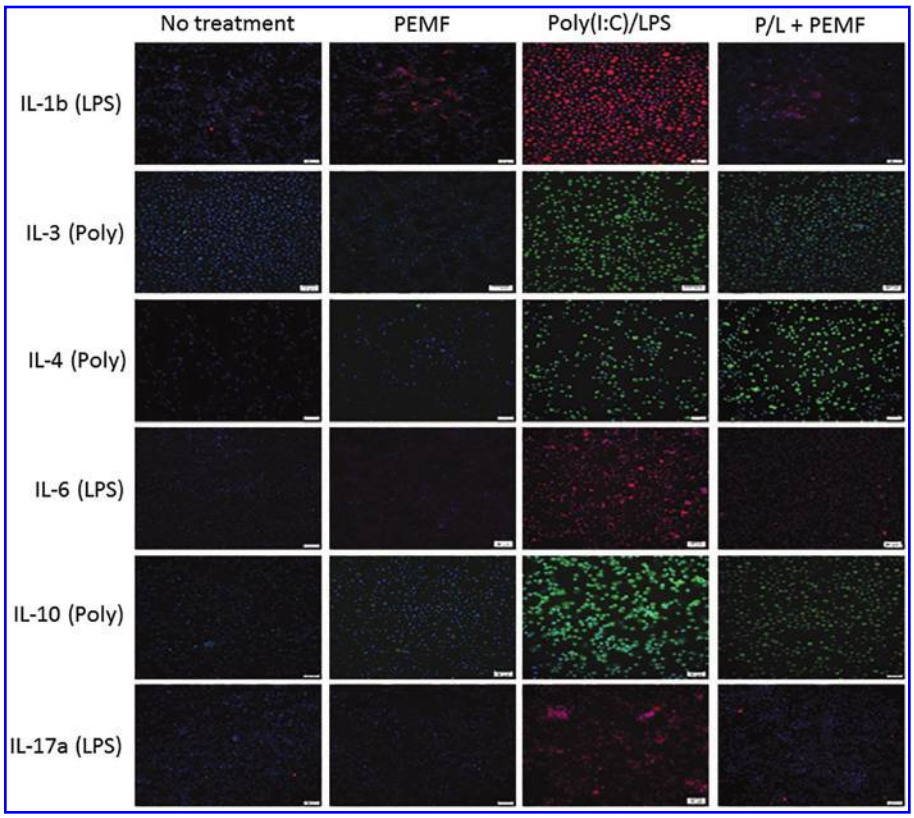

FIG. 1. MSCs were for both the TLR-3 (Poly), stimulating anti-inflammatory cytokine signaling; and TLR-4 (LPS) receptors, stimulating proinflammatory cytokine signaling. After PEMF exposure, activated MSCs show decrease of proinflammatory cell signaling (IL-1β, IL-6, and IL-17A) and stabilization of anti-inflammatory cytokine signaling (IL-3, IL-4, and IL-10). $N=5$ trials for IL-1b, IL-6, and IL-10; and $N=3$ trials for IL-3, IL-4, and IL-17A. IL, interleukin; L, LPS for proinflammatory cytokine activation; LPS, lipopolysaccharide; MSCs, mesenchymal stem/stromal cells; P, Poly (I:C) for anti-inflammatory cytokine activation; TLR, Toll-like receptor.

myoblasts into myotubes with or without TNF-α were assessed after PEMF treatment. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to measure changes in the NF-κB inflammatory pathway in TNF-α-treated cells 25 min after PEMF exposure. TNF-α exposure increased the response of the NF-κB fluorescent mKATE reporter in treated C2C12 cells as indicated by fluorescent staining, which was significantly reduced when cells were exposed to PEMF (Figs. 6 and 7). Furthermore, PEMF treatment significantly increased the expression of the NF-κB inhibitor alpha gene (*Nfkbia*) (Fig. 8).

PEMF and Inflammation

Promoting the return to homeostasis between pro- and anti-inflammatory signaling mechanisms is instrumental in bringing the immune response back to normal function and preventing an acute-phase inflammatory response from becoming chronic. In this phase of the inflammatory response, PEMF has the potential for regulating immune cell signaling mechanisms to promote tissue regeneration. Immunomodulatory cells such as MSCs mediate their immunosuppressive effects through a variety of mechanisms, including the induction of macrophages, which also have immunomodulatory capabilities.[54] Our data showed a significant decrease in IL-1β secretion in both the human MSCs and THP-1 macrophages after activation with LPS and exposure to PEMF. This proinflammatory cytokine causes a number of autoinflammatory conditions, such as type 2 diabetes mellitus, pulmonary fibrosis, Crohn's disease, and atherosclerosis.[55] In the THP-1 cells, PEMF showed a significant decrease in TNF-α, a proinflammatory cytokine involved in systemic inflammation, produced mainly by activated macrophages.[56] In activated MSCs, cytokine IL-3 and IL-4 results showed a stabilization of these signaling molecules after cells were exposed to PEMF (Fig. 1). IL-3 is an anti-inflammatory cytokine associated with macrophages reported to improve the body's natural defense to disease as part of the immune response.[36] IL-4 is an anti-inflammatory cytokine that stimulates activated B cell and T cell proliferation and is a key regulator in humoral and adaptive immunity.[57] Results of our data also showed significant reduction of IL-6 production in activated MSCs and THP-1 cells (Figs. 1–4) after PEMF exposure. IL-6 acts as both a proinflammatory cytokine and an anti-inflammatory myokine.[58,59] Skeletal muscle produces and releases significant levels of IL-6, which has been associated with stimulation of hypertrophic muscle growth and myogenesis through regulation of muscle satellite cells.[59] Paradoxically, deleterious actions for IL-6 have been proposed for atrophy and muscle wasting.[59]

To test IL-10 signaling in the MSCs, cells were activated with Poly(I:C) via the TLR-3 receptor. In the THP-1 cells, IL-10 was activated with LPS. PEMF showed a stabilization/increase in IL-10 production in both MSCs and THP-1 cells (Figs. 1–4). IL-10 is an anti-inflammatory cytokine with multiple pleiotropic effects in immunoregulation and inflammation, including the blocking of the NF-κB activity.[60] IL-10 predominantly inhibits LPS and bacterial product-mediated induction of the proinflammatory cytokines TNF-α and NF-κB.[61] IL-10 is a cytokine with potent anti-inflammatory properties that play a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis. Dysregulation of IL-10 is associated with enhanced immunopathology in response to infection as well as increased risk for development of many autoimmune diseases.[62]

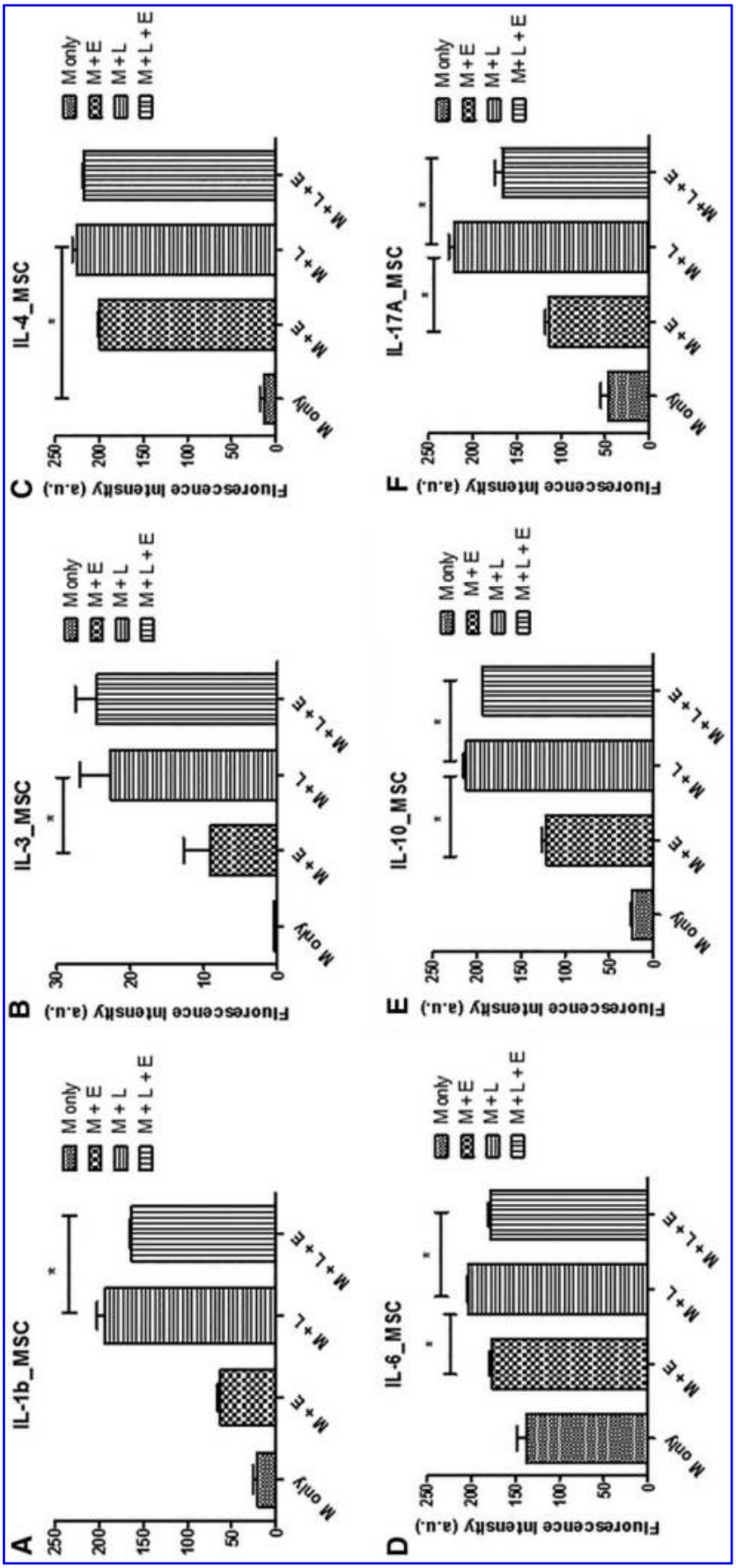

FIG. 2. Quantitative analysis of Figure 1 shows decrease in signaling of proinflammatory cytokines (**A**) IL-1b, (**D**) IL-6, and (**F**) IL-17A in activated MSCs; and stabilization or increase of anti-inflammatory cytokines (**B**) IL-3, (**C**) IL-4, and (**E**) IL-10 after activation of MSCs. $N = 5$ independent trials run for $^*p < 0.05$.

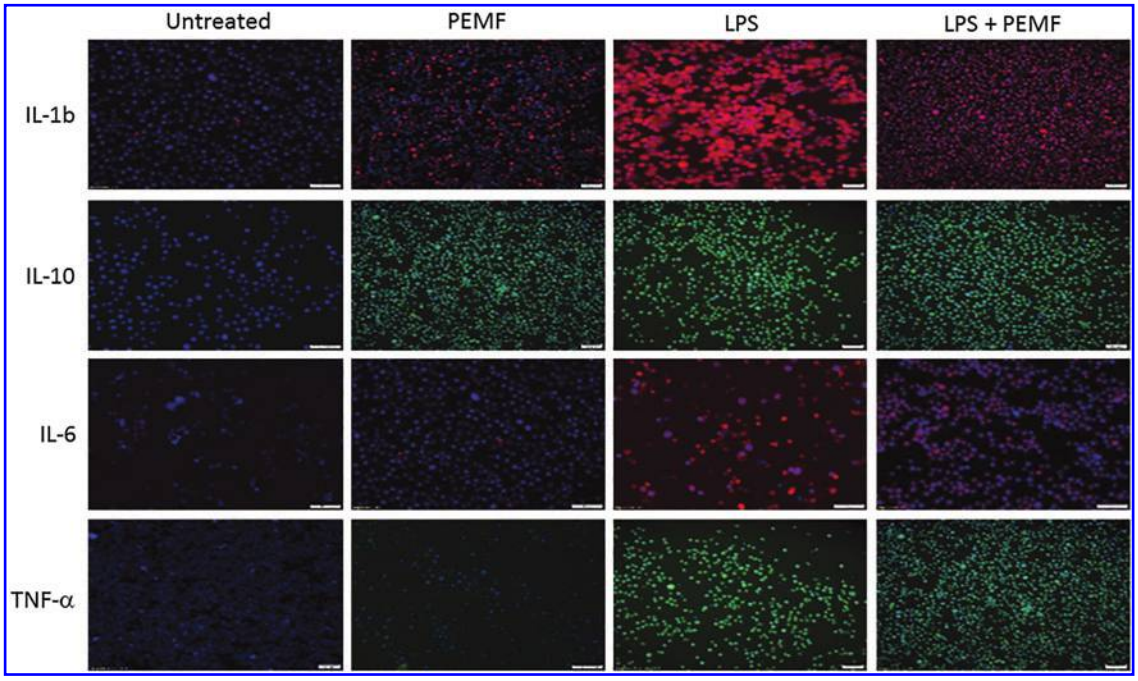

FIG. 3. THP-1 cells show decrease in proinflammatory cytokines signaling for IL-1b, IL-6, and TNF-α and increase/stabilization of anti-inflammatory cytokine signaling (IL-10) after exposure to PEMF ($N=5$ trials). PEMF, pulsed electromagnetic field. TNF-α, tumor necrosis factor-α.

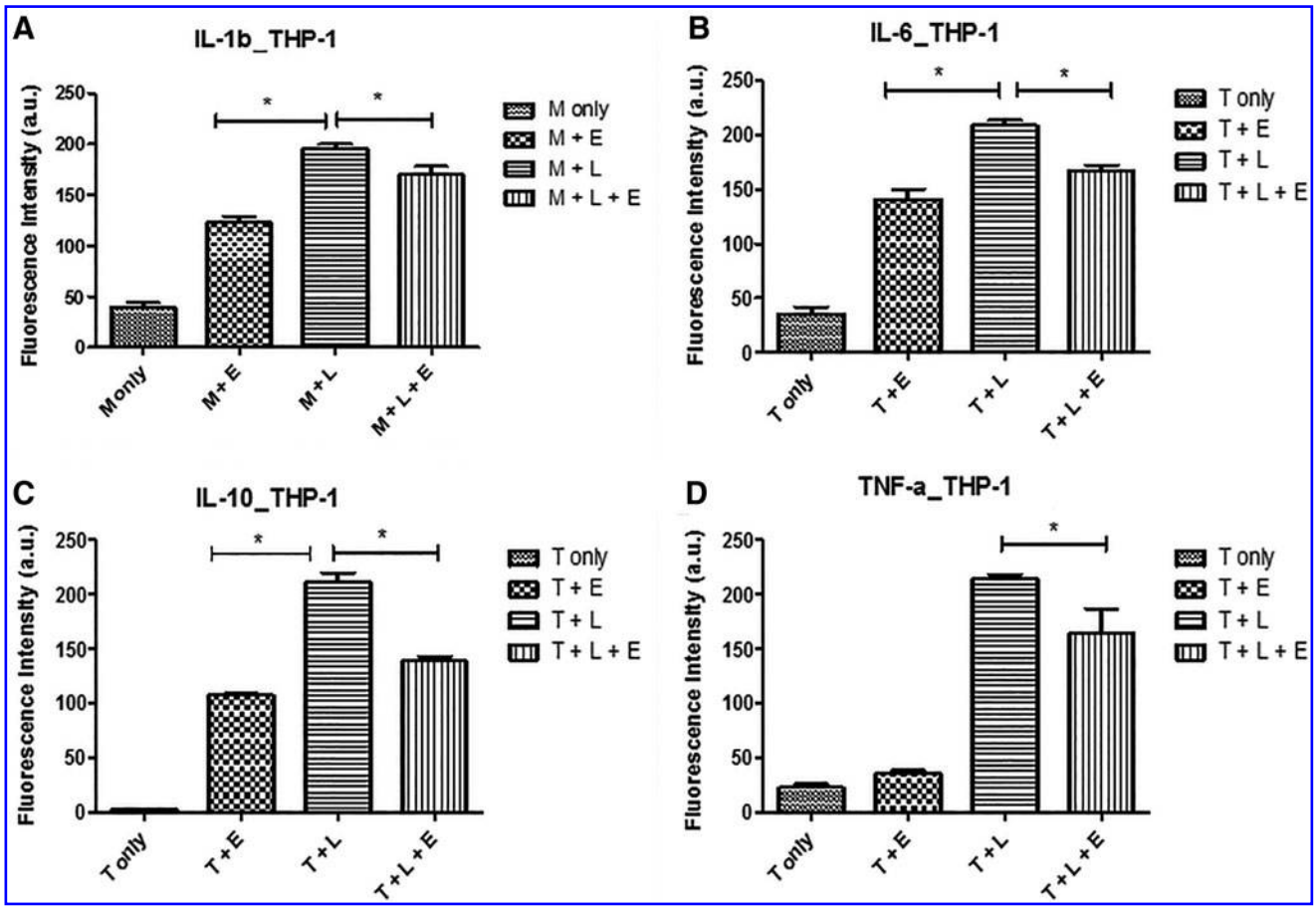

FIG. 4. Quantitative analysis of Figure 3 shows activated THP-1 cells exposed to PEMF and measured for proinflammatory cytokines and anti-inflammatory cytokine IL-10. Results show statistically significant decreased **(A)** IL-1b, **(B)** IL-6, and **(D)** TNF-α signaling. Anti-inflammatory cytokine **(C)** IL-10 showed increase in signaling before activation and decrease in signaling after activation. $N=5$ independent trials run for cytokines, with $^*p<0.05$.

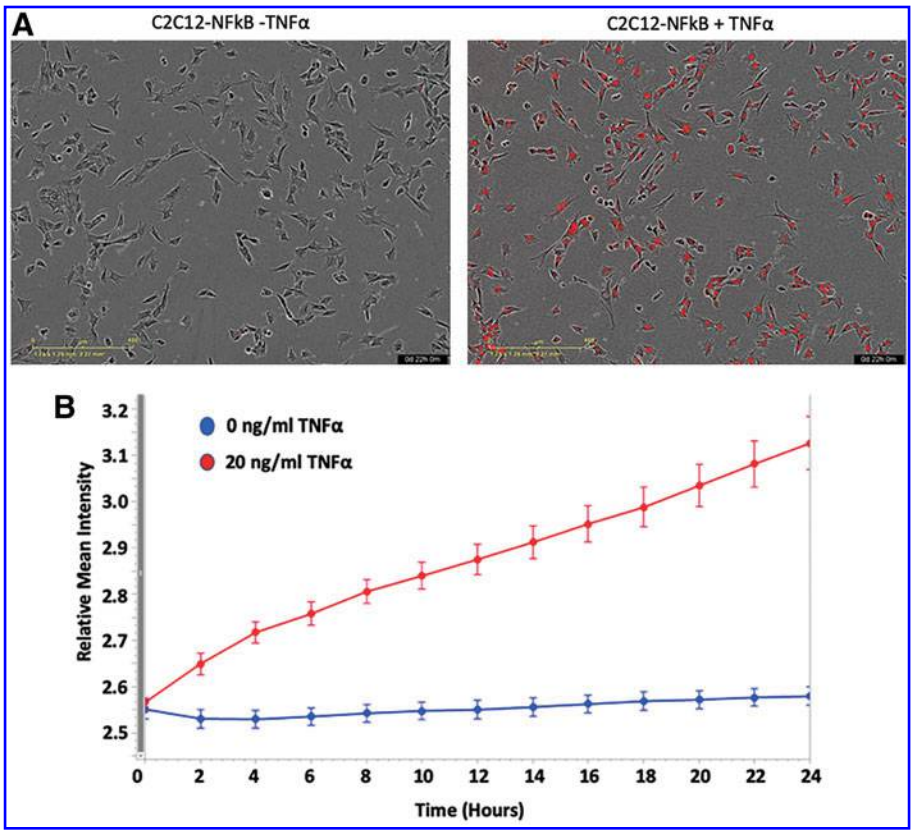

FIG. 5. A C2C12 cell line expressing an NF-κB fluorescent mKATE reporter was used for these experiments. Treatment with 20 ng/mL TNF-α induced mKATE expression. **(A)** Images of C2C12 cells treated with and without TNF-α. **(B)** Quantification of red fluorescence.

Our data also showed that PEMF decreased the production of IL-17A secretion in MSCs activated with LPS (Figs. 1 and 2). IL-17A is a proinflammatory cytokine that regulates the activities of NF-κB and mitogen-activated protein kinases and can stimulate the production of IL-6 and nitric oxide (NO).[63] IL-17A plays an important role in host defense against bacterial and fungal infection and also contributes to the pathogenesis of various autoimmune inflammatory diseases, including psoriatic and rheumatoid arthritis (RA).[64,65] Evidence has shown that targeting MSCs with PEMF can modulate IL-1b, IL-4, IL-6, IL-10, and IL-17A and has the potential to offer a novel treatment for RA.[18] These cytokines play key roles in the function of MSCs/M_Φ as they respond to pathogens and modulate the adaptive immune response to

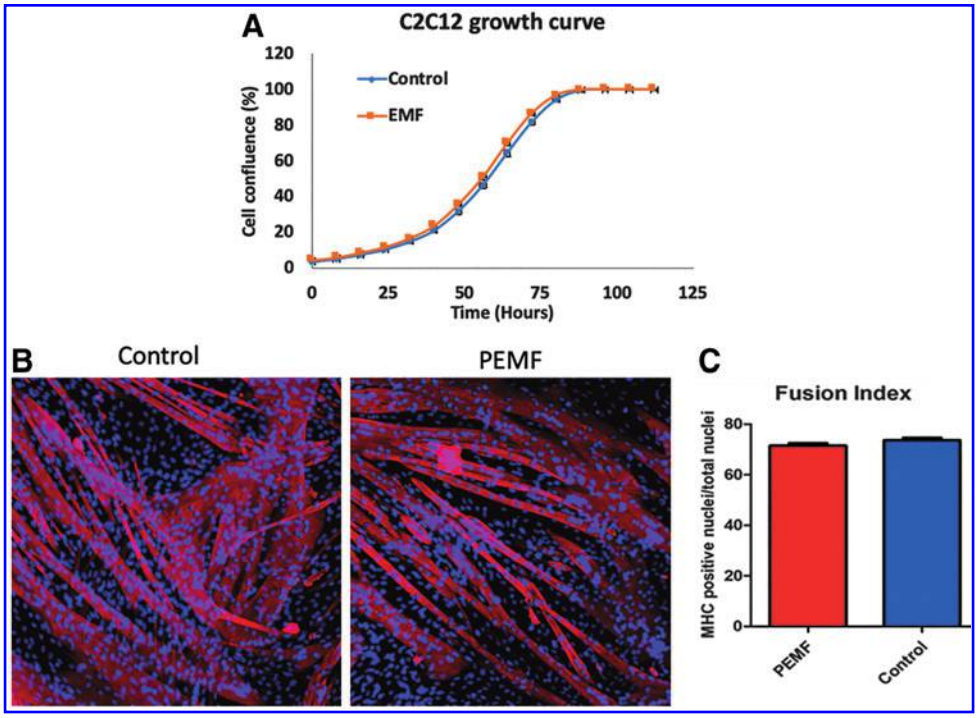

FIG. 6. PEMF treatment did not show significant effects on C2C12 proliferation or myotube differentiation. **(A)** C2C12 proliferation curves over time with (red) and without (blue) PEMF treatment. **(B)** Myosin heavy chain (MF20) immunofluorescence staining of C2C12 cells after growth in differentiation media for 5 days with and without 30 min PEMF treatment daily. **(C)** Fusion index (MHC+ nuclei/total nuclei) was calculated using an InCell 2000 from 128 images for each treatment. Scale bar = 100 μm.

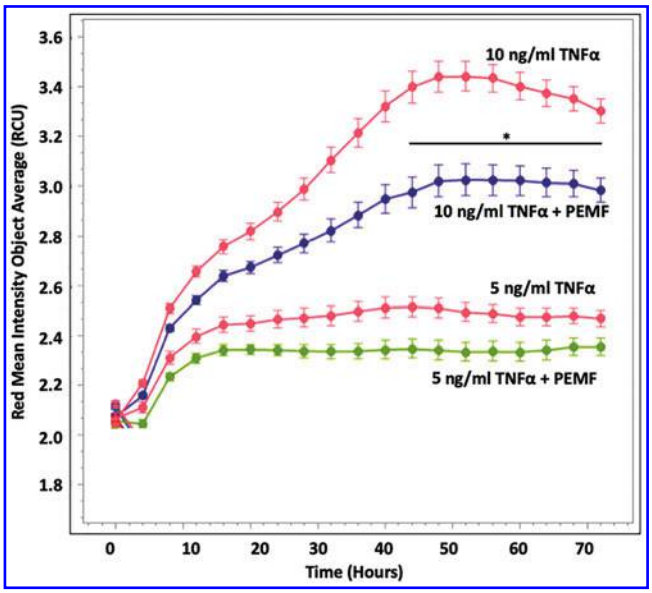

FIG. 7. PEMF significantly decreased NF-κB reporter expression in C2C12 cells treated with 10 ng/mL TNF-α (*$p < 0.05$). There was a trend toward significance in cells treated with PEMF and 5 ng/mL TNF-α.

provide resolution of inflammation, tissue repair, and homeostasis.[66] Our data also show that PEMF significantly decreased the production of TNF-α in the THP-1 cells. M_Φ are a major producer of TNF-α and are highly responsive to this cytokine. Aberrant production of TNF-α and its signaling receptor has been associated with the pathogenesis of several diseases, including RA, Crohn's disease, atherosclerosis, psoriasis, sepsis, diabetes, and obesity.[67] Due to its pivotal role in orchestrating the cytokine cascade in many inflammatory diseases, TNF-α has been called the *master-regulator* of inflammatory cytokine production.[67]

With regard to C2C12 cells, our results showed an increase in transcription of the NF-κB inhibitor alpha gene (*Nfkbia*) (Fig. 8). NF-κB is a well-recognized TLR-4 transcription factor that directs the production of TNF-α and other proinflammatory cytokines that are major mediators of protein breakdown and atrophy in skeletal muscle.[68,69] NF-κB is a critical activator of genes involved in inflammation, where proinflammatory cytokines activate the IκB kinase (IKK) complex that phosphorylates NF-κB inhibitors, triggering their conjugation with ubiquitin and subsequent degradation.[70] NF-κB signaling in tissue M_Φ, and the interaction of these M_Φ with muscle myofibers, has been shown to promote fibrosis associated with muscular dystrophy.[71] However, NF-κB signaling has also been shown to play an anti-inflammatory role in leukocytes recruited to sites of injury after the acute response is resolved.[72] Through the inflammatory/immune response, the immune system is able to maintain dynamic equilibrium (homeostasis) by activating and inhibiting signals, while at the same time adapting to environmental cues,[73] this would include PEMF signals. *Acute* inflammation is initiated by resident immune cells already present in the body, whereas *chronic* inflammation leads to progressive shifts in the type of cells present at the site of inflammation. This shift involves mononuclear cells, which are characterized by simultaneous destruction and healing of tissue involved in the inflammatory process.[73] For example, chronic inflammation is associated with disruptions of anabolic signals initiating muscle growth and has been implicated as part of the cause of muscle loss that occurs with aging.[74,75]

It has been suggested that PEMF can be propagated and effectively amplified along the entire signal transduction pathway, thereby modifying cell behavior.[76–78] This feasibility study proposed to influence the inflammatory/immune response using PEMF to potentially stimulate tissue regeneration. Therapeutic dosimetry involved in this model would involve treatment with 5 Hz to first modulate inflammation and then adjusting to the frequency appropriate for tissue regeneration at the site of insult, in this case 15 Hz for muscle cells. Field intensity would be dependent on depth of tissue targeted (using the inverse square $[1/r^2]$ law). Necessary time of exposure would be dependent on the severity of tissue injury.

Mechanisms of Action

The immune response is a tightly regulated process where any imbalance in its strict regulation could lead to pathological conditions. The important role of ion channel stability in immune function is becoming more apparent. After immune activation, changes in the cells' microenvironment are integrated into a survival response by complex signal transduction mechanisms.[79] Lipid nanopores forming stable ion

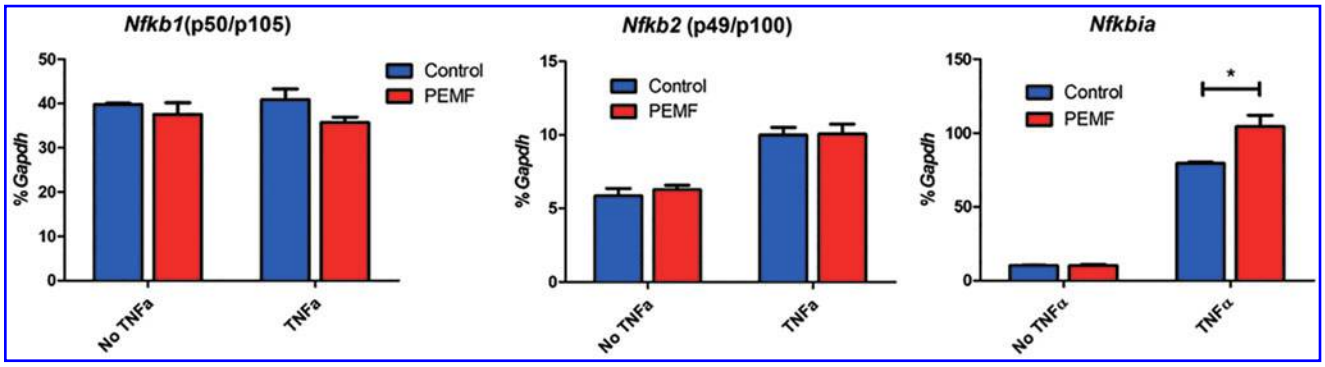

FIG. 8. NF-κB inhibitor alpha transcript (Nfkbia) was significantly higher in PEMF-treated cells compared with untreated control cells. C2C12 cells were treated with 10 ng/mL TNF-α for 20 min, followed by PEMF treatment for 30 min. Cell lysates were collected 1 h after TNF-α treatment for RNA isolation and RT-qPCR analysis. *$p < 0.05$. RT-qPCR, reverse transcription quantitative polymerase chain reaction.

channel conduction pathways in the plasma membrane of cells[80] explain the conduction of ions into the cell from the extracellular space.[80] It has been postulated that a direct effect of PEMF on phospholipids within the plasma membrane stimulates the production of second messengers, initiating multiple intracellular signal transduction pathways.[81–83] PEMF can alter cell function by triggering the forced vibration of free ions on the surface of the plasma membrane, causing external oscillating field disruptions in the electrochemical balance of transmembrane proteins (ion channels).[24,84]

The formation of a complex multicellular organism from a single cell is one of the most amazing processes of biology. Embryonic development is characterized by the careful regulation of cell behaviors such as cell proliferation, migration, differentiation, and tissue formation at the perfect time and place. These processes are dependent on the activities of genetics, signaling pathways, and information processing that coordinate cellular interactions leading to organogenesis.[85] During human development, lineage-committed cells of the three embryonic germ layers migrate and proliferate in the form of endogenous ionic currents, giving rise to EFs.[86] While endogenous EFs are present in all developing and regenerating animal tissues, their existence in inflammatory/immune modulation and tissue regeneration has been largely ignored. Ion flux is closely involved in differentiation control as stem cells migrate and proliferate in specific directions to form tissues and organs, each having their own signature characteristics to form specific cell and tissue types. Applying the PEMF would modulate mechanisms of action that play significant roles in action potential/voltage-gated ion regulation. The density of the musculoskeletal system versus the delicacy of the immune system shows two very different characteristics in human physiology; therefore, the targeted tissue would require different dosimetry.

The mechanisms through which PEMF exchanges information between cells, and how the conversion of this biochemical signaling is translated, have been researched for decades showing that the PEMF can permeate both the plasma and nuclear membranes of cells, thereby affecting a variety of cell functions and tissue types.[87–89] For example, PEMF can induce depolarization in the cell membrane, followed by an increase or decrease of intracellular calcium (Ca^{2+}).[90] While Ca^{2+} release from voltage-gated Ca^{2+}channels (VGCCs) regulates immune responses to pathogens,[91] inhibiting VGCCs in infected macrophages can reduce calcium influx, upregulating the expression of proinflammatory genes.[91] As biophysicists point out, a very important factor for regulating cell homeostasis is the level of the resting potentials, generated on the cell membrane.[19,92] VGCCs are activated by membrane depolarization in action potentials,[93] and when regulated by physical stimuli, VGCCs play a pivotal role in MSC differentiation. Levin and colleagues have shown that human MSC differentiation is accompanied by progressive hyperpolarization of voltage-gated ion channels.[94] Artificial depolarization keeps these cells in an undifferentiated state, whereas artificial hyperpolarization accelerates differentiation.[95] Poor regenerative capacity of musculoskeletal tissue has been the focus of regenerative medicine for many years. VGCCs are a group of membrane proteins that are predominantly found in excitable cells, such as cardiomyocytes, muscle, neurons and glial cells. VGCCs are known for their involvement in electrical current generation but are also expressed in nonexcitable cells including osteoblasts and chondrocytes.[96] VGCCs increase intracellular Ca^{2+} concentration, which leads to the initiation of different physical stimuli, such as electrical, electromagnetic/magnetic, and mechanical function in regenerative processes.[97] The bioelectric properties of a cell are mainly defined by the cellular membrane potential that controls different cell functions, which depend on the particular cell type.[98] Electrically charged membranes tightly regulate the concentration of ions such as electrically charged Ca^{2+}, sodium (Na^{+}), and/or potassium (K^{+}), which MSCs use as potent signal mediators.[99] Here is where the effects of PEMF in cells occur, triggered at the membrane level. Evidence shows that PEMF can act on Ca^{2+} concentrations,[100–102] Ca^{2+}-dependent pathways,[103] as well as Na^{+} and K^{+} pathways.[104] PEMF can affect action potentials and hyperpolarization to modulate endogenous electrical potentials in plants, animals, and humans.[7,95,105,106] Multiple factors cause discrepancies in the outcomes of PEMF-exposed cells during the inflammatory response. These variations include frequency, intensity, time of exposure and waveform, as well as the biological sample. The goal is to find the optimal PEMF dosimetry for creating homeostasis of cytokine signaling, transcription factors, and ion-flux-driven action potentials.

Conclusion

Poor regulation of inflammatory/immune function can allow acute-phase inflammatory response to become chronic, initiating disease and inhibiting tissue regeneration. Current theories of damage-associated molecules released by injured/infected cells or secreted by innate immune cells generate danger signals, activating the immune response. These signaling mechanisms are important to the subsequent activation of homeostatic mechanisms that control the immune response in pro- and anti-inflammatory reactions that allow for therapeutic treatments.[9] In this review, we described the effects of PEMF on inflammatory/immune regulators and transcription factors relevant to the activation of danger signals and innate immune cells. Achieving homeostasis in the face of acute inflammatory/immune challenges in the human body involves maintaining a balance of highly complex biochemical and cellular interactions, such as cytokine expression and signal transduction. When this delicate balance is upset, acute inflammatory and immune responses designed to quickly eliminate a transient threat become chronic, and inflammatory and/or autoimmune disease sets in. The importance of maintaining healthy cytokine expression during this impactful time cannot be overstated. Our feasibility study shows that PEMF has the potential to regulate this very delicate balance. More investigative research is needed to discover therapies to regulate signaling molecules involved in inflammation and tissue regeneration. We propose PEMF as such a therapy and performed a proof-of-concept study using MSCs, M_{Φ}, and C2C12 cells, exposing them to PEMF to investigate its effect on expression of inflammatory molecules after insult. Results show that the immunomodulatory effect of this therapy has the potential to decrease the production of proinflammatory secretion, while stabilizing or increasing anti-inflammatory cytokine production, and NF-κB expression during activated response. By

modulating the expression of various signaling cascades and cellular information processing networks to restore them to homeostatic (healthy) production levels, PEMF is showing promise as a treatment for inflammatory regulation to be used to promote tissue regeneration.

Authors' Contributions

C.L.R. wrote the article, ran ICC experiments for MSCs and THP-1s, and provided expertise on the therapeutic effects of PEMF. Y.Z. performed RT-qPCR for C2C12 cells and provided expertise on muscle cells. C.E.M. provided expertise and contributed editorial and written content on inflammatory and immune responses in cells (MSCs, M_Φ, and C2C12 cells). S.S. provided editorial content. T.L.C. provided expertise on C2C12 muscle cells and contributed editorial and written content on cell biology. All authors have seen and agree with the contents of the article and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication, is not published, nor in press.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

The authors wish to acknowledge the Guth Family Fund 120330-740196 and the Department of Defense (USAMRAA AFIRM W81XWH-08-2-0032) for funding this project.

References

1. Julier Z, Park AJ, Briquez PS, et al. Promoting tissue regeneration by modulating the immune system. Acta Biomater 2017;53:13–28.
2. Abnave P, Ghigo E. Role of the immune system in regeneration and its dynamic interplay with adult stem cells. Semin Cell Dev Biol 2019;87:160–168.
3. Matyushov D. Protein electron transfer: Is biology (thermo)dynamic? J Phys Condens Matter 2015;27: 473001.
4. Wisneski L, Anderson L. The Scientific Basis of Integrative Medicine, 2nd ed. Boca Raton: CRC Press; Taylor & Francis Group, 2009:205.
5. Bassett C. Fundamental and practical aspects of therapeutic uses of pulsed electromagnetic fields (PEMFs). Crit Rev Biomed Eng 1989;17:451–529.
6. Adey W. Potential therapeutic applications of non-thermal electromagnetic fields: Ensemble organization of cells in tissue as a factor in biological field sensing. In: Rosch PJ, Markov M, eds. Bioelectromagnetic Medicine. New York: Marcel Dekker, 2004.
7. Ross C, Siriwardane ML, Almeida-Porada G, et al. The effect of low-frequency electromagnetic field on human bone-marrow derived mesenchymal stem/progenitor cell differentiation. Stem Cell Res 2015;15:96–108.
8. Marino A, Becker RO. Piezoelectric effect and growth control in bone. Nature 1970;228:473–474.
9. Rosado M, Simkó M, Mattsson M-O, et al. Immune-modulating perspectives for low frequency electromagnetic fields in innate immunity. Front Public Health 2018; 6:85.
10. Jasti A, Wetzel B, Aviles H, et al. Effect of a wound healing electromagnetic field on inflammatory cytokine gene expression in rats. Biomed Sci Instrum 2001;37:209–214.
11. Sun W, Gan Y, Fu Y, et al. An incoherent magnetic field inhibited EFG receptor clustering and phosphorylation induced by a 50 Hz magnetic field in cultured FL cells. Cell Physiol Biochem 2008;33:508–514.
12. Goodman R, Lin-Ye A, Geddis MS, et al. Extremely low frequency electromagnetic fields activate the ERK cascade, increase hsp70 protein levels and promote regeneration in planaria. Int J Radiat Biol 2009;85:851–859.
13. Nie K, Henderson A. MAP kinase activation in cells exposed to a 60 Hz electromagnetic field. J Cell Biochem 2003;90:1197–1206.
14. Bekhite M, Finkensieper A, Abou-Zaid FA, et al. Static electromagnetic fields induce vasculogenesis and chondro-osteogenesis of mouse embryonic stem cells by reactive oxygen species-mediated up-regulation of vascular endothelial growth factor. Stem Cells Dev 2010;19:731–743.
15. Paleolog E. Angiogenesis in rheumatoid arthritis. Arthritis Res Ther 2002;4(Suppl 3):S81–S90.
16. McGonagle D, McDermott MF. A proposed classification of the immunological diseases. PLoS Med 2006;3: e297.
17. Prevosto C, Zancolli M, Canevali P, et al. Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction. Haematologica 2007; 92:881–888.
18. Ross C, Ang DC, Almeida-Porada G. Targeting mesenchymal stromal cells/pericytes (MSCs) with pulsed electromagnetic field (PEMF) has the potential to treat rheumatoid arthritis. Front Immunol 2019;10:1–12.
19. Funk R. Coupling of pulsed electromagnetic fields (PEMF) therapy to molecular grounds of the cell. Am J Transl Res 2018;10:1260–1272.
20. Masieri F, Schofield JR, Velloso CP, et al. Pulsed electromagnetic fields modulate metabolic activity, myokine release and differentiation into myotubes of myoblasts grown in vitro. Orthopaed Proc 2018;100B(Suppl 15):2049–4416.
21. Tasbih-Forosh M, Zarei L, Saboory E, et al. Effects of pulsed electromagnetic field with predatory stress on functional and histological index of injured-sciatic nerve in rat. Bull Emerg Trauma 2017;5:96–103.
22. Mohammadi R, Mahmoodzadeh S. Combination of local transplantation of in vitro bone-marrow stromal cells and pulsed electromagnetic fields accelerate functional recovery of transected sciatic nerve regeneration: A novel approach in transected nerve repair. Curr Neurovasc Res 2015;12:222–231.
23. Orgel M, O'Brien WJ, Murray HM. Pulsing electromagnetic field therapy in nerve regeneration: An experimental study in the cat. Plast Reconstr Surg 1984;73:173–183.
24. Ganesan K, Gengadharan A, Balachandran C, et al. Low frequency pulsed electromagnetic field—A viable alternative for arthritis. Indian J Exp Biol 2009;47:939–948.
25. Antonsson E, Mann RW. The frequency content of gait. J Biomech Eng 1985;18:39–47.
26. Serhan C. Resolution of inflammation: Novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Ann Rev Immun 2007;25:101–137.
27. Ariel A, Serhan CN. Resolvings and protections in the termination program of acute inflammation. Trends Immunol 2007;28:176–183.

28. Weidenbusch M, Anders H-J. Tissue microenvironments define and get reinforced by macrophage phenotypes in homeostasis or during inflammation, repair and fibrosis. J Innate Immun 2012;4:463–477.
29. Medzhitov R. Origin and physiological roles of inflammation. Nature 2008;454:428–435.
30. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 2005;105:1815–1822.
31. Mun C, Kang MI, Shin YD, et al. The expression of immunomodulation-related cytokines and genes of adipose- and bone marrow-derived human mesenchymal stromal cells from early to late passages. Tissue Eng Regen Med 2018;15:771–779.
32. Holloway J. Harnessing the immune response to improve functional healing. Sci Transl Med 2018;10:eaav3889.
33. Parisi L, Gini E, Baci D, et al. Macrophage polarization in chronic inflammatory diseases: Killers or builders? J Immunol Res 2018. DOI: 10.1155/2018/891780.
34. Betancourt A. New cell-based therapy paradigm: Induction of bone marrow-derived multipotent mesenchymal stromal cells into pro-inflammatory MSC1 and anti-inflammatory MSC2 phenotypes. Adv Biochem Eng Biotechnol 2013;130:163–197.
35. Genin M, Clement F, Fattaccioli A, et al. M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide. BMC Cancer 2015;15:577.
36. Zhang J-M, An J. Cytokines, inflammation and pain. Int Anesth Clin 2007;45:27–37.
37. Németh K, Leelahavanichkul A, Yuen PS, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 2009; 15:42–49.
38. Li Y, Liu J, Liao G. Early intervention with mesenchymal stem cells prevents nephropathy in diabetic rats by ameliorating the inflammatory microenvironment. Int J Mol Med 2018;41:2629–2639.
39. Vasandan A, Jahnavi S, Shashank C, et al. Human Mesenchymal stem cells program macrophage plasticity by altering their metabolic status via a PGE2-dependent mechanism. Sci Rep 2016;6:38308.
40. Ren G, Zhao X, Wang Y, et al. CCR2-dependent recruitment of macrophages by tumor-educated mesenchymal stromal cells promotes tumor development and is mimicked by TNFα. Cell Stem Cell 2012;11:812–824.
41. Aurora A, Olson EN. Immune modulation of stem cells and regeneration. Cell Stem Cell 2014;15:14–25.
42. DelaRosa O, Dalemans W, Lombardo E. Toll-like receptors as modulators of mesenchymal stem cells. Front Immunol 2012;3:1–8.
43. Romieu-Mourez R, François M, Boivin MN, et al. Cytokine modulation of TLR expression and activation in mesenchymal stromal cells leads to a proinflammatory phenotype. J Immunol 2009;182:7963–7973.
44. Waterman R, Tomchuck SL, Henkle SL, et al. A new mesenchymal stem cells (MSC) paradigm: Polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. PLoS One 2010;5:e10088.
45. Zhang B, Zhao N, Zhang J, et al. Mesenchymal stem cells rejuvenate cardiac muscle through regulating macrophage polarization. Aging (Albany NY) 2019;11: 3900–3908.
46. Xu C, Fu F, Li X, et al. Mesenchymal stem cells maintain the microenvironment of central nervous system by regulating the polarization of macrophages/microglia after traumatic brain injury. Int J Neurosci 2017;127:1124–1135.
47. Perdiguero E, Sousa-Victor P, Ruiz-Bonilla V, et al. p38/MKP-1-regulated AKT coordinates macrophage transitions and resolution of inflammation during tissue repair. J Cell Biol 2011;195:307–322.
48. Shapouri-Moghaddam A, Mohammadian S, Vazini H, et al. Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol 2018;233:6425–6440.
49. Waterman R, Henkle SL, Betancourt AM. Mesenchymal stem cell 1 (MSC1)-based therapy attenuates tumor growth whereas MSC2-treatment promotes tumor growth and metastasis. PLoS One 2012;7:e45590.
50. Sadiku M. Elements of Electromagnetics, 4th ed. New York & Oxford: Oxford University Press. 2007:386. ISBN 0-19-530048-3.
51. Ross C, Harrison BS. Effect of pulsed electromagnetic field on inflammatory pathway markers in RAW 264.7 murine macrophages. J Inflamm Res 2013;6:45–51.
52. Noh M, Lim SM, Oh K-W, et al. Mesenchymal stem cells modulate the functional properties of microglia via TGF-β secretion. Stem Cells Transl Med 2016;5:1538–1549.
53. Yoon Y-K, Woo H-J, Kim Y. *Orostachys japonicus* inhibits expression of the TLR4, NOD2, iNOS, and COX-2 genes in LPS-stimulated human PMA-differentiated THP-1 cells by inhibiting NF-κB and MAPK activation. Evid Based Complement Alternat Med 2015;2015. DOI: 10.1155/2015/682019.
54. Eggenhofer E. Mesenchymal stem cell-educated macrophages. Transplant Res 2012;1784:119–126.
55. Masters S, Simon A, Aksentijevich I, et al. Horror autoinflammaticus: The molecular pathophysiology of autoinflammatory disease. Annu Rev Immunol 2009;27:621–668.
56. Locksley R, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: Integrating mammalian biology. Cell 2001;104:487–501.
57. Vazquez M, Catalan-Dibene J, Zlotnika A. B cells responses and cytokine production are regulated by their immune microenvironment. Cytokine 2015;74:318–326.
58. Hojman P, Brolin C, Nørgaard-Christensen N, et al. IL-6 released from muscles during exercise is stimulated by lactate-dependent protease activity. Am J Physiol Endocrinol Metab 2019. Epub ahead of print; DOI: 10.1152/ajpendo.00414.2018.
59. Muñoz-Cánoves P, Scheele C, Pedersen BK, et al. Interleukin-6 myokine signaling in skeletal muscle: A double-edged sword? FEBS J 2013;280:4131–4148.
60. Wang P, Wu P, Siegel MI, et al. Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes. IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. J Biol Chem 1995; 270:9558–9563.
61. Murthy P, DennisVA, Lasater BL, et al. Interleukin-10 modulates proinflammatory cytokines in the human monocytic cell line THP-1 stimulated with *Borrelia burgdorferi* lipoproteins. Infect Immun 2000;68:6663–6669.
62. Iyer S, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit Rev Immunol 2012;32:23–63.
63. Onishi R, Gaffen SL. Interleukin-17 and its target genes: Mechanisms of interleukin-17 function in disease. Immunol Suppl 2010;129:311–321.

64. Qian Y, Kang Z, Liu C, Li X. IL-17 signaling in host defense and inflammatory diseases. Cell Mol Immunol 2010;7:328–333.
65. Kirkham B, Kavanaugh A, Reich K. Interleukin-17A: A unique pathway in immune-mediated diseases: Psoriasis, psoriatic arthritis and rheumatoid arthritis. Immunology 2014;141:133–142.
66. Huang X, Li Y, Fu M, et al. Polarizing macrophages in vitro. Methods Mol Biol 2018;1784:119–126.
67. Parameswaran N, Sonika Patial S. Tumor necrosis factor-α signaling in macrophages. Crit Rev Eukaryot Gene Expr 2010;20:87–103.
68. Garg A, Aggarwal BB. Reactive oxygen intermediates in TNF signaling. Mol Immunol 2002;39:509–517.
69. Schakman O, Dehoux M, Bouchuari S, et al. Role of IGF-I and the TNFα/NF-kB pathway in the induction of muscle atrogenes by acute inflammation. Am J Physiol Endocrinol Metab 2012;303:E729–739.
70. Liu T, Zhang L, Joo D, et al. NF-κB signaling in inflammation. Signal Transduct Target Ther 2017;2: 17023.
71. Acharyya S, Villalta SA, Bakkar N, et al. Interplay of IKK/NF-Kappa B signaling in macrophages and myofibers promotes muscle degeneration in Duchenne muscular dystrophy. J Clin Investig 2007;117:889–901.
72. Lawrence T, Gilroy DW, Colville-Nash PR, et al. Possible new role for NC-kappaB in the resolution of inflammation. Nat Med 2001;7:1291–1297.
73. Chen L, Deng H, Cui H, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2018;9:7204–7218.
74. Toth MJ, Matthews DE, Tracy RP, et al. Age-related differences in skeletal muscle protein synthesis: Relation to markers of immune activation. Am J Physiol Endocrinol Metab 2004;288:E883–E891.
75. Visser M, Pahor M, Taaffe DR, et al. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: The Health ABC Study. J Gerontol A Biol Sci Med Sci 2002; 57:M326–M332.
76. Delle-Monache S, Angelucci A, Sanità P, et al. Inhibition of angiogenesis mediated by extremely low-frequency magnetic fields (ELF-MFs). PLoS One 2013; 8:e79309.
77. Gordon G. Designed electromagnetic pulsed therapy: Clinical applications. J Cell Physiol 2007;212:579–582.
78. Ross C. The use of electric, magnetic, and electromagnetic field for directed cell migration and adhesion in regenerative medicine. Biotechnol Prog 2016;33:5–16.
79. Ladoux B, Mège RM. Mechanobiology of collective cell behaviours. Nat Rev Mol Cell Biol 2017;18:743–757.
80. Pakhomov A, Bowman AM, Ibey BL, et al. Lipid nanopores can form a stable, ion channel-like conduction pathway in cell membrane. Biochem Biophys Res Commun 2009;385:181–186.
81. Semenov I, Xiao S, Pakhomov AG. Primary pathways of intracellular Ca(2+) mobilization by nanosecond pulsed electric field. Biochim Biophys Acta 2013;1828:981–989.
82. Tolstykh G, Beier HT, Roth CC, et al. Activation of intracellular phosphoinositide signaling after a single 600 nanosecond electric pulse. Bioelectrochemistry 2013;94: 23–29.
83. Pilla A, Fitzsimmons R, Muehsam D, et al. Electromagnetic fields as first messenger in biological signaling: Application to calmodulin-dependent signaling in tissue repair. Biochim Biophys Acta 2011;1810:1236–1245.
84. Liboff A, McLeod BR. Kinetics of channelized membrane ions in magnetic field. Bioelectromagnetics 1988; 9:39–51.
85. Sanz-Ezquerro J, Münsterberg AE, Stricker S. Editorial: Signaling pathways in embryonic development. Front Cell Dev Biol 2017;5:76.
86. Levin M. Bioelectric mechanisms in regeneration: unique aspects and future perspectives. Semin Cell Dev Biol 2009;20:543–556.
87. Luben R. Membrane signal-transduction mechanisms and biological effects of low-energy electromagnetic fields. Adv Chem Ser 1995;250(Chapter 24):437–450.
88. Volpe P. Interactions of zero-frequency and oscillating magnetic fields with biostructures and biosystems. Photochem Photobiol Sci Review 2003;2:637–648.
89. Sun S, Liu Y, Lipsky S, et al. Physical manipulation of calcium oscillation facilitates osteodifferentiation of human mesenchymal stem cells. FASEB J 2007;21:1472–1480.
90. Foletti A, Ledda M, De Carlo F, et al. Calcium ion cyclotron resonance (ICR), 7.0 Hz, 9.2 microT magnetic field exposure initiates differentiation of pituitary corticotrope-derived AtT20 D16V cells. Electromagn Biol Med 2010;29:63–71.
91. Gupta S, Salam N, Srivastava V, et al. Voltage gated calcium channels negatively regulate protective immunity to *Mycobacterium tuberculosis*. PLoS One 2009;4: e5305.
92. Bose T, Cieślar-Pobuda A, Wiechec E. Role of ion channels in regulating Ca2+ homeostasis during the interplay between immune and cancer cells. Cell Death Dis 2015;6:e1648.
93. Zamponi G, Striessnig J, Koschak A, et al. The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. Pharmacol Rev 2015;67:821–870.
94. Sundelacruz S, Levin M, Kaplan DL. Membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells. PLoS One 2008;3: e3737.
95. Levin M. Large-scale biophysics: Ion flows and regeneration. Trends Cell Biol 2007;17:261–270.
96. Kawano S, Shoji S, Ichinose S, et al. Characterization of Ca2+ signaling pathways in human mesenchymal stem cells. Cell Calcium 2002;32:165–174.
97. Pall M. Electromagnetic fields act via activation of voltage-gated calcium channels to produce benficial or adverse effects. J Cell Mol Med 2013;17:958–965.
98. Levin M. Molecular bioelectricity: How endogenous voltage potentials control cell behavior and instruct pattern regulation in vivo. Mol Biol Cell 2014;25:3835–3850.
99. Pchelintseva E, Djamgoz MBA. Mesenchymal stem cell differentiation: Control by calcium-activated potassium channels. J Cell Physiol 2017;233:3755–3768.
100. Zhang X, Liu X, Pan L, et al. Magnetic fields at extremely low-frequency (50 Hz, 0.8 mT) can induce the uptake of

intracellular calcium levels in osteoblasts. Biochem Biophys Res Commun 2010;396:662–666.
101. Tong J, Sun L, Zhu B, et al. Pulsed electromagnetic fields promote the proliferation and differentiation of osteoblasts by reinforcing intracellular calcium transients. Bioelectromagnetics 2017;38:541–549.
102. Li J, Lin JC, Liu HC, et al. Comparison of ultrasound and electromagnetic field effects on osteoblast growth. Ultrasound Med Biol 2006;32:769–775.
103. Wu S, Yu Q, Lai A, et al. Pulsed electromagnetic field induces Ca2+ -dependent osteoblastogenesis in C3H10T1/2 mesenchymal cells through the Wnt-Ca 2+/Wnt-β-catenin signaling pathway. Biochem Biophys Res Commun 2018; 503:715–721.
104. Wade B. A review of pulsed electromagnetic field (PEMF) mechanisms at a cellular level: A rationale for clinical use. Am J Health Res 2013;1:51–55.
105. Tyler S. Nature's electric potential: A systematic review of the role of bioelectricity in wound healing and regenerative processes in animals, humans, and plants. Front Physiol 2017;8:627.
106. Popp F, Zhang J. Mechanism of interaction between electromagnetic fields and living organisms. Sci China (Series C) 2000;43:507–518.

Address correspondence to:
Christina L. Ross, PhD
Center for Integrative Medicine
Institute for Regenerative Medicine
Wake Forest School of Medicine
Winston-Salem, NC 27101

Email: chrross@wakehealth.edu