

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

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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

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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-1 β , 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- κ B 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 μ g/mL) or Poly (I:C) (2 μ g/mL) was added as the MSC agonists for TLR-4 and TLR-3, respectively, for no longer than 1 h (per Waterman et al. protocol⁴⁴) 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 μ g/mL) per Yoon et al. protocol⁵³ 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 μ L/mg Poly (I:C)] MSCs increased secretion of anti-inflammatory cytokines IL-3, IL-4, and IL-10, and TLR-4-activated (2 μ L/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 μ L/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- κ B-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 .

^aLPS 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 .

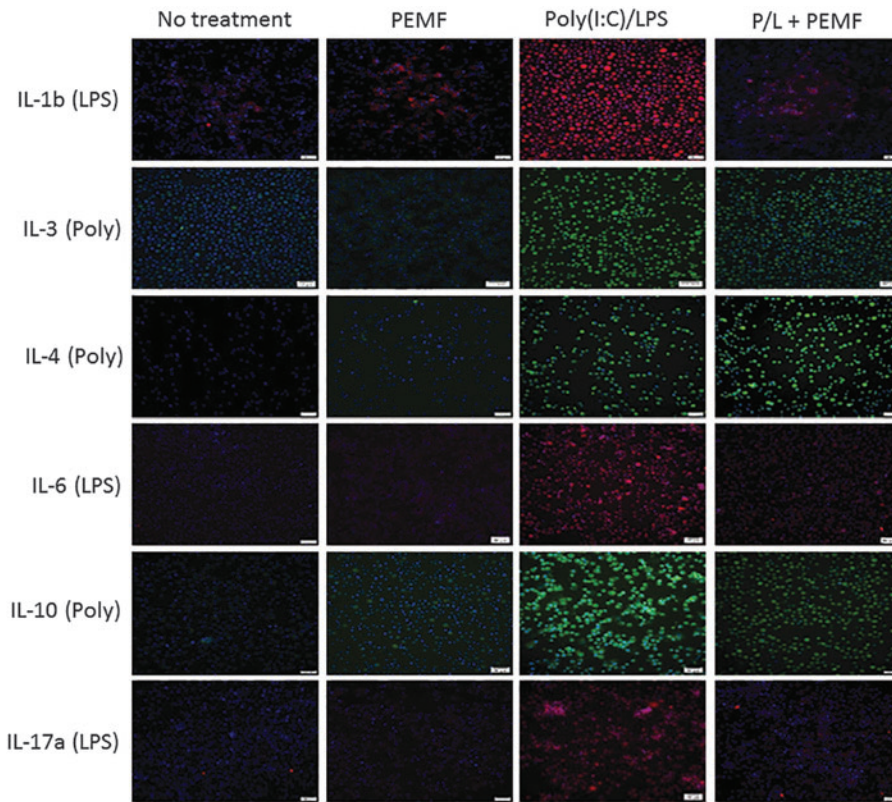


FIG. 1. MSCs were for both the TLR-3 (Poly), stimulating anti-inflammatory cytokine signaling; and TLR-4 (LPS) receptors, stimulating proinflammatory cytokine signaling. After PEMF exposure, activated MSCs show decrease of proinflammatory cell signaling (IL-1 β , IL-6, and IL-17A) and stabilization of 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- κ B inflammatory pathway in TNF- α -treated cells 25 min after PEMF exposure. TNF- α exposure increased the response of the NF- κ B 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- κ B inhibitor alpha gene (*Nfkb1a*) (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-1 β 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 mac-

rophages.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- κ B activity.⁶⁰ IL-10 predominantly inhibits LPS and bacterial product-mediated induction of the proinflammatory cytokines TNF- α and NF- κ B.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

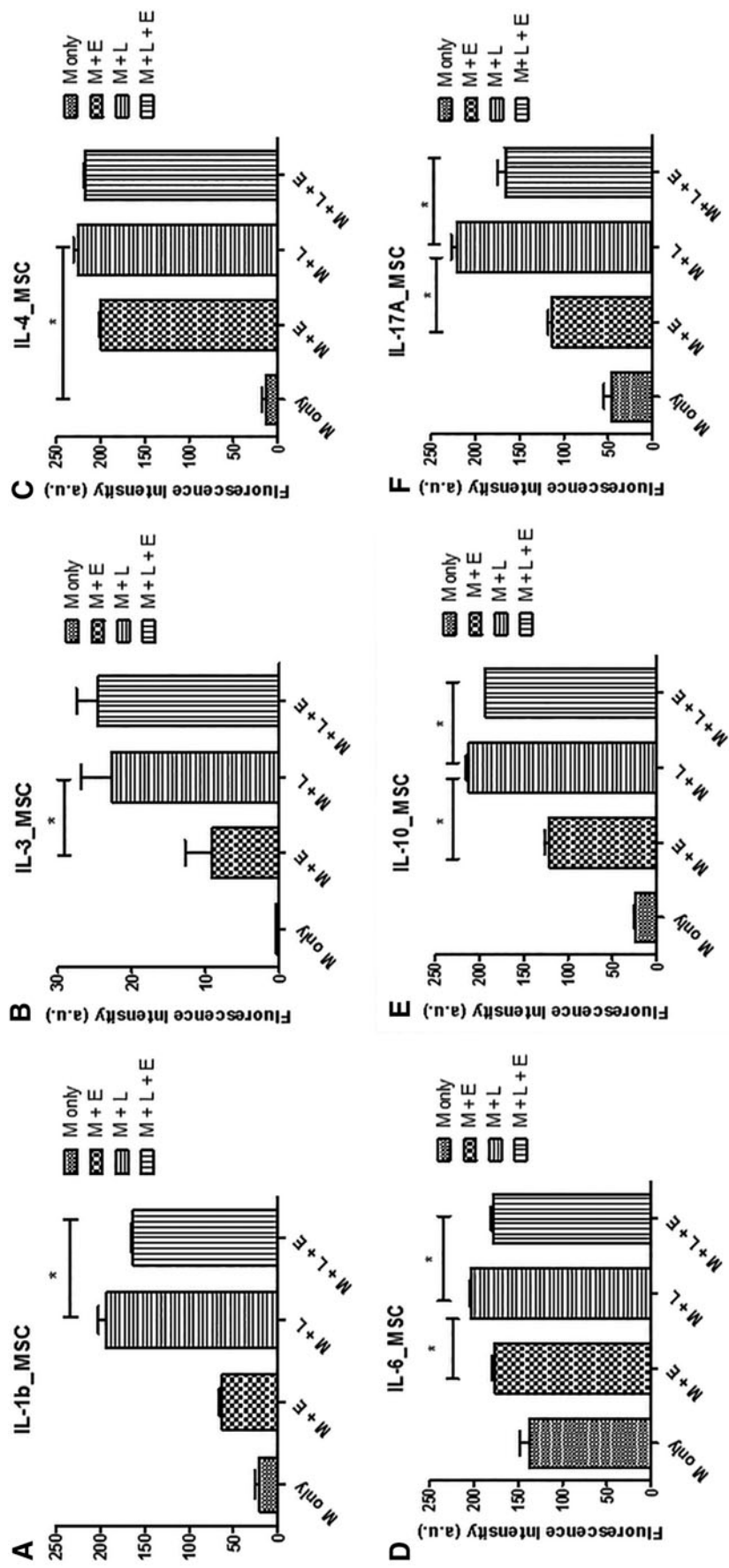


FIG. 2. Quantitative analysis of Figure 1 shows decrease in signaling of proinflammatory cytokines (A) IL-1b, (D) IL-6, and (F) IL-17A in activated MSCs; and stabilization or increase of anti-inflammatory cytokines (B) IL-3, (C) IL-4, and (E) IL-10 after activation of MSCs. *N* = 5 independent trials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

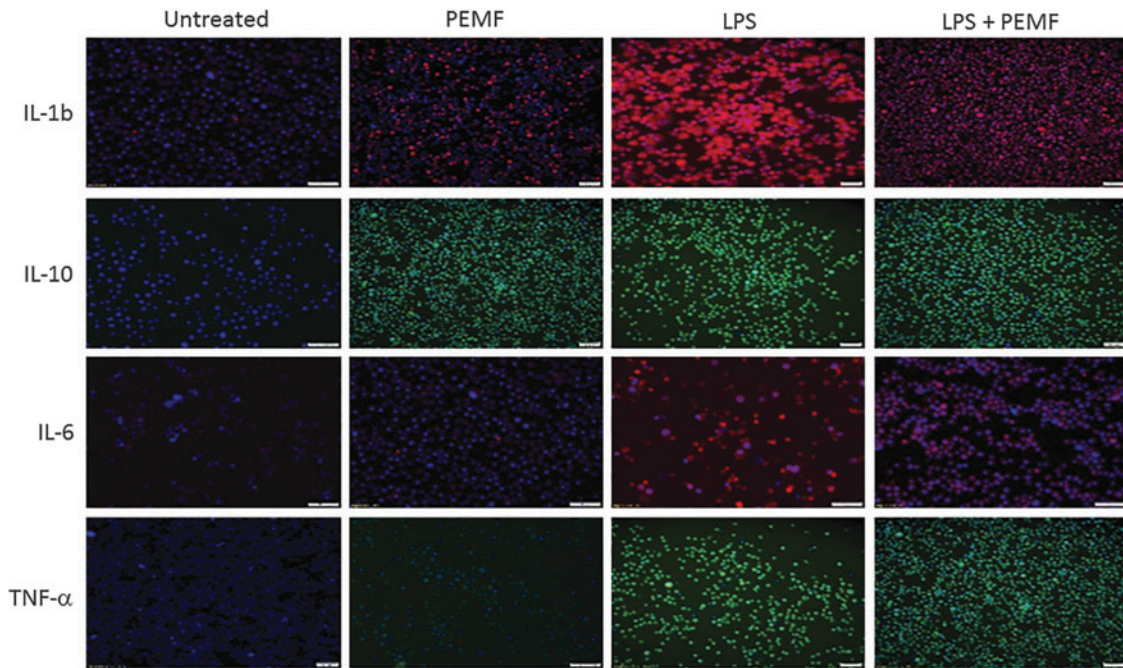


FIG. 3. THP-1 cells show decrease in proinflammatory cytokines signaling for IL-1b, IL-6, and TNF- α and increase/stabilization of anti-inflammatory cytokine signaling (IL-10) after exposure to PEMF ($N=5$ trials). PEMF, pulsed electromagnetic field. TNF- α , tumor necrosis factor- α .

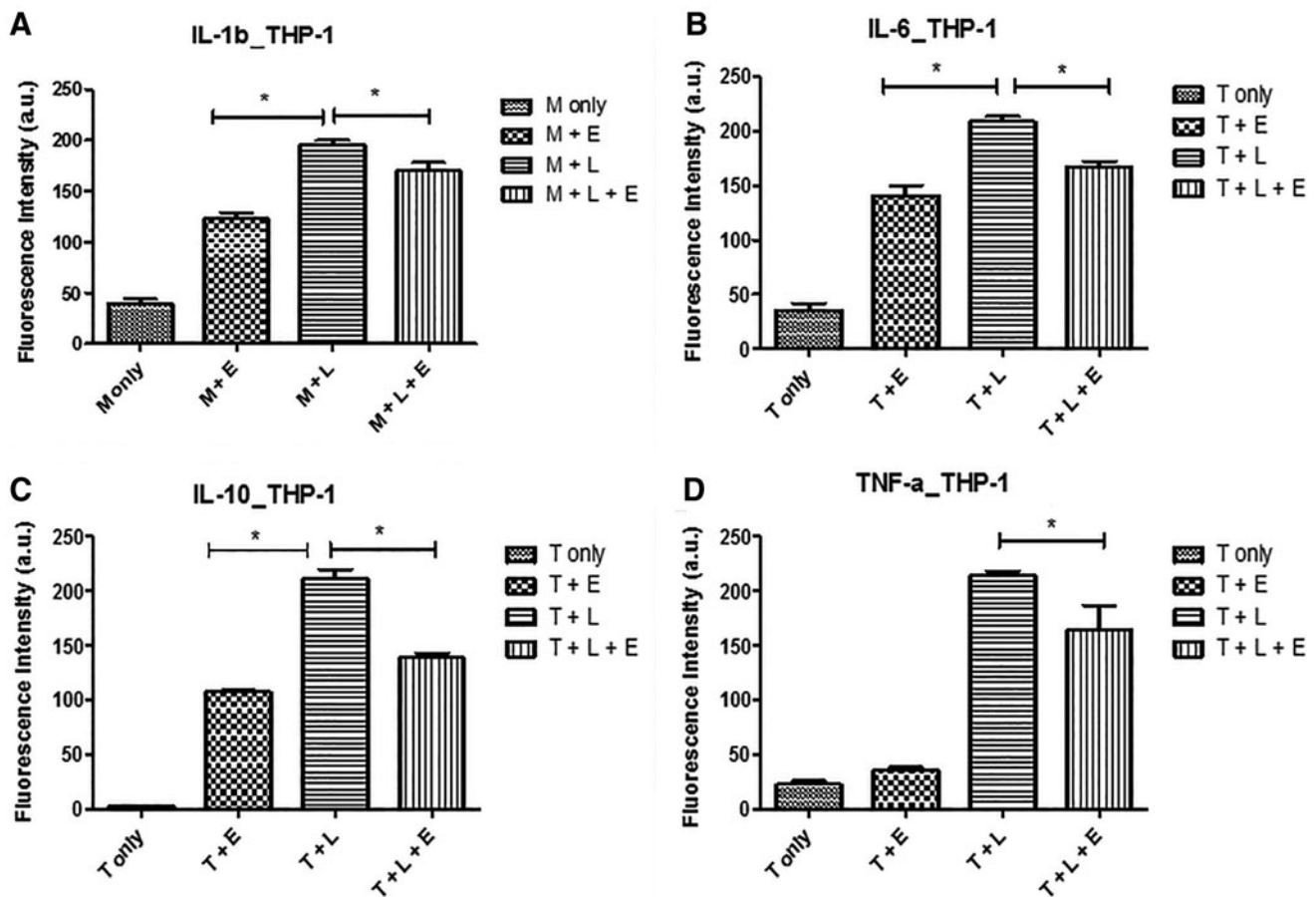


FIG. 4. Quantitative analysis of Figure 3 shows activated THP-1 cells exposed to PEMF and measured for proinflammatory cytokines and anti-inflammatory cytokine IL-10. Results show statistically significant decreased (A) IL-1b, (B) IL-6, and (D) TNF- α signaling. Anti-inflammatory cytokine (C) IL-10 showed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

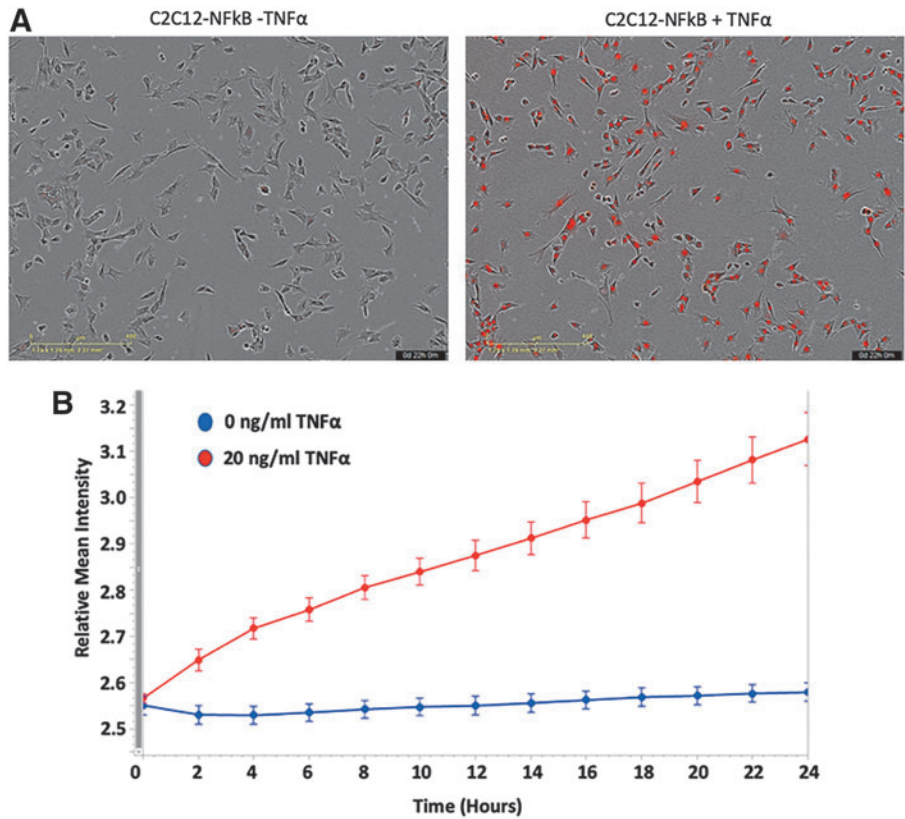
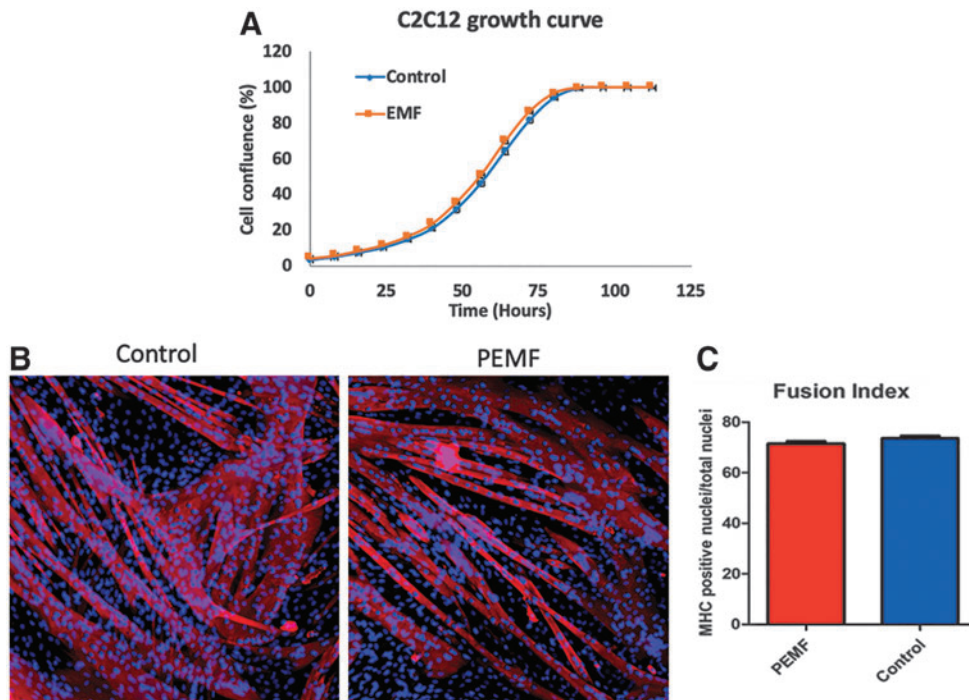


FIG. 5. A C2C12 cell line expressing an NF-κB fluorescent mKATE reporter was used for these experiments. Treatment with 20 ng/mL TNF-α induced mKATE expression. (A) Images of C2C12 cells treated with and without TNF-α. (B) Quantification of red fluorescence.

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/Mφ 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.



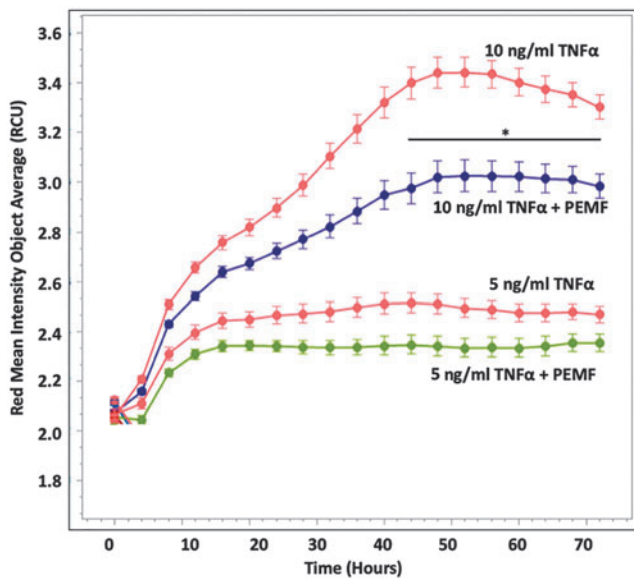


FIG. 7. PEMF significantly decreased NF- κ B reporter expression in C2C12 cells treated with 10 ng/mL TNF- α (* $p < 0.05$). There was a trend toward significance in cells treated with PEMF and 5 ng/mL TNF- α .

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- κ B inhibitor alpha gene (*Nfkb1a*) (Fig. 8). NF- κ B 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- κ B is a critical activator of genes involved in inflammation, where

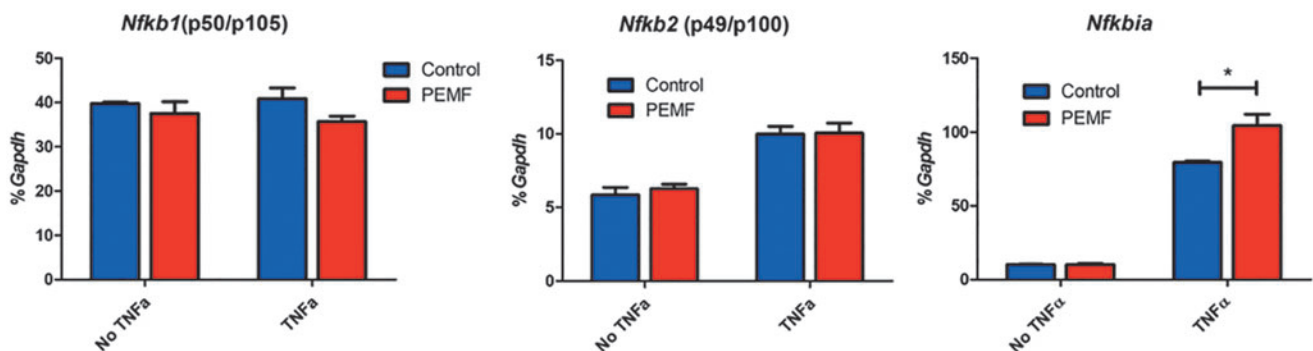


FIG. 8. NF- κ B inhibitor alpha transcript (*Nfkb1a*) was significantly higher in PEMF-treated cells compared with untreated control cells. C2C12 cells were treated with 10 ng/mL TNF- α for 20 min, followed by PEMF treatment for 30 min. Cell lysates were collected 1 h after TNF- α treatment for RNA isolation and RT-qPCR analysis. * $p < 0.05$. RT-qPCR, reverse transcription quantitative polymerase chain reaction.

proinflammatory cytokines activate the I κ B kinase (IKK) complex that phosphorylates NF- κ B inhibitors, triggering their conjugation with ubiquitin and subsequent degradation.⁷⁰ NF- κ B 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- κ B 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

channel conduction pathways in the plasma membrane of cells⁸⁰ 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, $\text{M}\Phi$, 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- κ B 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.

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