REVIEW ARTICLE

Extremely low frequency electromagnetic field and wound healing: implication of cytokines as biological mediators*

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ABSTRACT. Wound healing is a highly coordinated and complex process involving various cell types, chemical mediators and the surrounding extracellular matrix, resulting in a tightly orchestrated re-establishment of tissue integrity by specific cytokines. It consists of various dynamic processes including a series of overlapping phases: inflammation, proliferation, re-epithelialization and remodeling. One of the underlying mechanisms responsible for the disturbances in wound healing is an out-of-control inflammatory response that can cause pathological consequences, such as hypertrophic scars, keloids or chronic wounds and ulcers. Recently, several reports have evaluated the effects of extremely low frequency electromagnetic fields (EMFs) on tissue repair. In particular, the data analysis supports an anti-inflammatory effect of EMFs by the modulation of cytokine profiles that drive the transition from a chronic pro-inflammatory state to an anti-inflammatory state of the healing process. In this review, we focus on the effect of EMFs on skin wound healing showing emerging details of the anti-inflammatory effects of EMFs, with a view to cytokines as candidate biomarkers. Molecular clarification of the mechanisms involved in the modulation of inflammatory factors following exposure to EMFs will provide a better understanding of the cellular responses induced by EMFs and a potential, additional treatment in non-responding, chronic wounds.

Key words: wound healing, EMF

Wound healing is a dynamic process involving a series of coordinated events, including bleeding, coagulation, acute inflammatory response, regeneration, migration and proliferation of connective tissue and parenchyma cells, as well as synthesis of extracellular matrix (ECM) proteins and remodeling [1]. The repair process begins at the moment of injury that causes leakage of blood into the wound site and activation of the clotting cascade. Clotted blood provides a matrix that determines cell adhesion and migration. In particular, platelets provide a source of growth factors and pro-inflammatory cytokines that mediate the recruitment of inflammatory cells and fibroblasts into the wound site [2]. Neutrophils and macrophages combat invading microbes and also, critically, support the repair process by releasing a spectrum of cytokines and growth factors, which initiate the phase of granulation tissue formation. This tissue is composed of endothelial cells, macrophages, fibroblasts, and new extracellular matrix, and exerts its function in covering and filling the wound area. The components of the provisional extracellular wound matrix facilitate cell adhesion, migration and proliferation. Tissue integrity is restored by re-epithelialization, following keratinocyte proliferation and migration at the wound edge [3]. Finally, during the remodeling phase, a balance is reached between the synthesis of new components of scar matrix and their degradation by proteases in determining granulation tissue regression and its transformation into scar tissue. Typical features of these events include regression of vascular structures, transformation of fibroblasts into myofibroblasts, substitution of provisional ECM by a permanent, collagenous matrix and importantly, resolution of the inflammatory response [4-6].

Wounds can be categorized as acute or chronic according to their healing time-frame [7]. Acute wounds repair themselves and heal normally following the correct pathway. An example of a common acute wound is a clean and uninfected surgical incision wound closed by surgical sutures.

When wounds do not heal in a timely and orderly manner, they result in chronic, non-healing wounds (ulcers). Such wounds are those that have failed to progress through the normal stages of healing, and are characterized by chronicity and frequent relapse [7]. Ischemia, diabetes mellitus, venous stasis, and pressure can be at the root of the majority of non-healing wounds that are prone to complications including functional limitations, infections, and malignant transformation [8-10].

INFLAMMATION IN WOUND HEALING

The inflammatory response is the first stage in a number of overlapping processes that constitute wound healing.

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The normal function of inflammation in an acute wound is to prepare the wound bed for healing by removing necrotic tissue, debris, and bacterial contaminants, as well as recruiting and activating fibroblasts and keratinocytes. In particular, skin injury causes cell damage and injury to blood vessels. Damaged cells respond by activating several "stress signal" pathways within a few minutes [11, 12], and leaking endogenous molecules, including damage-associated molecular pattern molecules (DAMPs), which might act as activation cues and/or chemotactic factors for other cells in the area [13]. The inflammatory response starts during the late phase of coagulation and begins immediately with the passive leakage of circulating leukocytes (largely neutrophils) from damaged blood vessels into the wound [14]. The inflammatory response continues with active recruitment of neutrophils and then macrophages from nearby vessels, which is orchestrated by growth factor signals from serum [15, 16], release of platelet granule content, activation of cells resident at the wound site and presence of foreign epitopes from invading organisms.

Inflammation is mediated by a variety of soluble factors, including a group of secreted polypeptides known as cytokines. Inflammatory cytokines can be divided into two groups: those involved in acute inflammation and those responsible for chronic inflammation: some cytokines, such as IL-1, significantly contribute to both acute and chronic inflammation. Cytokines play an important role in the communication between cells, and their actions can be auto-, para- or endocrine, via specific cell-surface receptors on their target cells, which are cells of the same or similar type as the cytokine-producing cell. As intercellular mediators, they regulate survival, growth, differentiation and effector functions. Cytokines, along with other proteins, play regulatory roles in wound healing. As part of this process, inflammation involves platelet activation and recruitment of neutrophils, macrophages, and fibroblasts to the wound site. The activated platelets release a wide range of biologically active mediators, known to be key players in inflammation, such as: growth factors [17, 18], chemokines such as IL-8, MCP-1, MIP-1α, RANTES [19], MIP-2 (CXCL2), LIX (CXCL6), GRO-α (CXCL1), ENA-78 (CXCL5), SDF-1α (CXCL12), MCP-3 (CCL7), PF4 (CXCL4), and cytokine transforming growth factor (TGF)-β1, TGF-β2 and IL-1. Thrombin is another important and early mediator of clotting. It is released by platelets, and is a serine protease that mediates clot formation and also plays a role in inflammation [20]. Indeed, thrombin stimulates the release of pro-inflammatory cytokines, such as MCP-1, IL-6 and IL-8 by endothelial cells, which induce neutrophils and monocyte chemotaxis [21].

At the same time, there is activation of immune cells that are already resident within the tissue, such as mast cells [22], γδ T cells [23] and Langerhans cells [24], which, in turn, release a rapid pulse of chemokines and cytokines. Following injury, residential mast cells degranulate within hours, contributing to neutrophil recruitment, vascular permeability and wound closure rate [25]. Skin γδ T cells are strictly limited in their distribution to the epidermis and are described as γδ dendritic epidermal T cells (γδDETC). These cells have a role in improving the healing response following mechanical injury, having been identified as a source of key growth factors such as FGF-7 and -10, IGF-1 and keratinocyte growth factors (KGFs), thereby regulating keratinocyte proliferation and differentiation [26]. Finally, foreign epitopes such as the lipopolysaccharides (LPS) and formyl-methionyl peptides of invading microorganisms play a key role in active recruitment of neutrophils and subsequently of monocytes [27].

Together, these signals trigger local endothelial cell 'activation' and thus expression of selectins. These molecules control the rolling and then tethering of leukocytes to the vessel wall and subsequent crossing of the endothelial barrier [28]. At this point, recruited and activated neutrophils begin the debridement of devitalized tissue and phagocytosis of infectious agents, utilizing bursts of reactive oxygen species (ROS), release of cationic peptides and eicosanoids [29, 30]. Microarray analysis shows that change in the expression profile is induced in neutrophils upon recruitment to a wound site, and that these cells also influence many other aspects of repair, such as resolution of the fibrin clot and provisional ECM, promotion of angiogenesis, and re-epithelialization [31]. Also, an *in vitro* study demonstrated that neutrophils contribute to modulate the expression profile of macrophages at wound sites, regulating innate immunity in wound healing [32].

Macrophages appear in the wound 48-72 hours after injury [33]. Circulating monocytes mature into macrophages at the wound site and act with a specific expression profile according to their stimuli [34]. These cells clear up matrix and cell debris, including apoptotic neutrophils [35]. The phagocytosis of apoptotic neutrophils or other cells have been shown to induce an anti-inflammatory phenotype in macrophages. This phenotype includes the release of transforming growth factor-beta (TGF-β) and prostaglandin E_2 (PGE_2) and a reduced ability to produce pro-inflammatory mediators, such as tumor necrosis factor (TNF)-α, after LPS stimulation [36]. Accordingly, Deonarine *et al.* showed that both classically- and pro-inflammatory-activated macrophages (M1) and alternatively-activated (anti-inflammatory and pro-angiogenic) macrophages (M2) are present in the earlier phase of healing [37]. Subsequently, M2 become the predominant inflammatory cells. Macrophages play a key role in the late stage of the inflammatory response, thereby releasing cytokines and growth factors that have activated the keratinocytes, fibroblasts and endothelial cells [35, 38]. In addition, these cells generate nitric oxide (NO) and large amounts of ROS [39], which are known to drive the same aspects of repair [40].

The inflammatory response ends once wound healing is complete and several mechanisms have been proposed for resolution of the inflammatory response. These mechanisms include the drainage of inflammatory cells via lymphatic vessels [41, 42], down-regulation of chemokine expression by anti-inflammatory cytokines such as IL-10 and TGF-β1 [43, 44], up-regulation of anti-inflammatory molecules [45-47] and apoptosis [48].

An exaggerated and prolonged inflammatory response at the wound site is a cardinal feature of non-healing conditions and excessive scarring [49]. In the wound site, bacterial overgrowth, leukocyte trapping and necrotic tissue can cause a persistent recruitment and activation of inflammatory cells [50-53], inducing the predominant presence of pro-inflammatory cytokines, such as TNF-α [54, 55]. The physiological feedback mechanisms that

drive towards resolution of the inflammatory response are short-circuited, leading to an uncontrolled, inflammatory, positive feedback loop. In addition, pro-inflammatory cytokinesactivated neutrophils, macrophages, and resident cells, inducing expression and activity of several classes of matrix metalloproteases (MMPs), such as gelatinases (MMP-2, -9) and collagenases (MMP-1, -8) [56]; furthermore chronic wounds present elevated levels of serine protease, particularly elastase of neutrophilic origin [57]. As a result, fibroblasts are unable to make progress in depositing extracellular matrix because degradation of collagen occurs more rapidly than its synthesis. Tissue degradation further recruits inflammatory cells, continuing the inflammatory cycle.

In chronic wounds, the inflammatory cycle is also sustained by generation of a pro-oxidant microenvironment. Leukocytes and resident cells, particularly some fibroblasts that show premature senescence [58], are sources of ROS [59]. These molecules actively induce expression of pro-inflammatory cytokines, chemokines, MMPs and serine proteases.

Under normal conditions, the bioavailable NO has highly beneficial effects on wound healing, influencing angiogenesis and proliferation. Furthermore, NO has a scavenging effect on superoxide anion (O_2^-), which is the main component of oxidative stress. However, under conditions of excessive and prolonged production of O_2^- in wounds, the increase in NO might evolve into significantly increased nitroxidative stress due to the production of peroxynitrite ($ONOO^-$) and peroxynitrous acid (ONOOH). ONOOH can trigger a cascade of events leading to the generation of highly reactive and damaging radicals and oxidative species [60]. These species can impair the process of wound healing. Indeed, increased inducible nitric oxide synthase (iNOS) activity and nitrite levels have been shown to be responsible for diabetic foot and chronic venous ulcers [61, 62].

In summary, the high protease and pro-oxidant environment results in a chronic inflammatory state and in a significantly delayed time to complete wound healing. The implication is that despite the different underlying pathophysiology of the various ulcer types [63, 64], all ulcers have a final common pathway that leads to similar behaviors, in which chronic inflammation ubiquitously plays a key role.

EMFS/PEMFS AND WOUND REPAIR

Electromagnetic fields have been studied extensively as electro-pollutants, for example, cell phones, as well as a therapy. The ELF-EMF represent a form of non-ionizing, low-energy, electromagnetic field radiation capable of inducing physiological effects. We will herein refer to ELF-EMF of extremely low frequency sine waves (up to 300 Hz) and low amplitude (0.2-20 mT) as EMFs. Low frequency fields with specific wave shapes and amplitude are referred to as pulsed electromagnetic fields (PEMFs), a subset of ELF-EMF. In particular, therapy waves are grouped under the general heading of PEMF technology.

In general, EMFs have been found to produce a variety of biological effects. Although the mechanism interaction remains obscure, it has been shown that EMFs can cause changes in cell proliferation, cell differentiation, cell cycle, apoptosis, DNA replication and expression, and cytokine expression. Effects of EMFs are quite heterogeneous with regard to the cell type studied, intensity and type of field used. For more than three decades, the therapeutic efficacy of various forms of electrical stimulation, including capacitive coupling, direct current, combined magnetic fields, and PEMFs have been intensely investigated. PEMFs are usually more effective if less than 3 mT, and frequencies are commonly less than 100 Hz, below which they are referred to as ELF [65, 66].

Therapy with EMFs has been used for quite a long time in several medical therapeutic protocols, and the efficacy of low intensity EMFs has been demonstrated in several clinical applications. Although controversial, electromagnetic forces are believed to play a role in the normal repair of human tissues and have been investigated for this ability.

Repair stimulation is one of the stronger and better documented biological effects of EMFs. Human clinical studies have highlighted that PEMFs act in reducing healing time and the rate of recurrence of venous leg ulcers [67, 68]. In particular, Stiller *et al.* observed that exposure to PEMFs induced a significant decrease in wound depth and pain intensity in patients with venous ulcers and none of the patients treated exhibited worsening of the lesions [67]. Also, patients exposed to PEMFs showed significantly higher rate of healing of venous leg ulcers and protection from ulcer recurrence when compared to the control group [68]. Canedo *et al.* reported that field exposure of ulcers of venous etiology, reduced or eliminated pain, edema and weeping up to six weeks after the initiation of the therapy. However, the worsening of lesions is present only in patients with ulcers associated with a concomitant cofactor, such as obesity or arterial occlusion [69].

Encouraging results have also been suggested by several studies on rats and mice. Some of the *in vivo* studies have shown that the wound site decreased significantly in size in the group of animals treated with PEMFs compared to the control group [70, 71]. In addition, PEMF exposure supports a significantly faster progression of the healing of wounds in animals exposed at the end of therapy [71]. Histological organization was also assessed after PEMF exposure, as supported by experiments based on a non-wounded rat model exposed to PEMFs. Indeed, PEMF treatment stimulated early formation of connective tissue and a vascular network, early collagen synthesis and better maturation, all leading to complete re-epithelialization after 12 days of exposure [69, 72, 73]. However, a more recent study showed no benefits, suggesting the need to determine more accurately the appropriate parameters for electromagnetic fields in tissue repair [74].

EMFS/PEMFS, CYTOKINES AND WOUND REPAIR

The EMF effects on the expression of cytokines have been mostly investigated with *ex vivo* and *in vitro* experiments on different cell types involved in tissue repair. These reports contribute to the explanation for the positive effects of such a physical agent in human clinical studies and in studies with animal models. Cytokines are messenger molecules whose actions are vary varied yet overlapping. Cytokines affecting different target cell populations and involved as regulators of immune and inflammatory reactions may rep-

resent an interesting therapeutic target. The synthesis of different cytokines, induced by several stimuli, is responsible for activating different immune mechanisms. The outcome of interaction between the stimulus and tissues is dependent on the particular cytokine response. There are a number of reports investigating the effect of EMFs/PEMFs in the regulation of cytokine expression.

It is well know that EMFs regulate cytokine gene expression by calcium flux-modulation. Regulation of intracellular Ca^{2+} concentrations during exposure to EMFs has been reported by various investigators [75-77]. It has been suggested that PEMFs control the release of calcium from intracellular stores. This represents a cellular response to homeostatic challenge that prompts mitochondria to produce free radicals and heightens the DNA response [78]: a first order effect of this stimulus is to prevent the onset of inflammatory dynamics. In addition, the impact of EMFs on the conformational adaptive response of calcium channel proteins has been repeatedly cited [79-81].

Nevertheless, the effects of EMFs on cytokine expression are elicited immediately by up-regulation of antioxidant efficacy, as opposed to waiting for natural transcription to restore this balance. The time-delay needed to establish a time-varying equilibrium between free radicals and antioxidants in the secretory or constitutive phase of injury, determines whether there is activation of the entire inflammatory cascade including cytokine release [82]. Also, EMF investigators have established that gene up-regulation modeling takes place [83, 84]. These authors, accordingly, reported the up-regulation of HSP70, as just a part of a cluster of cyto-protective and restorative dynamics that EMFs set into play when tissue is oxidatively compromised.

We have identified a series of reports investigating the effect of EMFs/PEMFs in the regulation of cytokine expression and release of other pivotal mediators of inflammation (*table 1*). This experimental evidence can be related to wound healing that involves promotion of the initial pro-inflammatory stage, including PBMC influx and activation, and establishment of the anti-inflammatory stage that predisposes the resolution of the lesion.

Inflammation of skin can be determined at several, mutually nonexclusive checkpoints of the process with varying degrees determined by organ specificity. The most specific ones are those mediated by T cells that have specificity toward skin-specific antigens. The second checkpoint is at the stage of trafficking/chemotaxis/retention that dic-

Table 1
Overview of the *ex vivo* and *in vitro* studies on the effect of EMFs/PEMFs on cytokines and inflammation mediators expressed by cells involved in the skin repair process.

Mediator	Cell type	Stimulus	Wave	Frequency (Hz)	Intensity (mT)	Length of exposure (h)	Effect	Ref.
IL-1	hPBMC	ns or LPS	*s*	50	2.5	24	Increase	*[89]*
IL-1β	Mouse macrophage	ns	*s*	50	1	24	Increase	*[87]*
	Human fibroblast	ns	*p*	50	2.25	15 min a day (3 days)	Decrease	*[119]*
IL-2	hPBMC	ns	*p*	50	2.5	24	Not affected	*[86]*
	hPBMC	PHA	*p*	50	3	48	Increase	*[97]*
IL-2R	hPBMC	ns	*p*	50	2.5	24	Increase	*[86]*
IL-6	hPBMC	ns or LPS	*s*	50	2.5	24	Increase	*[89]*
	Human fibroblast	IL-1β	*p*	75	1.5	24	Decrease	*[118]*
IL-8	Human keratinocyte	ns	*s*	50	1	4 to 72	Decrease	*[107]*
	Human fibroblast	IL-1β	*p*	75	1.5	24	Decrease	*[118]*
IL-10	hPBMC	PHA or LPS	*p*	50	45 ± 5	3 h a day (3 days)	Increase	*[105]*
	Human fibroblast	IL-1β	*p*	75	1.5	24	Increase	*[118]*
	Human fibroblast	ns	*p*	50	2.25	15 min a day (3 days)	Increase	*[119]*
TNF-α	hPBMC	ns	*s*	50	1 to 30	71	Decrease	*[102]*
	hPBMC	ns or PHA or ionomycin	*s*	50-60	1-2	1 to 3 days	Decrease	*[103]*
	Mouse macrophage	ns	*s*	50	1	24	Increase	*[87]*
	Human fibroblast	ns	*p*	50	2.25	15 min a day (3 days)	Decrease	*[119]*
INF-α	hPBMC	ns	*s*	50	10	71	Decrease	*[102]*
INF-γ	hPBMC	PHA or LPS	*p*	50	45 ± 5	3 h a day (3 days)	Decrease	*[105]*
MCP-1	Human monocyte	ns or LPS	*s*	50	1	o.n.	Increase	*[98]*
	Human monocyte	PHA	*s*	50	1	24	Decrease	*[106]*
	Human keratinocyte	ns	*s*	50	1	4 to 72	Decrease	*[107]*
RANTES	Human monocyte	PHA	*s*	50	1	24	Decrease	*[106]*
	Human keratinocyte	ns	*s*	50	1	4 to 72	Decrease	*[107]*
MIP-1α	Human keratinocyte	ns	*s*	50	1	72	Decrease	*[107]*
PGE_2	Human keratinocyte	ns or LPS	*s*	50	1	1 to 48	Decrease	*[108]*
	human fibroblast	IL-1β	*p*	75	1.5	24	Decrease	*[118]*
NO	Human monocyte	ns or LPS	*s*	50	1	o.n.	Increase	*[98]*
	Human keratinocyte	ns or LPS	*s*	50	1	3	Increase	*[108]*
	Human keratinocyte	ns or LPS	*s*	50	1	18 to 48	Decrease	*[108]*

Abbreviations: IL, interleukin; IL-2R, interleukin-2 receptor; TNF, tumor necrosis factor; INF, interferon; MCP-1, monocyte chemotactic protein-1; RANTES, regulated upon activation, normal T-cell expressed and secreted; MIP-1α, macrophage inflammatory protein-1α; PGE_2, prostaglandin E_2; NO, nitric oxide; hPBMC, human peripheral blood mononuclear cells; ns, not stimulated; LPS, lipopolysaccharide; PHA, phytohaemagglutinin; s, sine; p, pulsed; Hz, hertz; mT, milliTesla; o.n., overnight; Ref, reference.

tates the entrance and duration of the inflammation in the skin. Lymphocyte and neutrophil recruitment is followed by subsequent recruitment of monocytes/macrophages that are enabled in the microenvironment of the lesion. The dynamic processes of leukocyte rolling and adhesion to the venular endothelium are considered to be effected by the microenvironment between leukocytes and the endothelium. Ushiyama *et al.*, through real-time, confocal laser-scanning microscopy, showed *in vivo,* that EMFs affect this process, reporting that whole body exposure (50 Hz, 3 mT, 30 min) significantly influences cell-to-cell interaction between venular endothelial cells and leukocytes in the mouse subcutaneous microvasculature [85].

EMFs also induce PBMC activation and pro-inflammatory cytokine production. Some authors have shown a significant increase in the percentage of activated T lymphocytes after PEMF exposure [86]. Frahm *et al.* proposed that EMFs functionally activate differentiated mouse macrophages by increasing their phagocytic activity and production of ROS, enabling the killing of microbes within their phagosomes. In addition, activation also causes the secretion of cytokines such as IL-1β and TNF-α [87], which further induces expression of the cell adhesion molecules on endothelial cell surfaces and recruitment of leucocytes to the wound site [88]. These data suggest the ability of fields to sustain the inflammatory process at the beginning of wound healing.

Cossarizza *et al.* demonstrated that PEMF exposure of PBMCs increased both the spontaneous and the phytohemagglutinin (PHA)- and TPA-induced production of interleukin-1 (IL-1) and IL-6. These findings suggest that cells of the monocytic lineage can be important cellular targets for PEMFs. Since these cytokines are among the most pleiotropic, these data first contributed to the understanding of the effects of PEMFs on the proliferation of human lymphocytes, and the effects exerted by such fields on human tissues, whose physiological activity is highly dependent on IL-1 and IL-6 [89].

Interleukin-2 (IL-2), originally identified as T cell growth factor [90], has been recently recognized for its critical role in the generation and maintenance of regulatory T cells [91-94]. Indeed, IL-2 deficiency reduces regulatory T cells levels [91, 95], leading to spontaneous lymphocyte proliferation, polyclonal activation of T and B cells, and autoimmune disease. Also, IL-2 provides essential signals for survival and expansion of γδDETC precursors in the fetal thymus and after migration to the skin. Of note, T cell stimulation increases the efficiency of tissue repair in wounded human skin cultured *in vitro*. In contrast, T cells isolated from chronic wounds do not produce growth factors, such as IGF-1, and are not responsive to stimulation. These cells are unable to produce IL-2 and other cytokines on *ex vivo* stimulation, suggesting that the normal TCR signaling pathway is impaired in patients with non-healing wounds [96]. The effect of EMFs on IL-2 and IL-2R expression on T-lymphocytes was first described by Cossarizza *et al.* 1989 [86]. Their results suggest that PEMFs (50 Hz, 2.5 mT) do not increase IL-2 production after 24 h of exposure, but reported that expression of IL-2R on lymphocyte cell membranes was markedly increased in PEMF-exposed cells, suggesting that field exposure could increase lymphocyte proliferation by increasing utilization of IL-2. To this end, Pessina and Aldinucci, showed increased levels of this cytokine in PBMCs exposed for longer periods (48 h) and stimulated with PHA. They proposed that the proliferation indexes were also significantly increased as a consequence of IL-2 production, at the same time as PEMFs treatment, comparing biological activity with cytokine antigen presence [97].

MCP-1 represents another target of EMFs. This chemokine is released from platelet granules and is produced in the wound area by resident cells, such as endothelial cells, keratinocytes at the wound edge and macrophages. It represents an important mediator of monocyte/macrophage recruitment and activation at the injury site. Reale *et al.* showed that exposure of LPS-stimulated human monocytes to EMFs, up-regulates MCP-1 both at the mRNA level and the protein level. Also, EMFs act in determining NO production and bioavailability. Treatment of the monocytic cell line (THP-1 cells) resulted in down-regulated expression of iNOS [98, 99], but in increased bioavailable NO, as confirmed by the correlated increment of cGMP in exposed compared to non-exposed control cells. Bioavailable NO is critical to ensure good wound closure. Indeed, NO participates in the orchestration of wound healing, influencing macrophages themselves, fibroblasts, and keratinocytes within the intercellular communication network during repair [100].

The anti-inflammatory effects of EMFs depend upon decreased pro-inflammatory cytokine production and increased anti-inflammatory cytokines. Recently, modulation of cytokines expression by PEMF therapy was reported in a clinical study for the first time. In particular, concentrations of the pro-inflammatory cytokine, IL-1β, in post-operative surgical wound exudates, were three-fold reduced [101]. Previously, Jonai *et al.* reported decreases in the spontaneous production of TNF-α in the intensity range of 1 mT to 30 mT, and in interferon-α (IFN-α) at 10 mT in human PBMCs [102]. Accordingly, Petrini *et al.* showed that sinusoidal 50 Hz EMFs suppresses TNF-α production in human PBMCs [103]. In contrast, Ikeda *et al.* suggested no effects from 50/60 Hz EMF exposure either as regards cytotoxic activity or cytokine production in human PBMCs [104].

Other data show decreased INF-γ levels and increased expression of the anti-inflammatory cytokine IL-10 in PBMCs of healthy volunteers [105]. Di Luzio *et al.* proposed that EMFs, through cytokine expression regulation, could modulate monocyte/macrophage transition. They reported significant inhibition by EMFs of the production of MCP-1 and RANTES in cultured human macrophages stimulated with PHA [106].

In addition to *ex vivo* monocytes/macrophages and the monocytic cell line, the EMF anti-inflammatory effects, significantly involved a keratinocyte cell line. This property was elicited by down-regulation of specific chemokines of the inflammatory phase of wound healing. Vianale *et al.*, showed that exposure of human keratinocytes (HaCat cell line) to 50 Hz EMFs, induced an early reduction of NF-kB levels, down-regulating mRNA expression and release of IL-8, MCP-1, MIP-1α and RANTES. Also, they reported an increase in keratinocyte growth [107], helping to explain the *in vivo* evidence that suggests improvement in the wound closure rate. More recently, Patruno *et al.* showed that the exposure of human keratinocytes to EMFs increased iNOS and eNOS expres-

sion levels after three h, with different decrease in time for the two NOS isoforms,suggesting their different roles in the repair process [108]. These effects of EMFs on the increase expression levels of NOS were paralleled by increased NOS activities, and increased NO production. Increased levels of NO could explain the down-regulation of RANTES by EMF exposure. Indeed, Frank *et al.* purposed that increased levels of NO may contribute to the down-regulation of RANTES *in vitro* and possibly *in vivo*. They demonstrate that NO very efficiently suppressed IL-1β and TNF-α-induced RANTES expression in keratinocytes. Furthermore, they observed the strongest RANTES-immunopositive labelling in epithelial areas that were characterized by an NO-mediated low cellularity [109]. Also, increased levels of NO could explain the down-regulation of MCP-1 in hyper-proliferative keratinocytes during the inflammatory phase of wound healing [110]. NO regulates skin wound healing, acting in both the early and the late phase, and it is likely that the timing and level of NO production in the healing wound must be carefully balanced to ensure a beneficial effect [109]. An excess of NO in specific phases of wound healing may be just as damaging as underproduction. Therefore, fine adjustments of NO levels are essential for the spatial and temporal progression of tissue repair. NO-mediated down-regulation of pro-inflammatory cytokines may represent the beginning of the transition from the inflammatory to the regenerative phase of wound healing.

Pilla *et al.* proposed a model that contributes to explain the EMF-mediated activity of eNOS reported by Patruno *et al.* on keratinocytes, as previously discussed. They suggest that EMFs modulate Ca^{2+} binding to CaM, and therefore the production of activated CaM, and subsequently activated eNOS [111]. Also, several studies argue that different cell types, such as endothelial cells, respond to EMFs by producing HSP [112]. The effect of EMFs on HSP can be induced by CaM-dependent NO signaling, even at low levels [113]. Moreover, HSP induced prior to injury, is poised to cause, upon injury, an immediate release of NO from eNOS, contributing to the down-regulation of pro-inflammatory cytokines, such as IL-1β [114], and protecting tissues from inflammation damage [111].

One of the early responses to inflammatory stimuli in cells involved in the repair processes of keratinocytes, is the induction of COX-2, promoting the release of PGs. Up-regulation of COX-2 appears to be significantly involved in the persistent inflammation seen in chronic wounds [115]. Contradictory data on the role of COX-2 in wound repair have been reported. Some authors affirm that COX-2 inhibition suppresses wound inflammation and reduces granulation/scar tissues [116], while others indicate that COX-2 is not essential for wound repair, probably because of the presence of compensatory pathways [117]. Patruno *et al.* showed that a COX-2 expression-reduction following EMF exposure reduced PGE_2 production associated with a decrease in catalase activity and O^{2-} production in human keratinocytes [108]. These experiments indicate that EMF exposure accelerates the switching from the inflammatory phase to the final repair phase during wound healing.

Several studies show that field exposure also has anti-inflammatory effects on fibroblast-like cell populations. To this end, Ongaro *et al.* demonstrated that EMFs decreased PGE_2 and the production of pro-inflammatory cytokines IL-6 and IL-8 in human fibroblasts first activated with IL-1β. Also, they observed EMF activity in increasing IL-10 levels and they speculate that these effects could be partially dependent on synergistic effects of EMFs and adenosine receptors stimulation, inhibiting the pro-inflammatory NF-kB signaling pathway [118]. These results are in accordance with early mediated reduction of NF-kB levels by EMFs described by Vianale *et al.* on keratinocytes. Similarly, a recent study concluded that PEMF irradiation, not altering the cell immune-phenotype of the fibroblast-like cell population, provokes a decrease in the production of inflammatory-type cytokines (IL-1β, TNF-α) and an increase in cytokines of lymphocytic origin (IL-10) [119].

CONCLUSION

In this review, we report a summary of experimental works that describe the effects of EMFs in regulating the expression and modulation of inflammation in relation to pathological conditions, particularly chronic wound healing. It emerged that EMFs can increase the initial inflammatory response, improving recruitment and activation of PBMCs at wound sites. In particular, fields act by increasing ROS, NO and pro-inflammatory cytokines production in macrophages and following this can contribute to the establishment of a switch toward the resolution of the inflammatory response, and thus wound healing. Accordingly, EMFs induce anti-inflammatory cytokines and contribute to the down-regulation of pro-inflammatory ones. This event can be explained by the increase in the bioavailability of NO induced by exposure to EMFs in cell types involved in the reparative process. Indeed, it has been reported that EMFs activating the CaM lead to increased activity of eNOS and bioavailable NO. At this level, the NO is able to activate both guanylate cyclase (sGC) and adenylate cyclase (sAC). The first activation is confirmed by increased levels of cGMP caused by exposure to EMFs and might explain the NO-mediated effects observed *in vivo* and *in vitro* proliferation, tissue repair and angiogenesis, while the activation of sAC, which was confirmed by a reduction of the effects of EMF exposure through the use of antagonists for AR receptors, may explain the anti-inflammatory effects of fields treatment. The activation of this transduction signaling could explain the modulation effect of EMFs on cytokine expression profiles, through synergy with adenosine receptors and induction of an early decrease in the activity of NF-kB.

In conclusion, EMFs might have a possible therapeutic application in diseases such as ulcers, in which chronic inflammation is an important component. However, although numerous *in vitro* experiments have allowed us to understand partially the evidence described *in vivo*, an optimal range of wave parameters, in particular shape, frequency, amplitude and intensity, remains to be delineated.

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