Extremely low-frequency electromagnetic fields accelerates wound healing modulating MMP-9 and inflammatory cytokines

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Abstract

Objectives: In our previous reports, we have demonstrated that extremely low-frequency electromagnetic fields (ELF-EMF) exposure enhances the proliferation of keratinocyte. The present study aimed to clarify effects of ELF-EMF on wound healing and molecular mechanisms involved, using a scratch in vitro model.

Materials and methods: The wounded monolayer cultures of human immortalized keratinocytes (HaCaT), at different ELF-EMF and Sham exposure times were monitored under an inverted microscope. The production and expression of IL-1 β , TNF- α , IL-18 and IL-18BP were measured by enzyme-linked immunosorbent assay and quantitative real-time PCR. The activity and the expression of matrix metalloproteinases (MMP)-2/9 was evaluated by zymography and Western blot analysis, respectively. Signal transduction proteins expression (Akt and ERK) was measured by Western blot.

Results: The results of wound healing in vitro assay revealed a significant reduction of cell-free area time-dependent in ELF-EMF-exposed cells compared to Sham condition. Gene expression and release of cytokines analysed were significantly increased in ELF-EMF-exposed cells. Our results further showed that ELF-EMF exposure induced the activity and expressions of MMP-9. Molecular data showed that effects of ELF-EMF might be mediated via Akt and ERK signal pathway, as demonstrated using their specific inhibitors.

Conclusions: Our results highlight ability of ELF-EMF to modulate inflammation mediators and keratinocyte proliferation/migration, playing an important role in wound repair. The ELF-EMF accelerates wound healing modulating expression of the MMP-9 via Akt/ERK pathway.

1 | INTRODUCTION

Wound healing is a complex biological process characterized by a series of events responsible of the maintenance of homeostasis in the repair of damaged tissue.¹ The normal healing response starts when the tissue is injured, and the earliest events trigger the release of numerous inflammatory mediators. The inflammatory response influences each subsequent phase of healing; excessive or prolonged inflammation is a hallmark of non-healing wounds and formation of fibrotic tissue.^{2,3} Cytokines such as IL-1 β , IL-18 and TNF- α are critical

for the skin's inflammatory response to tissue injury. Upon injury, pre-stored IL-1 is immediately released by keratinocytes, IL-18 mRNA is rapidly translated into protein, and all act as the initial signalling for inflammatory phase and resolution of wounds by its paracrine and autocrine effect.⁴⁻⁶

During wound healing, basal keratinocytes break their contact with the basement membrane through a break-up of their hemidesmosomes allowing cells to migrate in the wound area. Thus, a successful repair implies the synthesis and organization of an extracellular matrix appropriate to the function of tissue in its biophysical environment.⁷

The matrix metalloproteinases (MMPs) are a family of zinc- and calcium-dependent endopeptidases that play a fundamental role in many physiological processes such as the remodelling of extracellular matrix structures and keratinocytes migration in wound healing. *In vitro* data have demonstrated that human keratinocyte cell lines, metabolically highly active during wound healing, express and secrete several cytokines, MMPs and growth factors.⁸

Between different MMPs know, has been widely documented the involvement of MMP-2 and MMP-9 in the remodelling of the matrix in the wound healing process. In addition to predominant activity to degrade structural components of the ECM, MMP-2 and -9 exert their action on cytokines and chemokines involved in inflammatory and repair processes by cleaving their biologically inactive forms.⁹

Extremely low-frequency electromagnetic field (ELF-EMF) represent a form of non-ionizing and low-energy radiation capable to induce a variety of biological effects. For several years, the significance of study of the influences of electromagnetic fields on the organism is increasing because it has been accumulating evidence of the effectiveness of these in several clinical applications, especially for tissue repair processes. The mechanisms of the ELF-EMF-induced effects are quite heterogeneous and not completely known. In fact, the electromagnetic field effects on living systems are complicated by various nonlinearities (intensity, frequency and time windows of the fields) and peculiarities (cell type, age, treatment).^{10,11}

In previous reports, our research group was able to demonstrate that ELF-EMF exposure enhances keratinocyte proliferation and accelerates the switch from the inflammatory to proliferative phase, through the modulation of the inflammatory mediators and the modification of the transcriptomal profile.^{12,13} Therefore, in the present study, we have analysed the effects of ELF-EMF on wound closure, using an *in vitro* wound model on a confluent monolayer of human keratinocyte HaCaT cell line, investigating molecular mechanisms involved in the process.

2 | MATERIALS AND METHODS

2.1 | ELF-EMF exposure system and cell culture

All experiments were performed using an exposure system producing an oscillating magnetic field (AC MF) consisting of: (i) a generator of sinusoidal signal at 50 Hz (Agilent mod. 33220A Santa Clara, CA, USA); (ii) a power amplifier (mod. 216; NAD Electronics Ltd, London, UK); (iii) an oscilloscope (ISO-TECH mod. ISR658; Vicenza, Italy) dedicated to the monitoring of output signals from the Gaussmeter (MG-3D; Walker Scientific Inc., Worcester, MA, USA) and the AC MF generator; (iv) a 160-turn solenoid (22 cm length, 6 cm radius, 1.25×10^{-5} cm copper wire diameter) generating a horizontal magnetic field. The achieved MF intensity was 1 mT (rms), and was measured continuously during exposure using a Hall-effect probe connected to the Gaussmeter.¹⁴

HaCaT keratinocyte cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and 1000 U penicillin, streptomycin (Sigma, Milan, Italy). Cells were grown to approximately 80% confluence before experiments were carried out. Cell

cultures were maintained at $37 \pm 0.3^\circ\text{C}$ in a humidified atmosphere of 5% CO_2 and placed within the central region of the solenoid that was characterized by the greatest field homogeneity (98%). A digital thermometer (HD 2107.2; Delta OHM, Padua, Italy) was placed inside the solenoid directly alongside the cell cultures, and temperature of the cell medium was recorded using a specially designed thermoresistor (HD 9216; Delta OHM). No significant temperature changes were observed associated with application of the ELF field ($\Delta T = 0.1^\circ\text{C}$). A set of experiments (ELF-EMF-exposed) was then performed placing the cells within the central region of the solenoid characterized by the greatest field homogeneity (98%). Simultaneously, another set of experiments (Sham-exposed) was performed placing HaCaT cells within the central region of the identical electrically disconnected solenoid. In both exposure conditions, cells were maintained at $37 \pm 0.3^\circ\text{C}$ in a humidified atmosphere of 5% CO_2 in 2 identical incubators (HERAcell, Heraeus, Germany). At the end of incubation, both ELF-EMF- and Sham-exposed cells were harvested using trypsin-EDTA. Cell-free supernatants and pellets were pooled and stored at -80°C until further analysis.

2.2 | Wound healing scratch assay

The scratch wound assay is a well-developed method to investigate cell migration *in vitro*.¹⁵ HaCaT cells were seeded at a density of about 1×10^6 cells/well (10^4 cells/cm², in 6-well plates) in complete medium at 37°C and 5% CO_2 (v/v), and grown for 24 hours to allow them to reach about 90% confluence. In a different set of plates, the medium was removed and replaced with medium containing 5 $\mu\text{g/mL}$ mitomycin C for 3 hours to inhibit cell proliferation. Scratch wounds were created mechanically with a sterile pipette tip ($\varnothing = 0.1$ mm) on cell monolayer. We were careful to produce uniformly sized wounds of approximately 0.5 mm, and debris was removed from the culture and cells were then cultured with fresh medium. The initial scratch (T0) was observed to have almost the same area in both Sham and ELF-EMF exposure conditions. Wound closure were assessed every 2 hours using an inverted microscope (Leica DM IL D-35578 Wetzlar, Germany) at a magnification of x10 and photographed with a Colour View II digital camera to measuring the remaining cell-free area in triplicates wells. Statistically significant differences in cell-free areas of Sham and ELF-EMF exposure ($*P < .05$) were detected after 8 and 24 hours and expressed as percentage of the cell-free area of the T0 and represented as mean $\pm$ SD of 3 independent experiments.

2.3 | Analysis of MMP-2 and MMP-9 activities by gelatin zymography

Gel zymography was performed as previously described.¹⁶ Cellular supernatants were collected and protein content was determined by Bradford assay (Bio-Rad, Hercules, CA, USA). Samples were loaded and subjected to 8.5% SDS-PAGE (Bio-Rad) containing porcine gelatin at 4.5 mg/mL (Sigma-Aldrich, St Louis, MO, USA) in non-denaturing, non-reducing conditions. The MMP-2/9 band density quantification was performed using Gel Doc 1000 (Bio-Rad).

2.4 | Western blot

For Western blot analysis, HaCaT cells were collected and lysed with RIPA buffer.¹⁷ Total protein extracts were separated on a 4%-12% NuPAGE gradient gel (Gibco Invitrogen, Paisley, UK).¹⁸ Blots were probed and incubated overnight with primary antibodies for MMP-2 (sc-13594), MMP-9 (sc-12759), Akt (G-5, sc-55523), p-Akt (Thr 308, sc-135650), p-ERK (Thr202 sc-101760), ERK (sc-271269) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and α -actin (A5441; Sigma-Aldrich). Detection was performed by Super Signal Ultra chemiluminescence detection reagents (Pierce Biotechnology, Rockford, IL, USA). The blot images were analysed with a gel analysis software package (Gel Doc 1000; Bio-Rad).

2.5 | mRNA extraction and qRT-PCR analysis

Total RNA was extracted from HaCaT cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. Total RNA concentration was estimated by measuring the absorbance at 260 nm using a Bio-Photometer (Eppendorf AG, Hamburg, Germany), and RNA samples were kept frozen at -80°C until use. Quantitative real-time PCR assay was carried out in an Eppendorf Mastercycler ep realplex (Eppendorf AG). Preliminary PCR reactions were run to optimize the concentration and ratio of each primer set. For all cDNA templates, 2 μL was used in a 20 μL real-time PCR amplification system of SYBR Green Real Master Mix Kit following the manufacturer's protocol. Specific cytokine primers and 18S rRNA as control were designed using GeneWorks software (IntelliGenetix, Inc., World Wide Corporation, Mountain View, CA, USA) (Table 1). The fluorescence intensity of the double strand-specific SYBR Green, reflecting the amount of formed PCR product, was monitored at the end of each elongation step. Melting curve analysis was performed to confirm the purity of the PCR products. Changes in cytokines gene expression were determined using the ($\Delta\Delta\text{Ct}$) method (relative expression = $2^{\Delta\text{Ct}}$, where $\Delta\text{Ct} = \text{C}_{\text{T(cytokines)}} - \text{C}_{\text{T(18S)}}$) with a threshold cycle value of 0.2 normalized to 18S rRNA. Predicted cycle threshold values were exported directly into Excel worksheets for analysis. Relative changes in gene expression were reported as a calibrator-normalized ratio relative to the calibrator cDNA (Sham value = 1) prepared in parallel with the experimental cDNAs. The experiments were repeated twice with consistent results and data are presented as means $\pm$ SEM from triplicates.

2.6 | ELISA

The levels of cytokines, in cell-free supernatants, were evaluated by commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D,

Minneapolis, MN, USA), following the manufacturer's instructions. The detection limit of the assay was 0.5 pg/mL for TNF α and IL-18BP, 1 pg/mL for IL-1 β and 12.5 pg/mL for IL-18 evaluated by commercial ELISA kit (MBL, Woburn, MA, USA). Intra- and inter-assay reproducibility were $>90\%$. Duplicate values that differed from the mean by greater than 10% were not considered for further analysis.

2.7 | Statistical analysis

All results were expressed as mean $\pm$ standard deviation. Repeated measures ANOVA was performed to compare means between groups. A probability of null hypothesis of $<5\%$ ($P < .05$) was considered as statistically significant.

3 | RESULTS

3.1 | Effect of ELF-EMF on wound healing closure

We analysed the effect of 50 Hz 1mT ELF-EMF on the healing process and observed that in ELF-EMF-exposed scratch, cell-free area was significantly decrease. The results shown in Figure 1A reveal that the reduction of cell-free area was time-dependent, with early reduction in ELF-EMF-exposed with respect to Sham-exposed cells. Quantitative evaluation performed measuring the cell-free area showed that after 8 hours of ELF-EMF or Sham exposure, the cell-free area was, respectively, 23.33% and 66.6% and after 24 hours was, respectively, 0% and 16.6% (Figure 1B). To eliminate the possibility that decreased wound area is dependent on proliferation, we treated the cells with mitomycin C, a potent DNA crosslinker and inhibitor of cell proliferation. We observed a reduction in mitomycin C-treated cells, with more evident effect in ELF-EMF-exposed cells (Figure 2)

3.2 | Effect of ELF-EMF on expression and production of IL-1 β , IL-18, IL-18BP and TNF α

Wound healing is a process that begins with inflammatory reactions mediated by cytokines gene expression and production. In our previous study, we showed that ELF-EMF exposure was associated with a shift towards anti-inflammatory chemokine profiles in parallel with a decrease of pro-inflammatory chemokines.¹⁹

In this study, we have analysed the expression and production of IL-1 β , IL-18, IL-18BP and TNF α , as marker of inflammatory response, and we have found the early increase of expression and production of

TABLE 1 Human primer sequences used in qRT-PCR

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
18s	CTTTGCCATCACTGCCATTAAG	TCCATCCTTTACATCCTTCTGTC
IL-1 β	TGAGGATGACTTGTCTTTGAAG	GTGGTGGTCGGAGATTCCG
TNF α	CCTTCCTGATCGTGGCAG	GCTTGAGGGTTTGCTACAAC
IL-18	CAGTCAGCAAGGAATTGTCTC	GAGGAAGCGATCTGGAAGG
IL-18 BP	CAACTGGACACCAGACCTCA	AGCTCAGCGTTCCATTCACT

FIGURE 1 Scratch assay: time-course of wound closure. We show a representative result of wound immediately after the (A) scratches (T0), and after 8 and 24 h of Sham and ELF-EMF exposure. (B) Wound closure was evaluated measuring the remaining cell-free area and expressed as percentage of the initial cell-free area. Scale bar = 100 μ m. The results of 3 independent experiments are expressed as mean $\pm$ SD of percentage of cell-free area **P*-value <.05 ELF-EMF vs Sham

FIGURE 2 Effect of mitomycin C on wound closure. HaCaT cells were either untreated or treated with mitomycin C and then exposed to Sham or ELF-EMF. Photographs show representative results of wound immediately after the scratches (T0), and closure after 8 and 24 h of Sham and ELF-EMF exposure in cells treated or not with mitomycin C. Wound closure was evaluated measuring the remaining cell-free area and expressed as percentage of the initial cell-free area. Scale bar = 200 μ m. Results are representative of 3 independent experiments. **P* < .05 vs mitomycin-treated cells

cytokines analysed. Afterwards, a progressive reduction of IL-1 β , IL-18 and TNF α expression until 24 hours after scratch was observed in both ELF-EMF- and Sham-exposed cells. The higher reduction of IL-1 β

expression was observed in ELF-EMF-exposed cells confirming its role in inflammatory response during wound healing (Figure 3). Data showed in Figure 3 evidenced the early increase of IL-1 β , IL-18 and TNF α and

FIGURE 3 Cytokines gene expression. Gene expression of IL-1 β , IL-18, IL-18BP and TNF α in ELF-EMF- or Sham-exposed scratched HaCaT cells monolayer. Changes in cytokines gene expression were determined by qRT-PCR assay, using the ($\Delta\Delta C_t$) method and 18S as housekeeping gene. *P-value <.05 8 and 24 h vs 1 h, **P-value <.01 1 h ELF-EMF vs 1 h Sham

FIGURE 4 Cytokines production. IL-1 β , IL-18, IL-18BP and TNF α , levels in cell-free supernatants of basal (0), or after 1, 8 and 24 h of Sham- or ELF-EMF-exposed scratched HaCaT cells monolayer. Samples of 3 independent experiments were evaluated by enzyme-linked immunosorbent assays (ELISA) using commercially available kits, and analysed in duplicate at the same time, and plotted as mean $\pm$ SD. *P-value <.05 ELF-EMF vs Sham

IL-18BP production in ELF-EMF-exposed cells with respect to Sham exposure. At the same time of a complete healing (24 hours), the reduction of pro-inflammatory cytokines and an increase of IL-18BP were observed in ELF-EMF-exposed cells (Figure 4).

3.3 | Effect of ELF-EMF on expression and activation of MMP-2 and MMP-9

Extracellular matrix remodelling and induction of keratinocyte migration were determined by secretion of MMPs. Among MMPs, we focused on MMP-2 and MMP-9 that are associated with inflammation

and remodelling.²⁰ The Figure 5A shows a representative gelatin zymography analysis performed on cellular supernatants. The results demonstrated that the latent MMP-9 enzyme characterized by a lytic band at 92 kDa, in keratinocytes exposed to ELF-EMF peaked at 8 hours and then decrease at 24 hours in exposed cells in contrast to Sham-exposed cells where MMP-9 levels increase depending on time. Instead, the levels of latent MMP-2 form were not modified by EMF exposure. Protein levels assay showed that MMP-9 expression is in accord with activity assay. In fact after 8 hours of ELF-EMF exposure, MMP-9 peaked and then after 24 hours decreased significantly (Figure 5B).

FIGURE 5 EMF-ELF effects on activity and protein expression of MMP. A, Cell-free conditioned media were assayed for MMP-2 and MMP-9 activity by gelatin zymography. The activity of MMP-9 (92 kDa) (bottom) was reported with fold induction values compared to Sham MMP-9 activity. One-way ANOVA, values represent mean $\pm$ SD ($n = 6$); * $P < .05$ compared to Sham exposure cells. Zymogram (top) is representative of 6 gels using 3 separate pools of total protein extracted from HaCaT cell line. We performed the cell counts for each experimental condition to assure that the supernatants were obtained from equal cell numbers. B, Western blot analysis of MMP-9 protein levels from HaCaT cells in Sham and ELF-EMF exposure. Relative MMP-9 protein levels after normalization to β -actin from 3 separate experiments are presented as mean $\pm$ SD. * $P < .05$ compared with the Sham exposure

FIGURE 6 Effect of ELF-EMF exposure on Akt and ERK activity. Representative image of immunoblotting for p-AKT (A) and p-ERK (B) of gels using 3 separate pools of protein extracted from HaCaT cells in Sham and ELF-EMF exposure. At the bottom, in the densitometric analysis ($n = 3$), averaged band density of p-AKT and p-ERK immunoreactive is expressed as relative expression in both Sham and ELF-EMF exposure (mean $\pm$ SD; * $P < .05$ vs Sham-exposed cells)

3.4 | ELF-EMF accelerates wound healing via PI3K/Akt, ERK/MAPK pathways

To clarify whether the PI3K/Akt and ERK signalling pathways are involved in the enhanced ELF-EMF-induced HaCaT migration/proliferation, first of all we tested the phosphorylated protein levels of threonine kinase Akt (Thr 308) and p-ERK at 10 minutes, 30 minutes, 1 hour and 3 hour. A similar time-dependent expression trend of Akt was observed in both ELF-EMF- or Sham-exposed cells, with higher levels of phosphorylation at 1 hour. In ELF-EMF-exposed cells, levels of phosphorylated Akt expression were higher compared to Sham condition at all time studied (Figure 6A).

The densitometric analysis of the time-course experiments reported in Figure 6B shows a time-dependent induction of p-ERK in ELF-EMF-exposed cells that peaked early at 1 hour. Instead, in Sham-exposed HaCaT cells, no significant differences in p-ERK levels were observed at 10, 30 minutes and 1 hour, while a significant increase was observed after 3 hours.

To verify if MMP-9 activity and HaCaT proliferation were differently modulated by Akt/ERK activation in ELF-EMF-exposed cells, we have pre-treated HaCaT cells with a specific inhibitor of ERK

(PD98059) and PI3K/Akt (LY294002). As highlighted by densitometry analysis of Western blot assay, both specific inhibitors, at all time-points examined (1-8-24 hours), reduced MMP-9 protein levels in HaCaT cells (Figure 7). In detail, in ELF-EMF-exposed HaCaT cells, the MMP-9 protein expression is significantly modulated by ERK, otherwise in Sham-exposed cells, it is Akt that plays a key role in the MMP-9 protein expression levels, as evidenced by treatment with the respective inhibitors. In addition, the effect of Akt/ERK inhibitors on wound healing was evaluated by measuring the cell-free area after 8 and 24 hours of Sham- or ELF-EMF exposure. The pre-treatment with both specific inhibitors reduced wound healing calculated as the percentage of cell-free area. Results showed in Figure 8 highlight that ERK inhibition is decisive for delayed wound healing in ELF-EMF-exposed cells, as observed at 8 and 24 hours.

4 | DISCUSSION

The exposure to electromagnetic fields (EMF) can lead to different biological effects depending on the intensity, frequency, dose, exposure time and cell type. In fact, recent evidences showed that different

FIGURE 7 Effects of pharmacological inhibitors on MMP-9 protein expression. Representative image of immunoblotting for MMP-9 in Sham- and ELF-EMF-exposed HaCaT cell at 1, 8 and 24 h, pre-treated or not with selective inhibitor of Akt (Ly294002, 1 μ mol/L) and ERK (PD980559, 1 μ mol/L). Data are reported as relative expression of MMP-9 vs β -actin (mean $\pm$ SD, $n = 6$). # $P < .05$ treated cells vs cells not treated with selective inhibitors

FIGURE 8 Akt and Erk inhibitors delayed wound closure. Wound closure in presence of PD980559 1 μ mol/L, and Ly294002 1 μ mol/L, was evaluated by measuring the cell-free area after 8 and 24 h in ELF-EMF- or Sham-exposed scratched HaCaT cells monolayer. The results of 3 independent experiments are expressed as percentage of cell-free area. * $P < .05$ treated cells vs cells not treated with selective inhibitors

keratinocyte lines may respond differently to ELF-EMF, and our early reports showed that on HaCaT keratinocytes cells line the ELF-EMF (50 Hz, 1mT) triggers a change in the gene expression profile suggesting a switch towards an increase of "Proliferative" and "Migration" function and modulation of inflammatory mediators.^{12,19,21}

It is well known that keratinocytes play an important role in covering the wound bed by migration and proliferation, and that at the migrating front of re-epithelization, during wound healing. They are

the predominant source of MMPs and their timing of expression, activation and regulation is fundamental for an efficacious healing. In the present study, using an in vitro wound healing model, we demonstrated the effect of ELF-EMF to accelerate wound healing associated to a modulation of several cytokines expression/production and to an increase of MMP-9 activity/expression.

MMP-9, a 92-kDa gelatinase that degrades collagen IV, was widely investigated for its ability to regulate a large spectrum of physiology and pathophysiology processes involved in tissue remodelling.²² The early up-regulation of MMP-9 activity/expression in ELF-EMF-exposed wound model may represent a mechanism to promote the migration of keratinocyte and induce phagocytosis in the inflammatory phase of wound healing. At more long time, during a proliferative phase, the decreased activity and level of MMP-9, are important to control the excessive degradation of extracellular matrix and avoid the formation of chronic wounds. Our results obtained by zymography analysis demonstrated the effectiveness of ELF-EMF to come early the up-regulation of MMP-9 level that was higher in ELF-EMF-exposed cells already at 1 hour peaking at 8 hours, and decreasing at 24 hours, in accordance to the reduction of percentage of cell-free area and the completely filled 24 hours after wounding as detected by microscopic observation.

Several reports have demonstrated that MMP-9 activation and proliferative process are critically mediated by MAPK or PI3K/Akt signalling pathway, in response to different stimulators.^{23,24}

Therefore, first of all, we examined the effect of ELF-EMF on the activity of Akt and ERK signalling pathways. The results showed that, while in Sham-exposed HaCaT cells the higher phosphorylated ERK levels reached after 3 hours match with a decline of phosphorylated Akt levels; interestingly, in ELF-EMF exposure the relationship between Akt and ERK was changed. In fact, both Akt and ERK reached the higher phosphorylation levels simultaneously at 1 hour, highlighting that the ELF-EMF exposure quickened ERK activation.

These results suggest that in Sham-exposed wound healing, in line with other study, the coordinated action of Akt and ERK signalling pathways is determined by mutual influence to each other, resulting in a dynamic and complex crosstalk.²⁵ The detected early EMF-induced ERK signalling activation raises the questions as to how interacting with biological function. A plausible cellular mechanism of this activation could be an EMF-induced rearrangement of membrane surface proteins/receptor resulting in a modulation of transduction system such as Ras/Raf/EK/ERK and PI3K/PTEN/Akt signalling pathways activation.^{26,27} The importance of an early activation of ERK, in ELF-EMF-exposed cells, is underlined by expression and functional analysis performed with its specific inhibitors, revealing as ERK pathway participates in the very first steps of the epidermal healing process. We have confirmed the predominant role of ERK in our in vitro scratching experiments and MMP-9 activity induction, using ERK and Akt inhibitors.

In addition, as a consequence of a tissue injury, pro-inflammatory danger signals in keratinocytes, may induce the activation of the Nod-like receptor protein (NLRP)-3 inflammasome and the release of IL-1 β and IL-18. In this study, we have observed an early increase of IL-1 β , IL-18 and TNF α production in ELF-EMF-exposed wound healing

scratch, in line with the observed rapidly increased levels of IL-18 protein following cutaneous wounding in mice.^{28,29} The early IL-18 expression and production was balanced by IL-18BP that increased time-dependently, suggesting that ELF-EMF may potentiate regulatory network of bioactive IL-18 and its counter-regulator IL-18BP and corroborating the potential use of ELF-EMF to support wound healing.

IL-18 has been described as an inducer of TNF α and IL-1 β , and in vivo study showed delayed wound healing associated with reduced levels of IL-1 β and dampened inflammatory response, stressing that IL-1 β plays an important role during the early stages of wound healing inducing the production of MMPs.^{30,31} Our results showed that ELF-EMF exposure induced an early IL-1 β expression and MMP-9 activity in accordance with quickened wound healing and led us to hypothesize that ELF-EMF-accelerated conversion of inflammatory phase into wound healing involves modulated activity of cytokines and MMP-9 in keratinocytes. Also, Akt and ERK pathways both contribute to tissue repair process and provide signals for pathways cooperation accelerating wound healing. Support for our conclusions includes the observation that MMP-9 and Akt/ERK pathways are activated upon ELF-EMF exposure, and their inhibition prevents ELF-EMF-induced wound healing.

In this study, we observed a time-dependently accelerated healing in scratch wound exposed to ELF-EMF and hypothesize the mechanisms by which ELF-EMF accelerates wound closure. Collectively, our findings improve the understanding of the ELF-EMF effects on inflammatory phase of wound healing, giving insight into potential management of the healing process using ELF-EMF treatment as well as the signalling molecules that it modulates.

Although many studies have shown the effect of magnetic fields on human and animal model,³²⁻³⁷ further and more comprehensive studies are still required. This study revealed that EMF might serve as a potential tool for manipulating activity of cells and it opens a new investigation field valuable for future new therapeutic approach to accelerate skin regeneration and wounds closure.

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CONFLICT OF INTEREST

None declared.

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