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Histological Observation of Acute Inflammation During Wound Healing in Mice Using Low-Frequency Electromagnetic Field (LF-EMF) Therapy

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ABSTRACT

Background and Objective: Electromagnetic Field (EMF) therapy has emerged as a non-invasive approach to enhance wound healing, yet its effects on the acute inflammatory phase remain unclear. This study aimed to assess the impact of low-frequency EMF (LF-EMF) on wound healing in mice, focusing on this phase.

Materials and Methods: Eighteen mice were divided into two groups: The LF-EMF-treated and control. A full-thickness wound was created on each mouse, with treatment (LF-EMF: 0.8 mT, 15 Hz, 15 min. Control: sham-exposure) administered daily from day 1 to 6 post-wounding.

Results: LF-EMF treatment consistently showed higher percentage reduction in wound area compared to the control group at all time points. The inflammatory response resolved quickly in the LF-EMF group compared to the control group as reflected by a steady decline in neutrophil percentage and remarkably low percentage by Day 6 compared to the control. Monocyte percentages increased gradually in LF-EMF-treated wounds, while lymphocytes declined from day 1 to 3 in both groups before rising again from Day 4 onward, although LF-EMF group was more pronounced. The H&E staining revealed that LF-EMF exposure accelerates wound healing dynamics, demonstrating enhanced angiogenesis, rapid fibrin resolution and early collagen deposition compared to the control group. Masson's trichrome staining further confirmed increased fibroblast migration and elevated cellularity within the granulation tissue. By day 6, the extracellular matrix displayed improved structural integrity with collagen fibres appearing denser, more aligned and morphologically mature.

Conclusion: These findings underscore the capacity of LF-EMF to modulate key reparative pathways by promoting cellular activation, amplifying fibroproliferative responses and facilitating efficient matrix remodelling.

INTRODUCTION

Wound healing is a complex physiological response to tissue injury involving various cell types, cytokines, chemokines, growth factors and the vascular system. The process is typically divided into four tightly regulated and overlapping phases: Haemostasis, inflammation, proliferation and remodelling. Immediately after tissue injury, haemostasis occurs, marked by initial vasoconstriction of blood vessels and the formation of blood clots to stop the bleeding. The inflammatory phase soon follows, where mast cells release histamine-filled granules, contributing to the classic signs of inflammation, such as redness (rubor)[1]. Then, inflammatory cells such as neutrophils extravasate to the wound site, followed by macrophages to clean up debris and pathogens. The cells also release cytokines, chemokines and growth factors to recruit other cells, such as fibroblasts and epithelial cells, thus laying the framework for the next phase of wound healing, which is the proliferative phase.

New tissue and blood vessels (angiogenesis) are created during this phase[1]. Concurrently, matrix construction is performed to fill the wounded area. The final remodelling phase overlaps with the previous phase, during which the collagen fibres are reorganised and cross-linked to enhance the tensile strength of the Extracellular Matrix (ECM) along with the reduction of blood supply at the damaged area as angiogenesis declines[2].

Although, inflammation is the most integral part of healing, this phase must be tightly regulated. Excessive or prolonged inflammation can impair the healing process and lead to chronic wounds, a condition often seen in patients with underlying issues such as diabetes. One significant complication of diabetes is the development of diabetic foot ulcers. It poses a significant healthcare burden, leading to chronic wounds that often recur. Ultimately, if not addressed, these ulcers can require amputations. This not only impacts patients' quality of life but also creates a financial strain on the healthcare system and, most importantly, on the family. A study by Ng et al.[3] at Segambut Hospital in Johor, Malaysia (2016-2018), highlighted this severity. The research revealed that 36% of the orthopaedic ward surgeries dealt with diabetic foot infections, with a concerning rate of amputations (25% digits, 9% below the knee, 4% above the knee).

This leads to the exploration of other promising solutions that have the potential to enhance the inflammatory phase to allow faster transition to the proliferative phase. One promising solution is the use of Electromagnetic Fields (EMF). EMF is a form of non-ionising, low-energy electromagnetic field capable of inducing physiological effects. The concept underlying EMF therapy is the electric charge from the cells involved in wound healing. Endogenous bioelectricity plays an essential role in cellular migration to wound sites and may be involved with angiogenesis and tissue repair. In delayed wound healing, these bioelectric signals are often disrupted but externally applied electrical simulations, such as EMF, can mimic or amplify these effects, thereby reactivating the wound-healing process[4].

EMFs at frequencies lower than 300 Hz lack the energy to ionise, cause DNA damage, break molecular bonds, or even have thermal effects on tissues and cells[5]. Pesce et al.[6] referred to this EMF as low frequency EMF (LF-EMF), with waves as being sinusoidal in shape (up to 300 Hz) and of low amplitude (0.2-20 mT). EMF therapy has demonstrated its ability to influence molecular pathways involved in wound healing, which includes modulating inflammatory signalling, promoting cell proliferation and improving angiogenesis[5]. However, there is limited research specifically addressing the role of LF-EMF in modulating the acute inflammatory response during wound healing and more studies are needed to clarify its therapeutic potential.

MATERIALS AND METHODS

Animal acclimatisation: Animal experiments were approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC). CD-1 mice aged 8 to 12 weeks old and weighing approximately 30 g were obtained from the Laboratory Animal Resource Unit, National University of Malaysia. They were acclimated to the laboratory environment at least 5 days prior to initiating the experiment at 24±1 and 55% ±5% humidity with a 12 hrs light-dark cycle. Food and water were given ad libitum.

Mice wound model: The mice were anaesthetised with Zoletil® 100 (Virbac, France). Then, a serrated forceps was used to lift the skin and iris scissors were used to create a full-thickness wound that extended through the subcutaneous tissues. The wound area was measured daily by taking several pictures of the wound with a ruler placed next to it for scale. Then, the wound area was measured using ImageJ version 1.54i (NIH, Maryland, USA).

LF-EMF treatment: LF-EMF was created by wrapping copper wires around a PVC tube and connected to a power supply. The magnetic field density (mT) was evaluated using a Teslameter (Fig. 1). Mice began receiving treatment one day after wound induction. The mice were exposed to

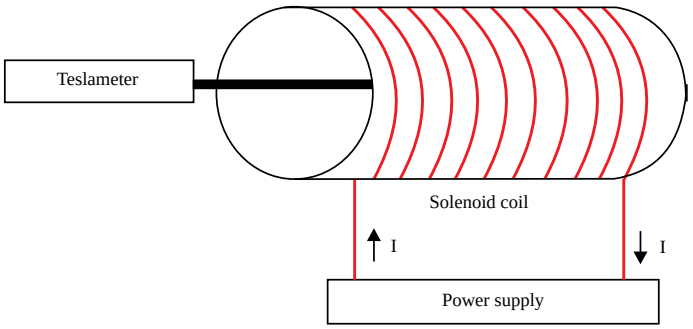

Fig. 1: LF-EMF set-up, consisting of copper wires (red) wrapped around a PVC tube and connected to a power supply
A Teslameter was used to evaluate the magnetic field density inside the tube

0.8 mT at 15 Hz for 15 min each day. Control groups were placed inside the solenoid with inactive generators (sham-exposure).

White blood cell (WBC) differential count: Before tissue retrieval, blood was collected via cardiac puncture. Mice were heavily sedated with Zoletil® 100 and placed on their backs. The chest was cleaned with an alcohol swab and a 21 G needle attached to a 1 mL syringe was inserted into the heart. Around 1 mL of blood was drawn and transferred into an EDTA tube. To analyse white blood cells (WBCs), slides were stained with Wright's stain. A differential count was performed by examining 20 High-Power Fields (HPF) under a 40× objective (total magnification 400×). At least 100 WBCs were counted per sample, focusing on monocytes, neutrophils and lymphocytes. Three slides per sample (N = 3) were analysed and the percentages of each type of WBC were calculated with the following formula:

$$\text{Each type of WBC}(\%) = \frac{\text{No. of specific WBC type}}{\text{Total WBC}} \times 100\%$$

Histological analysis: Histological analysis was performed on mice at day 1, 3 and 6. Euthanasia was carried out using an overdose of Zoletil®. The entire wound bed tissue was carefully excised using iris scissors and fixed in 10% formalin overnight to ensure proper tissue preservation. The samples were prepared using the Formalin-fixed, Paraffin-Embedded (FFPE) method. Following fixation, the tissues underwent processing, embedding, sectioning and staining with Haematoxylin and Eosin (H&E) and Masson's trichrome to evaluate overall tissue morphology and cellular structure.

RESULTS

Percentage decrease of wound area (%): The percentage reduction in wound area was compared between the LF-EMF-treated and control groups from day 1-6. The LF-EMF group consistently showed greater wound reduction at all time points. The difference between the groups became more pronounced over time.

On day 1, wound reduction was significantly higher in the LF-EMF group (12.55±5.61%) than that of the control group (3.19±1.45%) ($p<0.05$). By Day 2, both groups showed marked improvement; however, the LF-EMF group maintained a higher reduction (25.98±6.74%) compared to the control group (20.18±1.67%), though the difference was less pronounced. On Day 3, wound reduction increased steadily in the LF-EMF-exposed group (30.91±5.88%), while the control group showed a slower rate (22.86±1.58%).

On Day 4, the gap between the groups became more evident, as the percentage reduction of wound area increased (41.48±4.29%) for the LF-EMF-exposed group, compared

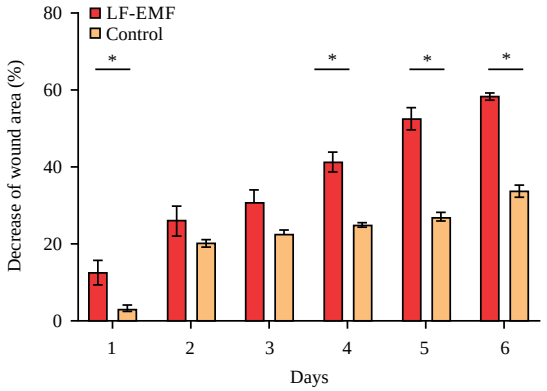

Fig. 2: Bar chart showing the percentage decrease of wound area of LF-EMF and control groups from day 1 to 6
Results were presented as average±standard error of the mean (SEM) based on three experimental replicates (N = 3) Statistical significance is indicated by * = $p<0.05$

to the control group (25.50±0.86%) ($p<.05$). By day 5, the LF-EMF group showed a markedly greater reduction (52.72±4.78%), in contrast with the control group (27.10±1.88%), with a significant difference of 25.62% ($p<0.05$). By Day 6, LF-EMF-treated wounds achieved a reduction of 58.44±1.59%, while the control group reached only 33.79±2.68%, showing a significant difference of 24.65% ($p<0.05$) (Fig. 2).

Percentage of neutrophils: The neutrophils percentage of the LF-EMF-exposed group gradually declined from day 1 to 6 while the control group exhibited an initial decrease from day 1 to 2, followed by a rise on Day 3 before declining steadily to day 6. LF-EMF-exposed groups had a higher neutrophil percentage than the control group only on days 1 and 2. From day 3 onward, the control group had a higher neutrophil percentage and the gap between groups became more pronounced over time.

On day 1, neutrophil percentages were slightly higher in the LF-EMF-exposed group (58.45±1.75%) than that of the control group (56.02±3.40%). By day 2, both groups showed a slight decrease (LF-EMF: 51.08±4.87%, Control: 47.01±1.45%). On day 3, the LF-EMF group continued declining (49.49±3.82%), while the control group increased (57.73±2.80%). By Day 4, the LF-EMF group dropped further (42.50±3.02%), in contrast to the persistently elevated percentages in the control group (56.12±2.33%), resulting in a statistically significant difference ($p<0.05$). This trend persisted on Day 5, where the LF-EMF-exposed group demonstrated further declined of neutrophils percentage (35.11±1.31%), whereas the control group retained a higher percentage (50.58±2.46%), reaching statistical significance ($p<0.05$). By day 6, neutrophils levels in the LF-EMF group were markedly reduced (25.35±1.21%) compared to the control group (46.03±1.68%), showing a substantial difference of 20.68% ($p<0.05$) (Fig. 3).

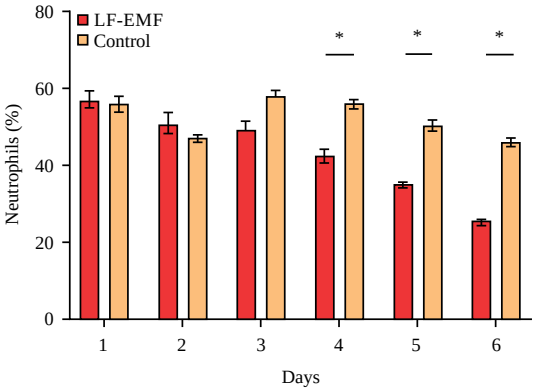

Fig. 3: Graph showing the percentage of neutrophils for LF-EMF and control groups from day 1 until day 6
The results were displayed as average±SEM, *Significant difference at p<0.05

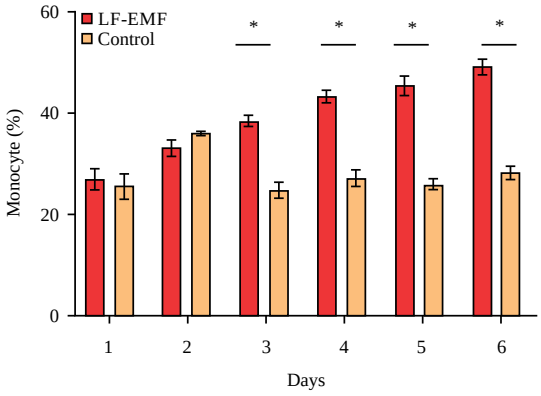

Fig. 4: Bar chart showing the percentage of monocytes for LF-EMF and control groups from day 1 to 6
Results were presented as average ± standard error of the mean (SEM) based on three experimental replicates (N = 3). Statistical significance is indicated by * = p<0.05

Percentage of monocytes: The LF-EMF group showed a steady increase in monocyte percentage from day 1 to 6, while the control group increased initially (day 1-2) but declined from day 3 onward before remaining stable until day 6. The LF-EMF group consistently had higher monocyte levels, except on day 2, with statistically significant differences from day 3 onward (p<0.05).

On day 1, both the LF-EMF and control groups exhibited similar monocyte percentages (26.27±2.27% and 25.48±4.31%, respectively). By day 2, the percentages increased in both groups, with the control group showing a slightly greater rise (36.19±0.53%) compared to the LF-EMF-exposed group (33.28±2.96%). From day 3 onward, the LF-EMF-exposed group maintained a higher monocyte percentage, while the control group markedly declined. The percentage increased to 38.57±1.96% in the LF-EMF-treated group, whereas the control group dropped to 24.88±2.62%, with a statistically significant difference (p<0.05).

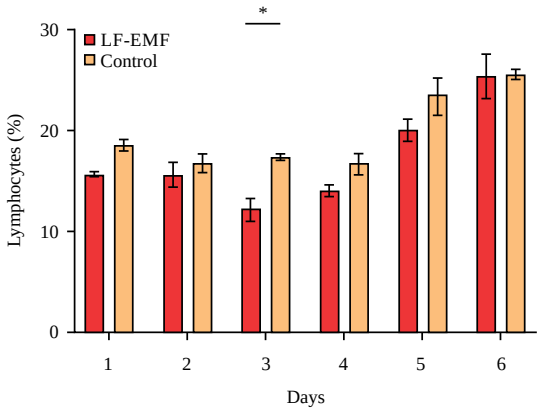

Fig. 5: Bar chart showing the percentage of lymphocytes for LF-EMF and control groups from day 1 to 6
Results were presented as average±standard error of the mean (SEM) based on three experimental replicates (N = 3). Statistical significance is indicated by * = p<0.05

The growing trend of monocyte percentage persisted through day 4, with the LF-EMF-exposed group reaching 43.44±2.11%, noticeably higher than the control group (27.20±2.97%) (p<0.05). By Day 5, monocyte levels in the LF-EMF-exposed group continued to rise (45.47±3.29%) and remained higher than the control group (25.98±1.73%) (p<0.05). On day 6, the pattern was still evident, with the LF-EMF-exposed group reaching a peak of 49.26±2.70%, while the control group showed only a small increase to 28.33±2.46%. The difference between groups widened to 20.93% (p<0.05) (Fig. 4).

Percentage of lymphocytes: The LF-EMF-exposed group had consistently lower lymphocyte percentages than the control group. Lymphocyte levels decreased from day 1 to 3 in the LF-EMF group, then increased until day 6. The control group showed a similar trend but fluctuated slightly, with levels rising from day 4 onward.

On day 1, the percentage of lymphocytes was lower in the LF-EMF group (15.27±0.70%) compared to the control group (18.49±0.98%), although the difference was not statistically significant. On day 2, a small increase was observed in the LF-EMF-exposed group (15.64±2.08%), while the control group exhibited a decline to 16.81±1.77%. By day 3, lymphocyte levels in the LF-EMF-exposed groups showed a steep decline to 12.14±1.95%, whereas the control group remained stable at 17.39±0.61%, showing a statistically significant difference (p<0.05) (Fig. 5).

On day 4, a slight recovery in lymphocyte percentage was observed in the LF-EMF-exposed group (14.06±0.94%), while the control group demonstrated a slight decrease (16.68±1.75%). On day 5, both groups showed a marked rise in lymphocyte percentages, with the LF-EMF-exposed group increasing to 19.42±2.65% and the

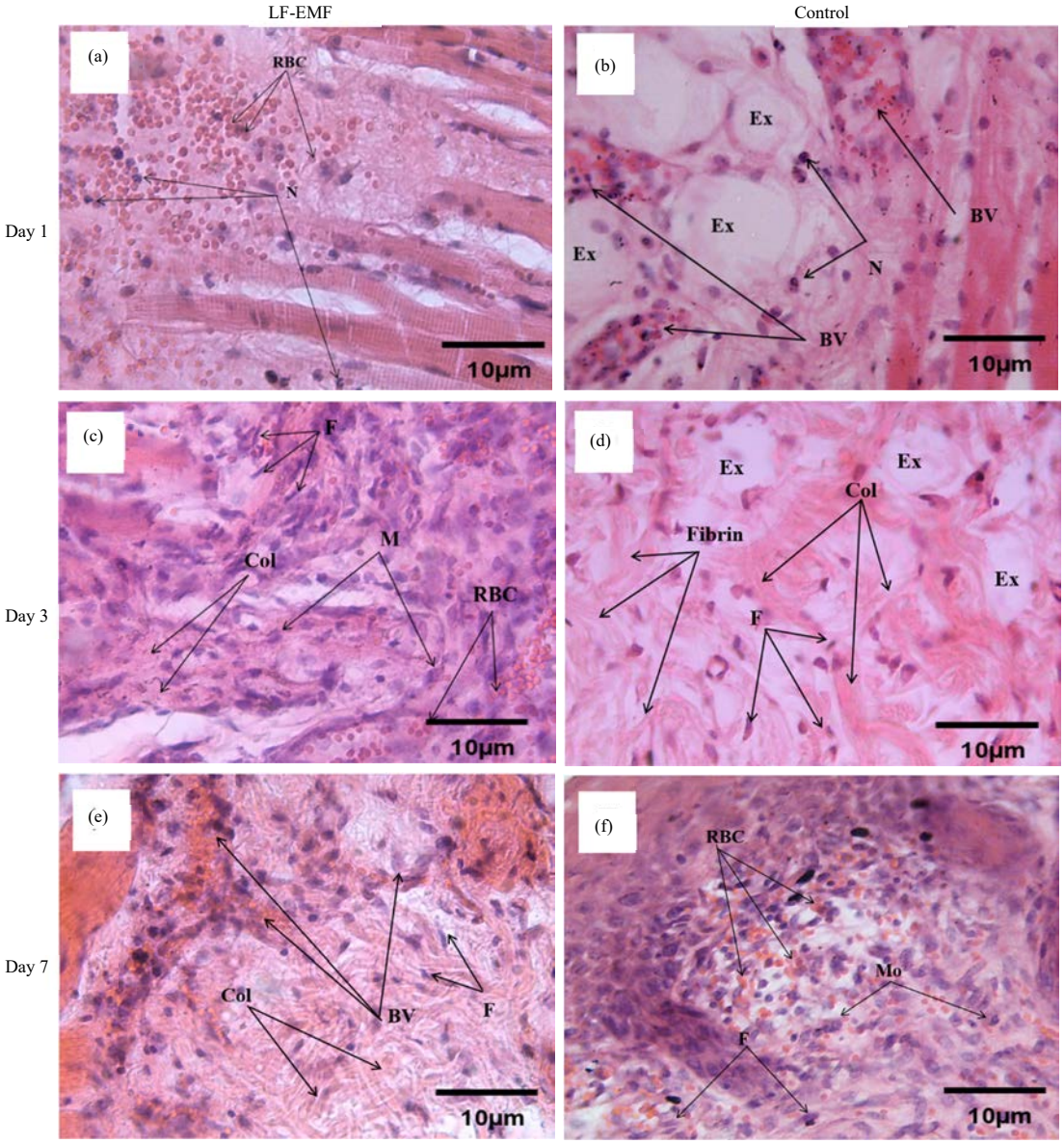

Fig. 6(a-f): Histological observation of wound tissue treated with LF-EMF (a, c and e) and Control (b, d and f) on Day 1, 3 and 6 at 40x magnification

RBC: Red blood cells, N: Neutrophil, Ex: Extracellular space, Col: Collagen, F: Fibroblast, BV: Blood vessel, Mo: Monocyte

control group to 23.44±3.15%. By day 6, lymphocyte levels in both groups peaked, with the LF-EMF group at 25.40±0.85% and the control group at 25.63±0.85%. The difference between groups narrowed by day 6.

Microscopic observation of wound

H&E staining: On day 1 post-injury, histological examination of LF-EMF-exposed tissue (Fig. 6a) revealed significant neutrophilic migration infiltration concentrating at the wound margins. Numerous mature neutrophils with segmented nuclei were identified in close proximity to fibrinous exudates and necrotic debris, suggesting an ongoing phagocytic activity and inflammatory response. Well-formed fibrin meshwork with numerous extravasated erythrocytes was evidence and was consistent with early thrombus formation. Control tissue (Fig. 6b) exhibited prominent vascular dilation, perivascular oedema and erythrocytes congestion, indicative of vascular stasis with mild neutrophilic infiltration.

On day 3 post-injury, LF-EMF tissue (Fig. 6c) demonstrated newly formed blood vessels containing intraluminal erythrocytes, which was consistent with early angiogenesis. Collagen deposition accompanied by proliferating fibroblasts was observed within the extracellular matrix of the LF-EMF-treated wounds. Control tissue (Fig. 6d) showed a reduced inflammatory cell infiltrate with areas of fibrin gradually being replaced by denser collagen bundles, indicating early tissue remodelling.

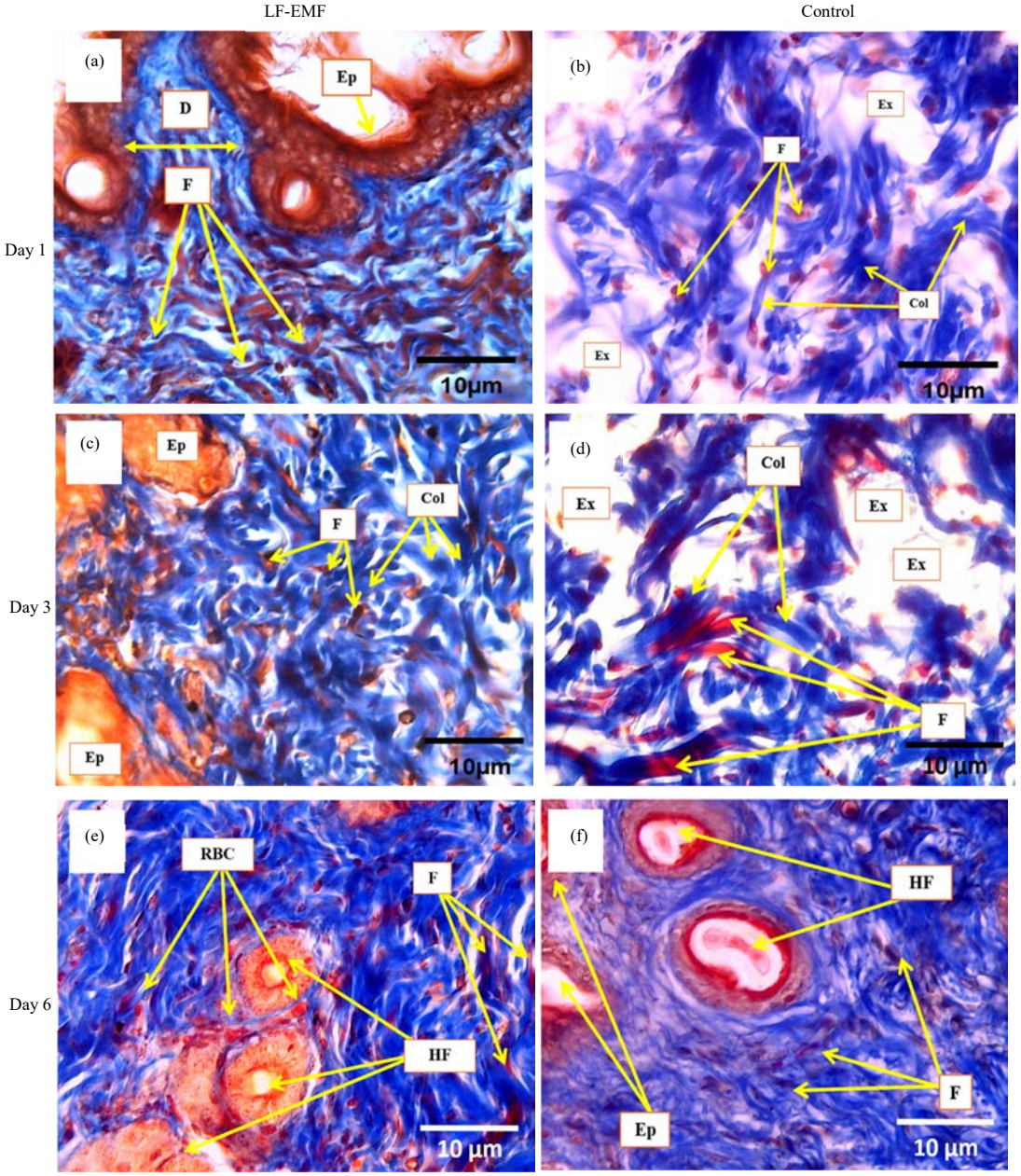

Fig. 7(a-f): Histological analysis of wound tissue treated with LF-EMF (a, c and d) and control (b, d and f) on day 1, 3 and 6 at 40x magnification.
Ep: Epidermis. D: Dermis, Ex: Extracellular space, F: Fibroblast. Col: Collagen, RBC: Red blood cell, HF: Hair follicle

On day 6 post-injury, LF-EMF-exposed tissue (Fig. 6e) demonstrated an overall reduction in inflammation with fewer immune cells infiltrated. Capillaries were prominent at the wound margins with branching vessels extending from the injury site, indicative of active angiogenesis. Denser collagen deposition and compact extracellular matrix were also observed. Control tissue (Fig. 6f) exhibited less collagen accumulation and fewer angiogenic features with deposition of fibroblasts along with extravasated erythrocytes, reflecting ongoing vascular disruption and early stages of tissue repair.

Masson's trichrome staining: On day 1 post-injury, the LF-EMF-treated wound (Fig. 7a) showed an intact epidermis and increased presence of spindle-shaped fibroblasts indicative of early cellular activation and migration. In the control wounds (Fig. 7b), the overall collagen architecture appeared disrupted, as shown by the fragmented fibres, widened stromal spaces and a significant reduction in fibroblast density.

On day 3 post-injury, the LF-EMF-treated wounds (Fig. 7c) showed an increase of collagen deposition with compact extracellular matrix and minimal interstitial

spacing. The collagen fibres remain irregularly arranged, reflecting an early-phase matrix remodelling. Fibroblast deposition appeared less elongated and was consistent with post-migratory positioning. The control tissue (Fig. 7d) displayed reduced spindle-shaped fibroblasts deposition compared to the LF-EMF-exposed tissues, indicating ongoing migration. Collagen fibres are loosely arranged with wider interstitial spaces, suggesting delayed matrix maturation.

On day 6 post-injury, the LF-EMF-treated tissues (Fig. 7e) demonstrated a well-organised extracellular matrix characterised by densely aligned collagen fibres. Newly formed blood vessels containing intraluminal erythrocytes were observed adjacent to hair follicles, indicating active angiogenesis and were consistent with the completion of the reparative process. Control tissue (Fig. 7f) demonstrated disordered collagen deposition with fibres appearing sparse and loosely arranged, suggesting a slower ongoing remodelling phase compared to the LF-EMF-treated tissues.

DISCUSSION

Wound healing is a dynamic and tightly regulated process consisting of four overlapping phases: haemostasis, inflammation, proliferation and remodelling. Disruptions or delays in these phases can lead to chronic wounds, such as diabetic foot ulcers. The inflammatory phase, or acute inflammation is particularly critical, as it sets the stage for subsequent healing processes. This study explores the potential of low-frequency electromagnetic field (LF-EMF) treatment as an alternative therapeutic approach to enhance wound healing, specifically by modulating the inflammatory phase. LF-EMFs are non-ionizing, low-energy electromagnetic fields that can induce various biological effects[5].

The percentage of wound area reduction decreased exponentially for LF-EMF groups from day 1 to 6. In contrast, the percentage decrease in wound area was less pronounced in the control group. This observation is consistent with the findings of Goudarzi et al.[7] and Callaghan et al.[8]. Goudarzi et al.[7] demonstrated that exposure to extremely low frequency-pulsed EMF (ELF-PEMF) significantly enhanced the wound healing process in diabetic rats compared to the control group. They observed that wound healing took significantly longer in the control group, whereas the healing rate was notably faster in the PEMF-treated group. Furthermore, the PEMF-treated wounds exhibited significantly higher wound tensile strength compared to the controls. Additionally, the healing duration in the PEMF-treated control group was shorter than in the control group[7].

Neutrophils play an important role in the early inflammatory phase of wound healing, acting as the first line of defence, responding to DAMPs and PAMPs released at the wound site. LF-EMF treatment modulates their recruitment and eventual resolution during the healing process, showing a peak on Day 1 (58.45±1.01%) and a gradual decrease until Day 6 (25.35±0.70%) for LF-EMF groups. In contrast, neutrophil recruitment to the wound site was delayed in the control groups and remained high at 46.03±0.97% on Day 6, indicating a slower response to the wound.

Ouyang et al.[9] supported this finding by showing that EMF treatment reduced the proportion of neutrophils in the synovial-like layer. At the same time, it increased the proportion of macrophages and enhanced their efferocytosis capacity, therefore allowing for more effective removal of neutrophils from the wound site. This process contributed to the faster resolution of inflammation observed in LF-EMF-treated groups. According to Peña and Martin[10], the phenotype switch of macrophages from a proinflammatory to a pro-resolving state is initiated through their efferocytosis, which also enhances the production and secretion of lipoxins, promoting the resolution of inflammation. Therefore, if EMF can enhance efferocytosis, it may also accelerate inflammation resolution by promoting the phenotypic switch of macrophages.

Monocytes are also important in acute inflammation, as their presence in the wound site is crucial for clearing apoptotic neutrophils, modulating inflammation and stimulating angiogenesis. As seen in Fig. 4, the percentage of monocytes was higher in LF-EMF groups at all investigated time points except for day 2, although the gap between the groups was not as pronounced as at other time points. The observed differences suggest that LF-EMF exposure influences monocyte recruitment, leading to accelerated wound healing by promoting a faster shift from an inflammatory to a pro-healing environment. This increase could be linked to greater expression of monocyte-recruiting cytokines, such as CCL2 (MCP-1), which is known to mediate monocyte extravasation from the bloodstream into inflamed tissues. According to Costantini et al.[11], EMF treatment was able to upregulate MCP-1 expression, leading to a reduction in the cell-free area observed in the in vivo scratch assay.

Additionally, Wang et al.[12] reported an increase in NF- B expression following EMF exposure, further suggesting that EMF can modulate vital inflammatory pathways. In astrocytes, NF- B signalling was shown to influence the expression of STAT2 and CCL2, thus regulating the infiltration of leukocytes upon injury[13]. These findings suggested that EMF could increase the expression of CCL2 by modulating the NF- B signalling, thus enhancing monocyte infiltration at the wound site. In addition, Patruno et al.[14] demonstrated that EMF treatment increased the expression of p-ARK and p-ERK, critical components required for stabilising the NF-B pathway. The

AKT contributes to NF-B activation by phosphorylating and activating IKK, therefore allowing NF-B to translocate to the nucleus and express pro-inflammatory cytokines, as indicated by the increased level of TNF-, IL-18 and IL-1 observed by the authors[14].

The significant difference in lymphocyte percentages on Day 3 ($p<0.05$), when the LF-EMF group had lower levels than the control group, could indicate that LF-EMF exposure modulated early immune cell recruitment, possibly by accelerating neutrophil clearance and promoting monocyte differentiation. Lymphocytes, particularly T-helper (Th) cells, play a crucial role in orchestrating immune responses during wound healing. A study by Sobhanifard et al.[15] found that EMF treatment reduced the level of IL-4 and interferon-gamma (IFN-γ), although the exposure only altered the sample pre-stimulated with inflammatory agents such as Human Serum Albumin (HSA). The treatment was also able to modulate the gene expression that is related to the differentiation of CD4+cells into Th1 and Th2 effector cells, such as T-bet and GATA-3 mRNA[15]. This suggests that EMF could suppress the Th1 response, as reflected in the reduction of the percentage of lymphocytes on Day 3. A rise in lymphocyte percentages was observed until Day 6, which might suggest an influx of Th2 cells to promote tissue repair and prevent excessive inflammation. However, as GATA-3 is a key regulator of Th2 differentiation, its reported reduction by Sobhanifard et al.[15] would not be expected to drive a significant Th2 response. However, the percentage decrease in lymphocytes was not statistically significant from day 3 onwards.

In histological analysis in Figure 6, RBCs interwoven with fibrin mesh were visible, likely forming a clot at the wound site in the LF-EMF group (Fig. 6a). In contrast, the control tissue (Fig. 6b) on Day 1 showed a lack of fibrin mesh or floating RBCs. According to Vallejo et al.[16], Extremely Low-frequency Magnetic Fields (ELF-MF) can stimulate the coagulation cascade, resulting in faster clot formation, as indicated by the shortened prothrombin time observed in their study.

On day 3, early angiogenesis was evident, accompanied by collagen deposition and the presence of fibroblasts. According to Delle Monache et al.[17], EMF can stimulate angiogenesis via VEGF receptor 2 (KDR/Flk-1), as demonstrated by increased endothelial cell proliferation and tubule formation. Additionally, Maiullari et al.[18] showed that EMF treatment enhances the expression of HIF-2, along with HSP70 and HSP90, which are involved in immune cell recruitment. A study by Tran et al.[19] found that HIF-2 triggers the expression of CXCR4 in endothelial progenitor cells, promoting their migration to the wound site and activating neovascularisation for wound healing. Therefore, it is likely that the modulatory effects of EMF on HIF-2 and VEGF production contributed to earlier angiogenesis in the LF-EMF group.

On day 6, LF-EMF wounds showed capillaries at the wound edge with branches extending distally, while collagen deposition was notably denser. This observation was supported by Delle Monache et al.[17]. They found that EMF treatment increased the capillary-like tube lengths and tube branch points compared to the control groups.

In tissue stained with Masson's trichrome, abundant fibroblast migration was observed in the LF-EMF-treated wound (Fig. 7a), compared to the control wound on day 1. Fibroblasts infiltrate the wound and degrade the fibrin clot by producing MMPs, replacing it with ECM components such as collagen and hyaluronic acid. As noted by Bainbridge[20], fibroblasts migrate along the orientation of collagen, which was already evident in the LF-EMF group on day 1.

Fibroblast migration to the wound site is stimulated by TGF- , which is released by macrophages. Elevated levels of TGF- 3 have been shown to reduce scarring in rat models[21]. According to Costantini et al.[22], TGF-expression increased with EMF treatment, suggesting that EMF may influence fibroblast migration through enhanced TGF-expression. The migration of fibroblasts could also be due to the electromagnetic field itself via a process called electrotaxis, although this observation was seen in an in vitro model of fibroblast migration[23]. Fibroblasts were observed migrating towards the anode and this finding was associated with increased levels of phosphorylated Akt (p-Akt), suggesting the involvement of the PI3K pathway in mediating this response[23].

Both fibroblasts and neutrophils secrete MMP-9, with macrophages being a potent source. MMP-9 breaks down ECM components, facilitating fibroblast and keratinocyte migration to the wound site[24]. The observed increase in MMP-9 production corresponds with the high percentage of neutrophils during the early phase of treatment. According to Patruno et al.[14], The EMF treatment was shown to increase MMP-9 expression, though this expression decreased after 24 hrs. This suggests that the EMF effect on MMP-9 is transient, thus preventing any harmful effects on wound healing.

Furthermore, LF-EMF treatment led to significantly higher collagen deposition compared to the control group from day 1 until day 6. On day 6, individual collagen strands were visible, indicating that the structure was transitioning to a more organised fibrillar arrangement, characteristic of the later stages of the proliferative phase. This suggests that LF-EMF not only accelerates the healing process but also promotes more organised collagen formation, contributing to more efficient tissue repair. According to Lin et al.[25], PEMF was able to enhance collagen type I and total collagen synthesis protein levels, even under inflamed conditions induced by IL-1.

H&E staining revealed that on Day 1, LF-EMF exposure led to a stronger inflammatory response, with

increased leukocyte migration and formation of fibrin mesh. By day 3, fibroblast activity and collagen deposition were more pronounced, accelerating the transition to the proliferative phase. On day 6, the LF-EMF tissues exhibited active angiogenesis and dense collagen formation, promoting faster wound closure, whereas control tissues showed slower healing progress. Masson's trichrome staining showed that collagen deposition and presence of fibroblasts were consistently higher in the LF-EMF-treated wounds compared to the control wounds from day 1 to 6. By day 6, the ECM appeared more structured with noticeable alignment in the LF-EMF-treated wounds, whereas in the control wounds, the collagen deposition was still sparse and the collagen appeared thin and loose. Overall, the EMC remodelling took place at a higher rate in the LF-EMF-treated wounds compared to the control wounds.

CONCLUSION

This study highlights the significant impact of Low-frequency Electromagnetic Field (LF-EMF) exposure on wound healing by modulating the inflammatory phase and accelerating the transition to the proliferative phase, ultimately promoting faster wound closure compared to the control group. LF-EMF treatment facilitated an earlier peak and a more rapid decline in neutrophil recruitment, accelerating the resolution of inflammation. The increased presence of monocytes in the LF-EMF group further supported a faster transition from the inflammatory to the pro-healing environment. Histological analysis using H&E and Masson's trichrome staining revealed enhanced angiogenesis, greater fibroblast migration and denser collagen deposition in LF-EMF-treated wounds. Additionally, LF-EMF exposure led to faster fibrin mesh replacement with collagen and increased capillary formation, whereas control tissues exhibited prolonged inflammation, delayed collagen deposition and slower tissue repair. These findings suggest that LF-EMF is a promising non-invasive therapeutic approach for enhancing wound healing by accelerating the transition from the inflammatory to the proliferative phase. Future studies should investigate the molecular mechanisms underlying LF-EMF's effects and assess its long-term implications in wound healing.

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