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Advances in Electromagnetic Therapy for Wound Healing

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ABSTRACT

Understanding the molecular basis of wound healing and tissue regeneration continues to remain as one of the major challenges in modern medicine. Wound healing is a complex procedure involving various cellular mechanism. Though high frequency electromagnetic fields are reported to cause cancer, birth defects and DNA damage, electromagnetic field at low intensity and low frequency can be effectively used for wound healing and for many more medical applications. Low intensity-low frequency pulsed electromagnetic therapy is evidenced to have a significant impact on wound repair and regeneration. It provides a non-invasive reparative technique to treat an injury. *In vitro* studies reported a significant effect of electromagnetic field on neovascularisation and angiogenesis. There are also many pieces of evidence which support its efficiency in reducing the duration of wound healing and improving the tensile strength of scars. Here, we compared the traditional stigma associated with pulsed electromagnetic fields and weighed them with its potential therapeutic effect on wound healing. Furthermore, emphasised the need for more focused research to determine the therapeutic strategies and optimised parameters of pulsed electromagnetic field that can assure efficient wound healing and regeneration.

Keywords: Electromagnetic field; Pulsed electromagnetic field; Extremely low frequency electromagnetic field

NOMENCLATURE

ICNIRP	International Commission on Non-Ionising Radiation Protection
TMS	Transcranial magnetic stimulation
IARC	International Agency for Research on Cancer
HUVECs	Human Umbilical Vein Endothelial Cells
FGF-2	Fibroblast Growth Factor 2
RANTES	Regulated on Activation, Normal T Cell Expressed and Secrete
MCP-1	Monocyte Chemoattractant Protein-1
MIP-1a	Macrophage Inflammatory Protein 1a
IL-8	Interleukin 8

1. INTRODUCTION

An accelerated charged particle produces magnetic field. Similarly, time varying magnetic field can produce electrical energy. Synchronised oscillations of varying electric and magnetic field, mutually perpendicular to one another, produce an electromagnetic field in a direction right angle to both the fields. We are constantly surrounded by these non-thermal, low power-frequency fields including radiofrequency/microwave radiation emissions (RF) in our daily life. They are deeply embedded in our lifestyle through power distribution networks, industrial machinery and electrical appliances, household electrical wiring, motor-driven instruments, computer screens, telecommunications and broadcasting facilities, wireless communication, and mobile telephones etc. It has been one of the major concerns of environmental health hazards since the 1970's. Studies have shown a possible role of high intensity Pulsed EMF in cancer incidence[1] and fetal loss[2] which continues to bewilder the scientific community. In contrast, low intensity extremely low-frequency electromagnetic field (ELF-EMF) is bearable by living organisms without any pernicious effects[3] and its contribution towards signal transduction, protein synthesis, and gene expression can have an ameliorating effect on cell growth and regeneration[4]. ELF-EMF is also suggested as a therapeutic alternative to Transcranial magnetic stimulation (TMS) in spinal cord injury[5]. In spite of the huge amount of data dealing with the biological effects of the electromagnetic fields, the potential therapeutic role of ELF-EMF in wound healing continue to remain a subject of exploration as shown in Fig. 1.

1.1 Wounds and Wound Healing

Clinically, a wound can be any tissue injury resulting in the disruption of the skin, damaging and penetrating through the epidermis and dermis layers exposing the underlying tissues or organs. Depending on the cause, site, and depth, wounds can be superficial or deep, acute or chronic ranging from simple to life threatening consequences. This anatomic discontinuity of the skin is restored meticulously by the cohesive process of wound healing which may vary significantly depending on the nature of the wound. However, the major activity of conventional wound healing remains the same which can be classified into

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haemostasis, inflammation, proliferation, epithelialisation and remodelling. Resident cells along the wound edges (including keratinocytes, fibroblasts, and endothelial cells) in collaboration with macrophages play a significant part in wound healing process[6].

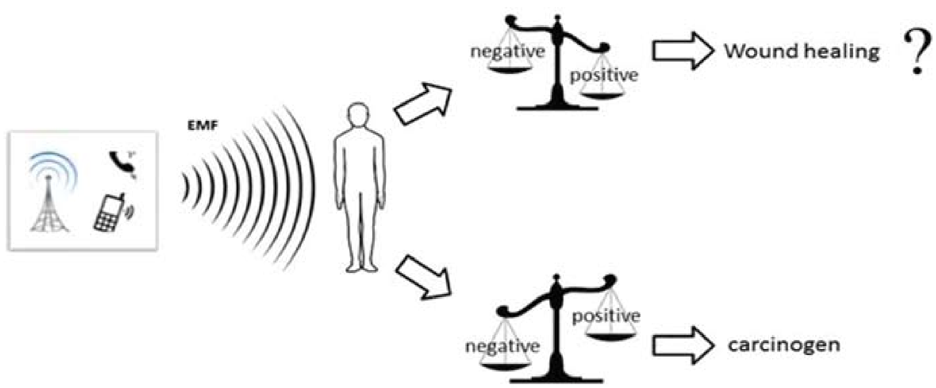

Figure 1. Effects of exposure to electromagnetic fields.

On investigating the chronic wound microenvironment, many physiological differences compared to a normally healing wound surfaced. In such wounds, the conventional course of wound healing may be arrested at any stage but most frequently it is arrested at the inflammatory stage, resulting in the accumulation of devitalised tissue, decreased angiogenesis, increased levels of proteases, imbalance of growth factors and cytokines, defective extracellular matrix and presence of infection at outer surface which prevents the adequate cellular response of chronic wounds to healing stimuli[7]. ELF-EMF has been reported to be effective in healing chronic wounds. An overview of the effect of ELF-EMF in wound healing emphasising the pressing need to standardise electromagnetic therapy for wound healing purposes is presented.

1.2 Effect of Electromagnetic Field on Biomolecules

Electromagnetic field plays a crucial role in the cascade of cellular processes involving cell migration, adhesion and differentiation. The passive uptake of Na^+ ions from the environment generates a current leading to an internally positive trans-epithelial potential difference (TEP), the gradient of which plays an important role in various cellular processes and neural impulses. Also the asymmetrical distribution of K^+/H^+-ATPase generates a crucial potential. These endogenous electric field also exist at the site of wound, which are disturbed due to the disruption of TEP in the epithelial layer. At the site of injury the electric potential collapses which in a healthy tissue varies between 1-5 V/cm, however, with an increase in distance from the wound this potential raises to that of healthy cells. EMF can interfere with these potentials at the wounded site and help restore them at an accelerated pace. It may interact by

(a) Energy transfer accelerates ions and charged proteins, modifies cell membranes and receptor proteins
(b) Electric fields induced inside the body exert force on charged proteins and ions
(c) Magnetic field induces an electric field (by Faraday's law) and interacts with free radical molecules.

However, it is important to realise that EMF can induce beneficial physiological effects only at extremely low frequency (8 Hz – 60 Hz) and low amplitudes (less than 1Gs)[8, 9].

2. ELF-EMF THERAPY

We are constantly surrounded by ELF-EMF radiations emancipating from various sources. The EM spectrum spans a huge range of frequencies and wavelengths. Literature suggests that the frequency range of 3 Hz - 300 Hz termed as extremely low frequency (ELF) is useful for medical applications. Pulsed electromagnetic field (PEMF) is a non invasive reparative technique commonly used by many practitioners and researchers for different biomedical applications. Every PEMF system essentially consists of 3 modules namely: waveform generator, coil driver circuit and magnetic field exposure system which is generally a pair of coils. Before designing an instrument, one must identify the various physical parameters which need to be optimised for different biological experiments. These parameters could include waveform type (sine, square, saw tooth and triangular), frequency, time of exposure and most importantly, the magnetic field intensity or strength. The required magnetic field strength is decided using ICNIRP guidelines. The most important part while designing and fabricating an exposure system is the coil system. The size, shape and type of coil system can be decided according to the biological system which needs to be exposed. The generated field must be uniform for whole body exposure in order to have consistent results. Circular coil systems such as Helmholtz coil are used for generating magnetic fields over a small volume whereas square coil systems such as Meritt and Ruben coil generate magnetic field over a large volume. Scientists generally prefer Helmholtz coil over other systems due to ease of construction and uniform magnetic field.

There are various EMF generators available in the market resulting in non-linearities in intensity, amplitude, frequency, and wave shape of the signal. This is creating a barrier in extrapolating the *in vitro* and *in vivo* studies to clinical set up.

3. CRITICAL EFFECTS OF EMF EXPOSURE

The growing electricity demand and ever-advancing technologies as shown in Fig. 2, created more and more artificial sources exposing the environment to man-made electromagnetic fields.

Scientific community explored the detrimental effects of these waves suggesting its carcinogenic potential. The essential cellular processes including proliferation[10] morphology[11], apoptosis[12], gene expression[13], and differentiation[14] are plausibly affected by EMF. Also, the increased intracellular Ca^{+2} levels[15], prolonged survival of reactive oxygen species and other free radicals[16] following EMF exposure are well documented. According to the Bio Initiative report[17], ELF-EMF exposure may culminate in health endpoints including childhood leukemia, brain tumors, genotoxic effect, neurological effects and neurodegenerative diseases, immune system deregulation, allergic and inflammatory responses, breast cancer, fetal loss and miscarriage, and some cardiovascular effect affecting every major organ/organ system in human body[17] as shown in Fig. 3.

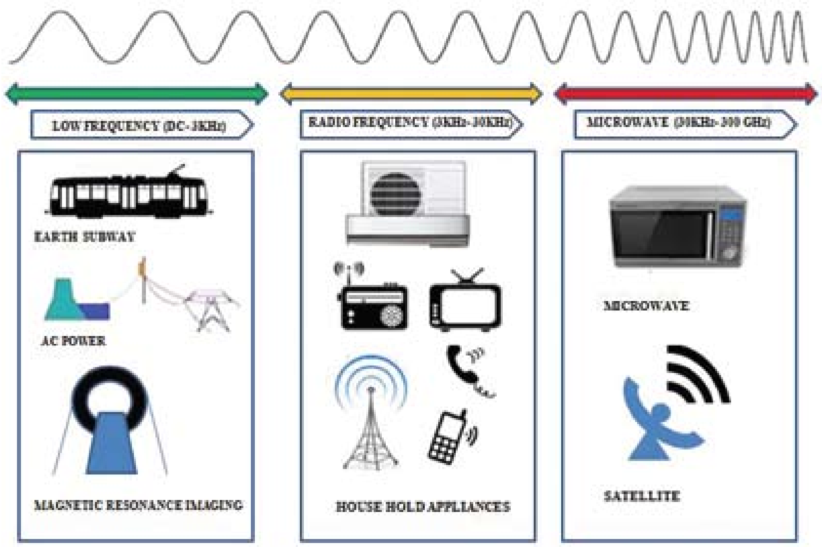

Figure 2. Common sources of electromagnetic fields.

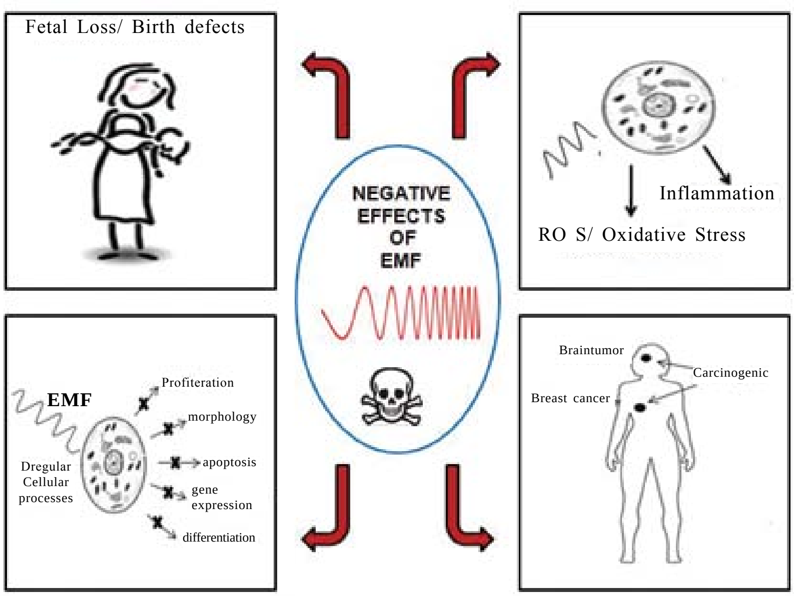

Figure 3. The adverse effects of electromagnetic field.

4. THERAPEUTIC ROLE OF ELECTROMAGNETIC FIELD

Though ELF-EMF is being scrutinised for it carcinogenic potential and its relationship with immune system functions, they are widely used for diagnosis (magnetic resonance imaging- MRI, scanner, and microwave imaging) and treatment of some pathological conditions to stimulate neural regeneration, tissue and bone repair[18]. Electromagnetic therapy has various medical applications; as a matter of fact, it provides an easy and noninvasive technique to treat an injury[19]. PEMF in low frequency and intensity range (micro-tesla) increases blood oxygenation, improves its circulation, enhances cell metabolism and function, and assuage pain and fatigue from fibromyalgia. It can effectively improve symptoms pertaining to nervous system including treatment-resistant depression and multiple sclerosis[20]. EMFs have shown to facilitate the re-establishment of normal potentials in damaged cells[21]; this property can be suavely exploited in the area of orthopedics in the treatment of non-uniform fractures and failed fusions. In fact, EMF improves osteogenic phase of the healing process[22] by favouring the bone formation and healing through increased blood flow[23], proliferation, differentiation and maturation of osteoblasts[24]. It can even accelerate the recovery of the tensile strength after tendon injury[25].

The physiological effects of EMF exposure depend not only on the wave form properties (frequency, amplitude, intensity and length of exposure) but also on the intrinsic susceptibility/ responsiveness of the cell type. In mononuclear blood cells EMF exposure had no effect on the cytokine levels[26] whereas a growth inhibitory effect has been observed in T-lymphocytes[27]. Several studies have reported that EMF exposure can alter intracellular Ca^{2+} homeostasis; significant increase in intracellular Ca^{2+} levels have been observed in various immune cell-models[28] whereas, contradicting observations have surfaced from studies of neuroendocrine cells[29]. Also, in contrast to the common belief that ELF-EMF exposure causes mutations to organisms, Chow and Tung have demonstrated that low frequency magnetic fields can actually enhance the efficiency of DNA repair through the induction of DnaK/J synthesis[30].

In general, regeneration and repair stimulation is one of the well-documented biological effects of EMFs. The variability in the therapeutic results on exposure of different tissues for repair by EMF has varying and sometimes contradicting results due to the involvement of several cell types that may differentially respond to the EMF stimulus. Bertolino[31], *et al.* highlighted the effect of vascularisation, reduction in wound depth by granulation tissue, and reduced inflammatory cell migration and infiltration as shown in Fig. 4.

4.1 Effect of ELF-EMF on Pain

Evidence supporting the analgesic effects of ELF-EMF has been growing lately, as it alleviates pain caused by psoriases, tendonitis and rheumatoid arthritis[32,33] showed that mice exposed to EMF (100 μT x 30 min) displayed increased latency towards the hot plate test. They also observed a decrease in pain following a brief EMF exposure (30 min) in humans. A burst firing ELF-EMF exposure for 30 min increased the pain threshold for 4h after a single exposure. Mathur[34], *et al.* as showed that naloxone (opioid antagonist) pretreatment reversed the effect of ELF-EMF exposure. Therefore, opioid-mediated factors are crucial for the electromagnetic anti-nociception research.

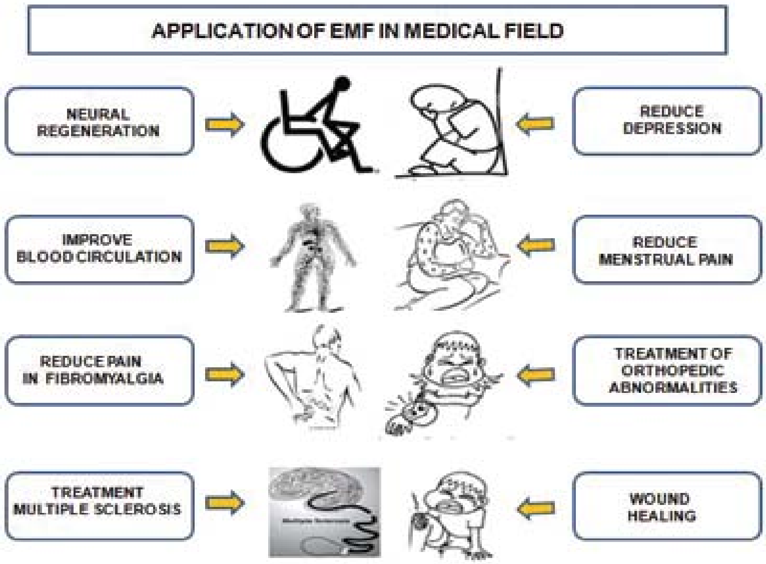

Figure 4. Electromagnetic field as medical aid.

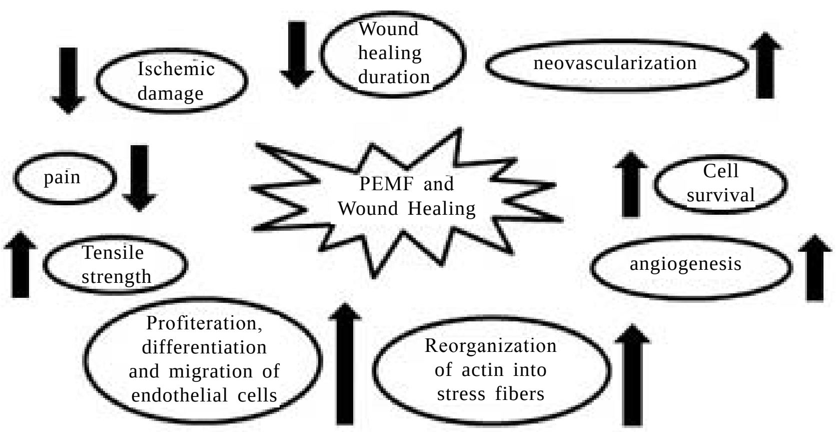

Figure 5. The potential effect of EMF on wound healing.

4.2 Effect of ELF- EMF on Wound Healing

Wound healing is an orderly, dynamic, integrated process occurring as a cellular response to injury and involves activation of skin cell components (platelets, macrophages, fibroblasts, endothelial cells, and keratinocytes).

Wounds can be categorised into acute and chronic wounds depending on their healing time. The non healing chronic wounds, resulting from ischemia, diabetes mellitus, and venous stasis and pressure are prone to complications including functional limitations, infections and malignant transformation. There are also many pieces of evidence that PEMF is efficient in reducing the wound healing duration[35] and improving the tensile strength of scars[36].

PEMF has also demonstrated to increase the proliferation and tuberculation of endothelial cell cultures and the expression of the stimulator of angiogenesis, the fibroblast growth factor (FGF-2). EMF has a significant effect on the cytokines which are responsible for the transition from the chronic pro-inflammatory to an anti-inflammatory phase which is crucial for wound healing process as shown in Fig. 5.

4.3 Effect of ELF-EMF on Angiogenesis

As the formation of new blood vessels is crucial for wound healing as well as in osteoarthritis or diabetic retinopathy, exploring the consequences of EMF exposure on the stimulation of angiogenesis becomes increasingly significant[37]. The use of EMFs to treat ulcers unresponsive to the conventional therapies as it increased the superficial vascular network on the skin[38] reinforcing the competence of EMF in wound healing[39,40].

The important events in angiogenesis, cell proliferation and cell migration, including the reorganisation of actin into stress fibers, long filaments that transverse the cell are positively affected by EMF stimulation. Delle Monache et al. demonstrated that sinusoidal EMF (1 mT, 50 Hz for up 12 h) induced an increase in the rate of capillary structure formation in cultured umbilical human vein endothelial cells (HUVECs). They observed an exposure and time-dependent increase in the number of connections and the proliferation rate of HUVECs, which confirmed increase in proliferation and tubulisation of endothelial cell cultures and the increase in the expression of fibroblast growth factor 2 (FGF-2), a potent stimulator of angiogenesis, after exposure to electromagnetic field. EMF also induced structural alterations in the cytoskeleton actin stress fibers and focal adhesions, elements crucial for the survival, proliferation, movement and differentiation of the endothelial cells[41,42].

4.4 Effect of ELF-EMF on Keratinocyte Cell Growth and Inflammatory Chemokines

The process of proliferation and differentiation of keratinocytes is the principle phase of tissue regeneration following injury resulting in the formation of a massive bulk of cells, covering the wounded area. EMF exposure facilitates wound healing through hindering the infiltration of inflammatory cells and promoting the replacement of granulation tissue with connective tissue[31].

In addition, several lines of evidence indicate that advanced stages of wound repair are associated with a shift towards anti-inflammatory chemokine profiles in parallel with a decrease in the levels of pro-inflammatory chemokines[43]. Vianale et al. showed that ELF-EMF exposure inhibits the NF-kB signaling pathway and thereby modulates the production of pro-inflammatory chemokines, RANTES, MCP-1, MIP-1a, and IL-8[44]. It also increased the proliferative activity of human keratinocytes (as tested on HaCaT cells) thereby accelerating the tissue regeneration.

It is widely accepted that an excessive and extended inflammatory response results in poor and prolonged healing process, any approach that could downregulate the proinflammatory chemokines may have potentially relevant therapeutic application; as EMF modulated the keratinocyte proliferation and the expression of inflammatory chemokine levels, EMF exposure a non-invasive therapy could be used to treat inflammatory skin conditions.

5. MODEL ORGANISM

Among the various model organisms available the conventional *Rattus norvegicus* and *Mus musculus* are the major ones. Laboratory rats (*Rattus norvegicus*) and mice (*Mus musculus*) were extensively used to study various physiological processes and interpret the pharmacological significance of EMF exposure because of their close genetic and physiological similarities to humans, as well as the ease with which its genome can be manipulated and analysed.

Drosophila melanogaster being the queen of genetics has been rigorously used to study the effect of EMF on life span, fecundity and neurodegeneration. It is one of the major model organisms to study the process of wound healing owing to the close resemblance between the 'purse-string' assembly of epithelial fusion during wound healing process and the naturally occurring morphogenetic movement of dorsal closure in *Drosophila* embryogenesis.

Towards the end of embryogenesis, germ band retraction leaves an epithelial hole on the dorsal side of the embryo, covered with an extraembryonic tissue, the amnioserosa. The two epidermal flanks converge dorsally without cell proliferation resulting in the complete removal of amnioserosa and progressive fusion of the epidermal layer. The phenomenon was first characterised in early 90's and it has been studied extensively since then due to its remarkable similarities with wound healing, making *Drosophila* an ideal model organism to validate the effect of ELF-EMF on wound healing[45].

5.1 ELF-EMF and Drosophila

For over a century now, Drosophila has been used productively as a model organism to study a diverse range of biological processes including genetics and inheritance, embryonic development, learning, behaviour, and aging.

Ramirez[46], *et al.* subjected fruit fly to relatively high field intensity (10 G - 45 G) at two different frequencies (50 Hz and 100 Hz) of mixed pulsated and sinusoidal fields without considering the alignment of the AC and the DC fields in the experimental design and claimed that the egg mortality rate of the exposed group was significantly higher than the control groups[46].

Kholy and Husseiny[47] through their experiments showed significant behavioural changes (reduced locomotor activity and behavioural response to odorants) in *Drosophila melanogaster* concomitant with its reduced viability. They, however, proposed no significant effect on adult fecundity on exposure to EMF emancipating from household electrical appliances. EMF exposure (30 G and 50 Hz) followed by transcriptomic analysis evidenced differentially expressed genes involved in the metabolic process, cell death, protein modification and proteolysis, apoptosis, aging, immunological stress and cell division[48].

However, based on the diversity of the experimental design, frequencies of the AC field intensity, qualities of the EMF, biological systems and the end points used by various investigators these results can be misleading as ELF-EMF exposure now promises applications that include mitigation of inflammation and stimulation of classes of genes following onset of illness and injury.

5.2 ELF-EMF and Rattus Norvegicus

Extensive research has been done using the rat as a model organism on the potential effects of ELF-EMF on wound healing. Injured area significantly decreased in PEMF exposed animals compared to the control groups[49]. This was further supported by Callaghan[50], *et al.* who evidenced through their findings that PEMF accelerated the overall healing of wounds, particularly during the early stages of tissue repair process (up to 9 days). PEMF exposure improved the histological organization of the wounded tissues resulting in the accelerated and complete re-epithelialisation aided by increased skin collagen synthesis after 12 days of PEMF exposure (25 Hz, 2 mT, 8 days, 2.5 h/day)[45].

A few studies were conducted on diabetic mouse models and PEMF exposure notably increased the tensile strength of scar resulting in increased rate of wound healing. PEMF exposure up - regulates key upregulates key angiogenesis factor (FGF-2) involved in tissue repair and prevents tissue necrosis and breakdown in diabetic animals in response to standard ischemic results[50].

In vivo results available so far suggest that PEMF has a significant impact on all the three levels in the process of wound healing. It reduces tissue inflammation (inflammatory phase); increases angiogenesis epithelialisation and neovascular network formation (proliferative phase); accelerates collagen formation, inducing better fiber organisation (remodelling phase)[50,51]. The literature reported so far indicate that the beneficial effects of PEMF in rebuilding the damaged tissue are limited to the subset of low-frequency (3-80 Hz) and intensity (up to a maximum of 20 mT) and anything above this range is reported to have a detrimental effect on the physiology of the animal.

5.3 ELF-EMF and Human Clinical Studies

The clinical studies performed so far have gravitated on the healing of ulcers and have reported a significant benefit in pulsed electromagnetic field (PEMF) exposed patients as compared to the control group.

The success rate of healing leg ulcers exposed to PEMF was significantly higher compared to patients in the control group. It was also demonstrated that PEMF treatment protected patients from ulcer recurrence compared to the placebo group. Stiller[52], *et al.* documented that patients with recalcitrant leg ulcers, when treated with PEMF, reported a decrease in pain intensity and wound depth compared to those in the control group. They also have reported that none of the patients exposed to PEMF exhibited worsening of lesions which made a significant difference compared to the placebo group.

Reports available so far suggest that the ELF-EMF treatment is effective only in patients with chronic ulcers, particularly venous origin which is also limited by the presence of associated co-morbidities. Treatment efficiency may also rely on the device and specific combination of field parameters which further have to be validated and established.

6. CONCLUSIONS

In the current review, we have presented an overview of the cellular, molecular and physiological impacts of ELF-EMF exposure with an emphasis on its potential therapeutic role and advantage in the process of wound healing. It has been reported in the *in vitro* studies to have a clinically beneficiary role in anti-inflammatory, pro-angiogenesis and collagen formation during tissue repair and regeneration. The *in vitro, in vivo* and the clinical studies, reported so far could not be pooled in a meta-analysis due to the disparate etiology of wound healing, diverse and varied therapeutic protocols, work parameters, and treatment duration. Electromagnetic therapy is still failing to have clinical applications mainly due to the nonlinearities in the intensity, amplitude, frequency parameters of the device used and the wave shapes of the signal advised. The wide-ranging commercially available ELF-EMF/PEMF devices make it difficult to standardise and compare its characteristics and delineate the biological and clinical effects they induce on the human physiology.

Conflict of Interest: None

REFERENCES

1. Armstrong, B.; Sim, M.; McNamee, J.; Leszczynski, D.; Juutilainen, J.; Dasenbrock, C.; Miyakoshi, J.; Shirai, T.; Mevissen, M.; Röösli, M. & Melnick, R. Carcinogenicity of radiofrequency electromagnetic fields. 2011. doi: 10.1016/S1470-2045(11)70147-4
2. Patelarou, E. & Kelly, F.J. Indoor exposure and adverse birth outcomes related to fetal growth, miscarriage and prematurity—A systematic review. *Int. J. Environ. Res. Public Health*, 2014, **11**(6), 5904-5933. doi: 10.3390/ijerph110605904
3. Kroupova, J.; Bartova, E.; Fojt, L.; Strasak, L.; Kozubek, S. & Vetterl, V. Low-frequency magnetic field effect on cytoskeleton and chromatin. *Bioelectrochemistry,* 2007, **70(1)**, 96–100. doi: 10.1016/j.bioelechem.2006.03.034
4. Ross, C. L;, Siriwardane, M.; Almeida-Porada, G.; Porada, C. D.; Brink, P.; Christ, G. J. and Harrison, B.S.The effect of low-frequency electromagnetic field on human bone marrow stem/progenitor cell differentiation. *Stem Cell Res.*, 2015, **15**(1), 96-108.
5. Kumar, S.; Dey, S. & Jain, S. Extremely low-frequency electromagnetic fields: A possible non-invasive therapeutic tool for spinal cord injury rehabilitation. *Electromagn. Biol.Med*., 2016, **36**(1), 88-101. doi: 10.1080/15368378.2016.1194290
6. Costin, G. E.; Birlea, S. A. & Norris, D. A. Trends in Wound Repair: Cellular and Molecular Basis of Regenerative Therapy Using Electromagnetic Fields. *Curr. Mol. Med.*, 2012, **12**(1),14–26. doi: 10.2174/156652412798376143
7. Eltorai, I. M.; Montroy, R. E.; Kobayashi, M.; Jakowatz, J. & Guttierez, P. Marjolin's ulcer in patients with spinal cord injury. *Spinal Cord Med J.*, 2002, **25(3)**, 191–196. doi: 10.1080/10790268.2002.11753621
8. Valberg, P.A.; Kavet, R. & Rafferty, C.N.Can low-level 50/60 Hz electric and magnetic fields cause biological effects?. *Radiat. Res.*, 1997, **148**(1), 2-21. doi: 10.2307/3579533
9. Funk, R.H. & Monsees, T.K. Effects of electromagnetic fields on cells: physiological and therapeutical approaches and molecular mechanisms of interaction. *Cells Tissues Organs*, 2006, **182**(2), 59-78. doi: 10.1159/000093061
10. Tsai, M. T.; Chang, W.H. S.; Chang, K.; Hou, R. J. & Wu, T.W. Pulsed electromagnetic fields affect osteoblast proliferation and differentiation in bone tissue engineering. *Bioelectromagnetics*, 2007, **28**(7), 519–28. doi: 10.1002/bem.20336
11. Noriega –Luna, B.; Sabanero, M.; Sosa, M. & Avila-Rodriguez, M. Influence of pulsed magnetic fields on the morphology of bone cells in early stages of growth. *Micron,* 2011, **42**(6), 600–607. doi: 10.1016/j.micron.2011.02.005
12. Grassi, C.; D'Ascenzo, M.; Torsello, A.; Martinotti, G.; Wolf, F.; Cittadini, A. & Azzena, G. B. Effects of 50 Hz electromagnetic fields on voltage-gated Ca^{2+} channels and their role in modulation of neuroendocrine cell proliferation and death. *Cell Calcium*, 2004, **35**(4), 307–315. doi: 10.1016/j.ceca.2003.09.001
13. Mayer-Wagner, S.; Passberger, A.; Sievers, B.; Aigner, J.; Summer, B.; Schiergens, T. S.; Jansson, V. & Müller, P.E. Effects of low frequency electromagnetic fields on the chondrogenic differentiation of human mesenchymal stem cells. *Bioelectromagnetics,* 2011, **32**(4), 283–290. doi: 10.1002/bem.20633
14. Piacentini, R.; Ripoli, C.; Mezzogori, D.; Azzena, G. B. & Grassi, C. Extremely low-frequency electromagnetic fields promote in vitro neurogenesis via upregulation of Cav1-channel activity. *J. Cell. Physiol*., 2008, **215**(1), 129–139. doi: 10.1002/jcp.21293
15. Walleczek, J. Electromagnetic field effects on cells of the immune system: the role of calcium signaling. *The FASEB J.,* 1992, **6**(13), 3177-3185. doi: 10.1096/fj.1530-6860
16. Mannerling, A.C.; Simko, M.; Mild, K.H. & Mattsson, M.O. Effects of 50-Hz magnetic field exposure on superoxide radical anion formation and HSP70 induction in human K562 cells. *Radiat. Environ. Biophys*., 2010, **49**(4), 731–741.
17. Hardell, L. & Sage, C. Biological Effects from Electromagnetic Field Exposure and Public Exposure Standards. *Biomedicine & Pharmacotherapy*, 2008, **62**(2), 104-109. doi: 10.1016/j.biopha.2007.12.004
18. Kheifets, L. & Shimkhada, R. Childhood leukemia and EMF: Review of the epidemiologic evidence. *Bioelectromagnetics*, 2005, **166**(2), 303-313. doi: 10.1002/bem.20139
19. Sutbeyaz, S.T.; Sezer, N.; Koseoglu, F. & Kibar, S. Low-frequency pulsed electromagnetic field therapy in fibromyalgia: a randomized, double-blind, sham-controlled

clinical study. *Clin. J. Pain*, 2009, **25**(8), 722–728. doi: 10.1097/AJP.0b013e3181a68a6c

20. Martiny, K.; Lunde, M. & Bech, P. Transcranial low voltage pulsed electromagnetic fields in patients with treatment-resistant depression. *Biol. Psychiatry*, 2010, **68**(2), 163–169. doi: 10.1016/j.biopsych.2010.02.017
21. Fiorani, M.; Biagiarelli, B.; Vetrano, F.; Guidi, G.; Dacha, M.; & Stocchi, V. In vitro effects of 50 Hz magnetic fields on oxidatively damaged rabbit red blood cells. *Bioelectromagnetics*, 1997, **18**(2), 125–131. doi: 10.1002/(SICI)1521 - 186X(1997)18:2<125::AID - BEM5>3.0.CO;2-4
22. Cane, V.; Botti, P.; & Soana, S. Pulsed magnetic fields improve osteoblast activity during the repair of an experimental osseous defect. *J. Orthop. Res.,* 1993, **11**(5), 664–670. doi: 10.1002/jor.1100110508
23. Gmitrov, J.; Ohkubo, C. & Okano, H. Effect of 0.25 T Static Magnetic Field on Microcirculation in Rabbits. *Bioelectromagnetics*, 2002, **23**(3), 224–229. doi: 10.1002/bem.10007
24. Wei, Y.; Xiaolin, H. & Tao, S.; Effects of extremely low-frequency-pulsed electromagnetic field on different-derived osteoblast-like cells. *Electromagn. Biol. Med.,* 2008, **27**(3), 298–311. doi: 10.1080/15368370802289604
25. Strauch, B.; Patel, M. K.; Rosen, D. J.; Mahadevia, S.; Brindzei, N. & Pilla, A. Pulsed magnetic field therapy increases tensile strength in a rat Achilles' tendon repair model. *J. Hand Surg. Am.,* 2006, **31**(7), 1131–1135. doi: 10.1016/j.jhsa.2006.03.024
26. Ikeda, K.; Shinmura, Y.; Mizoe, H.; Yoshizawa, H.; Yoshida, A.; Kanao, S.; Sumitani, H.; Hasebe, S.; Motomura, T.; Yamakawa, T.; Mizuno, F.; Otaka, Y. & Hirose, H. No Effects of Extremely LowFrequency Magnetic Fields Found on Cytotoxic Activities and Cytokine Production of Human Peripheral Blood Mononuclear Cells In Vitro. *Bioelectromagnetics*, 2003, **24**(1), 21–31. doi: 10.1002/bem.10062
27. Norimura, T.; Imada, H.; Kunugita, N. & Yoshida, N. M. Effects of strong magnetic fields on cell growth and radiation response of human T-lymphocytes in culture. *Uoeh.* 1993, **15**(2), 103–112.
28. Barbier, E.; Dufy, B. & Veyret, B.; Stimulation of Ca^{2+} influx in rat pituitary cells under exposure to a 50 Hz magnetic field. *Bioelectromagnetics,* 1996, **17**(4), 303–311. doi: 10.1002/(SICI)1521-186X(1996)17:4<303::AID-BEM6>3.0.CO;2-7
29. Takao, J.; Yudate, T.; Das, A.; Shikano, S.; Bonkobara, M.; Ariizumi K. & Cruz P. D. Expression of NF-jB in epidermis and the relationship between NF-jB activation and inhibition of keratinocyte growth. *Br J Dermatol.* 2003, **148**(4)**,** 680–688. doi: 10.1046/j.1365-2133.2003.05285.
30. Chow, K. C. & Tung, W. L. Magnetic field exposure enhances DNA repair through the induction of DnaK/J synthesis. *FEBS Lett.*, 2000, **478**, 133–136. doi: 10.1016/S0014-5793(00)01822-6
31. Bertolino, G.; De Freitas Braga, A.; De Oliveira Lima Do Couto Rosa, K.; De Brito L. C. & De Araujo J. E. Macroscopic and histological effects of magnetic field exposition in the process of tissue reparation in Wistar rats. *Arch. Dermatol. Res.*, 2006, **298**(3), 121–126.
32. Thomas, W.; White, K. P.; Drost D. J.; Cook C. & Prato F. S. A comparison of rheumatoid arthritis and fibromyalgia patients and healthy controls exposed to a pulsed (200 microT) magnetic field: effects on normal standing balance. *Neurosci. Lett.*, 2001, **309**(1), 17–20. doi.org/10.1016/j.neulet.2004.05.054
33. Shupak, N. M.; Hensel, J. M.; Cross-Mellor, S. K.; Kavaliers, M.; Prato, F. S. & Thomas, A. W. Analgesic and behavioral effects of a 100 microT specific pulsed extremely low frequency magnetic field on control and morphine treated CF-1 mice. *Neurosci Lett.,* 2004, **354**(1), 30-3. doi.org/10.1016/j.neulet.2003.09.063
34. Mathur, R. ; Dhawan, L. & Upadhyay, R.; Pain responses in rats exposed to 50 Hz magnetic field for varied durations. *In: Mathur, R. Pain Updated: Mechanisms and Effects,* 2006, 187–213.
35. Cheing, G. L.Y; Li, X.; Huang, L.; Kwan, R. L. C & Cheung, K. K. Pulsed electromagnetic fields (PEMF) promote early wound healing and myofibroblast proliferation in diabetic rats. *Bioelectromagnetics,* 2014, **35**(3), 161–169. doi: 10.1002/bem.21832
36. Goudarzi, I.; Hajizadeh, S.; Salmani & M. E.; Abrari, K. Pulsed electromagnetic fields accelerate wound healing in the skin of diabetic rats. *Bioelectromagnetics,* 2010, **31**(4), 318–323. doi: 10.1002/bem.20567
37. Strauch , B.; Patel, M. K.; Navarro, J. A.; Berdichevsky, M.; Yu, H-L. & Pilla, A. A. Pulsed magnetic fields accelerate cutaneous wound healing in rats. *Plast. Reconstr. Surg,* 2007, **120**(2), 425–430 doi: 10.1097/01.prs.0000267700.15452.d0
38. Canedo-Dorantes, L.; Garcia-Cantu, R.; Barrera, R.; Mendez-Ramirez, I.; Navarro V. H. & Serrano, G. Healing of chronic arterial and venous leg ulcers through systemic effects of electromagnetic fields. *Arch. Med. Res,* 2002, **33**(3), 281–289. doi: 10.1016/S0188-4409(02)00357-0
39. Ottani, V.; De Pasquale, V.; Govoni, P.; Franchi, M.; Ruggeri, A. & Zaniol P. Effects of pulsed extremely low frequency magnetic fields on skin wounds in the rat. *Bioelectromagnetics*, 1988, 9(1), 53–62. doi: 10.1002/bem.2250090105
40. Okano, H.; Onmori, R.; Tomita, N. & Ikada Y. Effects of a moderate-intensity static magnetic field on VEGF-A stimulated endothelial capillary tubule formation in vitro. *Bioelectromagnetics*, 2006, **27**(8), 628–640. doi: 10.1002/bem.20246
41. Tepper, O. M.; Callaghan, M. J.; Chang, E. I.; Galiano, R. D.; Bhatt, K.; Baharestani, S.; Gan, J.; Simon, B.; Hopper, R.; Levine, J. P. & Gurtner, G. C. Electromagnetic fields increase in vitro and in vivo angiogenesis through

endothelial release of FGF-2. *FASEB J,* 2004,**18**(11), 1231–1233.
doi: 10.1096/fj.03-0847fje

42. Delle Monache, S.; Alessandro R.; Iorio R.; Gualtieri G. & Colonna R. Extremely low frequency electromagnetic fields (ELF-EMFs) induce in vitro angiogenesis process in human endothelial cells. *Bioelectromagnetics,* 2008, **29**(8), 640-648.
43. Fivenson, D. P.; Faria, D. T.; Nickoloff, B. J; Poverini, P. J.; Kunkel, S.; Burdick, M. & Strieter, R. M. Chemokine and inflammatory cytokine changes during chronic wound healing. *Wound Repair Regen*. 1997, **5**(4), 310–322.
doi: 10.1046/j.1524-475X.1997.50405.x
44. Vianale, G.; Reale, M.; Amerio, P.; Stefanachi, M.; Di Luzio, S. & Muraro, R. Extremely low frequency electromagnetic field enhances human keratinocyte cell growth and decreases proinflammatory chemokine production. *Br. J. Dermatol.* 2008, **158**(6), 1189–1196.
doi: 10.1111/j.1365-2133.2008.08540.x
45. Hayes, P. & Solon, J. Drosophila dorsal closure: An orchestra of forces to zip shut the embryo. *Mech. Dev*., 2017, **144**, 2-10.
doi: 10.1016/j.mod.2016.12.005
46. Ramirez, E.; Monteagudo J.L.; Garcia-Gracia M. & Delgado J.M.R. Oviposition and development of Drosophila modified by magnetic fields. *Bioelectromagnetics*, 1983, **4**(4), 315-326.
doi: 10.1002/bem.2250040404
47. Kholy, S.E. & Husseiny, E.M. Effect of 60 Minutes Exposure to Electromagnetic field on fecundity, learning and memory, speed of movement and whole body protein of the fruit fly drosophila melanogaster. *J. Egypt. Soc. Parasitol.* 2013, **42**(3), 639–648.
doi: 10.12816/0006347
48. Li, S.; Zhang, Z.Y.; Yang, C. J.; Lian, H.Y. & Cai, P. Gene expression and reproductive abilities of male Drosophila melanogaster subjected to ELF-EMF exposure. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.*, 2013, **758**(1), 95–103.
doi: 10.1016/j.mrgentox.2013.10.004
49. Patino, O.; Grana, D.; Bolgiani, A.; Prezzavento, G.; Mino, J.; Merlo, A. & Benaim, F. Pulsed electromagnetic fields in experimental cutaneous wound healing in rats. *J Burn Care Rehabil,* 1996, **17**(suppl_6_pt_1), 528-531.
50. Callaghan, M.J.; Chang, E.I.; Seiser, N.; Aarabi, S.; Ghali, S.; Kinnucan, E.R.; Simon, B. J. & Gurtner, G.C. Pulsed electromagnetic fields accelerate normal and diabetic wound healing by increasing endogenous FGF-2 release. *Plast. Reconstr. Surg.*, 2008, **121**(1), 130–141.
doi: 10.1097/01.prs.0000293761.27219.84
51. Athanasiou, A.; Karkambounas, S.; Batistatou, A.; Lykoudis, E.; Katsaraki, A .; Kartsiouni, T.; Papalois, A. & Evangelou, A. The effect of pulsed electromagnetic fields on secondary skin wound healing: An experimental study. *Bioelectromagnetics,* 2007, **28**(5), 362–368.
doi: 10.1002/bem.20303
52. Stiller, M. J.; Pak, G. H.; Shupack, J. L.; Thaler, S.; Kenny, C. & Jondreau, L. A portable pulsed electromagnetic field (PEMF) device to enhance healing of recalcitrant venous ulcers: a double-blind, placebo-controlled clinical trial. *Br. J. Dermatol*., 1992, **127**(2), 147–154.
doi: 10.1111/j.1365-2133.1992.tb08047.x

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