EMF-ELF and Wound-Repair

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Wound healing is a complex, staged process. It involves extensive communication between the different cellular constituents of various compartments of the skin and its extracellular matrix (ECM). Different signaling pathways are determined by a mutual influence on each other, resulting in a dynamic and complex crosstalk. It consists of various dynamic processes including a series of overlapping phases: hemostasis, inflammation response, new tissue formation, and tissue remodeling. Interruption or deregulation of one or more of these phases may lead to non-healing (chronic) wounds. The most important factor among local and systemic exogenous factors leading to a chronic wound is infection with a biofilm presence. In the last few years, an increasing number of reports have evaluated the effects of extremely low frequency (ELF) electromagnetic fields (EMFs) on tissue repair. Each experimental result comes from a single element of this complex process. An interaction between ELF-EMFs and healing has shown to effectively modulate inflammation, protease matrix rearrangement, neo-angiogenesis, senescence, stem-cell proliferation, and epithelialization.

Keywords: ELF-EMF ; wound ; healing ; fibroblasts ; keratinocytes ; non-healing wounds

1. Introduction

Wound healing is a complex and well-regulated process controlled by extensive communication between cells, with a dynamic and complex crosstalk between different signaling pathways [1].

This process is composed of many phases, which include, consecutively, the hemostasis, inflammation response, new tissue formation, and tissue remodeling phases [2][3]. The hemostatic phase results from the immediate activation of platelets. These cells release molecules, such as a growth factor and cytokines, that prevent bleeding and initiate wound repair. The second step is inflammation, which is characterized by the afflux 24 to 48 h after injury of different immune cells, such as neutrophils, monocytes, and lymphocytes. These cells work together and in close coordination to prevent infection and to remove dead tissue [3][4]. Two to ten days following tissue injury, cellular proliferation, and migration of different cell types, such as fibroblasts, keratinocytes, and endothelial cells, new tissue formation occurs [5][6]. Fibroblasts are known as the main actors regulating the wound repair process and, in the presence of the wound microenvironment, migration, proliferation [6][7][8] and synthesis, and secretion of many factors, such as Matrix metalloproteinase-14 (MMP-14), basic fibroblast growth factor (bFGF), and fibroblast growth factor-9 (FGF-9), collagen homeostasis and angiogenesis increase [9][10].

Finally, in the re-modelling phase, two to three weeks after injury, fibroblasts differentiate into myofibroblasts [11], which produces an extracellular matrix leading to a mature scar [12]. The tissue remodeling process may last for a year or more. At this stage, all the processes started by injury will turn off through apoptosis of involved cells, fibroblasts, macrophages, and endothelial cells [10][11][12][13][14]. The wound will be repaired only if all these classic healing steps work correctly and in close coordination.

The process is highly efficient, but sometimes it can deviate from its physiological course, resulting in an ulcerative skin defect (chronic wound) or an excessive scar formation (hypertrophic scar or keloid). Chronic wound development may be common in various conditions including pressure, diabetes, venous pathology (venous, arterial, mixed, and vasculitis), trauma, and surgery, with significant morbidity and mortality risk [15][16] as well as impact for a healthy economy [17][18].

ELF-EMFs are non-ionizing, low-energy, electromagnetic fields capable of inducing several biological effects. Frequencies considered to be ELFs range from 3 Hz to 300 Hz. The study of the interaction between ELF-EMFs and the tissue is not always easy since different biological effects are related to EMFs' time of exposure, waveform, frequency, amplitude, cell type, and cell status [19][20].

The ELF-EMFs are commonly produced by electrical devices, high tension electrical distribution networks, from residential and occupational sources, and by power lines. Low-frequency electric fields influence all systems characterized by

charged particles as the human body. In fact, tiny electrical currents exist in the human body due to the chemical reactions that occur as part of normal bodily functions, even in the absence of external electric fields.

The interest in the biological interaction of ELF-EMFs with tissues has, nevertheless, increased due to their possible effect on human health as well as their potential therapeutic use. ELF-EMFs with frequencies less than 300 Hz do not have enough energy to break molecular bonds, nor cause DNA damage, ionization, or even to have thermal effects on cells and tissues [21]. Biological effects modulated by EMFs are very wide and include cell migration, proliferation and differentiation, cytokine and growth factors expression, and nitric oxide signaling alteration [20][21][22][23][24][25]. ELF-EMFs can interact with the chemical and biological processes modulating the physiological homeostasis, and, thus, can interact in wound healing.

Despite some studies reporting potential negative effects of ELF-EMFs, such as increased risk of childhood cancer, breast cancer, neoplastic development, neurodegenerative diseases, and in fertility, cardiovascular disorders, disease promotion, and progression [26][27][28][29][30][31][32][33], no convincing evidence was ever provided for a direct relationship between ELF-EMFs and disease development. In the last few years, an increasing number of reports have evaluated the effects of ELF-EMFs on tissue repair.

2. EMF-ELF and Wound-Repair: Mechanism of Action

Despite the high ability of the innate reparative process, multiple cellular aspects of an individual's injury response can be disturbed, compromising wound closure, and leading to chronic wound development. Recent studies have shed light on bioeffects induced by the EMF and how they might control tissue regeneration and wound healing, suggesting that EMF has a positive impact on all the different stages of healing. In fact, a promising novel strategy for treating the chronic wound may be the local delivery of ELF-EMFs to target resident cells to improve their ability in modulating immune responses and tissue healing.

2.1. ELF-EMFs and Hemostatic Phase

The initial phase of wound repair after injury is characterized by blood vessel damage and formation of a blood clot. The relationship between ELF-EMFs and platelets, as principal contributors to haemostasias and coagulation, has recently drawn interest. Platelets represent the inducers for the bleeding prevention mechanism and for the initiation of repair systems, supporting the recruitment of immune cells, cytokines, and growth factors necessary for early wound repair. A previous in vivo study reported contrasting results. Lai et al. observed no significant variation in platelet count after 100-μT ELF-EMFs exposure [34], while Liu et al. reported that exposure to ELF-EMFs increases the number of white blood cells (WBCs) and lymphocytes but decreases the mean platelet volume (MPV) levels at a bandwidth of 5 Hz to 32 KHz [35]. The mechanism of platelet activation is complex and required the increase of calcium levels, protein kinase C stimulation, and free oxygen radical generation.

ELF-EMFs may be generated with frequencies close to the resonant patterns of calcium (Ca^{2+}), sodium (Na^{+}), and other ions. Numerous studies reported that Ca^{2+} ions are the main target of ELF-EMFs [36]. Many studies have shown that voltage-dependent calcium channels may account for the biological effects of ELF-EMFs exposure. As an indirect proof of calcium role, it has also been shown that calcium channel blockers can greatly reduce the effects of 1 mT and 50-Hz exposure, and cause interference in cell differentiation and neurogenesis [37]. It is well documented that Ca^{2+} ions affect activity-dependent gene expression [38] and this effect is mediated by signaling pathways activating Ca^{2} -responsive DNA regulatory elements. Due to this direct cellular interaction, electromagnetic fields have been demonstrated to increase healing rates much faster than other therapies, as they may reach the deep tissue more quickly immediately after the insult [39] (Figure 1).

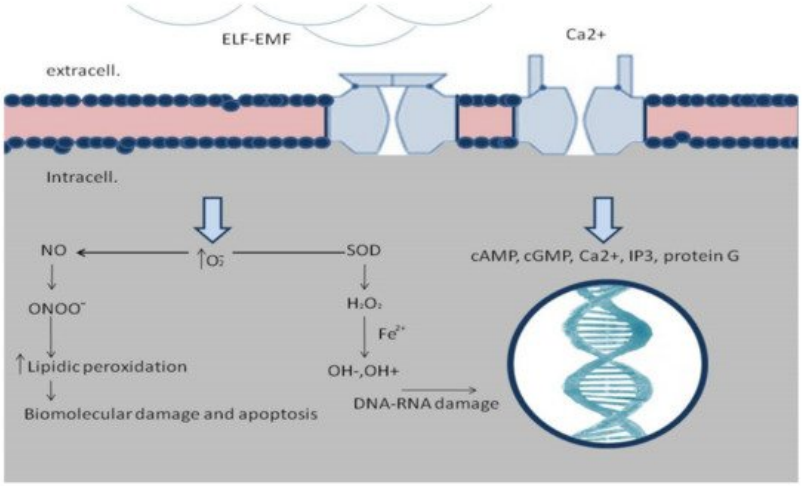

Figure 1. Molecular mechanisms of ELF-EMFs' effects on cell function. ELF-EMFs open voltage-dependent calcium channels, causing interference in cell differentiation with Ca^{2+} influx into cells. It is well documented that Ca^{2+} ions affect activity-dependent gene expression, and this effect is mediated by signaling pathways activating Ca^{2+}-responsive DNA regulatory elements. Decreasing antioxidants concentration has a defense mechanism against free radicals. The ELF-EMFs could also induce the production of oxygen (O_2) in the cellular environment, which plays a major role in oxidative damage that, subsequently, led to biomolecular damage, DNA double strand breaks, DNA/RNA damage, and cell death.

EMF quickly restores the balance between free radicals and antioxidants to stop the cascade of inflammatory progression and biochemical degradation in traumatized tissue [40]. Free radicals can be mitogenic or cytotoxic depending on levels, antioxidant system efficiency, and cell types. Previous studies have shown that reactive oxygen species (ROS), generated after brief exposure to ELF-EMFs, play a key role in cell proliferation as a possible initial cell event. Conversely, the continuous generation of ROS by long-term 50-60 Hz ELF-EMFs exposures can induce the accumulation of DNA damage and slow cell cycle progression [41][42]. ELF-EMFs have also been reported to up-regulate clusters of protective and restorative gene loci as well as down-regulate deregulatory and apoptotic gene loci [39].

2.2. ELF-EMFs and Inflammatory Phase

A large body of evidence on chronic wound tissue and fluids demonstrates an unstable competition between pro-inflammatory and anti-inflammatory signals that lead to the misbalanced environment favoring the development of chronic wounds [43][44].

It has also been shown that increased pro-inflammatory cellular infiltrates, composed largely of neutrophils and macrophages, contribute to delayed healing in chronic ulcers [45][46]. As a result, deregulation of several key pro-inflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor-α (TNF-α), prolong the inflammatory phase and delay healing [44][47]. IL-1β and TNFα are increased in chronic wounds, and this increase has been shown to cause elevated levels of metalloproteinases that excessively degrade the local ECM and, thus, impair cell migration [48].

EMFs effects on the expression of cytokines have been mostly investigated with ex vivo and in vitro experiments on different cell types involved in tissue repair. Several reports have supported the anti-inflammatory effects of EMFs on tissue repair. Vianale et al. demonstrate that 50 Hz ELF-EMFs exposure may inhibit inflammatory processes by producing Regulated upon Activation, Normal T Cell Expressed and Presumably Secreted (RANTES), Macrophage chemotactic protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α and IL-8, and activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) inflammatory signaling pathways in keratinocytes in vitro [49].

The effect of ELF-EMFs on transition from a chronic pro-inflammatory to an anti-inflammatory state of the healing process was also reviewed by Pesce et al. [50]. In the epidermal wound healing process, ELF-EMFs exposure is reported to mediate keratinocyte proliferation, up-regulation of the Nitric Oxide Synthase (NOS) activities, and down-regulation of Cyclooxygenase-2 (COX-2) expression and Prostaglandin E2 (PGE-2) production, involved in the inflammatory response modulation [51].

The effects of ELF-EMFs on the inflammatory molecules are timing and cell type dependent. In fact, 50 Hz of exposure induced an early increase of IL-1β, IL-18, and TNFα production and secretion by keratinocytes and fibroblasts, while a later inhibition of inflammatory mediators led to healing, mirroring the physiological process of wound repair [52][53][54]. In accordance, in vivo and in vitro studies reported the influence of ELF-EMFs exposure on inflammatory state promotion, with increased production of pro-inflammatory cytokines, such as IL-6, IL-9, and TNF-α, and increased levels of ROS [55]

[56]. Furthermore, ex vivo studies reported the ability of ELF-EMFs exposure in activating the anti-inflammatory response by down-modulating pro-inflammatory cytokines, inducing IL-10, and transforming growth factor β (TGFβ), as a key antagonist of pro-inflammatory mediators [55][57], which enhanced the immune system response in the damaged areas and favored early wound healing. A clinical and experimental investigation showed that ELF-EMF speed up the switch from an inflammatory phase to a proliferative phase in the wound healing.

2.3. ELF-EMFs and Proliferative Phase

In the healing process, the proliferative phase is characterized by the activation of a wide array of cells: keratinocytes, fibroblasts, macrophages, and endothelial cells. This leads to complete wound healing, through matrix deposition and angiogenesis. This phase starts 12 h after the damage, with release of MMPs and migration stimulation, while new ECM proteins reconstitute the basement membrane.

MMPs, such as collagenase and gelatinases A and B, are more elevated in chronic wounds as compared to acute wounds [43]. ELF-EMFs were shown to upregulate MMP-9 release early with a physiologically late decrease [57]. Also, Wang et al., in a study on scleral fibroblasts, report that 0.2 mT of ELF-EMFs might act by increasing the expression of MMP-2 and reducing collagen I synthesis, which can be involved in pathological matrix remodeling [58].

This early up-regulation of MMPs activity and/or expression may represent a mechanism to promote the migration of keratinocytes and induce phagocytosis to eliminate cell debris during the inflammatory phase of wound repair [59].

To support the metabolic needs of the highly proliferative healing phase, blood vessels are essential, supporting cells with nutrition and oxygen. Both angiogenesis (sprouting of capillaries from existing blood vessels) and vasculogenesis (mobilization of bone marrow—derived endothelial progenitors) are essential to tissue repair [60].

Chronic wounds exhibit a higher expression of proteins with anti-angiogenic properties, such as myeloperoxidase, while angiogenic stimulators, such as extracellular superoxide dismutase, are generally decreased [61]. Overall, proteolytic degradation of pro-angiogenic factors, such as members of the vascular endothelial growth factor (VEGF) family, and a subsequent decrease of their bioactivity in the chronic wound microenvironment, has been suggested to be responsible for impaired tissue healing [62][63]. ELF-EMFs have been shown to influence cell migration and proliferation, and regulate mitogen activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK), promoting the following angiogenic process. In vitro and in vivo experiments revealed that ELF-EMFs impact the modulation of VEGF-dependent signal transduction pathways and increase the number of angiogenesis factors [64][65][66][67]. Furthermore, Fan et al. showed that ELF-EMFs may increase the proliferation of Human Bone Marrow Stromal Osteoprogenitor Cells (hBM-SCs), promoting DNA synthesis and increasing the proportion of cells in the S phase, and up-regulating the expressions of hematopoietic growth factors both in hBM-SCs and mesenchymal stem cells (MSC) [68]. The regulation of the differentiation and proliferation processes, necessary for optimal healing of bone fractures, is linked to the production of cytokines and growth factors. ELF-EMFs are capable of up-regulate the expressions of growth factors involved in the regenerative process, such as the macrophage colony-stimulating factor (M-CSF), stem cell factor (SCF), thrombopoietin (TPO), fibroblast growth factor 2 (FGF2), and VEGF [68][69]. Overall, changes in the wound microenvironment, with the production of several growth factors, are increased by ELF-EMF exposure, as reported by several results. These data support that the ELF-EMF exposure can be a valuable insight for angiogenesis during normal tissue repair.

2.4. ELF-EMFs and Remodeling Phase

Wound closure is considered the tissue injury endpoint. However, remodeling or tissue maturation can last several months or even years. This last phase of wound healing consists of a regression of the neovasculature, accompanied by deposition to the ECM and subsequent granulation tissue modification into scar tissue. In the physiological remodeling phase, collagen I synthesis, collagen III lysis, and reorganization of the ECM all take place [70]. The effect of ELF-EMFs exposure in the re-modelling phase is responsible for a change in the biological needs of the tissue. The EMF effects on collagen synthesis depend on factors including frequency, level of magnetic induction, cell density, and type. Studies reported how the application of 50 or 60 Hz ranges can modulate collagen synthesis, according to the type of skin damage and the cells involved in the remodeling mechanism [71][72]. As reported in animal models, ELF-EMFs were capable to promote healing of skin ulcers through the increase of skin collagen synthesis, but not in heat-damaged skin tissue or after skin fibroblasts activation [71][72]. The overview of experimental studies provided rational support of ELF-EMF involvement in all wound healing steps. Several studies have addressed that the ELF-EMF is differently efficient within the frequency and the intensity window, as well as the exposure time and clinical conditions, such as soft and hard tissue injury.

3. ELF-EMFs and Non-Healing Conditions

The onset of chronic inflammatory processes in pathological conditions, such as diabetes, hypertension, and arteriosclerosis, which may favor the onset of arterial and venous ulcers of the lower limbs. Pathological conditions may act through the induction of an insufficient blood supply, anoxia, edema, cell death, and infection, resulting in an alteration of the balance between the structural components of the affected tissues and the immune cells, which prevents wound healing [72]. Infection and alteration in biofilm composition and cellular senescence are commonly considered as an exogenous and endogenous factor that can act detrimentally in the physiological process of wound healing, leading to chronic wound development [73][74][75][76] (Figure 2).

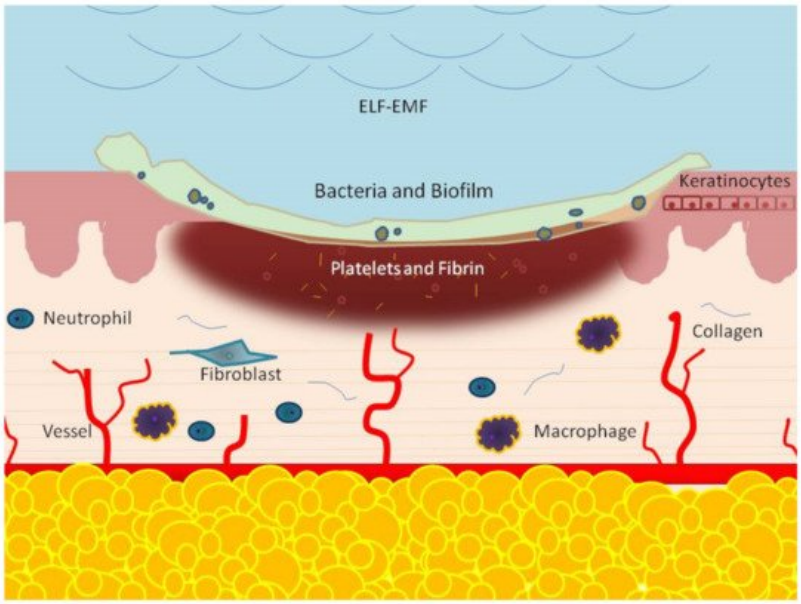

Figure 2. The chronic wound shows the presence of infection and biofilm formation, a hyperproliferative and nonmigratory epidermis, and an inflammatory state with an increase in inflammatory cells (neutrophils and macrophages) not properly functioning. Fibroblasts and keratinocytes become senescent while there is a reduction of angiogenesis, stem cell recruitment and activation, and ECM remodeling. ELF-EMFs has been shown to regulate the inflammatory response, induce senescence of fibroblasts, and keratinocytes through increased proliferation and migration. The regulation of MMP and collagen synthesis improves the ECM microenvironment. Proangiogenic and vasculogenic activity support cells with nutrition and oxygen. The role on biofilm and infection is still controversial.

References

1. Alonso, N.; Nebreda, A.D.; Monczor, F.; Gutkind, J.S.; Davio, C.; Fernandez, N.; Shayo, C. PI3K pathway is involved in ERK signaling cascade activation by histamine H2R agonist in HEK293T cells. Biochim. Biophys. Acta 2016, 1860, 1998–2007.
2. Aarabi, S.; Longaker, M.T.; Gurtner, G.C. Hypertrophic Scar Formation Following Burns and Trauma: New Approaches to Treatment. PLoS Med. 2007, 4, e234.
3. Martin, P.; D'Souza, D.; Martin, J.; Grose, R.; Cooper, L.; Maki, R.; McKercher, S.R. Wound healing in the PU.1 null mouse–tissue repair is not dependent on inflammatory cells. Curr. Biol. 2013, 13, 1122–1128.
4. Martin, P.; Leibovich, S.J. Inflammatory cells during wound repair: The good, the bad and the ugly. Trends Cell Biol. 2005, 15, 599–607.
5. Schmidt, B.A.; Horsley, V. Intradermal adipocytes mediate fibroblast recruitment during skin wound healing. Development 2013, 140, 1517–1527.
6. Xie, C.; Shi, K.; Zhang, X.; Zhao, J.; Yu, J. MiR-1908 promotes scar formation post-burn wound healing by suppressing Ski-mediated inflammation and fibroblast proliferation. Cell Tissue Res. 2016, 366, 371–380.
7. Amin, Z.A.; Ali, H.M.; Alshawsh, M.A.; Darvish, P.H.; Abdulla, M.A. Application of Antrodia camphorata promotes rat's wound healing in vivo and facilitates fibroblast cell proliferation in vitro. Evid. Based Complement. Altern. Med. 2015, 2015, 317693.
8. Park, J.; Hong, Y.; Kwon, S.H.; Park, J.; Park, J. Anti-aging effects of Piper cambodianum P. Fourn. extract on normal human dermal fibroblast cells and a wound-healing model in mice. Clin. Interv. Aging 2016, 11, 1017–1026.

9. Upadhyay, A.; Chattopadhyay, P.; Goyary, D.; Mazumder, P.M.; Veer, V. Ixora coccinea Enhances Cutaneous Wound H ealing by Upregulating the Expression of Collagen and Basic Fibroblast Growth Factor. ISRN Pharmacol. 2014, 2014, 7 51824.
10. Zheng, Z.; Kang, H.Y.; Lee, S.; Kang, S.W.; Goo, B.; Cho, S.B. Up-regulation of fibroblast growth factor (FGF) 9 expres sion and FGF-WNT/beta-catenin signaling in la-ser-induced wound healing. Wound Repair Regen. 2014, 22, 660–665.
11. Nakamichi, M.; Akishima-Fukasawa, Y.; Fujisawa, C.; Mikami, T.; Onishi, K.; Akasaka, Y. Basic Fibroblast Growth Facto r Induces Angiogenic Properties of Fibrocytes to Stimulate Vascular Formation during Wound Healing. Am. J. Pathol. 2 016, 186, 3203–3216.
12. Zigrino, P.; Brinckmann, J.; Niehoff, A.; Lu, Y.; Giebeler, N.; Eckes, B.; Kadler, K.E.; Mauch, C. Fibroblast-Derived MMP- 14 Regulates Collagen Homeostasis in Adult Skin. J. Investig. Dermatol. 2016, 136, 1575–1583.
13. Opalenik, S.R.; Davidson, J.M. Fibroblast differentiation of bone marrow-derived cells during wound repair. FASEB J. 2 005, 19, 1561–1563.
14. Werner, S.; Krieg, T.; Smola, H. Keratinocyte–Fibroblast Interactions in Wound Healing. J. Investig. Dermatol. 2007, 12 7, 998–1008.
15. Briggs, M.; Closs, S. Patients' perceptions of the impact of treatments and products on their experience of leg ulcer pai n. J. Wound Care 2006, 15, 333–337.
16. Monari, P.; Pelizzari, L.; Crotti, S.; Damiani, G.; Calzavara-Pinton, P.; Gualdi, G. The Use of PRISM (Pictorial Represent ation of Illness and Self Measure) in Patients Affected by Chronic Cutaneous Ulcers. Adv. Skin Wound Care 2015, 28, 489–494.
17. Gurtner, G.C.; Werner, S.; Barrandon, Y.; Longaker, M.T. Wound repair and regeneration. Nat. Cell Biol. 2008, 453, 314 –321.
18. Eming, S.A.; Martin, P.; Tomic-Canic, M. Wound repair and regeneration: Mechanisms, signaling, and translation. Sci. T ransl. Med. 2014, 6, 265sr6.
19. Geng, D.Y.; Li, C.H.; Wan, X.W.; Xu, G.Z. Biochemical kinetics of cell proliferation regulated by extremely low frequency electromagnetic field. Biomed. Mater. Eng. 2014, 24, 1391–1397.
20. Pirozzoli, M.C.; Marino, C.; Lovisolo, G.A.; Laconi, C.; Mosiello, L.; Negroni, A. Effects of 50 Hz electromagnetic field ex posure on apoptosis and differentiation in a neuroblastoma cell line. Bioelectromagnetics 2003, 24, 510–516.
21. Repacholi, M.H.; Greenebaum, B. Interaction of static and extremely low frequency electric and magneticfields with livi ng systems: Health effects and research needs. Bioelectromagnetics 1999, 20, 133–160.
22. Kaszuba-Zwoinska, J.; Chorobik, P.; Juszczak, K.; Zaraska, W.; Thor, P.J. Pulsed electromagnetic field affects intrinsic and endoplasmatic reticulum apoptosis induction pathways in MonoMac6 cell-line culture. J. Physiol. Pharmacol. 2012, 63, 537–545.
23. Cheng, K.; Zou, C. Electromagnetic field effect on separation of nucleotide sequences and unwinding of a double helix during DNA replication. Med. Hypotheses 2006, 66, 148–153.
24. Jasti, A.C.; Wetzel, B.J.; Aviles, H.; Vesper, D.N.; Nindl, G.; Johnson, M.T. Effect of a wound healing electromagnetic fie ld on inflammatory cytokine gene expression in rats. Biomed. Sci. Instrum. 2001, 37, 209–214.
25. Köbbert, C.; Berndt, A.; Bierbaum, T.; Sontag, W.; Breithardt, G.; Weissen-Plenz, G.; Sindermann, J.R. Low-energy ele ctromagnetic fields promote proliferation of vascular smooth muscle cells. Electromagn. Biol. Med. 2008, 27, 41–53.
26. Huo, R.; Ma, Q.; Wu, J.J.; Chin-Nuke, K.; Jing, Y.; Chen, J.; Miyar, M.E.; Davis, S.C.; Li, J. Noninvasive electromagnetic fields on keratinocyte growth and migration. J. Surg. Res. 2010, 162, 299–307.
27. Yokus, B.; Akdag, M.Z.; Dasdag, S.; Cakir, D.U.; Kizil, M. Extremely low frequency magnetic fields cause oxidative DNA damage in rats. Int. J. Radiat. Biol. 2008, 84, 789–795.
28. Akdag, M.Z.; Dasdag, S.; Uzunlar, A.K.; Ulukaya, E.; Oral, A.Y.; Çelik, N.; Akşen, F. Can safe and long-term exposure t o extremely low frequency (50 Hz) magnetic fields affect apoptosis, re-production, and oxidative stress? Int. J. Radiat. Biol. 2013, 89, 1053–1060.
29. Khaki, A.; Imani, S.A.M.; Golzar, F.S. Effects of rosmarinic acid on male sex hormones (testosterone-FSH-LH) and testi s tissue apoptosis after exposure to electromagnetic field (EMF) in rats. Afr. J. Pharm. Pharmacol. 2016, 6, 248–252.
30. Murbach, M.; Christopoulou, M.; Achermann, P.; Kuster, N.; Crespo-Valero, P. Exposure system to study hypotheses of ELF and RF electromagnetic field interactions of mobile phones with the central nervous system. Bioelectromagnetics 2012, 33, 527–533.
31. Akdag, M.Z.; Dasdag, S.; Ketani, M.A.; Sagsoz, H. Effect of extremely low frequency magnetic fields in safety standard s on structure of acidophilic and basophilic cells in anteriorpituitary gland of rats: An experimental study. J. Int. Dent. Me

d. Res. 2009, 2, 61–66.

32. Akdag, M.Z.; Dasdag, S.; Ulukaya, E.; Uzunlar, A.K.; Kurt, M.A.; Taşkin, A. Effects of extremely low-frequency magnetic field on caspase activities and oxidative stress values in rat brain. Biol. Trace Elem. Res. 2013, 38, 238–249.

33. Akdag, M.Z.; Dasdag, S.; Cakir, D.U.; Yokus, B.; Kizil, G.; Kizil, M. Do 100- and 500-μT ELF magnetic fields alter beta-amyloid protein, protein carbonyl and malondialdehyde in rat brains? Electromagn. Biol. Med. 2013, 32, 363–372.

34. Lai, J.; Zhang, Y.; Zhang, J.; Liu, X.; Ruan, G.; Chaugai, S.; Tang, J.; Wang, H.; Chen, C.; Wang, D.W. Effects of 100-μT extremely low frequency electromagnetic fields exposure on hematograms and blood chemistry in rats. J. Radiat. Res. 2015, 57, 16–24.

35. Liu, X.; Zhao, L.; Yu, D.; Ma, S.; Liu, X. Effects of extremely low frequency electromagnetic field on the health of workers in auto-motive industry. Electromagn. Biol. Med. 2013, 32, 551–559.

36. Liboff, A. The cyclotron resonance hypothesis: Experimental evidence and theoretical constraints. In Interaction Mechanisms of Low-Level Electromagnetic Fields with Living Systems; Norden, B., Ramal, C., Eds.; Oxford University Press: London, UK, 1991; pp. 130–147.

37. Piacentini, R.; Ripoli, C.; Mezzogori, D.; Azzena, G.B.; Grassi, C. Extremely low-frequency electromagnetic fields promote in vitro neurogenesis via upregulation of Ca(v)1-channel activity. J. Cell Physiol. 2008, 215, 129–139.

38. Carrasco, M.A.; Hidalgo, C. Calcium microdomains and gene expression in neurons and skeletal muscle cells. Cell Calcium 2006, 40, 575–583.

39. Gordon, G.A. Designed electromagnetic pulsed therapy: Clinical applications. J. Cell. Physiol. 2007, 212, 579–582.

40. Han, J.; Shuvaev, V.V.; Muzykantovv, V.R. Targeted interception of signaling reactive oxygen species in the vascular endothelium. Ther. Deliv. 2012, 3, 263–276.

41. Wolf, F.I.; Torsello, A.; Tedesco, B.; Fasanella, S.; Boninsegna, A.; D'Ascenzo, M.; Grassi, C.; Azzena, G.B.; Cittadini, A. 50-Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: Possible involvement of a redox mechanism. Biochim. Biophys. Acta 2015, 1743, 120–129.

42. Huang, C.Y.; Chang, C.W.; Chen, C.R.; Chuang, C.Y.; Chiang, C.S.; Shu, W.Y.; Fan, T.C.; Hsu, I.C. Extremely Low-Frequency Electromagnetic Fields Cause G1 Phase Arrest through the Activation of the ATM-Chk2-p21 Pathway. PLoS ONE 2014, 9, e104732.

43. Eming, S.A.; Koch, M.; Krieger, A.; Brachvogel, B.; Kreft, S.; Bruckner-Tuderman, L.; Krieg, T.; Shannon, J.D.; Fox, J.W. Differential proteomic analysis distinguishes tissue repair biomarker signatures in wound exudates obtained from normal healing and chronic wounds. J. Proteome Res. 2010, 9, 4758–4766.

44. Beidler, S.K.; Douillet, C.D.; Berndt, D.F.; Keagy, B.A.; Rich, P.B.; Marston, W.A. Inflammatory cytokine levels in chronic venous insufficiency ulcer tissue before and after compression therapy. J. Vasc. Surg. 2009, 49, 1013–1020.

45. Sindrilaru, A.; Peters, T.; Wieschalka, S.; Baican, C.; Baican, A.; Peter, H.; Hainzl, A.; Schatz, S.; Qi, Y.; Schlecht, A.; et al. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. J. Clin. Investig. 2011, 121, 985–997.

46. Loots, M.A.; Lamme, E.N.; Zeegelaar, J.; Mekkes, J.R.; Bos, J.D.; Middelkoop, E. Differences in cellular infiltrate and extracellular matrix of chronic diabetic and venous ulcers versus acute wounds. J. Investig. Dermatol. 1998, 111, 850–857.

47. Barrientos, S.; Stojadinovic, O.; Golinko, M.S.; Brem, H.; Tomic-Canic, M. Growth factors and cytokines in wound healing. Wound Repair Regen. 2008, 16, 585–601.

48. Tarnuzzer, R.W.; Schultz, G.S. Biochemical analysis of acute and chronic wound environments. Wound Repair Regen. 1996, 4, 321–325.

49. 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 pro-inflammatory chemokine production. Br. J. Dermatol. 2008, 158, 1189–1196.

50. Pesce, M.; Patruno, A.; Speranza, L.; Reale, M. Extremely low frequency electromagnetic field and wound healing: Implication of cytokines as biological medi-ators. Eur. Cytokine Netw. 2013, 24, 1–10.

51. Patruno, A.; Amerio, P.; Pesce, M.; Vianale, G.; Di Luzio, S.; Tulli, A.; Franceschelli, S.; Grilli, A.; Muraro, R.; Reale, M. Extremely low frequency electromagnetic fields modulate expression of inducible nitric oxide syn-thase, endothelial nitric oxide synthase and cyclooxygenase–2 in the human keratinocyte cell line HaCaT: Potential therapeutic effects in wound healing. Br. J. Dermatol. 2010, 162, 258–266.

52. Costantini, E.; Sinjari, B.; D'Angelo, C.; Murmura, G.; Reale, M.; Caputi, S. Human Gingival Fibroblasts Exposed to Extremely Low-Frequency Electromagnetic Fields: In Vitro Model of Wound-Healing Improvement. Int. J. Mol. Sci. 2019, 2

0, 2108.

53. Patruno, A.; Ferrone, A.; Costantini, E.; Franceschelli, S.; Pesce, M.; Speranza, L.; Amerio, P.; D'Angelo, C.; Felaco, M.; Grilli, A.; et al. Extremely low-frequency electromagnetic fields accelerate wound healing modulating MMP-9 and inf lam-matory cytokines. Cell Prolif. 2018, 51, e12432.
54. Gomez-Ochoa, I.; Gómez-Ochoa, P.; Gómez-Casal, F.; Cativiela, E.; Larrad-Mur, L. Pulsed electromagnetic fields decr ease proinflammatory cytokine secretion (IL-1beta and TNFalpha) on human fibroblast-like cell culture. Rheumatol. Int. 2011, 31, 1283–1289.
55. Mahaki, H.; Mahaki, H.; Jabarivasal, N.; Sardanian, K.; Zamani, A. Effects of various densities of 50 Hz electromagnetic field on serum IL-9, IL-10, and TNF-α levels. Int. J. Occup. Environ. Med. 2020, 11, 24–32.
56. Yager, D.R.; Zhang, L.-Y.; Liang, H.-X.; Diegelmann, R.F.; Cohen, I.K. Wound Fluids from Human Pressure Ulcers Cont ain Elevated Matrix Metalloproteinase Levels and Activity Compared to Surgical Wound Fluids. J. Investig. Dermatol. 1 996, 107, 743–748.
57. Mahdavinejad, L.; Alahgholi-Hajibehzad, M.; Eftekharian, M.M.; Zaerieghane, Z.; Salehi, I.; Hajilooi, M.; Mahaki, H.; Za mani, A. Extremely Low Frequency Electromagnetic Fields Decrease Serum Levels of Interleukin-17, Transforming Gro wth Factor-β and Downregulate Foxp3 Expression in the Spleen. J. Interferon Cytokine Res. 2018, 38, 457–462.
58. Wang, J.; Cui, J.; Zhu, H. Suppression of type I collagen in human scleral fibroblasts treated with extremely low-freque ncy electromagnetic fields. Mol. Vis. 2013, 19, 885–893.
59. Blanpain, C.; Fuchs, E. Epidermal homeostasis: A balancing act of stem cells in the skin. Nat. Rev. Mol. Cell Biol. 2009, 10, 207–217.
60. Krisp, C.; Jacobsen, F.; McKay, M.J.; Molloy, M.P.; Steinstraesser, L.; Wolters, D.A. Proteome analysis reveals antiangi ogenic environments in chronic wounds of diabetes mellitus type 2 patients. Proteomics 2013, 13, 2670–2681.
61. Hoffmann, D.C.; Willenborg, S.; Koch, M.; Zwolanek, D.; Müller, S.; Becker, A.K.; Metzger, S.; Ehrbar, M.; Kurschat, P.; Hellmich, M.; et al. Proteolytic processing regulates placental growth factor activities. J. Biol. Chem. 2013, 288, 17976–17989.
62. Leu, A.J.; Leu, H.J.; Franzeck, U.K.; Bollinger, A. Microvascular changes in chronic venous insufficiency—A review. Car diovasc. Surg. 1995, 3, 237–245.
63. Lauer, G.; Sollberg, S.; Cole, M.; Flamme, I.; Stürzebecher, J.; Mann, K.; Krieg, T.; Eming, S.A. Expression and proteol ysis of vascular endothelial growth factor is increased in chronic wounds. J. Investig. Dermatol. 2000, 15, 12–18.
64. Cichon, N.; Bijak, M.; Czarny, P.; Miller, E.; Synowiec, E.; Sliwinski, T.; Saluk-Bijak, J. Increase in Blood Levels of Growt h Factors Involved in the Neuroplasticity Process by Using an Extremely Low Frequency Electromagnetic Field in Post-stroke Patients. Front. Aging Neurosci. 2018, 10, 294.
65. 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, 640–648.
66. Ozgün, A.; Marote, A.; Behie, A.; Salgado, L.; Garipcan, A.; Ozgun, B. Extremely low frequency magnetic field induces human neuronal differentiation through NMDA receptor activation. J. Neural Transm. 2019, 126, 1281–1290.
67. Peng, J.; Zhao, J.; Long, Y.; Xie, Y.; Nie, J.; Chen, L. Magnetic Materials in Promoting Bone Regeneration. Front. Mater. 2019, 6, 268.
68. Fan, W.; Qian, F.; Ma, Q.; Zhang, P.; Chen, T.; Chen, C.; Zhang, Y.; Deng, P.; Zhou, Z.; Yu, Z. 50 Hz electromagnetic fie ld exposure promotes proliferation and cytokine production of bone marrow mesen-chymal stem cells. Int. J. Clin. Exp. Med. 2015, 8, 7394–7404.
69. Trzyna, A.; Pikula, B.; Ludwin, A.; Kocan, B.; Banas-Zabczyk, A. The influence of an electromagnetic field on adipose-d erived stem/stromal cells' growth factor secretion: Modulation of FGF-2 production by in vitro exposure. Arch. Biol. Sci. 2020, 72, 339–347.
70. Rodrigues, M.; Kosaric, N.; Bonham, C.A.; Gurtner, G.C. Wound Healing: A Cellular Perspective. Physiol. Rev. 2019, 9 9, 665–706.
71. Ahmadian, S.; Zarchi, S.R.; Bolouri, B. Effects of extremely-low-frequency pulsed electromagnetic fields on collagen sy nthesis in rat skin. Biotechnol. Appl. Biochem. 2006, 43, 71–75.
72. Godina-Nava, J.J.; Eduardo-Ambrosio, P.; Sanchez-Dominguez, D. Comparative analyzes of 120 Hz Electromagnetic F ield, respect the interferon-β and Transfer Factor effect in the recovery of chronic ulcers measuring the frequency of the lymphocytes CD4+ and CD8+ in an animal model. J. Phys. Conf. Ser. 2019, 1221, 012056.
73. Percival, S.L.; Vuotto, C.; Donelli, G.; Lipsky, B.A. Biofilms and Wounds: An Identification Algorithm and Potential Treat ment Options. Adv. Wound Care 2015, 4, 389–397.

74. International Wound Infection Institute. Wound Infection in Clinical Practice; International Wound Infection Institute: London, UK, 2016.
75. Malone, M.; Bjarnsholt, T.; McBain, A.J.; James, G.A.; Stoodley, P.; Leaper, D.; Tachi, M.; Schultz, G.; Swanson, T.; Wolcott, R.D. The prevalence of biofilms in chronic wounds: A systematic review and meta-analysis of published data. J. Wound Care 2017, 26, 20–25.
76. Ganesh, K.; Sinha, M.; Mathew-Steiner, S.S.; Das, A.; Roy, S.; Sen, C.K. Chronic wound biofilm model. Adv. Wound Care 2015, 4, 382–388.