Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2023**; 28(11): 285
https://doi.org/10.31083/j.fbl2811285

Review

Preconditioning of Mesenchymal Stem Cells with Electromagnetic Fields and Its Impact on Biological Responses and "Fate"—Potential Use in Therapeutic Applications

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Academic Editor: Viviana di Giacomo
Submitted: 29 July 2023 Revised: 26 September 2023 Accepted: 12 October 2023 Published: 8 November 2023

Abstract

Mesenchymal stem cells (MSCs) offer great potential for use in stem cell-based therapies due to their unique regenerative potential via reconstructive and paracrine capacities. These therapies offer new hope for patients suffering from conditions that have no cure. Currently, mesenchymal stem cells (from adipose tissues, bone marrow, and umbilical cords) are most interesting for application in those therapies. Nevertheless, the development of MSC-based medical products requires thorough research and standardization that maximizes the therapeutic effect while minimizing side effects. One of the interesting novel approaches to achieving this goal is combining MSC therapy with an electromagnetic field (EMF). Many studies have shown that EMF can enhance the regenerative properties of MSCs by influencing stem cell fate through modulating differentiation, proliferation, cell cycle regulation, metabolism, and cytokine and growth factor secretions. Combination therapy of EMF-MSCs is a promising perspective; however, it is important to select appropriate EMF parameters to obtain beneficial therapeutic effects. Therefore, understanding the mechanisms involved in the EMF impact on MSCs is crucial. In this study, we provide an overview of the effects of EMF on the biological response and "fate" of MSCs, paying attention to the gaps in research that remain unfilled and discuss the clinical application of this approach.

Keywords: MSCs; EMF; proliferation; metabolism; mitochondria; ion influx; trophic activity; secretome; stem cell-based therapy

1. Introduction

The development of science over the previous decades has added to the understanding of the biological basis involved in many diseases and their pathogenesis, which, together with technological breakthroughs, enables the development of more effective and precise treatment methods. Regenerative medicine is a relatively new branch of medicine, which is an interdisciplinary field that deals with the development of treatment techniques aimed at restoring health through the optimization and implementation of methods that ensure the restoration and/or replacement of damaged cells, tissues, and organs [1,2].

Stem cell-based therapy, as a branch of regenerative medicine, provides a potential therapeutic option via stem cells and their unique properties, such as an ability to self-renew and the potential to differentiate. Stem cell-based therapies bring new hope to patients with incurable diseases and disorders. Stem cells can be categorized by their differentiation potential (totipotent, pluripotent, multipotent, and unipotent) or by their source of origin (embryos, adult tissues, or reprogramed somatic cells) [3,4]. Currently, the greatest interest for use in regenerative therapy is induced pluripotent stem cells (iPSCs) and adult stem cells [1,4]. The iPSCs have great potential because of their pluripotency and lack of ethical concerns (in contrast to pluripotent embryonic stem cells). However, they have higher tumorigenic potential than multipotent stem cells, which is one reason (next to their unique advantages) why multipotent mesenchymal stem cells (MSCs) are currently most widely used in clinical and preclinical studies [5].

MSCs—first discovered by Friedenstein *et al.* [6] in bone marrow—are a type of stem cell found in adults that are of great interest in regenerative medicine owing to their unique properties, which offer potential use in stem cell-based therapies [1,7]. Importantly, the therapeutic application of MSCs is based on their reconstructive effect on damaged cells and tissues and relates to their differential properties. However, their paracrine function and ability to secrete cytokines, enzymes, growth factors, and chemokines can also promote regeneration processes [4,7]. To standardize the concept of applying MSCs in regenerative medicine, the International Society for Cellular Therapy (ISCT) published a minimum criteria for defining MSCs. According to the ISCT, they should be plastic-adherent and capable of differentiation into chondroblasts, osteoblasts, and adipocytes *in vitro* as well as express specific surface markers $\geq$95% CD105, CD90, and CD73 but stay negative for CD45, CD34, CD14 or CD11b, CD79alpha or CD19, and HLA-DR [8]. Currently, the greatest interest is focused on the use of adipose-derived mesenchymal stem cells (ASCs), and bone marrow MSCs (BM-MSCs). However, adipose tissue seems to be a better source of MSCs for use in stem cell-based therapy due to their improved secretion of

paracrine factors, delayed aging *in vitro*, easier tissue acquisition for MSC extraction, and more efficient isolation [7].

The regenerative potential of MSCs has been proven by many research groups. When using MSCs in stem cell-based therapy, it is extremely important to standardize and optimize procedures that maximize the therapeutic effect, while concurrently minimizing side effects. One intensively researched tool to enhance stem cell-based therapy is electromagnetic fields (EMFs), which have been shown to impact MSC biology by influencing proliferation, symmetric and asymmetric cell division, differentiation, cell cycle progression, metabolism, and cytokine and growth factor secretions [9–13]. EMFs are areas of energy in the form of a combined effect of magnetic and electric fields. It can be grouped into two categories, which are based on its frequency: non-ionizing, which is characterized by low radiation (energy), such as static EMF (0 Hz), extremely low (1–300 Hz), and low-frequency EMF (300 Hz–100 kHz), radiofrequency (100 kHz–300 MHz), microwaves (300 MHz–30 GHz), and infrared; ionizing radiation, which is characterized by high radiation levels, such as ultraviolet, x-rays, and gamma-rays [14–16]. EMF can act externally and affect cells in the human body, although the cells are also characterized by their own natural endogenous, ultra-weak EMF, which is potentially generated by polar biological structures [11]. The discovery of electromagnetic radiation and the rapid improvement of technology has led to the development of medicine and new diagnostic techniques (e.g., magnetic resonance imaging) as well as therapies (e.g., tumor treating fields (TTF)) but also to improving the awareness of the possible positive and negative impacts by EMFs on cells and the human body [14]. In the context of regenerative medicine, which is a relatively new branch of medicine, most of the scientific papers that have published positive effects of EMFs have tested low-intensity EMFs, thereby showing that non-ionizing EMFs can be a useful tool in stem cell-based therapies e.g., bone and cartilage repair or wound healing [15]. EMF preconditioning (with appropriately selected parameters), which modulates the trophic activity of MSCs, may stimulate proliferation, chondrogenesis, osteogenesis, and neurogenesis, thereby enhancing the regenerative properties of those cells [10,17].

Non-ionizing EMFs are currently being studied, and their impacts will be observed in the future. Thus, EMF can provide a very helpful, and, importantly, non-invasive biophysical tool to support stem cell-based therapy. The selection of appropriate EMF parameters, which can result in a positive biological effect that supports its therapeutic potential is crucial. In this review paper, we discuss the biological effects of extremely low-frequency electromagnetic fields (LF-EMFs) on MSCs and consider their safety and use in clinical applications.

2. Source Tissues for Mesenchymal Stem Cells

Currently, mesenchymal stem cells are often the most considered stem cells for use in clinical applications [5]. MSCs can be found in different human tissues, such as adipose tissue (AT), bone marrow (BM), skin, dental pulp, peripheral blood, lung, heart, hair, umbilical cord, Wharton's jelly, umbilical cord blood, and the placenta [1,7,18]. However, the longest utilized and most commonly considered adult tissue sources of MSCs in clinical and preclinical applications are bone marrow and adipose tissues [7,18], which we briefly describe below.

2.1 Adipose Tissue

Adipose tissue contains a heterogeneous population of cells, which only consist of approximately 20% lipid-rich adipocytes [19]. The rest of the cell population comprises stem cells, neural and vascular progenitor cells, multipotent progenitor cells, pericytes, fibroblasts and endothelial cells, eosinophils, macrophages, and innate lymphoid cells (ILCs), T cells, and B cells [7,19]. Adipose tissues also contain adipokines, including leptin and adiponectin, which can be considered as stem cell inducers [19,20].

There are two main types of adipose tissue, which differ in function—white (WAT) and brown (BAT) adipose tissue. WAT is responsible for the main energy storage in the organism, while BAT is an energy expenditure and protective tissue [7,20]. Moreover, it has been shown that adipocytes from WAT can undergo differentiation in adaptive responses and reversible change functions that make them similar to BAT cells (beige AT), thereby demonstrating the plasticity of adipose tissue [20]. WAT is localized subcutaneously, intra-abdominally, epicardially, and gonadally, while BAT is interscapular, paravertebral, perirenal, cervical, and supraclavicular [21]. However, due to the ease and non-invasive harvesting procedure by liposuction, subcutaneous AT is most widely considered as a source of adipose-derived mesenchymal stem cells rather than its other localizations in the human body [7]. Importantly, uncultured stromal vascular fractions (SVFs) from adipose tissues can usually obtain up to 3% of the ASCs. However, it has been shown that SVFs from subcutaneous liposuction aspirates can contain even 10% of the ASCs [19,22].

In clinical use, the type of adipose tissue, its harvesting area, tissue origin, age, weight, disease state, race and ethnicity, and body mass index of the donor are important factors in providing high-quality ASCs [7,22]. It has previously been shown that ASC proliferation, differentiation potential, and growth factor secretion from older donors are lower [23–26]. Moreover, the more the patient weighs may also affect lower self-renewal and angiogenic capacity but can also provide a higher adipogenic potential in isolated ASCs [7,27,28]. It has been also shown that smoking, diabetes mellitus, hypercholesterolemia, and hypertension have negative effects on the regenerative potential of ASCs [27,29].

2.2 Bone Marrow

Bone marrow is a source tissue from which mesenchymal stem cells are isolated, as defined for the first time by Friedenstein *et al.* [6]. Although it is commonly used for research in regenerative medicine, BM as a source of MSCs also has its limitations. Obtaining tissue to isolate MSCs is an invasive procedure that can cause the patient pain and infection [24,30].

Bone marrow, as a site for hematopoiesis, contains hematopoietic (blood cells lineages committed progenitors, stem cells) and non-hematopoietic cells (nervous system cells, adipocytes, fibroblasts, osteoblasts, osteocytes, osteoblastic precursors, mesenchymal stem cells) [6,31]. There are two types of BM that differ in function—red (where hematopoiesis occurs) and yellow (fat storage) [32].

It is worth noting that bone marrow is a less effective source of MSCs than adipose tissue. While MSCs form 3% of adipose tissue, their content is even lower in BM (0.002%) [19]. Moreover, BM-MSCs are characterized by faster senescence and lower proliferation capacities than ASCs during expansion, and it is very important to provide medical products of high quality as fast as possible to treat the patient [1]. Adipose tissue is also a better candidate for use in regenerative medicine due to its similar properties for differentiation as BM-MSCs, yet has an easier obtaining procedure and culture expansion. However, some studies have reported better osteogenic potential in BM-MSCs than ASCs, although those studies were performed on MSCs acquired from different donors. Additionally, the MSC origin may be crucial and adaptation of the source tissue for medical purposes may depend on the disease [1,24].

3. Effect of Electromagnetic Fields on Mesenchymal Stem Cells

Low-frequency electromagnetic fields have been shown to influence the biological processes of mesenchymal stem cells by incorporating differentiation, proliferation, trophic activity, cell division, and metabolism, and thus, stem cell fate [10–13]. Since EMFs are one of the factors that impact stem cell biology, it is very important to understand the mechanism of action on MSCs by EMFs to anticipate both the positive therapeutic effects and possible negative side effects [10]. Below, we discuss the influence of EMFs on the proliferation and cell cycle, trophic factors secretion, metabolism, and ions flow of MSCs. To our knowledge, this review and data collection on this subject are the first to be reported in the literature. Extensive discussion on the effects of EMFs and the mechanisms on the differentiations of MSCs was deliberately omitted from this review as there are many up-to-date, well-written scientific papers that have already covered the subject [9,10,12,33].

3.1 Proliferation and Cell Cycle

Many studies have shown that appropriate EMF stimulation affects the proliferative potential and cell cycle of MSCs; however, various different effects have been observed, which suggests that the effects are dependent on the EMF parameters and stimulation times [12,13,34–47].

A frequency of 50 Hz is a recognized parameter of electromagnetic fields emitted by everyday devices and has aroused the interest of researchers [34]. Li *et al.* [35] showed that pulsed electromagnetic fields (PEMF) (50 Hz; 10 mT) stimulation can increase the proliferation of rat MSCs after 3 and 6 h of exposure but also influence the cell cycle by increasing the percentage of cells in the G1 phase. Importantly, in that study, EMF exposure did not influence stem cell viability. Results by Li *et al.* [35] were a one-time effect, since, after 16 h of EMF exposure, no effect was again observed on MSC proliferation and cell cycle. In other studies, MSCs were repeatedly exposed to EMFs during the culture time with different outcomes. Fan *et al.* [36] and Seo *et al.* [37] showed that MSC proliferation increased after EMF exposure (50 Hz; 1 mT). Conversely, in work conducted by Seo *et al.* [37], the observed changes in proliferation were not statistically significant. Interestingly, Fan *et al.* [36] showed smaller changes in G2-phase cell percentage, contrary to Li *et al.* [35]. However, in both works, the authors indicated changes in the percentage of MSCs in S-phase post-exposure; nevertheless, as noted by Li *et al.* [35], this effect appeared detectable and significant 1-hour post-EMF exposure but not after 16 h [36]. This observation suggests that MSCs undergo adaptive responses to environmental conditions, such as EMFs, thereby indicating that stem cell fate can be modulated.

Alongside the 50 Hz frequency, scientists have also often studied the effects of EMFs with a frequency of 15 Hz. The results published in various research papers showed that 15 Hz can increase proliferation. Data published by Jazayeri *et al.* [38] showed that proliferation, after EMF exposure (15 Hz; 0.2 mT), increased, and the longer the culture underwent EMF stimulation the higher the effect. It has been shown that seeding density does not influence proliferation changes by EMF (15 Hz) stimulation; however, Sun *et al.* [39] showed that the slight observed increase reflected the entry of more bone marrow MSCs into the G2/M-phase at the beginning of the experiment. The number of cells at the G2/M-phase increased at the beginning and decreased after 18 h along with the number of MSCs in the S-phase, although S-phase was not affected at the beginning. Sun *et al.* [39] also showed that after 18 and 24 h of EMF exposure, a statistically significant higher percentage of MSCs entered into G0/G1-phase, although this effect was not observed after 30 h of EMF exposure. Song *et al.* [40] also discovered that the proliferation of MSCs after EMF stimulation (15 Hz; 1 mT) increased significantly after 7 and 10 days, however, this effect did not last until day 14. Similarly, using the same EMF parameters (15 Hz; 1.5 mT), other studies have also shown increased proliferation after 7 days of EMF stimulation [12,41,42].

Other frequencies of EMF than 15 Hz and 50 Hz were also studied. Ross *et al*. [43] showed a slight increase in BM-MSC proliferation using EMFs (5 Hz; 0.4 mT) for 10 min per day. However, an improved biological effect was achieved by Bloise *et al*. [44] (75 Hz; 2 mT, 20 min/day). A slight increase in proliferation was also shown by Poh *et al*. [45] (26 Hz). Thus, several studies showed EMF can increase MSC proliferation, yet Zhang *et al*. [13] showed no significant changes in proliferation after EMF stimulation (7.5, 15, 30, 50, 75 Hz; 1 mT) alongside results presented by Parate *et al*. [46] (15 Hz; 2 mT). Moreover, Yan *et al*. [47] indicated that EMF treatment (50 Hz; 20 mT) can inhibit MSC proliferation. The different studies on the effects of the influence of EMFs on MSC proliferation are presented in Table 1 (Ref. [12,13,34–48]).

The mechanisms involved in EMFs (with different parameters) influencing MSCs remain unclear owing to a lack of exploration. However, it is known that the MEK/ERK signaling pathway is involved in cell proliferation, while it has been shown that EMFs (15 Hz; 1 mT) stimulate proliferation through this pathway [40]. Similarly, EMFs (26 Hz) can increase the number of phosphorylated ERK 1/2 proteins suggesting that EMFs activate the MEK/ERK pathway in MSCs [45]. EMFs can induce cellular stress and promote increased reactive oxygen species (ROS) generation, which can become free radical ions. ROS are highly reactive signaling factors that are naturally produced by cells, and depending on concentration may have different effects on cells, including inhibiting proliferation [11]. Moreover, ion influx changes (K^+ and Ca^{2+} affect activated potassium channels) after EMF treatment may affect G1- to S-phase cell cycle progression in undifferentiated MSCs, thereby promoting proliferation (cell control proliferation process mostly occurs at phase G1) [38,39,49]. These determinations correlate with the requirement of prolonged ERK 1/2 activation for cyclin D1 transcription, which promotes cells from G1- to S-phase [40,49]. As shown by Marędziak *et al*. [50], static magnetic fields (sMFs, 0 Hz; 0.5 mT) can also promote the proliferation of MSCs via the PI3K/Akt pathway. Similarly, Poh *et al*. [45] and Ferroni *et al*. [51] showed that activation of the Akt signaling cascade in MSCs after EMF exposure (26 Hz), alongside ERK 1/2 activation, can promote proliferation. Importantly, extrinsic factors, such as EMFs, can potentially influence symmetric and asymmetric stem cell divisions via the targeting of tubulin dipoles in the spindle microtubules during cell division [11,52]. Interestingly, it has been also shown that EMF (50 Hz; 1 mT) can increase the proliferation of human periodontal ligament mesenchymal stem cells (PDLSCs) *in vitro* [48].

Overall, EMFs can have different effects on the proliferation of MSCs depending on the parameters and duration of stimulation. EMFs have the capacity to enhance proliferation but only at specific parameters, which can be a useful tool in stem cell-based therapy, to accelerate the regeneration process via an increased number of cells capable of differentiation as well as cytokine and growth factor secretions.

3.2 Secretome Modulation

MSCs offer great potential for use in regenerative medicine because of their unique secretome. In particular, ASCs deserve special attention here in relation to cell therapy owing to the extensive number of factors that they secrete, especially cell-free therapy. ASCs secrete cytokines, proteins, growth factors, and non-coding RNAs, which are carried by exosomes, and all offer great therapeutic potential. Most importantly, it was shown that EMFs with appropriate parameters can modulate the secretion of these compounds [7,53].

EMFs can enhance regenerative properties by modulating specific signaling pathways, gene expression, and protein secretion (Table 2, Ref. [17,36,44,46,54–58]). Immunomodulatory properties can be also mediated by EMFs [36,46,54]. Fan *et al*. [36] showed increased expression of cytokines, such as IL-11, IL-7, LIF, SCF, M-CSF, and TPO by BM-MSCs after EMF (50 Hz; 1 mT) stimulation but no influence in IL-6, IFN-γ, and TNF-α was observed. In contrast, it was also shown that EMF (5.1 Hz; 0.4 mT) can reduce the secretion of proinflammatory cytokine IL-6 as well as other proinflammatory molecules, such as IL-1b, IL-17A, and TNF-α. Ross *et al*. [54] also showed that EMF stimulation may stabilize the secretion of anti-inflammatory cytokines (IL-3, IL-4, and IL-10). However, Parate *et al*. [46] showed that EMF stimulation can significantly increase IL-10 secretion as well as BMP-2, BMP-4, TSP-2, and IL-1Ra by MSCs in non-differential medium after EMF exposure (15 Hz; 3 mT). Importantly, conditioned medium (CM) after EMF stimulation showed properties to reduce inflammation and the cellular apoptosis of chondrocytes and naive MSCs [46].

EMFs can also modulate the trophic activity of MSCs. It has been shown that EMFs increase the secretion of IGF-2 [46], VEGF [17,46,50,55,56], FGF-2, and PDGF [17], although the efficiency was dependent on the EMF parameters being applied. Studies also have shown that EMFs can influence TGF and BMP protein secretion, which is linked to the capacity of EMFs to enhance MSC differentiation [46,57,58]. EMFs (75 Hz; 2 mT) have been shown to slightly increase the deposition of osteogenic proteins (ALP, COL, FN, OPN, OSC, and OSN) in the cell matrix in a proliferative medium [44]. EMFs can also impact BDNF and NGF secretion [17,55]. Huang *et al*. [55] showed that treating BM-MSCs with PEMFs (50 Hz, 1 mT) significantly increased BDNF and VEGF expression *in vitro* via the Wnt/β-catenin pathway. Moreover, they proved that the use of BM-MSCs and EMF stimulation increased BDNF, NGF, and VEGF expressions *in vivo* alongside enhancing neuron preservation and increasing axonal growth in mice with spinal cord injuries. It is important to note that the

Table 1. Effects of electromagnetic fields on proliferation and cell cycle progression by mesenchymal stem cells.

Biological model	EMF parameters	Exposure duration	Biological effect	Study reference
Rat MSCs	PEMF (50 Hz; 10 mT)	3 h and 6 h	Time-independent increase in proliferation. Increased percentage of cells in G1 phase.	[35]
Rat BM-MSCs	EMF (50 Hz; 1 mT)	4 h/day for 3 days	Increased proliferation. Increased percentage of cells in S-phase.	[36]
Rat BM-MSCs	EMF (50 Hz; 1 mT)	1 h/day for 5, 7 and 10 days	Slight but not statistically significant increase in proliferation.	[37]
Human BM-MSCs	EMF (7.5, 15, 30, 50, 75 Hz; 1 mT)	24 h	No effect on MSCs proliferation for any of the tested frequencies.	[13]
Human ASCs	PEMF (26 Hz)	3, 7, and 14 days	Slight but not statistically significant increase in proliferation.	[45]
Human BM-MSCs	PEMF (15 Hz; 2 mT, 3 mT)	30 min	No significant changes in proliferation.	[46]
Human BM-MSCs	EMF (50 Hz; 20 mT)	12 h/day	Inhibited proliferation.	[47]
Rat BM-MSCs	EMF (15 Hz; 0.2 mT)	6 h/day for 5, 10, and 14 days	Increase in MSC proliferation.	[38]
Human BM-MSCs	EMF (15 Hz)	8 h/day for 10 days	Slight increase in MSC proliferation. Significant, time-dependent changes in G2/M- and S-phases.	[39]
Rat BM-MSCs	EMF (15 Hz; 1 mT)	1 h/day for 14 days	Increased proliferation.	[40]
Rat BM-MSCs	EMF (15 Hz; 1 mT)	4 h/day for 7 days	Increase in MSC proliferation.	[12]
Rat BM-MSCs	EMF (15 Hz; 1mT)	4 h/day for 7 days	Increase in MSC proliferation.	[41]
Human ASCs	PEMF (15 Hz; 1 mT)	8 h/day for 7, 14, and 21 days	Increase in proliferation while culturing cells on self-assembled peptide hydrogel with and without nanoparticles.	[42]
Human BM-MSCs	EMF (5 Hz; 0.4 mT)	10 min/day for 15 days	Slight but not statistically significant increase in proliferation.	[43]
Human BM-MSCs	EMF (75 Hz; 2 mT)	20 min/day, 3 times per week for 14 days	Increase in MSC proliferation.	[44]
Human ASCs	EMF (0 Hz; 0.5 mT)	Continuous exposure for 7 days	Increase in MSC proliferation.	[34]
Human PDLSCs	EMF (50 Hz; 1 mT)	6 h/day for 28 days and 10 days	Increase in MSC proliferation.	[48]

EMF, electromagnetic field; MSCs, mesenchymal stem cells; BM-MSCs, bone marrow mesenchymal stem cells; ASC, adipose-derived mesenchymal stem cells; PEMF, pulsed electromagnetic fields.

MSC secretome is often enclosed in microvesicles, which encapsulate target molecules as a specific drug system delivery [58]. However, in the literature, to the knowledge of the authors of this manuscript, there is a lack of studies on the influences of EMFs on human MSCs exosome secretion and their cargo. Moreover, there is still a small number of studies showing the effect of EMFs on the biology of ASCs, while they are great candidates for stem cell-free therapy [7].

The ability to enhance the anti-inflammatory properties of MSCs may represent an important new approach for the treatment of autoimmune diseases. Moreover, modulating the concentrations of cellular trophic factors may provide a crucial improvement in cell and tissue regeneration. However, as described and summarized in Table 2, the effects may vary depending on the EMF parameters and exposure time.

3.3 Mitochondrial Function

Mitochondria are extremely important organelles since they undertake the main processes of energy metabolism and adenosine triphosphate (ATP) production. These organelles also control various processes, such as cell signaling, ROS production, as well as calcium ion flow and concentration, thereby playing an important role in the proper course of key biological functions, such as differentiation or apoptosis. In response to internal and external signals, mitochondria initiate dynamic processes, such as mitochondrial fusion and fission, increasing mtDNA copy and respiratory enzymes level, enhancing oxygen consumption rate (OCR), and intracellular ATP levels to meet the metabolic demands of the cell [59,60].

According to existing data, changes occurring under the influence of EMFs suggest that MSCs are vulnerable to this biophysical factor and try to adapt to new environmental conditions by changing their metabolism. MSCs are

Table 2. Electromagnetic fields affect the secretome of mesenchymal stem cells.

Biological model	EMF parameters	Exposure duration	Biological effect	Study reference
Rat BM-MSCs	EMF (50 Hz; 1 mT)	4 h/day for 3 days	Increase in IL-11, IL-7, LIF, SCF, M-CSF, and TPO expression. No influence on IL-6, IFN-γ, and TNF-α expression.	[36]
Mice BM-MSCs	PEMF (50 Hz; 1 mT)	3 h/day	Significant increase in BDNF and VEGF expression through Wnt/β-catenin pathway.	[55]
Mice BM-MSCs transplanted into spinal cord injury mice model	PEMF (50 Hz; 1 mT)	1 h/day for 8 weeks	Combined transplantation of BM-MSCs and PEMFs increases the expression of BDNF, NGF, and VEGF *in vivo*.	[55]
Human BM-MSCs	PEMF (3 Hz; 80 mT, 150 mT)	10 min/day for 14 days	Significant increase in VEGF secretion after 7 days.	[56]
Human BM-MSCs	PEMF (15 Hz; 1G)	21 days	Significant increase in TGF-ß after 9 days.	[57]
Human ASCs	EMF (50 Hz; 1.5 mT)	3 days	Significant increase in FGF-2 concentration after 1 and 2 days. Significant decrease in FGF-2 after 3 days. Slight increase in SCF, VEGF-D, VEGF-A, BDNF, and PDGF-BB after 48 h.	[17]
Human BM-MSCs	PEMF (15 Hz; 3 mT)	10 min	Significant increase in BMP-2, BMP-4, TSP-2, IL-1Ra, and IL-10 secretion. Slight increase in TGF-ß1, TGF-ß3, IGF-2.	[46]
Human BM-MSCs	EMF (75 Hz; 2 mT)	20 min/day, 3 times per week for 14 days	Slight increase in selected osteogenic proteins in proliferative medium (ALP, COL, FN, OPN, OSC, and OSN).	[44]
Equine ASCs	EMF (0 Hz; 0.5 mT)	7 days	Increase in BMP-2 and VEGF and decrease in TNF-α secretion via microvesicles.	[58]
Human BM-MSCs	EMF (5.1 Hz; 0.4 mT)	5 min	Decrease in IL-1b, IL-6, IL-17A, and TNF-α secretion. Increase in IL-3, IL-4, and IL-10 secretion and stabilization.	[54]

IL, Interleukin; LIF, leukemia inhibitory factor; SCF, stem cell factor; M-CSF, macrophage colony-stimulating factor; TPO, thrombopoietin; IFN, Interferon; TNF, tumor necrosis factor; FGF-2, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; TSP, thrombospondin; TGF, transforming growth factor; BMP, bone morphogenic protein; IGF, insulin-like growth factor; ALP, alkaline phosphatase; COL, Collagen; FN, fibronectin; OPN, osteopontin; OSC, osteocalcin; OSN, osteonectin.

characterized by low mitochondrial activity and glycolytic state due to their dormant form in stem cell niches unless cells undergo differentiation (changing their fate), which requires a high energy demand [9,60,61]. Hollenberg *et al.* [9] showed that EMFs (10 Gauss for 4 days) significantly increased mitochondrial membrane potential in BM-MSCs indicating increased oxidative phosphorylation (OxPhos), and thus, mitochondrial activity and ATP production. Furthermore, a study conducted by Ehnert *et al.* [62] showed that EMFs (16 Hz and 26 Hz) can significantly increase mitochondrial activity in an osteoblast and ASC co-culture after 7 and 14 days (5 times per week for 7 min each day). Interestingly, Celik *et al.* [63] showed that the X-directed application of PEMFs (1 mT) has a greater effect on mitochondrial respiration than Z-directed stimulation. EMFs can influence the influx of ions, and thus, the mitochondrial membrane potential [9,33]. Since mitochondria store Ca^{2+} and EMF can modulate calcium ion influx and disrupt Ca^{2+} homeostasis, electromagnetic fields are likely to affect mitochondrial activity through calcium ion oscilla-

Table 3. Electromagnetic fields affect the mitochondria of mesenchymal stem cells.

Biological model	EMF parameters	Exposure duration	Biological effect	Study reference
Human BM-MSCs	EMF (10 G)	4 days	Increased mitochondrial activity.	[9]
Rat BM-MSCs	EMF (50 Hz; 5 mT)	30 min, 1 h and 6 h/per day for 2 days	Increased mitochondrial DNA content.	[65]
Co-culture of human ASCs and osteoblasts	EMF (16 Hz and 26 Hz)	14 days (5 times per week for 7 min each day)	Increased mitochondrial activity.	[62]
Human BM-MSCs	PEMFs administrated at Z and X direction (1 mT)	10 min	Increased mitochondrial activity, greater for X-directed stimulation.	[63]

tions. Then, mitochondrial activation can lead to further changes in MSCs and new stem cell fate decisions [33,44]. It is also worth noting that ASCs, similar to BM-MSCs, use a mixed metabolism that is based mainly on glycolysis and mitochondrial activity, which is reflected in the secretion of lactate and citrate. However, it has been shown that even ASCs isolated from the same type of adipose tissue, i.e., white adipose tissue, may differ in metabolism, which indicates the heterogeneity of this tissue. Lefevre *et al*. [64] demonstrated that mesenchymal stem cells from visceral adipose tissue (V-ASC) secrete higher concentrations of lactate than those isolated from subcutaneous adipose tissue (S-ASC), indicating their greater preference for glycolysis. Moreover, they showed that S-ASCs in cell culture without glutamine limit the pyruvate pathway towards lactate synthesis, yet the same does not occur in the V-ASCs. These differences should be considered in further studies on the effects of EMFs on ASC metabolism.

EMFs can both influence mitochondrial activity by switching glycolysis to OxPhos and increase mitochondrial DNA (mtDNA) copy numbers. Bai *et al*. [65] showed that EMF (50 Hz; 5 mT) increased the mtDNA copy number of BM-MSCs in a time-dependent manner, which may suggest that cells prepare for an increase in mitochondrial activity and higher cell energy demand needed for stem cell differentiation or division. It is worth mentioning that EMFs might influence mitochondrial migration, and the results we obtained showed that cells stimulated with EMFs (50 Hz; 1.5 mT) had mitochondria located closer to the periphery of the cell [data in press]. This finding may be important in understanding the regenerative potential of EMF-stimulated MSCs via mitochondrial transfer to other cells [60] since there are not many studies investigating mitochondrial transfer in physiological conditions [66].

Studies have shown that EMFs can influence mitochondria functioning; however, the clear mechanism of action remains unknown (Table 3, Ref. [9,62,63,65]). It is suggested that EMFs induce mitochondrial ROS production on a non-toxic level (activating TRPC1-mediated calcium entry) as well as interfere in Ca^{2+} homeostasis in the mitochondria, which in turn may activate pathways responsible for mtDNA replication, OxPhos activation, and stem cell differentiation [33,62,63,65].

3.4 Ions Influx and Cellular Membrane Potential

Electromagnetic fields can affect the ion influx of MSCs by inducing the vibration of free ions and disturbing the electrochemical potential on the cell membrane surface. This process may in turn affect the transmembrane receptors, phospholipids, and in effect specific pathways that modulate stem cell proliferation, differentiation, viability, metabolism, apoptosis, cell–cell communication, and signaling with extracellular matrix components that influence the stem cell fate [33,67]. Below, we present studies (Table 4, Ref. [13,44,47,68–71]) and briefly describe the influence of EMFs on K^+, Na^+, and Ca^{2+} channels and ions influx only in MSCs. However, most studies have focused on the examination of calcium channels and Ca^{2+} flow after EMF exposure. Moreover, to the knowledge of the authors, no studies have been performed on the effects of EMFs on the flow of ions in ASCs.

Yan *et al*. [47] showed increased K^+ and Na^+ ion concentrations in BM-MSC culture supernatants after EMF exposure (50 Hz; 20 mT). Moreover, it has been shown on different cell lines that LF-EMFs affect the K^+ current flow through the cell membrane by affecting the A-type K^+, delayed rectifier K^+, M-type K^+, fast-inactivating transient (IK, A), and dominant-sustained (IK, V) potassium channels [33]. It is also suggested that calcium-activated potassium channels have a significant influence on the differentiation of MSCs via intracellular calcium oscillation and membrane potential [72].

EMFs can also increase calcium currents [72] and the intracellular concentration of calcium ions in MSCs [13,44,47,68] by activating the L-type voltage-gated calcium channels (VGCCs) through membrane depolarization [68,69] and significantly increasing the expression of VGCC-related genes (CACNA1E and CACNA1G) [67,69]. Interestingly, two independent studies showed that EMF exposure at different parameters did not influence CACNA1C gene expression related to the VGCC [69,70]. Moreover, it has been shown that EMFs increased the expression of the TRPC1 and TRPV4 genes involved in the entry of calcium through the cell membrane [70] as well as P2X7 purinergic receptors related to the transport of sodium, calcium, and potassium [71]. Influencing calcium channels may have an effect on the differentiation and migration of MSCs, while Zhang *et al*. [13] showed that EMF-

Table 4. Electromagnetic fields affect ion influx and membrane potential of mesenchymal stem cells.

Biological model	EMF parameters	Exposure duration	Biological effect	Study reference
Human BM-MSCs	PEMF (75 Hz; 2 mT)	10 min/day	Increased Ca^{2+} currents and concentration of intracellular Ca^{2+} via effecting L-type VGCCs.	[68]
Human BM-MSCs	PEMF (50 Hz; 1 mT)	24 h	Increased concentration of intracellular Ca^{2+} that activated the FAK/Rho GTPase migratory pathway.	[13]
Human BM-MSCs	EMF (50 Hz; 20 mT)	12 h/day	Increased concentrations of K^{+} and Na^{+} in cell culture supernatants. Slight difference in calcium deposition.	[47]
Human BM-MSCs	EMF (75 Hz; 2 mT)	20 min/day, 3 times per week for 14 days	Increased concentration of intracellular Ca^{2+} in proliferative and osteogenic medium while culturing on nano-TiO2 surfaces.	[44]
Human BM-MSCs	EMF (45 Hz; 1 mT)	Twice every 8 h/day for 7 days	Significantly increased expression of L-type VGCCs CACNA1E and CACNA1G but only slight increase of CACNA1C and CACNA1I.	[69]
Human BM-MSCs	PEMF (15 Hz; 2 mT)	10 min	Increased expression of TRPC1 and TRPV4 genes related to channels for calcium entry. No significant influence on L-type VGCC gene expressions (CACNA1C and CACNA2D1).	[70]
Human BM-MSCs	EMFs (7.5, 15, 30, 50, and 75 Hz; 1 mT)	8 h/day	Increased purinergic receptor P2X7 expression.	[71]

VGCCs, voltage-gated calcium channels; FAK/Rho GTPased, focal adhesion kinase/ras homologous guanosine triphosphatase.

mediated changes in Ca^{2+} concentration activated the Focal Adhesion Kinase/Ras homologous Guanosine Triphosphatase (FAK/Rho GTPased) migratory signaling pathway [33]. The importance of Ca^{2+} influx may be due to its possible modulation of calpains, which are involved in post-translational protein regulation, as well as epigenetic regulation via DNA and histone modifications [73].

Electromagnetic fields can influence ion influx, and thus, regulate stem cell functioning, which may be useful in the context of using MSCs as therapeutic treatments. Calcium has the potential to regulate stem cell fate mediated by EMF, while the disturbance of this concentration may lead to both desired and undesirable effects, such as faster differentiation, increased proliferation, or cell death. More research is needed on the effects of EMFs to explore the impact of EMFs on the flow of ions in MSCs, especially from sources other than BM-MSCs.

3.5 Differentiation

Many *in vitro* and *in vivo* studies have demonstrated that electromagnetic fields can also influence the differentiation of mesenchymal stem cells [9,10,12,33]. EMFs influence the influx of ions, and thus, the concentration across the cell membrane and transmembrane potential. In turn, this influences cell–cell communication and cell physiological processes, thereby modulating stem cell fate by triggering epigenetic changes and gene expression towards the activation of differentiation pathways [10,11,33,74]. Electromagnetic fields, as suggested by Bai *et al.* [65], may cause the MSCs to become more sensitive to environmental changes, which leads to easier differentiation. In fact, EMFs influence Ca^{2+} influx. Calcium is a known cyclic AMP activator—a crucial component that triggers metabolic processes [10]—indeed, studies have already demonstrated that during differentiation, the demand for energy increases, which causes the mitochondria to alter [9,62,63,65]. Moreover, the induction of ROS, which are reactive signaling factors, through EMF stimulation, can affect ATP production and activate differentiation pathways [9–11]. Easier differentiation of MSCs after EMF stimulation may also be affected by its influence on spindle microtubules (due to tubulin dipoles), thereby leading to asymmetrical cell divisions [11,74]. However, knowledge is still limited regarding the influence of EMFs on symmetric and asymmetric stem cell divisions. In this review paper, we do not extensively discuss the effect of EMFs and the mechanisms involved in MSC differentiation. There are many up-to-date and well-written papers that only focus on this topic already published [9,10,12,33].

Clinically, most interest is focused on the chondrogenesis and osteogenesis of MSCs, while neurogenesis has also been widely studied. Previously, Safavi *et al.* [10] pub-

lished a systematic review covering the topic of MSC differentiation based on literature published up to December 2021. In this review paper, we only collected research papers available in PubMed that studied MSC chondrogenic, osteogenic, and neural differentiations published between January 2022 and July 2023 (Table 5, Ref. [48,55,56,75–81]).

Recently, Huang *et al*. [55] showed that a combined treatment of EMF (50 Hz; 1 mT) and BM-MSCs may enhance and promote spinal cord injury treatment in a mice model by increasing the expression of NGF, BDNF, and VEGF. It has been also reported that EMFs can increase neuron preservation (NeuN and NF-200) and increase axonal growth (MBP, myelin sheath) [55]. Moreover, it has been shown that an approach combining EMFs and MSCs may be successful for use in cerebral ischemic models. Park *et al*. [75] showed that EMFs (60 and 75 Hz; 10 mT) can increase the protein expression of MAP-2 and NF-L in cell cultures of BM-MSCs, while BM-MSCs/EMF (60 Hz; 10 mT) treatment can reduce inflammation and significantly improve behavior and motor coordination in a cerebral ischemic mice model after treating for 13 days. Interestingly, Guo *et al*. [82] managed to provoke ASCs to undergo neuronal differentiation by using biodegradable graphene film, which acted like a wireless electrical signal generator led by electromagnetic induction.

Seonwoo *et al*. [76] studied the effects of applying reduced graphene oxide-incorporated natural calcium phosphate cements (RGO-CPCs) and EMF (50 Hz; 0.6 $\pm$ 0.05 mT) to improve the osteogenic differentiation of MSCs and found that this approach was not severely toxic and could enhance the differentiation process. Recently, research by Gerdesmeyer *et al*. [56] showed that EMF (3 Hz; 80 mT, 150 mT) stimulation can increase the expression of markers related to osteogenesis and chondrogenesis, although the effect was not statistically significant. Additionally, Ren *et al*. [77] showed that BM-MSCs cultured on Fe_3O_4-TNrs (titanium dioxide nanorods) presented with better pro-osteogenic properties after EMF treatment (15 Hz, 1 mT), showing higher expressions of osteogenic markers, such as ALP, OCN, RUNX2, and OPN. Moreover, this biomaterial improved osseointegration femur defects in a rat model after EMF stimulation *in vivo* [77]. Interestingly, EMFs (50 Hz; 1 mT) also affected human periodontal ligament mesenchymal stem cells (PDLSCs) osteogenesis. Costantini *et al*. [48] proved that EMFs increased calcium deposition in cells and upregulated RUNX-2, COL1A1, and OPN expression.

An interesting study that used ASC-derived exosomes in osteopathist treatment, was performed by Xu *et al*. [78]. They reported that EMF exposure reduced inflammation and extracellular matrix degeneration in ASC-derived exosomes as well as increased COL2A1, SOX9, and ACAN expression in chondrocytes. Moreover, using the osteoarthritis rat model, authors showed that injecting EMF (75 Hz)-exposed ASC-derived exosomes promoted the regeneration of osteoarthritic cartilage [78]. A different study, performed by Sun *et al*. [79], also showed that static magnetic fields can increase COL2A1 and SOX9 expression and improve the migration of BM-MSCs through SDF-1/CXCR4. Additionally, Zhang *et al*. [13] showed that LF-EMFs can promote MSC migration by accumulating Ca^{2+} and through the FAK/Rho GTPase signaling pathways. In 2023, two studies investigated the effects of EMFs on MSCs cultured on hydrogel scaffolds and published the resulting chondrogenesis data. Both studies showed that EMFs can enhance chondrogenesis and this new tissue engineering approach (hydrogels and EMF) may increase the clinical potential of MSCs in treating cartilage defects [80,81].

Studies have shown that EMFs have a positive effect on MSC differentiation and can improve as well as accelerate this process. Thus, EMFs represent an easy-to-apply tool that can be considered for use in stem cell therapy.

4. Safety Considerations and Clinical Applications

MSCs can be obtained from most human tissues but with different efficiencies. Currently, the main sources of MSCs for consideration in stem cell-based therapy are bone marrow, adipose tissue, and the umbilical cord [1,7,83]. Interestingly, the differentiation of iPSCs into MSCs represents a new approach to obtaining MSCs and may be useful in autologous treatment using MSCs in geriatric patients [66]. MSCs can be considered in the treatment of various diseases and conditions in the fields of dermatology, neurology, pulmonology, cardiology, orthopedics, and immunology [7]. According to the clinical trial experiences of Hoang *et al*. [1], the tissue from which the MSCs originate is important for the targeted therapies and clinical applications with MSCs. Hoang *et al*. [1] supported the hypothesis that bone marrow can be a good source of MSCs for treating brain and spinal cord injuries, while MSCs originating from adipose tissues are good for treating reproductive disorders and skin regeneration; MSCs from umbilical cords can be used to treat pulmonary disease and acute respiratory distress syndrome.

Safety and effectiveness are mandatory criteria for introducing stem cell-based therapies as a standard but these require validated clinical trial results [2]. The clinical use of MSCs has its limitations, which are ascribed to the variable immunocompatibility, stemness stability, heterogeneity, differentiation, and migratory and homing capacity of MSCs. The unexpected behavior of MSCs in clinical settings might occur from difficulties in the production and administration of MSC-based medical products. These challenges require an appropriate approach, and for this reason, the preconditioning of cell culture through the application of biochemical or physical inducers (such as drugs or EMFs) as well as genetic modification of MSCs is be-

Table 5. Electromagnetic fields affect the differentiation of mesenchymal stem cells.

Biological model	EMF parameters	Exposure duration	Biological effect	Study reference
Mice BM-MSCs	PEMF (50 Hz; 1 mT)	1 h and 3 h/day	Improvement of neural functions and axon connection. Recovery after spinal cord injury is promoted via the Wnt/β-catenin signaling pathway.	[55]
Human BM-MSCs	PEMFs (30, 45, 60, and 75 Hz; 10 mT)	30 min/day for 3 days	Upregulation of MAP-2 and NF-L after exposure to 60 and 75 Hz. Significant increase in neural-related protein expressions. Reduction in MMP-9, TNF-α, and INF-γ expression in the ischemic area.	[75]
Human BM-MSCs	PEMF (3 Hz; 80 mT, 150 mT)	10 min/day for 14 days	Increased expressions of COL-I, ALP, and BMP-2, although not significant.	[56]
Rat ASCs	PEMF (50 Hz; 0.6 ± 0.05 mT)	30 min/day for 3 weeks	Enhanced osteogenesis. Significant increase in calcium deposition in cells after PEMF on 1% RGO.	[76]
Rat BM-MSCs	EMF (15 Hz; 1 mT)	1 h/day for 21 days	Increased osteogenesis on Fe_3O_4-TNrs. Increased expressions of ALP, OCN, RUNX2, and OPN.	[77]
Human PDLSCs	EMF (50 Hz; 1 mT)	6 h/day for 28 days and 10 days	Statistically significant increase in calcium deposition. Upregulation of RUNX-2, COL-1A1, and OPN expression.	[48]
Rat ASCs	EMFs (0, 15, 45, and 75 Hz; 1 mT)	24 h	ASC-derived exosomes: suppressed inflammation and extracellular matrix degeneration; upregulated COL-2A1, Sox-9, and ACAN expressions and also improved regeneration of osteoarthritic cartilage *in vivo*.	[78]
Mice BM-MSCs	EMF (0 Hz; 200 mT)	14 days	Increased expressions of Sox-9 and COL-2. Induced MSC migration through SDF-1/CXCR4.	[79]
Human BM-MSCs	EMF (15 Hz; 10 T/s)	3 h/day for 14 days	Promoted Sox-9, ACAN, COL2A1, and matrix protein collagen type II expression. Inhibited expression of degeneration matrix protein collagen type X and hypertrophic genes.	[80]
Human and rat BM-MSCs	EMF (15 Hz; 10 T/s)	3 h/day for 21 days	Increased expression of Sox-9, ACAN, and COL2A1. Regulated chondrogenesis through ERK and p38 MAPK pathways.	[81]

MAP, microtubule-associated protein; NF-L, neurofilament light chain; MMP, metalloproteinase; TNF, tumor necrosis factor; RGO, reduced graphene oxide; OCN, osteocalcin; RUNX2, Runt-related transcription factor; ACAN, aggrecan.

ing considered. Several studies that have preconditioned the MSCs are currently registered in the ClinicalTrials.gov database [33,84,85]. It is worth noting that EMF preconditioning can be precise and efficient (if the appropriate EMF parameter is selected) and more cost-effective during the process of *in vitro* cell differentiation compared to using synthetic growth factors, which are expensive and often pleiotropic. EMF preconditioning may also be more time-efficient and more personalized in the context of preparing stem cell-based medical products by increasing the rate of cell proliferation and modulating differentiation, which are important in autologous therapies.

Intense and rapid technological development has raised public concerns about the safety of EMFs on human health. At this point, it should be noted that these concerns may indeed be compounded by the increasing number of electromagnetic field sources in the environment and the statement by the International Agency for Research

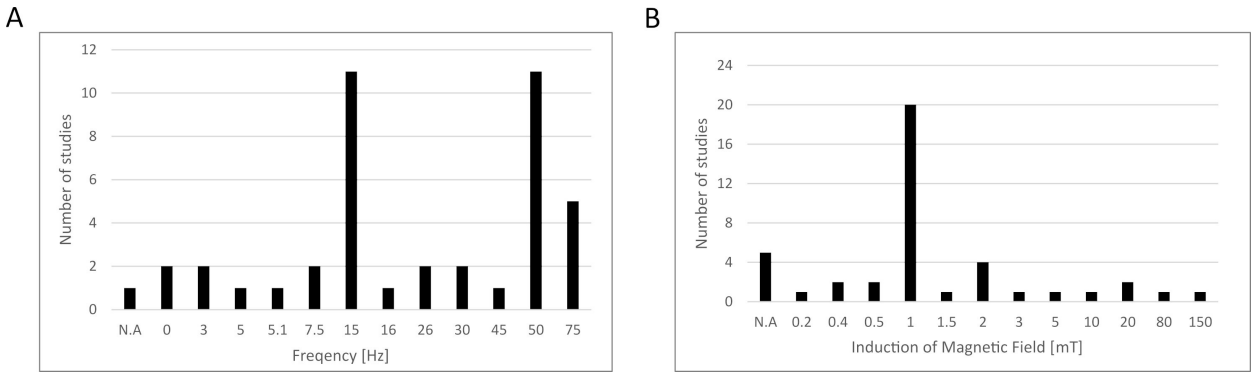

Fig. 1. Electromagnetic field parameters used in the studies collected in this review. (A) Frequency, (B) induction of magnetic field. N.A., data not available.

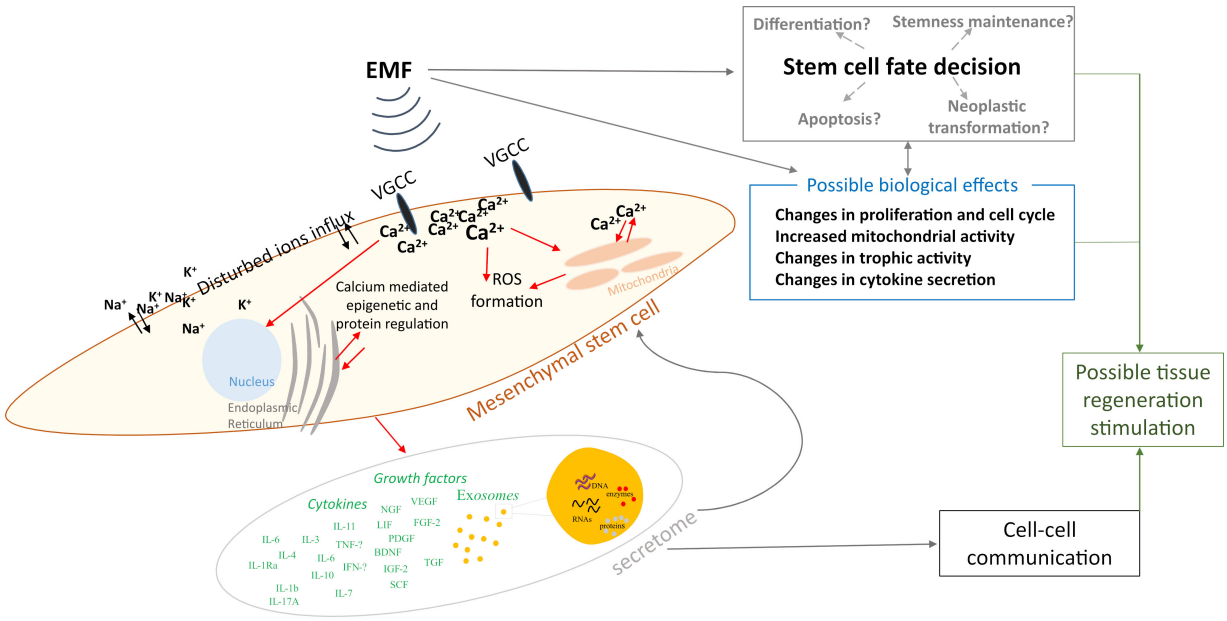

Fig. 2. Schematic representation of the possible influence of electromagnetic fields on mesenchymal stem cells. ROS, reactive oxygen species.

on Cancer (ICAR), which classified radiofrequency with the ability to induce potentially harmful effects, although low-frequency EMFs were not classified to this effect [15]. The World Health Organization (WHO) has also stated that there is currently no evidence of harmful effects being induced by low-level exposure to EMFs [86]. Moreover, the WHO is coordinating an international project to publish systematic reviews on the effects of radiofrequency EMFs on health outcomes [87]. Studies conducted *in vitro* showed no negative effect of LF-EMFs on the morphology or viability of MSCs [17,35,69], as well as on their karyotype [43]. However, the research was performed by researchers from around the world and was limited to only specific parameters of electromagnetic fields, thereby again reaffirming that the biological effect observed *in vitro/in vivo* is dependent upon the specific conditions of EMF exposure. Moreover, it is important to note that the MSC culture conditions are very important, while the composition of the culture medium may also influence the obtained results. It is suggested in protocols for medical products that MSCs should be cultured without fetal bovine serum (one alternative is culturing with human Platelet Lysate), although this can affect the cellular conditions and raises concerns regarding product safety and ethical problems [88,89]. Furthermore, the use of antibiotics in cell culturing may cause undesirable effects on the MSCs [90]. Skubis *et al.* [90] showed that amphotericin B can decrease cell viability, while a penicillin–streptomycin mixture can affect stemness phenotype, adipogenesis, and osteogenesis of human ASCs. In addition to streptomycin, amikacin has been shown to have a negative effect on MSCs when used at high concentrations [91].

Based on the data collected above in Chapter 2, we summarized the electromagnetic field parameters used in those studies (Fig. 1), concluding that most studies used testing frequencies of 15 and 50 Hz, while the induction of

Table 6. Clinical trials using the combined therapeutic approach of electromagnetic fields and mesenchymal stem cells registered in the clinicaltrials.gov database.

NCT number	Study title	Condition	Interventions
NCT04877067	Therapy of Toxic Optic Neuropathy Via Combination of Stem Cells with Electromagnetic Stimulation (Magnovision)	Toxic optic neuropathies	Wharton's Jelly-derived Mesenchymal Stem Cells and repetitive electromagnetic field stimulation for treating patients' eyes.
NCT05800301	Management of Retinitis Pigmentosa Via Combination of Wharton's Jelly-derived Mesenchymal Stem Cells and Magnovision	Retinitis pigmentosa	Wharton's Jelly-derived Mesenchymal Stem Cells and repetitive electromagnetic field stimulation for treating patients' eyes.

the magnetic field was 1 mT. However, the parameters used in the differentiation studies presented in this paper were not included due to the fact that this article does not present a broad analysis of this area of the literature, and instead, narrowed down the publication window to papers published between January 2022 and July 2023, as explained in Section 3.5. We also prepared a schematic representation of the possible influence of EMFs on MSCs based on the studies collected (Fig. 2).

Electromagnetic fields were initially approved as an effective health therapy by the Food and Drug Administration (FDA) in 1979 [56], while MSCs were first used in human subjects in 1995 [92]. Currently, two clinical trials are registered in the clinicaltrials.gov database that uses a combined therapeutic approach of EMFs and MSCs (Table 6), with both studies applying Wharton's jelly-derived MSCs (WJ-MSC) for ophthalmic use. A phase 3, open-labeled clinical study (NCT04877067) reported that treatment with MSCs and EMFs (42 Hz; 2 G for 30 min) can be effective in toxic optic neuropathy and can prevent permanent blindness [93]. The same group also tested a similar approach for treating retinitis pigmentosa (NCT05800301) but results are not yet available. Based on this, combination therapy using EMFs and MSCs appears to be well tolerated and effective, not only *in vitro* and *in vivo* but also in human subjects.

5. Conclusions and Future Perspectives

The use of LF-EMFs in preconditioning MSCs can be an effective approach to omitting the current limitations associated with the use of MSCs as a therapeutic treatment. Moreover, adjusting the EMF exposure parameters can control the stem cell fate and lead MSCs toward the desired therapeutic effect. LF-EMFs positively affect the proliferation, migration, differentiation, and immunomodulatory properties of MSCs and can be used in medicine for conditions where inflammation and tissue damage occur—for example—in wound healing, bone and cartilage regeneration, or regeneration after stroke. The exact mechanism through which EMFs act on MSCs is not yet known, although it is being intensively studied. It is currently known that EMFs affect the Ca^{2+} flux in the cell, which can cause a series of subsequent changes and a biological response manifested by accelerated proliferation or differentiation (Fig. 2). In addition, the use of EMFs may prove to be a faster and controllable method of preconditioning MSCs. However, EMFs can have different effects on stem cell biology depending on the parameters and duration of stimulation. Nevertheless, in the vast majority of *in vitro*, *in vivo*, and clinical studies, the biological effect is positive, which can be significant in increasing the effectiveness of therapy at the initial stage of preparation of a medical product containing stem cells, both, in cell therapy and cell-free therapy. Furthermore, understanding the mechanisms of action may be significant in improving this approach and the use of EMFs in regenerative medicine. It is important to conduct more studies with similar EMF conditions to provide reliable data on carefully sourced MSCs depending on the disease.

Author Contributions

AS conception of the project, collection, assembling, analysis and interpreting data, designing and writing the manuscript, tables and figures preparation. BP collection and assembling data, manuscript writing. ABZ conception of the project, coordinating and revising the manuscript critically for important intellectual content, final approval of manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest. Given her role as Guest Editor, Agnieszka Banaś-Ząbczyk had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Viviana di Giacomo.

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