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Review

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Electromagnetic field exposure as a plausible approach to enhance the proliferation and differentiation of mesenchymal stem cells in clinically relevant scenarios

Haslinda Abdul HAMID[1], Vahid Hosseinpour SARMADI[2,3], Vivek PRASAD[4], Rajesh RAMASAMY[4], Azizi MISKON[1]✉

[1]*Bio-artificial Organ and Regenerative Medicine Unit, National Defense University of Malaysia, Kuala Lumpur 57000, Malaysia*
[2]*Cellular and Molecular Research Centre, Iran University of Medical Sciences, Tehran 1449614535, Iran*
[3]*Institutes of Regenerative Medicine, Iran University of Medical Sciences, Tehran 1996714353, Iran*
[4]*Stem Cell and Immunity Research Group, Immunology Laboratory, Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM), Selangor 43400, Malaysia*

Abstract: Mesenchymal stem/stromal cell (MSC)-based therapy has been regarded as one of the most revolutionary breakthroughs in the history of modern medicine owing to its myriad of immunoregulatory and regenerative properties. With the rapid progress in the fields of osteo- and musculoskeletal therapies, the demand for MSC-based treatment modalities is becoming increasingly prominent. In this endeavor, researchers around the world have devised new and innovative techniques to support the proliferation of MSCs while minimizing the loss of hallmark features of stem cells. One such example is electromagnetic field (EMF) exposure, which is an alternative approach with promising potential. In this review, we present a critical discourse on the efficiency, practicability, and limitations of some of the relevant methods, with insurmountable evidence backing the implementation of EMF as a feasible strategy for the clinically relevant expansion of MSCs.

Key words: Electromagnetic field; Proliferation; Mesenchymal stem cell; Therapy

1 Introduction

Discovered around 50 years ago in the bone marrow (BM), mesenchymal stem cells (MSCs) are a heterogeneous group of spindle-shaped cells with unique self-renewing and differentiation properties (Friedenstein et al., 1966). In many studies, MSCs have been observed to differentiate into distinct cells of the mesodermal lineage, including osteocytes, chondrocytes, and adipocytes (Pittenger et al., 1999; Kolf et al., 2007; Thibault et al., 2010; Ang et al., 2014; Somoza et al., 2014). Such propensity allows MSCs to be present almost ubiquitously at different sites within the human body, including but not limited to, adipose, umbilical cord, and amniotic fluid tissues (el Omar et al., 2014; de Francesco et al., 2015; Mushahary et al., 2018). In addition, MSCs have been associated with various other biologically significant qualities, such as exerting anti-tumorigenic effects (Lu et al., 2008; Sarmadi et al., 2008, 2020; Bruno et al., 2013), potent immunomodulatory activity (Ramasamy et al., 2010; Volarevic et al., 2017; Maqbool et al., 2020; Zhang et al., 2021), and lower rates of immunogenicity (Aggarwal and Pittenger, 2005; Barry et al., 2005; Klyushnenkova et al., 2005). These characteristics, coupled with fewer ethical cues (Fung et al., 2017; Volarevic et al., 2018), have generated a widespread interest among scientists and researchers alike, thereby making MSCs one of the most clinically studied cells in the platform of experimental cell therapy. For instance, in musculoskeletal clinical trials, autologous and allogeneic transplantations of MSCs were found to yield better performance in terms of post-operative healing and functional outcomes (Hashimoto et al., 2019; Song et al., 2020; Chung et al., 2021). Similar observations have been reported in

✉ Azizi MISKON, azizimiskon@upnm.edu.my
Azizi MISKON, https://orcid.org/0000-0002-4267-4180

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several cases of psoriasis (Lee et al., 2017; Chen MS et al., 2019; Wang et al., 2020), ischaemic and non-ischaemic cardiomyopathy (Butler et al., 2017; Florea et al., 2017; Hare et al., 2017), and even in corona virus disease 2019 (COVID-19)-related pneumonia (Leng et al., 2020; Liang et al., 2020; Shu et al., 2020; Tang et al., 2020). Further examples involve direct application of MSCs in the treatment and management of skin and sensory organ-related injuries (Ude et al., 2018), post-kidney transplantation (Erpicum et al., 2019), diabetes (types 1 and 2) (Jiang et al., 2011; Carlsson and Svahn, 2018), graft-versus-host disease (GvHD) (Kurtzberg et al., 2020), and multiple sclerosis (Connick et al., 2012; Iacobaeus et al., 2019).

Despite their numerous positive attributes, freshly isolated MSCs are rare and have high heterogeneity, and are in limited quantities because of factors such as age and gender (Alt et al., 2012; Fossett and Khan, 2012; Yang et al., 2018); therefore, an extensive expansion of the isolated cells is necessary to meet the growing demands of clinical experimental trials. However, extensive in vitro passages may undergo morphological, phenotypic, and genetic changes (Yang et al., 2018). Considerable efforts have been made to enhance the proliferation of MSCs in vitro without modifying their capabilities of stemness and differentiation. These include providing additional supplements, mainly growth factors (ascorbic acid, fibroblast growth factor-2 (FGF-2), platelet-derived growth factor with two subunits BB (PDGF-BB), and epidermal growth factor (EGF)) into their cultures (Solchaga et al., 2005; Choi et al., 2008; Tamama et al., 2010; Sun et al., 2013). Nevertheless, Gharibi and Hughes (2012) demonstrated that, even if given all of the supplementations, MSCs showed a marked decline in their differentiation ability and expression of stem cell genes after as few as 100 d of culture (Gharibi and Hughes, 2012). In this context, MSCs reaching senescence early would no longer be able to accommodate the need for translational medical therapies. This is because senescent cells, albeit in small numbers, could affect the functionalities of other organs and tissues through the release of secretomes containing proinflammatory molecules (Childs et al., 2015). Thus, clinically practical and cost-effective protocols are imperative for the proper generation and maintenance of MSCs.

2 Potential of electromagnetic fields (EMFs) for MSC therapy

Over the last few years, EMF has garnered attention as one of the most promising tools in stem cell therapy (Esposito et al., 2013; Ongaro et al., 2014; Miskon et al., 2018). EMF is a combination of electrical and magnetic fields, which can create non-ionized waves and non-thermal fields. Magnetic therapy encompasses a variety of approaches, which include EMF or pulsed EMF (PEMF). The latter is a type of electromagnetic therapy in which a small electrical current and low-frequency EMFs with specific waveforms and amplitudes, ranging between 6 and 500 Hz, are intermittently applied to the body (Hu et al., 2020; Qiu et al., 2020). The application of EMF on living cells was found to induce a variety of effects not only at the cellular level, but also at the genomic level. In one study, the overexpression of p27 (cyclin-dependent kinase inhibitor), due to extremely low-frequency (ELF)-EMF exposure, was found to lead to cell cycle arrest (Geng et al., 2014), while in another study, PEMF caused significant changes in the expression of programmed cell-death-related genes during different stages of apoptosis and necrosis (Kaszuba-Zwoinska et al., 2012). EMF exposure has also been linked to changes in DNA replication (Cheng and Zou, 2006) and cytokine expression (Jasti et al., 2001). As a form of therapy, EMF has been widely utilized in the treatment of bone fractures, as well as musculoskeletal and neuronal disorders, with high rates of success (Shupak et al., 2003). Interestingly, some findings suggested that, at predetermined time intervals and frequencies, EMF may also promote the proliferation of MSCs (Sun et al., 2009; Fan et al., 2015; Miskon et al., 2018) and could drive their cellular state of differentiation into chondrogenesis (Parate et al., 2017), osteogenesis (Kim et al., 2015), and even neurogenesis (Seong et al., 2014).

In light of these data, this paper aims to provide a concise review of current strategies employed for the culture and maintenance of MSCs in experimental laboratories. This includes a comprehensive analysis of factors and culture conditions related to long-term in vitro expansion of MSCs without compromising their "stemness." In addition, the underlying effects of EMF exposure on the proliferative, regenerative, and differentiation capacities of MSCs will be thoroughly discussed. Finally, we make brief evaluation of the

deleterious impacts of EMF, if any, on cultures of MSCs.

2.1 Effects of EMFs on the proliferation of MSCs

In recent years, the effects of EMF exposure on the viability and proliferation of MSCs have been the topic of active research (Zhang et al., 2007, 2018; Sun et al., 2009; Song et al., 2014a; Fan et al., 2015; Marędziak et al., 2017; Ehnert et al., 2018; Ferroni et al., 2018; Miskon et al., 2018; Poh et al., 2018; Ross et al., 2018; Seo et al., 2018; Chen JY et al., 2019; Parate et al., 2020). In a study by Miskon et al. (2018), umbilical cord-derived MSCs were pulsed with EMF for 5 min, and the results indicated an increase in the numbers of monolayer and suspension MSCs by 1.39- and 2.41-fold, respectively, when compared with those of the control group after 5 d (Miskon et al., 2018). However, the underlying mechanism or EMF frequencies used in this study were not elucidated. In a different study, when BM-derived MSCs were exposed to 15 Hz of EMF for 8 h/d, Sun et al. (2009) reported a 59% and 40% increase in the viability of MSCs in low- (1000 cells/cm^2) and high-density (3000 cells/cm^2) seeded cultures, respectively. The reason for this could be due to the shortening of the lag phase, causing EMF to enhance the proliferation of MSCs during the exponential phase and leading the cells into the G2/M phase of the cell cycle. Nonetheless, enhanced cellular proliferation served as the outcome of increased DNA synthesis activity. This was made evident when exposing rat- and mouse-derived MSCs to 50 Hz of EMF for 4 h/d for three consecutive days. It was found that cell viability, DNA synthesis, and the proportion of MSCs in S phase in the EMF group were significantly elevated as compared with the SHAM group (Fan et al., 2015). This finding was also in accordance with a recent study by Bloise et al. (2018) who demonstrated increased DNA synthesis in PEMF-exposed BM-derived MSCs. The continuous expression of several cytokines is required for the process of MSC proliferation and differentiation, and the expression of these cytokines can be modulated by EMF. Some studies have shown that a shortened lag phase of the cell cycle induced by EMF may result in a higher cellular proliferation index, which could be mediated by the increased expression of cytokines (Sun et al., 2009; Zhang et al., 2013; Fan et al., 2015). MSCs exposed to EMF have exhibited the elevated messenger RNA (mRNA) expression of a group of hematopoietic cytokines, namely, macrophage colony-stimulating factor (M-CSF), stem cell factor (SCF), leukemia inhibitory factor (LIF), interleukin-7 (IL-7), and IL-11, while the mRNA expression of inflammatory cytokines, stromal cell-derived factor-1 (SDF-1), tumor necrosis factor-α (TNF-α), and IL-6 remained unchanged (Fan et al., 2015). These inflammatory cytokines are a part of the soluble factors secreted upon the instigation of signaling pathways activated by BM cells (Harmer et al., 2019). This dynamic event might be due to the pattern and time points of EMF application; it was suggested that the elevation of the aforementioned cytokines might enhance the proliferation of MSCs (Fan et al., 2015). The antigen expression profile of cytokines secreted by MSCs was further analyzed using an antibody fabricated array consisting of 120 antibodies reactive to 120 cytokines, where all MSCs were BM-derived (Park et al., 2009). The array was incubated with the secretion media of cells (from BM- and umbilical cord blood (UCB)-derived MSCs), followed by 1.5 h of incubation with biotin-conjugated antibodies. The hybridized antigen-antibody complexes were detected using streptavidin-conjugated peroxidase. A significant elevation of the signal was observed for six cytokines, IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), tissue inhibitor of matrix metalloproteinase-2 (TIMP-2), vascular endothelial growth factor (VEGF), and osteoprotegerin (OPG), which was consistent across BM-derived MSC donors of all races, ages, and genders. This profile pattern was found to be similar to that of UCB-derived MSCs, with IL-6 exhibiting the strongest signal. Furthermore, IL-8 with known mitogenic and angiogenic potential showed the highest signal in UCB-derived MSCs. These observations further elucidate the molecular mechanism underlying MSCs (Park et al., 2009). Although the expression levels of M-CSF, SCF, thrombopoietin (TPO), LIF, IL-11, and IL-7 were observed to be significantly upregulated in EMF-exposed MSCs, the causative relationship leading towards MSC proliferation remains unclear. It is also interesting to note that the proliferative effects of EMF on MSCs extend even beyond days after initial exposure, which suggests that the triggered underlying mechanism of action is long-lasting (Tu et al., 2018). The summary of recent studies on the proliferative effects of EMF and PEMF exposure on MSC culture is shown in Table 1 and Fig. 1.

Table 1 Recent studies on the proliferative effects of EMF/PEMF exposure on MSC cultures

Study	Biological model	Type of exposure	Exposure frequency (Hz)	Exposure intensity (mT)	Duration	Outcome
Jazayeri et al., 2017	rBM-MSCs	LF-EMF	15	0.2	6 h/d for 5, 10, and 14 d	Significantly increased MSC proliferation
Sun et al., 2009	hBM-MSCs	PEMF	15		8 h/d	Expedited the proliferation of BM-MSCs
Yan et al., 2010	hMSCs	ELF-MF	50	20		Inhibited the growth of hMSCs
Zhang et al., 2013	hESCs	LF-EMF	1, 10, and 50	5	30 min/d for 3, 5, and 7 d	Increased the proliferation hESCs
Song et al., 2014b	rBM-MSCs	SEMF	15	1	1 h/d	Increased MSC proliferation
Song et al., 2014a	BM-MSCs	EMF	15	1	4 h/d	Expedited the proliferation of BM-MSCs
Fan et al, 2015	rBM-MSCs	ELF-EMF	50	1	4 h/d	Increased the proliferation of MSCs and upregulated haematopoietic cytokines
Marędziak et al., 2017	hAD-MSCs	Static MF		0.5	24 h/d for 7 d	Improved the process of signaling associated with the proliferation of MSCs
Miskon et al., 2018	hUC-MSCs	EMF			300 s/d for 5 d	Increased the proliferation of hUC-MSCs in suspension culture
Ehnert et al., 2018	hAD-MSCs	ELF-PEMF	16–24	6×10^{-3}–282×10^{-3}	7 min/d, 5 times per week	Significantly increased mitochondrial activity
Ferroni et al., 2018	MSCs	PEMF			1, 3, 15, and 30 d	Significantly increased MSC proliferation
Seo et al., 2018	BM-MSCs	PEMF	50	1	1 h/d for 5, 7, and 10 d	Slight changes though not significant
Bloise et al., 2018	hBM-MSCs	PEMF	75	2	10 min/d for 1–15 d	Significantly increased MSC proliferation
Poh et al., 2018	hAD-MSCs	ELF-PEMF	26		3 d	No significant changes
Ross et al., 2018	hMSCs	ELF-EMF	5	0.4	20 min/d, 3 times per week for 2 weeks	Slight changes though not significant
Tu et al., 2018	rBM-MSCs	SEMF	15	1	4 h/d	Significantly increased MSC proliferation
Zhang et al., 2018	BM-MSCs	EMF	7.5, 15, 30, 50, and 75	1	24 h	Increased MSC migration but no proliferative changes
Chen et al., 2019a	rBM-MSCs	SEMF	15	1	4 h/d for 7 d	Significantly increased MSC proliferation
Parate et al., 2020	MSCs	PEMF	15	2	30 min	No significant changes

EMF: electromagnetic field; PEMF: pulsed EMF; MSC: mesenchymal stem cell; BM: bone marrow; rBM-MSCs: rat BM-MSCs; hBM-MSCs: human BM-MSCs; hMSCs: human MSCs; hESCs: human epidermal stem cells; hUC-MSCs: human umbilical cord-derived MSCs; hAD-MSCs: human adipose-derived MSCs; LF: low-frequency; ELF: extremely LF; MF: magnetic field; SEMF: sinusoidal EMF.

It was proposed that PEMF exposure may have triggered the release of free ions such as potassium ion (K^+) and calcium ion (Ca^{2+}) from the smooth endoplasmic reticulum (ER), which can subsequently affect the activity of activated K^+ channels, leading to enhanced cellular progression from G1 to S phases (Jazayeri et al., 2017). For instance, Ca^{2+} has been regarded as an important second messenger in a given cell, as it regulates a plethora of signal transduction in critical processes, such as adenosine triphosphate (ATP) synthesis, apoptosis, cellular motility, gene expression, and proliferation. In a study it was demonstrated that intracellular Ca^{2+} signaling mediates insulin-like growth factor-1 (IGF-1)-induced proliferation in MSCs (Wu et al., 2020). As non-excitable cells, Ca^{2+} oscillations in MSCs are typically initiated by the up-regulation of inositol-1,4,5-triphosphate receptor 2 (IP_3R2) and sarco/endoplasmic reticulum Ca^{2+}-ATPase 3 (SERCA3), resulting in Ca^{2+} release from the ER. It was outlined that Ca^{2+} oscillations may increase the

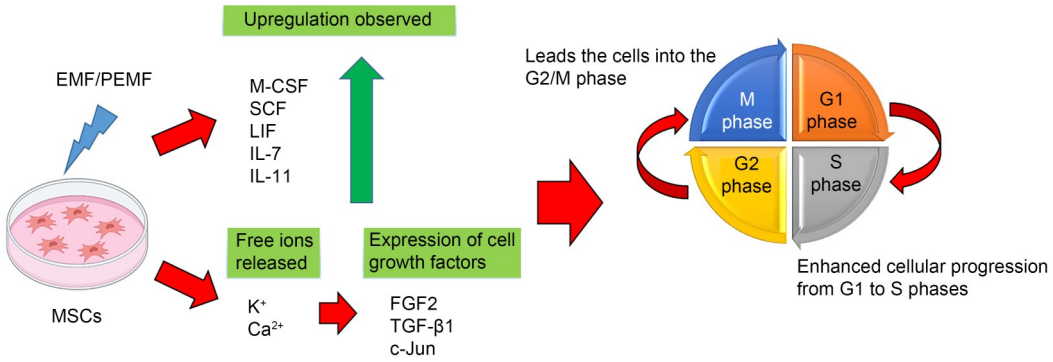

Fig. 1 EMF triggered the elevated mRNA expression of a group of hematopoietic cytokines that might enhance the proliferation of MSCs. EMF: electromagnetic field; mRNA: messenger RNA; MSC: mesenchymal stem cell; PEMF: pulsed EMF; M-CSF: macrophage colony-stimulating factor; SCF: stem cell factor; LIF: leukemia inhibitory factor; IL: interleukin; FGF-2: fibroblast growth factor-2; TGF-β1: transforming growth factor-β1.

levels of cyclins A and E that regulate cell cycle progression and proliferation (Hanna et al., 2017). Moreover, it was reported that elevated levels of extracellular Ca^{2+} may also directly promote MSC proliferation through the induced expression of FGF-2 and transforming growth factor-β1 (TGF-β1), as well as cell cycle regulator c-Jun (Lee et al., 2018). Given that cellular exposure to EMF has been shown to dramatically increase Ca^{2+} influx though enhanced presynaptic calcium channel expression, the proposed notion that the increased proliferation of EMF-exposed MSCs is mediated by the alterations of its Ca^{2+} current is well supported (Sun et al., 2016). Some investigators also proposed the function of signal transduction pathway as responsible for the proliferative effects of EMF exposure on MSC cultures. In their study, Fan et al. (2015) proposed that the induced FGF-2 may inhibit cellular senescence and promote cellular proliferation via the phosphoinositide 3-kinase (PI3K)/serine-threonine kinase (AKT)-mouse double minute 2 (MDM2) signaling pathway. This notion is in agreement with recent studies that proposed the involvement of PI3K/AKT signaling as one of key players in the maintenance of self-renewal and stemness of MSCs (Fong et al., 2007; Schaefer et al., 2020). The in-depth explanation regarding EMF interaction with biological cells may open a new perspective, in particular in the application of EMF to MSCs, as depicted in Fig. 2.

Though multiple studies have provided evidence on the proliferative effects of EMF on MSC cultures, some papers presented contradicting findings (Yan et al., 2010). These discrepancies are not immediately clear, though some scholars described that the effects of EMF depend on the cell type, cell state, frequency, amplitude, and exposure time (Ivancsits et al., 2005; Zhang et al., 2007; Song et al., 2014b). Prolonged EMF exposure, for instance, could negatively impact the viability and proliferation of MSCs. This has been made evident by Marmotti et al. (2018) who investigated the optimal exposure duration of EMF onto MSC culture per day and the longest time period of exposure it could withstand before exhibiting a decreasing trend in cell survival. Additionally, this study reaffirms the findings of earlier works that had also provided evidence supporting that the observed proliferative effects of MSCs post-EMF exposure are greatly time duration-dependent (Song et al., 2014b).

2.2 Effects of EMF on the differentiation of MSCs

Another important goal of the EMF exposure strategy is to positively reinforce the differentiative capability of MSCs. As mentioned previously, EMF therapy has been used to treat a broad range of diseases, such as non-union bone fractures, osteoporosis, and pseudarthrosis, as well as for cartilage and tendon repair (Shupak et al., 2003; Assiotis et al., 2012; Zhou et al., 2012). Although the exact mechanism by which EMF stimulation induces lineage commitment within MSCs remains inconclusive, it has been well established that Runt-related transcription factor 2 (*Runx2*)/core-binding factor α1 (*Cbfa1*) and osterix (*Sp7*) serve as predominant transcription factors that drive MSC osteogenic differentiation, while SRY-box transcription 9 (*Sox9*) and the modulation of the Wnt/β-catenin signaling pathway control chondrogenesis in cultures (Ross et al., 2015). Interestingly, the expression levels

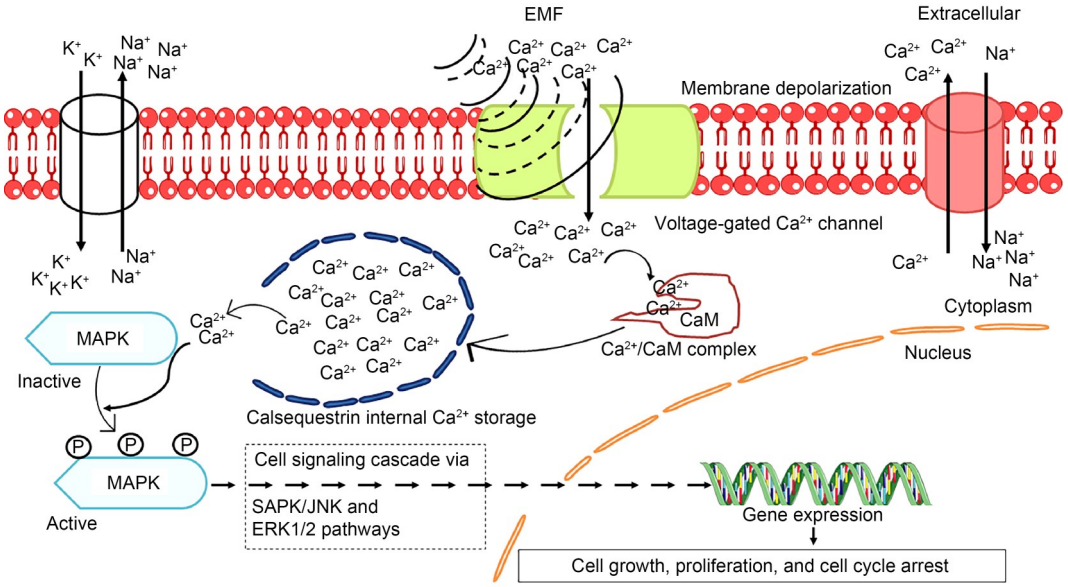

Fig. 2 Overview of cellular processes during exposure to EMF. EMF: electromagnetic field; MAPK: mitogen-activated protein kinase; SAPK: stress-activated protein kinase; JNK: c-Jun N-terminal kinase; ERK: extracellular signal-regulated kinase; CaM: calmodulin.

of osteoblastic marker genes *Runx2* and osteocalcin (*Ocn*) were significantly elevated when the MSCs were subjected to daily PEMF exposure when compared with unexposed cultures prior to transitioning to the mineralization phase between Day 10 and Day 14 (Jazayeri et al., 2017). Other studies have also highlighted the phosphorylation of the extracellular signal-regulated kinase 1/2 (ERK1/2) and protein kinase A (PKA) signaling pathways in osteogenesis induction by EMF as a result of elevated cyclic adenosine monophosphate (cAMP) levels. Yong et al. (2016) have shown that pretreatment of MSCs with ERK and PKA inhibitors prior to PEMF exposure resulted in the significant inhibition of the induction of osteogenic markers *Runx2*, bone morphogenetic protein 2 (*BMP2*), and *Ocn*. In a different study, increased osteogenic differentiation through PEMF exposure was accompanied by the strong expression of osteogenic markers including osteonectin, osteopontin, collagen I, and collagen III. It is noteworthy that alkaline phosphatase (ALP) activity was observed to be upregulated followed by the down-regulation of MSC markers cluster of differentiation 90 (CD90), CD73, and CD105 in cells (Kim et al., 2015).

In other examples, EMF was employed for cartilage and tendon repair via the chondrogenic and tenogenic differentiation potentials of MSCs, respectively, with varying degrees of success (Haddad et al., 2007; de Girolamo et al., 2013). Although several studies observed little to no effect of EMF stimulation during chondrification (Mayer-Wagner et al., 2011; Esposito et al., 2013; Ongaro et al., 2015), Parate et al. (2017) showed that brief single exposure of low-amplitude EMF worked best at inducing the chondrogenesis of MSCs. Obvious differences in the stimulation protocols or techniques used by different research groups may explain these inconsistent and conflicting results. Past studies had longer exposure time (hours per day for days or weeks), whereas Parate et al. (2017) applied EMF only once for 10 min for the chondrogenic stimulation of BM-derived MSCs. The major players implicated in the chondrogenic developmental process are Ca^{2+} influx and the mechano-sensitive transient receptor potential (TRP) channels TRPC1 and TRPV4 (Parate et al., 2017). With respect to the in vitro tendon commitment of umbilical cord-derived MSCs, exposure to PEMF for different periods leads to the increased syntheses of scleraxis and collagen type I. Moreover, an increase in the amounts of IL-10 and VEGF throughout the culture suggested the possibility of an indirect mechanism of tenogenic activation initiated by EMF for the healing and repair of tendons (Marmotti et al., 2018).

EMF exposure may also influence the differentiation of MSCs into non-mesodermal lineage cells,

such as neurons and astrocyte cells (Jeong et al., 2017; Asadian et al., 2021). At an intensity of 400 μT, BM-MSC can reach the highest degree of neuronal differentiation for EMF groups with sinusoidal frequencies of 50 and 75 Hz, while astrocytes are frequency-dependent, and 75 Hz square and 75 Hz EMF produce the most significant effect on the differentiation of BM-MSC to astrocytes (Asadian et al., 2021). In another study of astrocytes, ELF-EMF at 50 Hz upregulated sirtuin1 (*SIRT1*), promoting the astrocytic differentiation of human BM-MSCs (hBM-MSCs) through the regulation of downstream molecules of SIRT1 (HES1 and MASH1) (Jeong et al., 2017).

In addition to the use of PEMF, in bone tissue engineering applications, the incorporation of PEMF into titanium dioxide (TiO_2) surfaces cultured with hBM-MSCs in osteogenic medium also showed a good combination. This fusion is evidenced by the increased expression of early- and end-stage osteogenic genes and proteins (such as ALP, *COL-I*, *OCN*, and *OPN*) in PEMF-stimulated hBM-MSCs. Interestingly, the fact that osteogenesis significantly increased was further supported by the findings that cells treated with PEMF/TiO_2 produced secretions into media containing higher amounts of BMP2, decorin (DCN), and collagen type I, compared with untreated controls (Bloise et al., 2018). Table 2 shows a collection of studies on the effects of EMFs on the differentiation capacity of MSCs, and Fig. 3 depicts the effects of EMF on the differentiation of MSCs.

2.3 Effects of EMF on the immunoregulatory and regenerative activity of MSCs

MSCs exhibit potent immunomodulatory activity on almost all kinds of immune cells. This activity allows MSCs to act as an attractive tool for ameliorating autoimmune diseases (de Bari, 2015). For instance, the intravenous injection of MSCs derived from umbilical cord blood could constitute a therapeutic option for patients with rheumatoid arthritis (RA) through the

Table 2 Recent studies on the effects of EMF exposure on MSC differentiation

Study	Biological model	Type of exposure	Exposure frequency (Hz)	Exposure intensity (mT)	Duration	Outcome
Mayer-Wagner et al., 2011	hMSCs	Homogeneous SELF-MFs		5	3 weeks	EMF might be a way to stimulate and maintain the chondrogenesis of hMSCs
Esposito et al., 2013	WJ-MSCs	PEMF	75 (±5%)	1.8–3.0	8 h/d for 21 d	Significant early differentiation of WJ-MSCs into cartilaginous tissue
Ongaro et al., 2015	BM-MSC and AD-MSCs	PEMF	75	1500		PEMF stimulates the osteogenic differentiation of both BM-MSCs and AD-MSCs
Kim et al., 2015	hBM-MSCs	EMF	45	1	2× every 8 h/d for 7 d	Significantly increased osteogenic differentiation
Ross et al., 2015	hSSCs/ BMSCs	LF-EMF				Significantly enhanced osteogenic and chondrogenic differentiation of hSSCs/BMSCs
Yong et al., 2016	Rat MSCs	SEMF	15	1	8 h/d for 6 d	EMF promoted the osteogenic differentiation of sinus MSC
Parate et al., 2017	MSCs	PEMF	15	2	10 min/d	Significantly increased chondrogenic differentiation
Jazayeri et al., 2017	MSCs	PEMF	15	0.2	6 h/d for 10 d	Significantly increased osteogenic differentiation
Jeong et al., 2017	hBM-MSCs	ELF-EMF	50	1	12 d	Significantly promoted astrocytic differentiation
Bloise et al., 2018	hBM-MSCs	PEMF	75	2	10 min/d for 7 and 28 d	Significantly increased osteogenic differentiation
Asadian et al., 2021	BM-MSCs	SEMF	50 and 75	0.4	1 h/d for 7 d	Significantly increased differentiation to neurons and astrocyte cells

EMF: electromagnetic field; PEMF: pulsed EMF; MSC: mesenchymal stem cell; hMSCs: human MSCs; WJ-MSCs: Wharton's Jelly-derived MSCs; AD-MSCs: adipose-derived MSCs; BM: bone marrow; hBM-MSCs: human BM-MSCs; hSSCs/BMSCs: human skeletal stem cells/bone marrow stem cells; LF: low-frequency; ELF: extremely LF; SELF-MFs: sinusoidal ELF magnetic fields; SEMF: sinusoidal EMF.

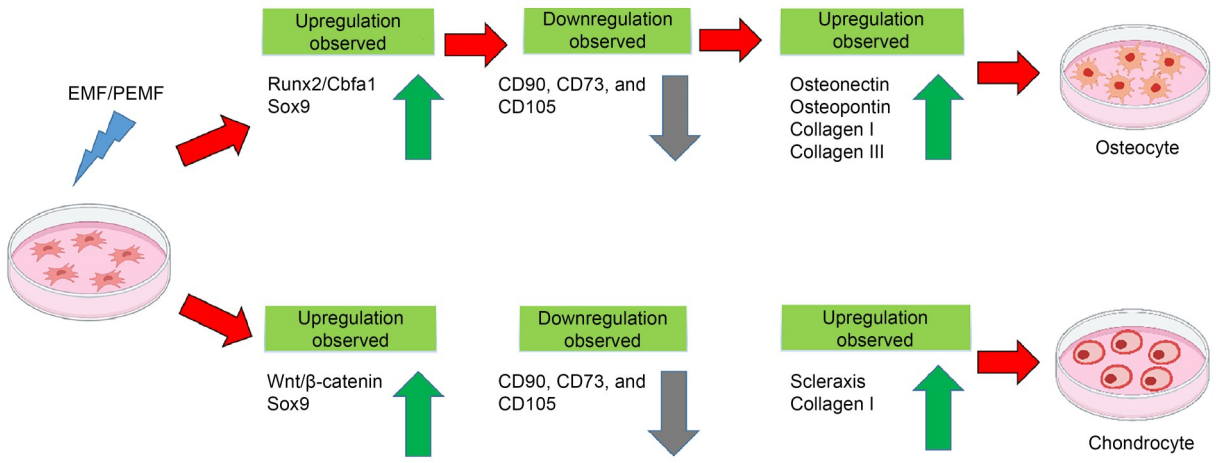

Fig. 3 Overview of the effects of EMF on the differentiation of MSCs. EMF: electromagnetic field; MSCs: mesenchymal stem cells; Runx2: runt-related transcription factor 2; Cbfa1: core-binding factor α1; Sox9: SRY-box transcription 9; CD: cluster of differentiation.

reduction of pro-inflammatory cytokine levels, such as those of IL-1β, IL-6, IL-8, and TNF-α (Park et al., 2018). MSCs activated by lipopolysaccharide (LPS) and exposed to 5.1 Hz EMF for 5 min showed the declined production of pro-inflammatory molecules (IL-1b, IL-6, and IL-17A) and enhanced secretions of anti-inflammatory cytokines (IL-3, IL-4, and IL-10) (Ross et al., 2019).

It was recently shown that EMF affects tissue regeneration and wound healing processes (Pesce et al., 2013; Geng et al., 2014). This includes reductions in total healing time, recurrence rate, wound size, as well as enhancement of the re-epithelialization process (Ieran et al., 1990; Ross and Harrison, 2013; Cheing et al., 2014). Zhang et al. (2018) employed different EMF frequencies (range of 7.5–75 Hz) to evaluate the migration activity of MSCs. They reported that EMF promoted MSC migration by accumulating intracellular Ca^{2+}. The activation of focal adhesion kinase (FAK) leads to the formation of focal contacts and Rho GTPase, which mediates the organization of the cytoskeleton via the FAK/Rho GTPase signaling pathways. Moreover, Wang et al. (2014) investigated the effects of EMF on rat osteoblast adhesion on implant surfaces (titanium), and found that EMF stimulation significantly increased the initial osteoblast adhesion to these surfaces. Furthermore, EMF promotes directional migration and adhesion for a variety of cells involved in regenerative medicine, tissue engineering, and wound healing (Ross, 2017).

3 Cytotoxic and genotoxic effects of EMF on MSCs

Safety and efficacy are two of the major facets of clinical-based trials and continue to serve as the obligatory criteria for any form of therapy intended for mass consumption (Bernardo and Fibbe, 2012; Wang et al., 2013; Freitag et al., 2016). EMF and its impacts on MSCs are a good example of emerging solutions for many ailments. Because of the negative connotations revolving around EMF and the effects of radiation, concerns have been raised regarding its usage at the therapeutic level (D'Angelo et al., 2015). To determine whether EMF is either dangerous or harmless, certain parameters such as amplitude, frequency, and exposure time are needed to be considered (Carpenter and Ayrapetyan, 1994; Miskon and Uslama, 2011). Two studies have shown that EMF exposure within a specified range does not cause any cytotoxic effects in MSCs. In the first, Kim et al. (2015) reported no morphological changes or necrosis to human BM-derived MSCs during osteogenesis upon exposure to 45 Hz of EMF (16 h/d with intervals) for a duration of 7 d. Furthermore, the lactate dehydrogenase (LDH) activity of MSCs treated with EMF did not increase, suggesting no damage to the cell membrane due to radio sensitization. Another study conducted by Ross et al. (2018) tested the cyto/genotoxic activity of 5 Hz, 0.4 mT ELF-EMF on human MSCs for 20 min/d three times a week for two weeks. Their experiment involved cell viability and proliferation assays along with

karyotype analysis, and the results showed no evidence of cytotoxicity or genotoxic chromosomal breaks in the nucleus of MSCs after EMF exposure. Nevertheless, the abovementioned findings are limited to EMF exposure within a small range of frequency, and the influence of other contributing variables, namely amplitude and length of exposure, remains to be investigated. Thus, the critical evaluation and optimization of the said parameters are vital prior to introducing EMF in the clinical therapy of MSCs.

4 Current strategies to trigger the proliferation of MSCs

During the clinical administration of MSCs, certain features such as suitable dose, proper routes of delivery, and correct functionalities of cells within the system are essential to ensure the most effective form of therapy (Kurtz, 2008; Golpanian et al., 2016; Florea et al., 2017). Nonetheless, primary human MSCs do not grow indefinitely in culture; hence an adequate repertoire of MSCs is necessary prior to conducting clinical-based transplantations. To achieve this aim, previous studies have performed the in-depth assessment and standardization of many aspects of MSC culture, including the type of culture media used, cell-plating density, addition of growth factors, and selection of appropriate culture flasks (Sotiropoulou et al., 2006; Bernardo et al., 2007; Ikebe and Suzuki, 2014). Regardless, it is worth mentioning that supplementation techniques and culture conditions are two of the most extensively studied areas on MSCs. The term culture conditions/environments here refer strictly to the physicochemical components of cell culture (temperature, pH, oxygen concentration, etc.). Thus, from this point onwards, focus will be given primarily to studies on these two parameters.

The proliferation and survival of MSCs depend heavily on the type and amount of nutrients contained in the culture media, which has been widely investigated in a number of recent studies (Solchaga et al., 2005; Choi et al., 2008; Tamama et al., 2010; Sun et al., 2013). The basic FGF (b-FGF) is one of the key ingredients for MSC culture due to its varied effector actions including the enhancement of proliferation and immunosuppressive activity of MSCs (Bianchi et al., 2003; Sotiropoulou et al., 2006; Ikebe and Suzuki, 2014). Besides b-FGF, other factors that may further ameliorate the expansion of MSCs are IGF (Doucet et al., 2005), EGF (Tamama et al., 2010), PDGF (Kumar et al., 2010), IL-6 (Pricola et al., 2009), and TGF-β (Doucet et al., 2005; Ng et al., 2008). However, whether MSCs could maintain their stem cell phenotype and differentiation potential following long-term culture with these supplements remains to be conclusive, since many of these studies were carried out within a short period of time. Some factors promoting cell division may elicit unwanted bystander impacts that could act on certain signaling pathways. The mitogen PDGF, for instance, could indirectly prevent the differentiation of MSCs (Pytlík et al., 2011). Furthermore, it has been shown that the addition of b-FGF could trigger a dose-dependent upregulation of histocompatibility markers, especially that of class I molecules (Sotiropoulou et al., 2006). This will inevitably render the cultured MSCs more prone to rejection during clinical transfer as a result of immune incompatibility.

When it comes to the viability and multipotency of stem cells, local niche conditions are deemed highly influential. Likewise, MSCs are sensitive to various types of physicochemical and mechanical changes in their microenvironment (Winer et al., 2009; Kelly and Jacobs, 2010). With regard to this matter, several studies have highlighted oxygen concentration as a major determinant of stem cell proliferation, as well as the maintenance of pluripotency (Ezashi et al., 2005; Ma et al., 2009; Widowati et al., 2014). In the case of MSCs, it was found that the low-oxygen (the volume fraction of O_2 is 2.5%) condition significantly increased the cell proliferation kinetics with reduced population doubling time (Widowati et al., 2014). This is in accordance with the observation made by Haque et al. (2013), which concluded that a hypoxic environment can greatly improve growth kinetics, genetic stability, and the expression of chemokine receptors during in vitro expansion, and eventually increase efficiency of MSC-based regenerative therapies. Hence, by manipulating the oxygenic settings of cells, the quantity and quality of MSCs may be improved for clinical use. One drawback to this approach is that the role of oxygen tension during MSC differentiation is largely influenced by the applied cell isolation technique, the presence of growth factors, and other physiologic requirements. Moreover, substantial discrepancies

exist in the differentiation capacities of hypoxic MSCs derived from in vitro and in vivo experimentations (Ma et al., 2009). Although seemingly promising, further laboratory validations are vital for a complete understanding of the effects of oxygen content on driving the underlying molecular mechanism of cell specialization.

Apart from oxygen concentration, mechanical strain and vibrations have also been associated with the modulation of MSCs, especially during cellular proliferation and differentiation. The response of MSCs to physical stimulation is influenced by the amount of frequency, acceleration, and duration used (McClarren and Olabisi, 2018). Low-intensity vibration (LIV) is usually applied to cells within the range of 10 and 800 Hz, which enhances their structure, leading to increased cellular proliferation and differentiation (Touchstone et al., 2019). When compared with the control group, the application of LIV on MSCs (about twice daily for 20 min at 90 Hz) increased the cell doubling rate by 28%, reduced the activity of β-galactosidase by about 39% in (related to senescence), and improved the rates of osteogenesis and adipogenesis at earlier passages (Bas et al., 2020). The application of LIV may also be beneficial for restoring the diminished proliferation of MSCs (Touchstone et al., 2019). Notwithstanding these findings, the reactivity of MSCs to mechanical loading appears to lack consistency and gave varying results in different culture environments, either in tissue culture plastics or two/three-dimensional scaffolds (McClarren and Olabisi, 2018).

5 Conclusions

Finding novel treatments to promote MSC proliferation and differentiation without cytotoxic and genotoxic effects can bring new ideas to the field of regenerative medicine and tissue engineering. With the recent advancement of cell culture methods and the emergence of specialized bioreactors, EMF therapy is clearly a promising method to further promote the proliferation and differentiation of MSCs. In the past, EMF therapy was successfully used as an effective, non-invasive approach for the treatment of bone diseases. As highlighted by the present review, various studies have demonstrated the positive effects of EMF of 0.2–5.0 mT and 15–75 Hz on the proliferation and differentiation of MSCs for various treatment time. The results of these studies also underscored that EMF could not only support expansion but also induce the commitment of MSCs into osteogenic, chondrogenic, and tenogenic lineages. Although EMF exposure has shown great potential as a novel strategy for the clinical expansion of MSCs, the further examination of the core properties of EMF is necessary to pinpoint the unique range of exposure that could enhance the proliferation of MSCs while maintaining their differentiation potential and stem cell phenotype.

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Author contributions

Haslinda Abdul HAMID drafted and wrote the manuscript with contribution from Azizi MISKON, Rajesh RAMASAMY, Vahid Hosseinpour SARMADI, and Vivek PRASAD. Azizi MISKON conceived the original idea. All authors have read and approved the final manuscript, and therefore, take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Haslinda Abdul HAMID, Vahid Hosseinpour SARMADI, Vivek PRASAD, Rajesh RAMASAMY, and Azizi MISKON declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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