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REVIEW ARTICLE

The Role of Low-Frequency Electromagnetic Fields on Mesenchymal Stem Cells Differentiation: A Systematic Review

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Abstract

BACKGROUND Low-frequency electromagnetic fields (EMFs) influence biological processes. This present study was aimed at the scientific literature on the use of EMFs in the mesenchymal stem cell differentiation process.

MATERIALS AND METHODS The electronic search was carried out in PubMed and Web of Science, a database with a combination of the sinusoidal and pulsed low- and extremely low-frequency electromagnetic fields stimulation and mesenchymal stem cells differentiation, considering the period of publication until December 2021. The literature search identified 118 references in PubMed and Web of Science of which 46 articles were selected, respectively, according to the eligibility requirements.

CONCLUSION The analysis of research indicated that EMFs are an easy-to-apply and practical way in cell therapy and tissue engineering when regulation of stem cells is required. Studies have shown that EMFs have positive effects on stem cell differentiation, accelerating its process regardless of the parameters and type of stem cells. However, the exact amplitude, frequency, duration of the electrical field, and application method remain elusive and need more study in future work.

Keywords Mesenchymal stem cells · Differentiation · Low-frequency electromagnetic fields

1 Introduction

Tissue engineering has gotten a lot of interest in the last few decades as a possible answer to clinical challenges and its purpose is to replace and restore biological tissue or organs [1]. Cells, bioreactors, scaffolds, tissue architecture techniques (3D printers), and chemical stimuli such as growth factors are the essential components of tissue engineering. Mesenchymal Stem cells are one of the most commonly used cell sources in tissue engineering because of their characteristics (self-renewal, differentiation, and immunomodulatory capacities) [2, 3]. Targeted differentiation of mesenchymal stem cells is significant. In light of recent improvements in stem cell differentiation, it has been demonstrated that chemical induction is not the only factor that influences stem cell fate. Physical factors such as electrical, and magnetic fields also play a significant role

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[4, 5]. Physical and mechanical stimuli are well recognized to affect biological systems, and the effects of electromagnetic fields (EMFs) have already been shown to play a significant role in this regard [6]. With the progress of electromagnetic theory in the last several decades, there has been an increased interest in the interaction between EMFs and many cell functions and behaviors. Low-frequency EMFs (0–100 Hz) have been shown to influence a variety of biological processes, including cell differentiation [7, 8], gene expression [9], protein secretion, proliferation, cell cycle [10], wound healing [11], and as a result, stem cell fate [12]. Furthermore, an essential component of the impact of these waves on the body, aside from mechanical and chemical factors, is the effective involvement of EMFs produced by cells in morphogenesis. These waves are produced during organ creation and direct the development of the fetus' primary organ in the early stages of development and formation [13]. Physiological activities, such as movements of the musculoskeletal system's structure, generate endogenous EMFs in living tissue. Mechanical stresses and currents due to human muscle vibrations have been observed during postural muscle activity (5–10 Hz) and walking (10 Hz) [12].

One of the biological processes which EMFs can influence is stem cell differentiation [7, 8]. The electrical characteristics of the plasma membrane are governed by the differences in the distribution of a few critical ions between intracellular and extracellular fluids, as well as their selective transport through the plasma membrane [7]. EMFs can influence ions influx and as a result ions concentration across the cell membrane and transmembrane potential (Fig. 1). In turn, this can induce physiological processes and affect stem cell fate by modulating epigenetic changes, gene expression, and differentiation pathways activation [13–15]. Calcium (Ca^{2+}) is a crucial regulator of various cellular functions, and it has been shown that EMFs influences its influx and concentration by interacting with voltage-sensitive Ca^{2+} channels and the EMFs effect may differ depending on its parameters [12, 14, 16]. Calcium is a cyclic AMP activator, which is a critical component in the triggering of intracellular metabolic processes and it is well known that the differentiation process increases energy demand, and alters the mitochondria. It also has been shown that EMFs can induce free radicals (ROS) forming which can influence ATP production and other chemical reactions. Moreover, it has been also shown that EMFs can affect spindle microtubules because of tubulin dipoles and thus can influence asymmetric cell divisions leading to stem cell differentiation [14, 15]. In summary, EMFs can alter stem cell fate and determine their differentiation by influencing charges in cell components and thus cell communication by regulating the signals delivered to cells. However, the EMFs effect depends on its parameters and it is important to verify which can have a positive impact and stimulate mesenchymal stem cells (MSCs) differentiation.

In this review, optimal conditions and specific EMFs parameters (frequency, intensity, and time of exposure) are discussed for effective differentiation of MSCs to osteogenic, chondrogenic, and neurogenic lineages in an *in vitro* setting.

2 Methods and materials

For this review study, the electronic databases PubMed and the Web of Science using keywords (mesenchymal stem cells, differentiation, low-frequency & extremely low-frequency electromagnetic field) were searched. The inclusion criteria were the year of publication from January 2010 to December 2021, studies on mesenchymal stem cell differentiation by using low- and extremely low & extremely low-frequency. The method of selecting the papers was carried out by perusing the titles and abstracts of the studies and completed articles, when necessary, importing all results from Mendeley library and then removing duplicates. Studies on other effects of electromagnetic fields on cells were excluded.

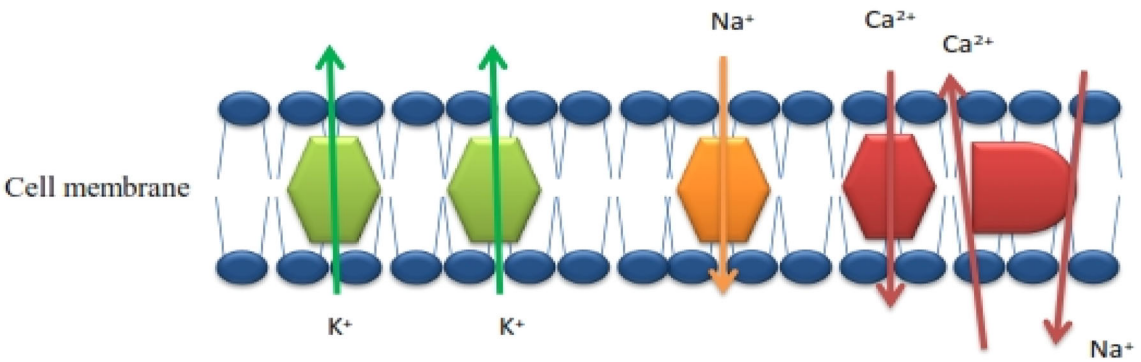

Fig. 1 Diagram summarizing the deformation of ion channels due to exposure to EMFs

The initial search in the PubMed and Web of Science databases found 118 articles, of which 52 were excluded because they did not meet the eligibility criteria and 20 duplicate reports were removed. After the analyzing of the titles and abstracts, 46 articles were selected for systematic review (Fig. 2).

3 Results

It has been confirmed that EMFs is a non-invasive and cost-effective method to facilitate or increase the differentiation of mesenchymal stem cells into different cell lines. The following review of the papers in detail.

3.1 EMFs and osteogenic differentiation

EMFs stimulation has been utilized successfully in the bone healing process for many years [10]. Aldebs et al. found that exposing human adipose-derived mesenchymal stem cells (hASCs) to a low-frequency pulsed electromagnetic fields (PEMFs) (15 Hz, 1 mT) for 8 h a day for 21 days in combination with super magnetic iron oxide nanoparticles (NPs) improved their osteogenic potential [17]. Wang et al. investigated the effect of EMFs (15 Hz/1 MT) on bone marrow MSCs (BM-MSCs) osteogenic differentiation. Low-frequency EMFs was found to improve osteogenic differentiation and bone repair in this investigation. Rabbit BM-MSCs were put onto a hydroxyapatite/collagen scaffold and subsequently stimulated with a 15 Hz/1mT low-frequency EMFs in their research. Low-frequency EMFs was found to improve osteogenic differentiation and bone repair in this investigation. Rabbit BM-MSCs were put onto a hydroxyapatite/collagen scaffold and subsequently stimulated with a 15 Hz/1mT low-frequency EMFs in their research. ALP activity and bone gene expression were used to measure MSC osteogenic differentiation. In addition, an *in vivo* assessment of a rabbit femur condyle defect model was carried out. The fate of MSCs influenced by EMF induction has been suggested as a possible therapeutic technique for bone tissue engineering based on this study. [18]. Coculture of human osteoblasts with hASCs and exposure to extremely low - frequency PEMFs were recommended by Ehnert et al. to promote osteogenic differentiation. The gene expression of hASCs subjected to two different very low- frequency PEMFs (16 and 26 Hz) was analyzed, and it discovered that exposure to 16 Hz PEMFs might result in bone formation, exposure to 26 Hz ELF-PEMF resulted in bone remodeling [19]. PEMFs therapy is shown to be an effective, non-invasive treatment for a Varous of clinical problems, particularly in the context of mesenchymal stem cell development. The effects of extremely low-frequency PEMFs on osteogenesis have been studied in certain research [19–24]. BM-MSCs tagged with super-paramagnetic iron oxide nanoparticles were used by Wu et al. to analyze the osteoblastogenesis influence (SPION). SPION-labeled rat BM-MSCs were subjected to a 50 Hz PEMFs at 1.1 mT. compared to a control group, low-frequency PEMFs exposure resulted in increased proliferation of SPION-labeled BM-MSCs [22]. Lim et al. conducted research on the effects of extremely low-frequency PEMFs

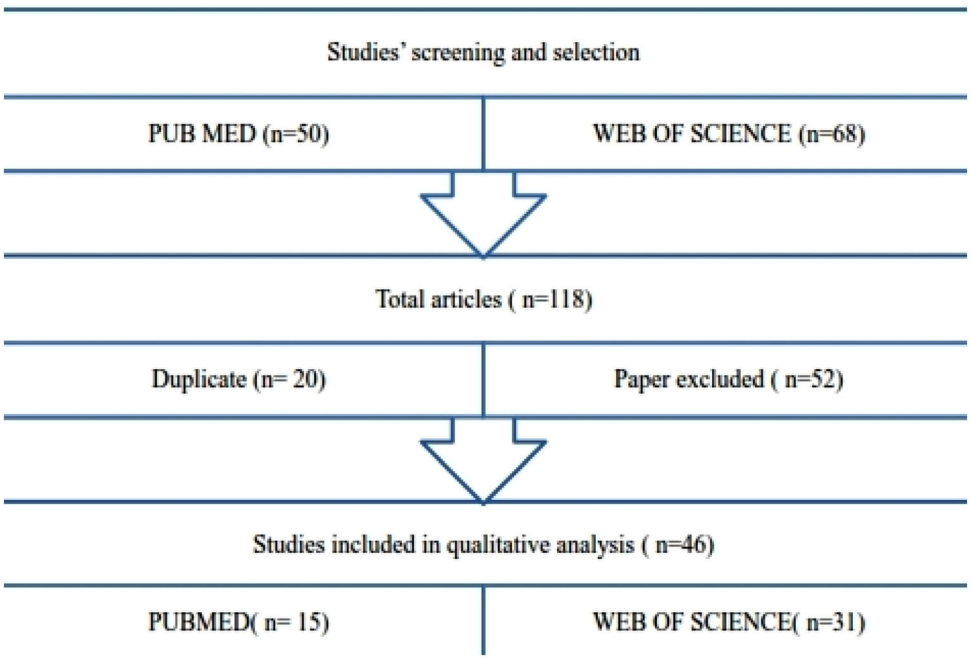

Fig. 2 Summary of the selection of articles for the review

on human alveolar bone-derived mesenchymal stem cells (hABMSCs) proliferation and differentiation. Osteogenesis is a complex series of events involving the differentiation of mesenchymal stem cells to produce new bone. The effects of extremely low-frequency PEMFs on cell proliferation, alkaline phosphatase (ALP) activity, and extracellular matrix mineralization were investigated in this work, as well as the expressions of vinculin, vimentin, and calmodulin (CaM) in hABMSCs throughout osteogenic differentiation. On day 5, PEMFs stimulation of hABMSCs increased proliferation by 15% relative to untreated cells. In addition, extremely low- frequency PEMFs considerably increased ALP expression during the early stages of osteogenesis and significantly improved mineralization towards the middle of osteogenesis in just 2 weeks. compared the control, PEMFs improved vinculin, vimentin, and CaM expression. CaM discovered that PEMFs significantly changed the expression of osteogenesis-related genes. extremely low-frequency PEMFs were found to increase early cell proliferation and accelerate osteogenesis in hABMSC-mediated osteogenesis [25]. PEMFs has been shown in this research to be a feasible option for bone tissue engineering applications. Yan et al. discovered that extremely low-frequency EMFs had no obvious influence on human mesenchymal stem cell development (hMSC). They tested the effect of extremely low-frequency EMFs (50 Hz, 20mT) on hMSCs for 23 days and discovered that EMFs inhibited hMSCs proliferation and metabolism. The alkaline phosphatase (ALP) assay, calcium assay, ALP staining, and Alizarin red staining, however, do not support the regulating influence of EMFs on osteogenic differentiation of hMSCs [26]. It could be because of the high magnetic flux density (20mT), different from prior research that used 1.0 mT or 1.1 mT. Mirzaee et al. found that the presence of the conductive polymer might increase the beneficial effects of PEMFs on the osteogenic development of dental pulp stem cells dental pulp stem cells (DPSCs). According to the findings, polyaniline (PANI) and PEMFs improved the osteogenic differentiation ability of human DPSCs in a synergistic manner [27]. Their results indicate that using an PEMFs during this loading regime causes the early phases of bone tissue formation. Many studies have demonstrated that an EMF can help MSCs differentiate into osteogenic cells. However, depending on the experimental and environmental conditions, the experimental outcomes have changed. These differences can be compensated for by optimizing electromagnetic field characteristics in a single designated machine [28–30]. *In vitro*, Kang et al. confirmed that various electromagnetic field parameters (frequency and magnetic flux density) fundamentally affect osteogenic differentiation of adipose-derived stem cells (ASCs). ASCs was determined before osteogenic differentiation, and the EMF became homogenous at the center of the solenoid coil. Then, by measuring alkaline phosphate (ALP) mRNA expression, positive (30/45 Hz, 1 mT) and negative (7.5 Hz, 1 mT) osteogenic differentiation conditions were chosen. compare to the non-stimulated group, the expression of osteogenic markers (RUNX2, COL-I, OSX, and OC) was higher in the 30/45 Hz condition than in the 7.5 Hz condition. Those findings were confirmed by both positive and negative modulation of ALP activity and mineralized nodule development. he effects of EMFs on osteogenic differentiation varied depending on the electromagnetic field's parameters. This finding lays the groundwork for future research into influencing stem cell fate by adjusting EMFs settings [31]. The synergistic effect of EMFs with other stimulus elements has been studied extensively. The synergistic effects of PEMFs signal with iron-ion-doped tricalcium phosphate bone substitute on osteogenesis of hMSCs *in vitro* were summarized by Habib et al. They stimulated hMSCs using PEMF (15 Hz) for 4 h daily for up to 10 days, with ALP activity increasing at a higher rate when combined with magnetic nano bone substitutes (MNBS). The findings demonstrate the synergistic effects of PEMFs and MNBS on osteogenesis and suggest that PEMFs and MNBS could be used to promote bone repair [32]. The effects of PEMFs (0.2 mT, 15 Hz) and biochemical stimulation on MSCs and their osteogenic pattern were studied by Jazayeri et al. After 10 days of EMF stimulation at 6 h each day, they found that a combination of chemical components and electromagnetic fields improves osteogenesis. Furthermore, in animal models, the use of differentiated osteoblasts seeded on collagen scaffolds promotes the formation of new bone tissues [33]. Meshkian et al. found that cell adhesion, proliferation, and differentiation were all regulated as the Nanofibers were influenced by EMFs, which had synergistic effects on the bone formation process [34]. The scaffold was combined with EMFs in different combinations. Many of these studies have found that using EMFs in conjunction with the scaffold has a synergistic effect. In analyzing osseointegration in osteoporosis, Ye et al. used 3D printed porous Ti (PTi) scaffolds with optimum pore size and porosity matching bone tissue with PEMF as an exogenous osteogenic induction stimulus. PEMF boosted the expression of osteogenic genes (ALP, RUNX2, BMP-2) on the surface of PTi scaffolds *in vitro*, resulting in increased BM-MSC proliferation and osteogenic differentiation [35].

Finally, EMFswith the suitable characteristics can help to stimulate osteogenesis. EMFs and PEMFs are potentially low-cost and widely applicable tissue engineering techniques that can heal and develop new bone. The studies discussed above are summarized in Table 1.

3.2 EMFs and chondrogenic differentiation

Cartilage is known for its low self-maintenance capacity, and currently, there are no efficient methods to improve cartilage repair [36]. Tissue engineering opens a new path to overcoming these limitations.

BM-MSCs exposed to low intensity (2mT) and extremely low- frequency (15 Hz) PEMFs for 10 min each day is ideal for chondrogenic differentiation, according to Parate et al. This finding emphasizes the complexities of calcium homeostasis during early chondrogenesis, as well as the limitations imposed on PEMFs-based healing approaches aiming at increasing MSCs chondrogenesis. The efficacy of optimized PEMFs for future cartilage regenerating techniques was suggested in this study [37]. Mayer-Wagner et al. developed a bioreactor system that allows the influence of low-frequency EMFs and simulated microgravity (SMG) *in vitro* chondrogenesis of human mesenchymal stem cells in 3D culture to be studied independently or in combination under controlled conditions. Gene expression was not affected by a single low-frequency EMFs. The expression of *COLXA1* and *COL2A1* was reduced by a single SMG. In comparison to SMG, low-frequency EMF/SMG resulted in considerably greater *COL2A1* expression. In comparison to SMG, low-frequency EMF/SMG increased *COLXA1* insignificantly. When compared to control culture levels treated with growth factors, the combination therapy EMFs/SMG was not substantially better. *COL2A1* expression was maintained by EMFs/SMG, which had been decreased by SMG [38]. In a three-dimensional (3D) MSCs alginate bead, Kavand et al. investigated the ability of EMFs with frequencies of 25 Hz and 50 Hz to control cartilage gene expressions. Six groups of cell-alginate constructs were tested and treated for 21 days. TGF-beta 1 treatment had a higher impact on *COL2* and *SOX9* gene expression in MSCs than PEMFs treatments alone, according to real-time polymerase chain reaction (PCR) data. *COL2* was found to have a larger transcriptional tendency to change after PEMF stimulation, however, there were no significant differences in *SOX9* gene expressions compared to the control group under the stated electromagnetic parameters used in this investigation. PEMFs increased extracellular matrix molecule deposition, and glycosaminoglycans stained favorably with Alcian blue [39]. Using magnetoresponsive stem cell spheroids (MR-SCS) 3D culture, Yoo et al. explored whether low-frequency EMF stimulates chondrogenic differentiation [40]. Mayer-Wanger et al. discovered that exposing the cultures to low-frequency EMFs (15 Hz, 5mT) for 45 min every 8 h boosted collagen type II (Col -II) expression and GAG content, but not *Aggrecan* or *SOX9* expression. Under electromagnetic stimulation, the Collagen type X gene expression was reduced. Based on these findings, it's been proposed that EMFs could be used to induce and maintain hMSCs chondrogenesis [41]. Low frequency PEMFs can stimulate chondrogenic differentiation of rat BM-MSCs *in vitro*, according to Oiu et al. the adhesion approach was used to extract the rat BM-MSCs, and the third generation of rat BM-MSCs was randomly divided into three groups: low-frequency PEMFs, chondrocyte-induced, and control. The whole medium PEMFs groups were subjected to 50 Hz, 1mT PEMFs for 30 min every day for 10, 15, and 20 days, respectively. The chondrocyte-induced group was given chondrogenic media, whilst the control groups were given only complete medium to culture. *Col-II* and *Aggrecan* mRNA and protein expression levels were considerably greater in the low-frequency PEMFs or chondrocyte-induced groups than in the control group, according to the findings. *In vitro*, low-frequency PEMFs have been shown to help rat BM-MSCs differentiate into chondrogenic cells [42]. EMFs stimulation (50 Hz, 30 mT) and 5% platelet-rich plasma (PRP) stimulated MSCs chondrogenesis, according to Hesari et al. [43].

All in all, cartilaginous tissue comprises a collagen protein that is considered a piezoelectric substrate and is affected by electric fields, making explicit chondrogenic qualities an intriguing possibility for bio-electromagnetic studies [44]. The studies discussed above have been summarized in Table 2.

3.3 EMFs and neurogenic differentiation

The most widely employed elements in nerve tissue engineering are biochemical cues like growth factors, but due to the structure of nerve cells and their sensitivity to electromagnetic fields, EMFs has recently been explored as a distinguishing factor. Several studies utilizing BM-MSCs have been carried out to investigate the effects of electromagnetic field settings in the range of 1–5 mT at a frequency of 50 Hz on the neuronal differentiation of MSCs. After 12 days of exposure, Cho et al. discovered that low-frequency EMFs (50 Hz; 1mT) reduced the growth of hBM-MSCs. Their gene expression levels changed, with the expression of neural stem cell markers like nestin decreasing while the expression of neural cell markers like *MAP2*, *NEUROD1*, *NF-L*, and Tau increased. They also confirmed the expression of each protein of neural cells, as well as oligodendrocyte and astrocyte-related proteins including O4 and GFAP, after extremely low-frequency EMFs stimulation in an immunofluorescence analysis. According to their findings, EMFs can stimulate neuronal differentiation in BM-MSCs without the need of pharmacological agents [45]. Kim et al. conducted a similar study in which they looked into the link between extremely low-frequency EMF exposure and neural differentiation.

Table 1 Characteristics of the osteogenic differentiation studies selected for review

Aim	Materials and methods	*In-vitro*	*In-vivo*	Results and conclusions	References
Checking if LF-EMFs influence osteogenic differentiation of hASCs in the recreated bone tissue microenvironment	Pulsed LF-EMF stimulation (15 Hz/1mT) 8 h a day for 21 days of hASCs on a three-dimensional (3D) hydrogel scaffold based on RADA16	Yes	No	EMF in conjugation with super magnetic iron oxide nanoparticles (NPs) increased the osteogenic capacity of hASCs	Aldebs et al. [17]
Checking if LF-EMFs is promoting osteogenic differentiation of rabbit BM-MSCs	Stimulation of BM-MSCs with EMF (15 Hz/1mT) on hydroxyapatite/collagen I scaffold	Yes	Yes	EMF can enhance osteogenic differentiation in cells on a hydroxyapatite/collagen I scaffold	Wang et al. [18]
Checking if LF-EMF has an effect on proliferation and osteogenic differentiation of co-cultured hASCs and osteoblasts	Pulsed LF-EMF stimulation (16 and 26 Hz) of co-cultured hASCs and osteoblasts	Yes	No	Improvement of osteogenic differentiation by LF-EMF (16 Hz – enhancing bone formation; 26 Hz – enhancing bone remodeling)	Ehnert et al. [19]
Checking the Effect of Pulsed LF-EMF on osteoblastogenesis	Rat BM-MSCs labeled with SPIONs exposed to pulsed LF-EMF (50 Hz/1.1mT)	Yes	No	The combination of LF-EMF SPIONs promotes migration and osteogenic differentiation of BM-MSCs	Wu et al. [22]
Checking the influence of ELF-PEMFs on proliferation and differentiation of hABMSCs	The hABMSCs were constantly exposed to ELF-PEMFs (10, 30 and 100 Hz)	Yes	No	Increased proliferation by 15% after 5 days; improved expression and mineralization of ALP near the midpoint of osteogenesis within 2 weeks. ELF-PEMFs can enhance early cell proliferation and accelerate osteogenesis	Lim et al. [25]
Checking if ELF-EMFs affect the growth, metabolism, and differentiation of hMSCs	hMSCs were stimulated with ELF-EMFs (50 Hz, 20mT) for 23 days	Yes	No	ELF-EMFs may inhibit the growth and metabolism of hMSCs. No significant effect on hMSCs differentiation was observed	Yan et al. [26]
Checking the synergistic effects of polyaniline and PEMF on the osteogenic differentiation of DPSCs	DPSCs were stimulated with PEMF (50 Hz, 1mT) for 14 days	Yes	No	The osteogenic differentiation potential of human DPSCs was synergistically enhanced by (PANI) and PEMF	Mirzaei et al. [27]
Checking the influence of EMF on ASCs osteogenesis	ASCs were exposed to EMF (7.5/30/45 Hz, 1 **mT**)	Yes	No	Exposure to EMF (30 and 45 Hz) of ASCs showed a higher expression of osteogenic markers (RUNX2, COL-I, OSX, and OC) than 7.5 Hz. The influence of EMF on the osteogenic differentiation of ASCs depends on the EMF parameters	Kang et al. [31]
Investigating the synergistic effect of PEMF with iron-ion-doped tricalcium phosphate bone substitute on hMSCs osteogenesis	Magnetic nano-bone substitutes were cultured with hMSCs and stimulated with PEMF (15 Hz) for 4 h daily up to 10 days	Yes	No	Faster increase in ALP activity while PEMF was combined with MNBS. Upregulation of the expression of the BMP-2, BGLAP and SPP1 gene. PEMF combined with MNBS may provide a new method for accelerating bone healing	Habib et al. [32]
Checking if EMF can influence osteogenic differentiation of MSCs	Rat MSCs were stimulated with EMF (15 Hz; 0.2 mT) for 10 days with 2, 4 and 6 h of exposure per day	Yes	Yes	EMF increased expression of osteogenic markers, most effectively after 6 h of stimulation per day for 10 days in osteogenic differentiation medium. Appropriate chemical factors combined with EMF resulted in a higher efficiency of osteogenic differentiation	Jazeyari et al. [33]

Table 1 continued

Aim	Materials and methods	*In-vitro*	*In-vivo*	Results and conclusions	References
Checking whether MSCs cultured on EMF stimulated nanofibers influence their growth, adhesion, and osteogenic differentiation	Mouse ASCs were cultured on fabricated nanofibers scaffold and were exposed to EMF (0.9 T, 30 s)	Yes	No	Increased proliferation and adhesion of ASCs cultured on nanofibers stimulated with EMF was observed as well as increased expression of osteogenic markers	Meshkian et al. [34]
Checking if 3D printed pTi scaffolds combined with PEMF influence osteogenic differentiation of BM-MSCs	The MSCs on 3D printed pTi scaffolds were combined with PEMF and stimulated (50 Hz; 1 mT; 2 h per day for 21 days)	Yes	No	Increased expression of osteogenic markers (ALP, RUNX2, BMP-2) has been observed after PEMF stimulation. PEMF can improve osteogenic differentiation and bone regeneration	Ye et al. [35]

During *in vitro* expansion, BM-MSCs were subjected to a 50 Hz EMFs reduced the rate of proliferation of BM-MSCs, which resulted in an increase in neural differentiation. Cells treated with extremely low-frequency EMF revealed higher levels of neuronal differentiation marker (MAP2), whereas the early neuronal marker (*Nestin*) was adversely regulated, similar to Cho et al. findings. [46]. Choi et al. then revealed that utilizing the same EMFs conditions (50 Hz; 1 mT), EMF is an effective means of differentiating into neural cells. PEG-phospholipid-encapsulated magnetite nanoparticles (Fe3O4) were employed in hBM-MSCs to increase intracellular absorption in this work. extremely low-frequency EMFs mixed with nanoparticles improved neuronal development by increasing the expression of *NeuroD1*, *MAP2*, *DCX*, *NF-L*, and *MBP*. Nanoparticles, on the other hand, can be cytotoxic, thus some considerations must be addressed [47]. Furthermore, Aikins et al. explored whether extremely low-frequency EMFs (50 Hz; 1mT) caused neuronal differentiation in hBM-MSCs. Cell proliferation was reduced and neural-like morphology developed after extremely low-frequency EMF stimulation, according to the researchers. At the mRNA level, neuronal markers such -tubulin3, pleiotrophin, and neurofilament-M were detected, as well as MAP2 at the protein level. Reduced expression of metal-response element-transcription factor 1 and MT3, as well as lower intracellular Zn content, were found to be associated with extremely low-frequency -EMF-induced neuronal differentiation. Additionally, upregulation of dihydropyrimidinase-related protein 2 was detected, although -enolase expression remained unchanged. These findings point to a potential MT3 regulation mechanism during neural differentiation [48]. Extremely low-frequency EMFs (50 Hz, 1mT) over 12 days influences the regulation of hBM-MSCs and stimulates astrocyte differentiation, according to Jeong et al. The astrocyte marker (GFAP) was upregulated in extremely low-frequency EMFs -treated cells, while the early neuronal marker (*Nestin*) and the stemness marker (OCT3/4) were down-regulated. Furthermore, after exposure to extremely low-frequency EMF, the number of reactive oxygen species (ROS) was found to be significantly raised, highlighting the modulatory involvement of sirtuin1 (SIRT1) and downstream SIRT1 molecules (TLE1, HES1, and MASH1) during astrocyte differentiation. These results suggest that extremelylow-frequencyEMFs induce astrocytic differentiation through activation of SIRT1 and SIRT1 downstream molecules [49]. In rats with BM-MSCs, Haghighat et al. investigated the effects of nitric oxide (NO) and physical factors (EMFs) on the expression of expression and neural differentiation markers. Cells exposed to high NO amounts in combination with EMFs began to differentiate [50]. Using *in vitro* and *in vivo* tests, Seo et al. evaluated the effects of low-frequency PEMF pretreatment on the proliferation and properties of BM-MSCs as well as the regeneration of the injured peripheral nerve. PEMFs increased not only the rate of BM-MSCs growth but also the expression of nerve growth factors *in vitro*. Additionally, when these treated PMSCs with PEMFs are introduced into a damaged mental nerve, they have a stronger influence on nerve regeneration than untreated BM-MSCs. This suggests that PEMFs pretreatment of BMSCs could be a more strategic tool in cell therapy for repairing damaged mental nerves [51]. In several investigations, graphene-based substrates were employed in conjunction with extremely low-frequency EMFs in nerve regeneration. So far, the findings of Lee et al. showed that the action of extremely low-frequency EMFs (50 Hz, 1 mT) and the graphene-coated substrate have a synergetic impact in increasing the biological efficacy of neuronal differentiation in hBM-MSCs. They claim that this increase in neurogenesis is due to a shift in the global gene expression profile, which up-regulated the gene expression profile, thereby up-regulating adhesion via intracellular calcium [52]. Moraveji et al. evaluated how extremely low-frequency EMFs (50 Hz, 1 mT) affected the expression of the

Table 2 Characteristics of the chondrogenic differentiation studies selected for review

Aim	Materials and methods	*In-vitro*	*In-vivo*	Results and conclusions	References
Checking if PEMF affects hBM-MSC chondrogenesis and calcium homeostasis	The hBM-MSCs were exposed to PEMF (15 Hz; 1–4 mT) for 5, 10, 20, 30, 60 min per day for 7 and 21 days	Yes	No	PEMF with parameters 15 Hz and 2 mT for 10 min per day is optimal for chondrogenic differentation. PEMF stimulation is effective only once at the onset of chondrogenic induction; repeated exposures diminished chondrogenic outcome probably due to the disturbance of calcium homeostasis	Parate et al. [37]
Checking if LF-EMF and SMG have influence on hMSCs chondrogenesis	hMSC pellets were cultured under the influence of LF-EMF (15 Hz; 5 mT) and SMG individually or in combination for 45 min three times a day for 21 days	Yes	No	LF-EMF did not show a significant influence on chondrogenesis, while SMG reduced the chondrogenic potential of hMSCs. The combined use of LF-EMF/SMG maintained chondrogenic potential (lowered by SMG) by reincreasing COL2A1 expression	Mayer - Wagner et al. [38]
Checking the influence of PEMF on the 3D (MSC)-alginate construct and SOX9 and COL2 expression	The 3D cultures of rabbit ASCs were treated with PEMF (25 and 50 Hz) 8 h per day for 21 days			PEMF (50 Hz) increases COL2 expression. Cotreatment with TGF-beta 1 has a greater influence on COL2 and SOX9 gene expression compared to PEMF alone	Kavand et al. [39]
Checking if LF-EMF stimulation promotes chondrogenesis using magnetoresponsive stem cell spheroid-based cartilage recovery platform	Magnetoresponsive mouse MSCs spheroids were stimulated with LF-EMF (15 Hz; 5mT) for 30 min per day for 21 days. MR-SCS were implanted in an ex vivo cartilage defect model	Yes	No	LF-EMF stimulation increased the expression of COL2, SOX9 and Aggrecan. *The* ex vivo model of the porcine femur showed an improvement in cartilage tissue regeneration	Yoo et al. [40]
Checking the Impact of EMF on hMSCs chondrogenesis	hMSCs cultured with FGF-2 and TGF -β(3) were stimulated with LF-EMF (15 Hz, 5mT) for 45 min every 8 h for 21 days	Yes	No	LF-EMF increased Col II and decrease Col X expression	Mayer - Wagner et al. [41]
Checking the influence of pulsed LF-EMF on rat BM-MSCs *in vitro*	Rat BM-MSCs were exposed to PEMF (50 Hz; 1mT) for 30 min per day for 10, 15 and 20 days	Yes	No	The levels of mRNA and protein expression of Col-II and aggrecan increased significantly in the chondrocyte-induced group, compared to the control group after PEMF stimulation	Qiu et al. [42]
Checking the influence of EMF on hASCs chondrogenesis	The hASCs were stimulated with EMF (50–400 Hz, 30 mT) for 6 h	Yes	No	LF-EMF stimulation upregulated SOX9 expression. LF-EMF (50 Hz) facilitate Chondrogenesis with minor inflammation and hypertrophic maturation	Hesari et al. [43]

MAP2 and *Nestin* genes in mesenchymal cells from the dermal papilla (DPCs). To see how chemical and electromagnetic elements affect gene expression, four experimental groups were created and treated for 5 days: chemical (cell exposure to chemical signals), EMFs (cell exposure to extremely low-frequency EMF), chemical-EMFs (cell exposure to chemical signals and extremely low-frequency EMF), and control (no treatment). Real-time PCR analysis proved that EMFs has a useful function in triggering neuronal differentiation. The expression of *MAP2* was higher after 14 days than it was after 5 days. The effect of prolonging the treatment period on neuronal differentiation has also been demonstrated by decreased in cell proliferation after 5 to 20 days of EMFs influence [53]. The impact of the synergistic action of EMFs and other physical stimuli has been studied in certain studies. Choi et al. conducted an experiment to determine whether PEMFs (60 Hz) and sound waves (1 kHz and 81 dB) have a synergistic effect on the neurogenic differentiation of hBM-MSCs. These findings suggest that a combination of

Table 3 Characteristics of the neural differentiation studies selected for review

Aim	Materials and methods	*In-vitro*	*In-vivo*	Results and conclusions	References
Checking if hBM-MSCs have the potential to differentiate into neural cells while stimulated with ELF-EMF	hBM-MSCs were continuously exposed to ELF-EMF (50 Hz; 1 mT) for 12 days	Yes	No	EMF inhibited hBM-MSC growth at 12 days of exposure. EMF induced the expression of MAP2, NEUROD1, NF-L, O4 and GFAP, and Tau, but decreased the expression of nestin. EMF can induce neural differentiation in hBM-MSCs	Cho et al. [45]
Checking if BM-MSCs have the potential to differentiate into nerve-type cells while stimulated with ELF-EMF	BM-MSCs were continuously exposed to ELF-EMF (50 Hz; 1 mT) for 12 days	Yes	No	ELF-EMF decreased the proliferation of BM-MSCs and increased the expression of MAP2 and decreased the expression of Nestin	Kim et al. [46]
Checking if hBM-MSCs with magnetic iron oxide (Fe_3O_4) nanoparticles have the potential to differentiate into nerve-type cells while stimulated with ELF-EMF	The PEGylated nanoparticles in the BM-MSCs were continuously exposed to the ELF-EMFs (50 Hz; 1mT) for 12 days	Yes	No	ELF-EMF combined with nanoparticles increased the expression of NeuroD1, MAP2, DCX, NF-L, and MBP. ELF-EMFs improved neural differentiation in hBM-MSCs incorporated with nanoparticles	Choi et al. [47]
Investigating the effect of ELF-EMF on the neural differentiation of hBM-MSCs on Zn-MT3 homeostatic interaction	hBM-MSCs were continuously exposed to ELF-EMF (50 Hz; 1 mT) for 12 days	Yes	No	ELF-EMF induced neural differentiation of hBM-MSCs, decreased proliferation, and enhanced neural-like morphology. Increased expression of β-tubulin3, pleiotrophin, neurofilament-M, and MAP2. ELF-EMF-induced neural differentiation correlated with decreased expression of metal-response element-transcription factor 1 and MT3, as well as decreased intracellular Zn concentration	Aikins et al. [48]
Checking the role of ELF-EMF in the enhancement of astrocytic differentiation of hBM-MSCs	hBM-MSCs were continuously exposed to ELF-EMF (50 Hz; 1 mT) for 12 days	Yes	No	ELF-EMF stimulation increased astrocyte marker (GFAP) levels and negatively regulated early neuronal marker (Nestin) and stem marker (OCT3/4). The increased level of ROS suggests astrocytic differentiation through the activation of downstream SIRT1 and SIRT1 molecules	Jeong [49] et al
Checking the effect of nitric oxide and LF-EMF on neuronal differentiation of rat BM-MSCs	Rat BM-MSCs treated with nitric oxide (50 μM and 1 mM) were exposed to LF-EMF (50 Hz; 20 mT) 4 h per day	Yes	No	Nitric oxide at low concentration helped cells protect the stem state, but at high concentration, together with EMF stimulation, directed cells into the differentiation pathway	Haghighat et al. [50]
Checking the influence of LF-PEMF on the proliferation and regeneration ability of BM-MSCs of crush-injured mental nerve	hBM-MSCs were exposed to LF-PEMF (50 Hz, 1 mT, 1 h/day) for 5, 7 or 10 days. Next, BM-MSCs were injected into an *in vivo* animal crush injury model	Yes	Yes	LF-PEMF increased S100, GFAP, NGF and BDNF expression levels.BM-MSCs pretreated with LF-PEMF when injected into the injured mental nerve showed higher efficiency in nerve regeneration than unpretreated BM-MSCs	Seo et al. [51]

Table 3 continued

Aim	Materials and methods	*In-vitro*	*In-vivo*	Results and conclusions	References
Checking the effect on hMSC neural differentiation of simultaneous use of ELF-EMF and a graphene-coated substrate	hMSCs cultured on a glass or graphene substrate were continuously exposed to ELF-EMF (50 Hz; 1 mT) for 14 days	Yes	No	ELF-EMF decreased Nestin expression but increased TUJ-1, MAP2, and NCAM. Those changes were more noticeable on the graphene substrate than on the glass substrate	Lee et al. [52]
Checking if ELF-EMF influence gene expression of dermal papilla mesenchymal cells	Papilla mesenchymal cells were continuously exposed to ELF-EMF (50 Hz; 1 mT) for 7 h for 5, 14 or 20 days			ELF-EMF reduced cell proliferation after 5 days and increased MAP2 expression after 14 days of EMF stimulation	Moraveji et al. [53]
Checking if simultaneous use of PEMF and sound waves can promote neural differentiation of hBM-MSCs	3D culture of BM-MSCs was exposed to PEMF(60 Hz for 12 h/day) and continuously sound waves (1 kHz and 81 dB)	Yes	No	Combined physical stimuli of PEMF and sound waves can enhance neural differentiation of BM-MSCs	Choi et al. [54]
Understanding the mechanism that mediates ELF-EMF-induced neuronal differentiation of hBM-MSCs	hBM-MSCs were exposed to ELF-EMF (50 Hz, 1mT) for 8 days	Yes	No	Erg-1 is one of the key factors in ELF-EMF-induced neuronal differentiation	Seong et al. [56]
Searching for the signaling pathway of hBM-MSCs neural differentiation stimulated by ELF-EMF	hBM-MSCs were exposed to ELF-EMF (50 Hz, 1mT)	Yes	No	ELF-EMF increased the expression of NF-L, MAP2, and NeuroD1 after 6 days, as the well as phosphorylation of Akt, CREB and EGFR. ELF-EMF can induce neural differentiation through	Park et al. [57]

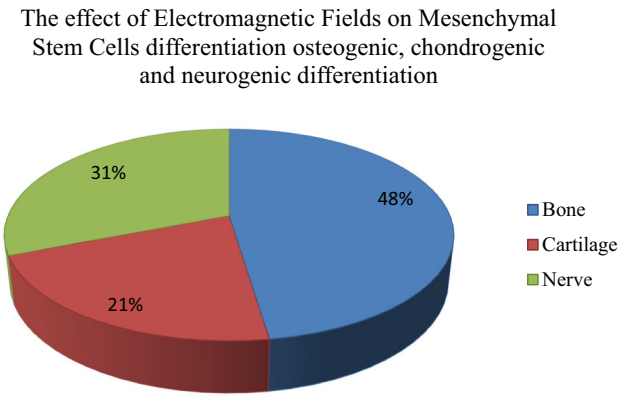

Fig. 3 Percentage of studies on the differentiation of MSCs stimulated with EMFs from 2010 to 2021

biophysical waves, PEMFs, and sound can help MSCs differentiate into neural cells [54]. Cruz et al. used a combination of flow-induced shear stress (FSS) and EMFs to boost neurogenesis for a brief period of time [55]. However, little is known about the molecular processes that regulate extremely low-frequency EMF-induced neuronal differentiation. Seong et al. used extremely low-frequency EMF (50 Hz frequency, 1 mT intensity) to stimulate neuronal differentiation in hBM-MSCs for 8 days and discovered that early growth response protein 1 (Egrl) is one of the main transcription factors in extremely low-frequency EMF-induced neuronal differentiation [56]. In addition, Park et al. employed hBM-MSCs treated with extremely low-frequency EMFs to investigate the signaling mechanism involved in neural differentiation (50 Hz, 1mT). EMFs exposure has been shown to influence cellular processes by increasing intracellular reactive oxygen species (ROS) levels. Researchers analyzed EMF-induced ROS production in BM-MSCs. Furthermore, pretreatment with *N*-acetylcystein, a ROS scavenger, and AG-1478, an EGFR inhibitor, inhibited phosphorylation of EGFR and downstream molecules. These findings imply that EMFs causes neuronal differentiation by activating EGFR signaling and causing a small amount of ROS [57]. The studies discussed above are summarized in Table 3.

4 Discussion/future prospects

Recent advancements in stem cell biology have opened the way for a new phase of tissue engineering and stem cell bio-engineering in the fast-growing field of regenerative medicine. Tissue engineers, on the other hand, face a challenge in accurately managing the timing and the result of this differentiation process. As a result, most strategies for regulating stem cell function have relied on chemical

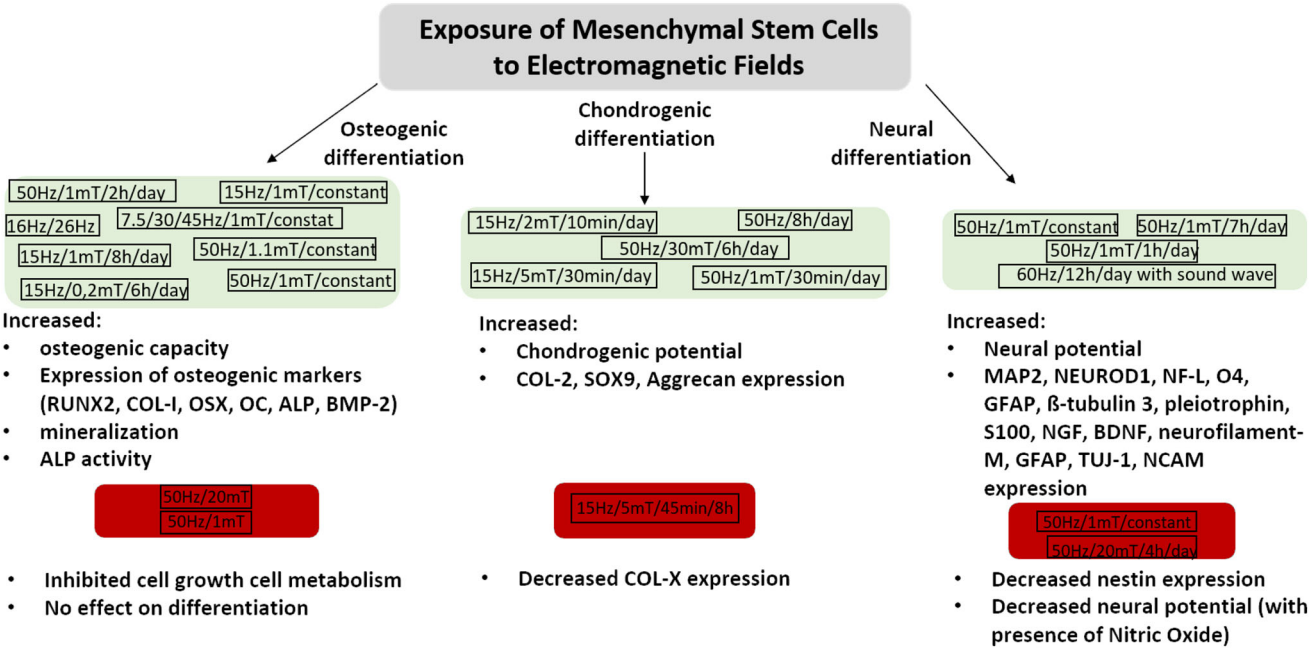

Fig. 4 Possible positive and negative effects on mesenchymal stem cells **A** osteogenic **B** chondrogenic and **C** neural differentiation depending on the electromagnetic field parameters and exposure time

inducers, however, physical stimulation, such as electromagnetic fields, is found to be effective in inducing or boosting growth.

In the last 11 years, 40 studies examined the effect of EMFs on MSCs (Fig. 3) osteogenic [17–19, 23, 26, 27, 32–36], chondrogenic [38–44], and neurogenic [46–58] differentiation, and it has been shown that it may be able to improve preimplantation culture methods for seeding MSCs in biomaterials fabrication [52]. Due to the vast range of (frequency, magnetic flux density) and exposure lengths utilized by different research groups, the parameter of EMFs is a complex topic. Although much of the EMF's research has focused on MSC differentiation to the bone, ASCs appear to be stimulated to commence chondrogenesis by the same 50 Hz frequency [24]. Furthermore, several studies have shown that exposing BM-MSCs to 50 Hz, 1 mT EMFs can successfully archive neurogenic differentiation and the use of EMFs to nerve regeneration [45–49, 52–54, 57, 58]. However, research on cartilage regeneration that has been conducted since 2010 shows the influence of EMFs on chondrogenesis with varied parameters, with the frequency of 15 Hz being the most frequently employed [38, 39, 41, 42]. Scaffolds have been approved as a platform for cell-biomaterial interactions, cell adhesion, proliferation, and differentiation. *In vitro*, it was given an extracellular microenvironment [1]. Because it matches the *in vivo* setting, some studies have found a synergistic effect of electromagnetic fields and 3D cell culture in stem cell differentiation [18, 28]. According to the culture condition, and the EMFs parameters, the effect on MSCs differentiation may vary (Fig. 4) and it is important to provide more appropriate reproducible studies with different EMF parameters in order to provide knowledge about its positive and negative effects on stem cell biology.

Many theories propose that EMFs have their principal effects on the plasma membrane due to their electrical characteristics. An EMF has been found to influence transmembrane signaling by modifying ion channels, ligand binding sites, and the density and distribution of receptors implanted in the cell membrane [47, 53]. Low-frequency EMF can considerably raise intracellular Ca^{2+} concentration, improving cell adhesion [53], as well as the modulator role of ferritin and thioredoxin-dependent peroxide reductase during neural differentiation [47]. In MSCs, electromagnetic stimulation increases Ca^{2+} flux and the expression/activity of Ca^{2+} binding proteins, including calmodulin, resulting in the activation of additional signaling pathways [9]. Considering the immunologic concerns raised by the use of bioactive molecules in tissue engineering, physical stimuli like ELF-EMFs may ensure no immune responses or, at the very least, fewer immune complications [59]. Additionally, it is less expensive, faster, and does not involve the use of expensive growth factors compared to other methods. However, it is very important to adjust the appropriate parameters of the electromagnetic field causing the therapeutic effect.

We were able to compare the effects of EMFs with different parameters on the osteogenic, chondrogenic, and neurogenic differentiation of stem cells in this analysis since we obtained groups of publications. Regardless of the parameters or biomaterials employed, studies have shown that EMFs and PEMFs increase lineage-specific gene expression. Standardization, on the other hand, is still faulty, and it should be further investigated to allow for more specific results regarding the EMFs protocols employed.

To define the best techniques, more research is needed to determine which types of EMFs and PEMFs stimuli (or their combinations with biomaterials) are most appropriate and when to initiate induction during culture. The responses found may improve the design of future EMFs and PEMFs systems.

In conclusion, EMFs and PEMFs are potential modulators of MSCs differentiation, and harnessing their effects may allow for improved pre-culture methods of MSCs in implantable constructs. Proper EMF parameters may provide faster and more effective mesenchymal stem cells differentiation and perhaps be of benefit to regeneration medicine.

Declarations

Conflict of interest We wish to confirm that there are no known conflicts of interest associated with this publication.

Ethical statement There are no animal experiments carried out for this article.

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