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REVIEW ARTICLE

Pulsed electromagnetic fields: promising treatment for osteoporosis

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Abstract

Osteoporosis (OP) is considered to be a well-defined disease which results in high morbidity and mortality. In patients diagnosed with OP, low bone mass and fragile bone strength have been demonstrated to significantly increase risk of fragility fractures. To date, various anabolic and antiresorptive therapies have been applied to maintain healthy bone mass and strength. Pulsed electromagnetic fields (PEMFs) are employed to treat patients suffering from delayed fracture healing and nonunions. Although PEMFs stimulate osteoblastogenesis, suppress osteoclastogenesis, and influence the activity of bone marrow mesenchymal stem cells (BMSCs) and osteocytes, ultimately leading to retention of bone mass and strength. However, whether PEMFs could be taken into clinical use to treat OP is still unknown. Furthermore, the deeper signaling pathways underlying the way in which PEMFs influence OP remain unclear.

Keywords BMSCs · Osteoblasts · Osteoclasts · Osteocytes · Osteoporosis · PEMFs

Introduction

Osteoporosis (OP) is a skeletal disease characterized by bone loss and deteriorating microarchitecture, accompanied by increased bone fragility and susceptibility to fragility fractures [1]. An imbalance between bone resorption and formation contributes to various types of osteoporosis, resulting in reduced bone mineral density and bone quality [2]. Currently, there are many clinical pharmacological therapies which can be used to treat OP, such as bisphosphonates, raloxifene, hormone replacement, parathyroid hormone (PTH), calcium, vitamin D, calcitonin, fluoride, testosterone, and anabolic steroids [3, 4]. However, long-term use of these antiosteoporosis drugs causes potential side effects, such as gastrointestinal complaints, osteonecrosis of the jaw, and atypical subtrochanteric or diaphyseal femoral fractures [5]. In addition to pharmacotherapy, physical therapy, comprising safe and noninvasive biophysical countermeasures, should be highly recommended for clinical application. Pulsed electromagnetic fields (PEMFs) are regarded as an efficient therapy for the treatment of various bone disorders, such as fresh fractures, delayed and nonunion fractures, diabetic osteopenia, and osteonecrosis compared to drug therapy [6, 7]. However, at present, the effects of PEMFs on OP patients are not clear. It has been demonstrated that properly applied PEMFs reduce discomfort such as pain and improve functional outcomes in patients with postmenopausal osteoporosis (PMOP). PEMFs, which exert positive effects as mechanical stimulation and drugs on maintaining bone mass, may have clinical application in the prevention and treatment of osteoporosis [7–10].

At last, the underlying mechanism of PEMFs on OP is not well-known (Fig. 1, Table 1). One group reported that PEMF stimulation could reverse bone loss and decrease without side effects in OP rats [59], acting via a process which is dependent on the Wnt/β-catenin signaling pathway. Certainly, PEMF stimulation may activate various intermediaries, such as parathyroid hormone pathways as well as insulin-like growth factor (IGFs) [59].

In this review, we summarize the effects of PEMF on OP and the underlying mechanism.

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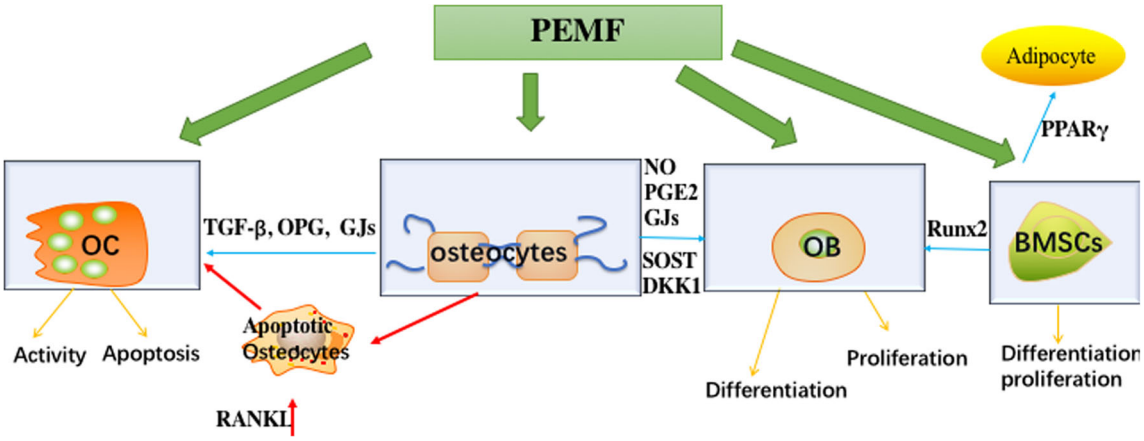

Fig. 1 Osteocytes act as positive regulators of osteoblasts, acting via gap junctions (GJs), nitric oxide (NO), and prostaglandin (PG) E2 in responding to mechanical loading and as a negative regulator of osteoblast activity through sclerostin (SOST) and Dickkopf-related protein 1 (DKK1). In addition, the relationship between osteoclasts and osteocytes is mainly dependent on paracrine signaling and GJs. Live osteocytes also secrete osteoprotegerin (OPG) and transforming growth factor (TGF)-β to influence osteoclastogenesis. It has been demonstrated that apoptotic osteocytes are the major source of receptor-activator of nuclear factor kappa B ligand (RANKL) to promote osteoclast resorption. Osteocytes can regulate bone mineralization, while bone marrow mesenchymal stem cells (BMSCs) can differentiate into osteoblasts and osteoclasts dependent on different stimuli. PEMFs are able to stimulate BMSCs to differentiate into osteoblasts, as well as enhancing osteoblast function through different mechanisms. Furthermore, PEMFs have an obvious influence on osteoclastogenesis, osteoclast apoptosis, and bone resorption. A previous study has shown that PEMFs can inhibit osteoclastogenesis by downregulating RANKL and upregulating OPG. PEMF stimulation of osteocytes induces production of cytokines such as PGE2, TGFβ1, and Connexin (Cx) 43, influencing communication between other cell types. OPG, osteoprotegerin; GJs, gap junctions; FGF23, BMSCS, bone marrow mesenchymal stem cells; OC, osteoclasts; OB, osteoblasts

PEMF

Characteristics

The pulsed electromagnetic field (PEMF) is a noninvasive physical therapy for skeletal diseases. PEMF therapy has achieved widespread application due to its rapid effect, ease of operation, and lack of adverse effects. PEMFs are characterized by frequencies at the low end of the electromagnetic spectrum, ranging from 6 to 500 Hz [60]. A higher rate of changes (Tesla/s) is capable of inducing biological currents in the tissue, with peculiar biological effects [61].

Effects of PEMF on osteoporosis

Clinical experiments

The clinical usage of PEMFs on OP patients is not well-known because of different design and small sample. One article comprehensively reviews recent studies regarding the effects of PEMFs on PMOP treatment in clinical. It summarized that PEMFs could significantly ease the pain in osteoporosis patients [62]. Bone mineral density (BMD) is the bone mineral content per volume. The effects of PEMFs on BMD are attracting attention, although controversy remains. Recently, PEMFs have also been confirmed to improve BMD in the distal radius, spine, and knees of patients with OP [63]. Tabrah et al. found that BMD in radii could increase in the sixth week but decrease during the next 36 weeks after exposure to PEMFs over a period of 12 weeks. The adopted parameters were 72 Hz, 2.85 mT PEMFs, and a duration of 10 h per day to treat 20 women with PMOP [64]. However, no long-term effects of PEMFs on BMD were observed over an 8-year follow-up [65]. Nevertheless, PEMF treatment with specific parameters (field frequency of 8 Hz, intensity of magnetism of 3.82 mT, and 40 min/day) was as effective as alendronate (70 mg/week) in treating postmenopausal osteoporosis within 24 weeks [7]. Garland et al. [9] found that PEMFs could delay bone loss in patients with complete spinal cord injury (SCI) of a minimum of 2 years duration.

Table 1 The effect of PEMFs on bone metabolism

Cells	Influences	Ref
BMSCs	PEMFs regulate BMSC proliferation, activity and mineralization	[11–27]
Osteoblasts	PEMFs have effects on osteoblast proliferation, differentiation and activity	[28–45]
Osteoclasts	PEMFs exert effects on osteoclastogenesis, osteoclast differentiation, and apoptosis	[46–56]
Osteocytes	PEMFs influence communication on between osteocytes and osteoblasts/osteoclasts, rescuing bone loss	[57, 58]

Specifically, the time of therapy of PEMFs lasted for 6 months, and at 3 months, BMD increased in the stimulated knees and declined in the control knees. By 6 months, the BMD returned to near baseline values, and at 12 months, both knees had lost bone at a similar rate. While the stimulation seems useful in preventing bone loss, the unexpected exaggerated decline in the control knees and reversal at 6 months indicates underlying mechanisms are more complex. However, no significant increase of BMD was detected in a single-blind, randomized pilot study [66]. Moreover, a randomized, sham-controlled study also did not observe long-term significant positive effects of PEMFs on BMD in patients with forearm disuse osteopenia [67]. There are a variety of reasons which could account for these conflicting results. First, different groups have applied PEMF treatment using different clinical designs and parameters. Secondly, the sample size of these studies was too small for a clinical trial.

PEMFs have stimulation on osteogenesis [68]. PEMFs have been reported to increase the bone formation biomarkers serum osteocalcin (OC) and serum carboxy-terminal propeptide of type I collagen (PINP) levels, along with decreased BMD [67]. Moreover, PEMF therapy maintained the expected normal level of serum bone-specific alkaline phosphatase (BSAP) and decreased serum C-terminal telopeptide (CTX) level, which was independent of BMD change [67]. Similarly, PEMF can significantly increase serum OC and serum PINP, which are biomarkers related with formation, independent of BMD change [66].

Animal experiments

PEMFs have been shown to prevent bone loss and deterioration of bone microstructure in different animal models of osteoporosis. PEMFs slowed ovariectomy-induced bone loss in rats [69] and led to markedly suppressed trabecular bone loss and improved cortical and trabecular bone structure in ovariectomized rats [70]. In addition, PEMFs greatly increased BMD in ovariectomized [71] and hindlimb-suspended [72] rats. PEMF was demonstrated to improve the fracture healing response in skeletally mature OVX rats [73].

PEMFs significantly upregulated levels of biomarkers of osteoblast-associated bone formation, such as serum BSAP, OC, and P1NP, but exerted only minor preventive effects on biomarkers of osteoclast-associated bone resorption, such as CTX and tartrate-resistant acid phosphatase 5b (TRAcP5b) [74–77].

The relationship between PEMFs and bone metabolism

PEMFs have been demonstrated to increase BMD in OP patients and prevent bone loss in animal models of disuse OP, tail-suspension OP, ovariectomy-induced OP (OVX), and diabetes-mellitus-induced OP [7, 78].

BMSCs

Adipocytes and osteoblasts are derived from the same progenitor cells: mesenchymal marrow stromal/stem cells (mMSCs), whose differentiation is controlled by peroxisome proliferator-activated receptor gamma (PPAR-γ) and runt-related transcription factor 2 (Runx2). PPAR-γ 2 is a transcription factor expressed predominantly and specifically in adipocytes which enhances the differentiation of BMSCs into adipocytes rather than osteoblasts, leading to increased marrow fat, decreased bone density, and higher risk of fracture. Runx2 expression can control the differentiation of mMSCs, stimulating osteogenesis and suppressing adipogenesis [11].

It has been reported that exposure to PEMFs stimulates BMSC proliferation and calcium accumulation in both low- and high-density cultures [12]. However, one group reported that PEMFs might have inhibitory effects on the proliferation of BMSCs [13]. These contradictory results might be due to differences in initial seeding density, with the high-seeding density of mMSCs used by Jansen et al. inhibiting the spreading of BMSCs. Since tension stimulates or enhances adipogenesis when BMSCs cannot develop tension in their actin skeleton under local adhesion, adipogenic differentiation will be affected [14–16]. This suggests that the initial-seeding density plays an important role in determining which molecular mechanism is induced by PEMF therapy. Cells have served as highly controllable model systems for treating osteoporosis. Although successful strategies for cells must ultimately be adapted to human subjects to be clinically relevant, human BMSCs are rarely employed in such studies.

PEMF treatment can induce earlier expression of osteogenesis markers in mMSCs by mediating alkaline phosphatase (ALP) activity and expression of Runx2/Cbfa1. PEMFs enhance ALP activity not only in the early phases of osteogenic differentiation but also throughout the whole differentiation period [17], accompanied by a delayed increase in cell proliferation. Moreover, PEMFs also enhance mineralization and have suppressive effects on the expression of adipogenic genes, such as the adipokine AP-2 in BMSCs [13, 18].

Currently, there are many hypotheses concerning the osteogenic mechanism of PEMFs. PEMFs are involved in osteogenic differentiation of BMSCs independently in the presence of bone morphogenetic protein 2 (BMP-2). Furthermore, treatment of BMSCs with the combination of PEMFs and BMP-2 seems to favor osteogenesis. The detailed mechanisms of how PEMFs influence osteogenic differentiation in BMSCs are not completely understood. Some groups have reported that PEMF-induced upregulation of adenosine receptors could at least partly mediate these effects [19, 20]. Furthermore, it is known that mMSCs can express A2A and A2B adenosine

receptors during osteogenic differentiation [21]. Upregulation of cyclic adenosine monophosphate, modulated by these two adenosine receptors, is able to mediate the expression of osteogenesis-associated genes, especially Runx2 and Osterix [22–24], indicating that these receptors can participate in osteogenesis. The mammalian target of rapamycin (mTOR) is involved in the regulation of various cell types such as osteoblasts and adipocytes. mTOR communicates with several proteins to form two different complexes named mTOR complex 1 (mTORC1) and 2 (mTORC2) which differ in their unique components, Raptor and Rictor. Suppression of mTOR signaling might stimulate osteoblastic differentiation and reduce adipogenic potential [25]. Excessive exposure to rapamycin, an inhibitor of TORC1, can also damage mTORC2 function [26]. One group reported that PEMF treatment could abolish the decreased mineralization of the extracellular matrix induced by rapamycin. Taken together, these data suggest that PEMFs might act via the mTOR pathway to induce commitment of BMSCs to the osteoblast lineage [27].

Osteoblasts

PEMFs can influence osteoblast activity in different ways. PEMFs are considered to play a dominant role in stimulating osteoblast function. However, the effects of PEMFs on cell proliferation and differentiation are contradictory. Most studies postulated that PEMFs enhanced osteoblast activity, resulting in increased cell differentiation [28]. In contrast, some groups reported that exposure to PEMFs could stimulate osteoblast proliferation but had no effect on differentiation [29]. Specifically, regarding ALP activity, Diniz et al. found that ALP activity was significantly increased by PEMF treatment (at 15 Hz and 7 mT) in the osteoblast-like MC3T3 cell line [28]. However, Chang et al. [29] reported that ALP activity could be suppressed when cells were exposed to PEMFs. The reasons for these contradictory results might lie in the different types of cells used, the different frequencies and intensities of the PEMFs used and the different time points chosen for analysis. Although the effects of PEMFs on osteoblast function are contradictory, the consensus is that PEMFs exert reproducible osteogenic effects with a window effect [68]. PEMFs also upregulate mRNA production of BMP2, transforming growth factor (TGF-β), osteoprotegerin (OPG), OC, Runx2/Cbfal, ALP, matrix metalloproteinase (MMP)-l and − 3, nuclear factor kappa B (NF-κB) ligand [30, 31], and bone sialoprotein. Such reports indicated that PEMFs directly stimulate osteogenic differentiation of osteoprogenitor cells. In addition, PEMFs can upregulate bone mass and TGF-β concentrations in rats. However, interleukin 6 (IL-6) concentration can be reduced by PEMFs [72], which can efficiently suppress bone loss. PEMFs have also been shown not only to upregulate various genes associated with the formation of bone and matrix components but also to downregulate several genes associated with degradation of the extracellular matrix (ECM) [32]. In vitro, PEMFs can enhance ECM production, IGF-II, and TGF-β secretion, as well as decreasing prostaglandin E2 (PGE2) secretion, and stimulating the sequence of events resulting in bone tissue formation [33, 34]. The intracellular calcium transient plays an important role in osteoblast proliferation and differentiation [35], and this can be stimulated by PEMF treatment. PEMFs upregulate expression of Ccnd 1 and Ccne 1, which are responsible for cell cycle progression from proliferation stage to differentiation and mineralization stages, resulting in enhanced osteogenesis [36]. Osteoblast morphology and orientation can also be mediated by PEMFs, inducing osteoblast differentiation by promoting a smaller, shorter, and more rounded morphology of osteoblasts compared to a sham treatment. Meanwhile, exposure to PEMFs induces osteoblasts to orient orthogonal to the application of the magnetic field [37].

There are various hypotheses concerning the mechanism of how PEMFs influence osteoblast lineage cells. Canonical Wnt signaling plays a key role in modulating bone homeostasis [38]. Canonical Wnt proteins are triggered through extracellular Wnt ligands which initially bind to the Frizzled and LRP5/6 coreceptors on the cell membrane, leading to stabilization of β-catenin, and upregulation of Wnt-targeted genes [38]. Osteoblastogenesis and osteoblast activity can be enhanced by activating the canonical Wnt signaling pathway. PEMFs increase the expression of genes related to the Wnt signaling pathway, both in vivo and in vitro, such as Wnt1a, Wnt3a, Lrp5, and Lrp6. In addition, PEMFs also downregulate dickkopf-related protein 1 (DKK1), which antagonizes the Wnt signaling pathway [39].

Other signaling pathways involved in the effects of PEMFs include the insulin receptor substrate-I (IRS-I) protein, the S6 ribosomal subunit protein, and the endothelial nitric oxide synthase, which trigger activation of PTH as well as insulin to the same degree as PEMFs. One group demonstrated that PEMF exposure could significantly upregulate three important components of the mTOR molecular pathway, such as p70 S6 kinase and ribosomal protein S6. Rapamycin and PI3-kinase inhibitor, an upstream regulator of the mTOR signaling pathway, could block this stimulatory effect [40].

PEMFs may stimulate osteoblast functions through the BMP2 signaling pathway [41], by promoting secretion of BMP-2 protein [41]. Thus, more BMP-2 binds to its receptor, which phosphorylates, triggering activation of the intracellular signaling molecules Smad 1 and Smad 5 [42]. This, in turn, causes the upregulation of expression of the transcription factors Runx2 and Osterix, influencing bone formation [43]. PEMFs have also been reported to increase expression of genes associated with bone formation, such as HOXA10 and AKTl, genes related to activation of transduction such as CALM 1 and P2RX7, as well as genes encoding extracellular organic matrix components such as COLlA2 and SPARC, and

genes related to cytoskeletal components such as FNI and VCL [32].

PEMFs may inhibit the genes involved in matrix degradation, such as downregulating matrix MMP-11 and DUSP4, which can participate in the suppression of osteoblast differentiation and proliferation. Additionally, PEMF treatment can influence the expression of c-myc and c-fos, acting as an activator for osteoblast proliferation and differentiation [44].

PEMF treatment can trigger activation of the extracellular regulated protein kinases (ERK)1/2 molecular pathway. U0126, an inhibitor of the ERK1/2 signaling pathway, can suppress ALP activity and matrix mineralization induced by PEMFs. Taken together, these findings demonstrate that the positive effects of PEMFs on osteoblast function are ERK1/2 signaling-dependent.

Low nitric oxide (NO) levels are associated with osteoblast proliferation and differentiation, which are inhibited by high concentrations of NO [45]. PEMFs have been demonstrated to stimulate NO synthesis by increasing nitrite concentration. In turn, the NO synthase (NOS) inhibitor, l-NMMA, suppressed this positive influence. Thus, PEMFs can stimulate osteoblast proliferation and differentiation through increasing NO synthesis.

Osteoclasts

It has been reported that PEMFs exert effects on osteoclastogenesis [46] and osteoclast apoptosis [47], as well as bone resorption [48]. Specifically, PEMF treatment can inhibit osteoclastogenesis in primary bone marrow cells derived from OVX rats [46]. Reduced concentrations of tumor necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), and IL-6 may account for this process.

Over the last several years, the receptor-activator of nuclear factor kappa B (RANK)/RANK ligand (RANKL)/OPG system has been shown to play an important role in bone remodeling [49]. Osteocytes and osteoblasts mainly express RANKL, a cell surface protein, which binds to its specific receptor, RANK, located on the membrane of osteoclasts, stimulating osteoclastogenesis. OPG, derived from osteoblasts, suppresses osteoclast maturation, blocking osteoclastogenesis [50]. PEMFs may exert their suppressive effects on the mediation of osteoclastogenesis via the OPG/RANK/RANKL signaling pathway. PEMFs suppress the expression of RANKL and meanwhile enhance expression of OPG, resulting in an increased ratio of OPG/RANKL [51]. In addition, PEMFs not only upregulate expression of OPG but also suppress the activity of NF-kB p65 subunit, induced by IL-1β. This process is dependent on the increased anti-inflammatory effect of A2A or A3ARs [20].

PEMFs have been demonstrated to increase cell viability and decrease osteoclast number as well as expression of cathepsin K (CTSK), and nuclear factor of activated T cells 1 (NFATC1). Moreover, both CTSK and NFATC1 are responsible for osteoclastogenesis [52].

PEMFs can also downregulate expression of carbonic anhydrase II (CA II), which is responsible for the resorptive activity of osteoclasts [53].

Furthermore, osteoclastic differentiation might be regulated by the Ca^{2+}–calcineurin–NFATc1 signaling pathway [54]. Using FK506, an inhibitor of calcineurin activity, suppresses bone resorption [55], accompanied by decreased expression of NFATc1 and CTSK. Additionally, PEMFs further decrease NFATc1 autoamplification and expression of CTSK in the presence of nifedipine or FK506. This suggests that PEMFs has a critical effect on the Ca^{2+}–calcineurin–NFAT signaling pathway [56].

Osteocytes

Osteocytes can mediate the activity of both osteoblasts and osteoclasts. More and more groups have reported that osteocytes should be considered as a therapeutic target for OP. Osteocytes influence osteoblasts directly through gap junction intercellular communication (GJIC), such as via Cx43, which is a positive regulator of osteoblast function and a negative regulator of osteoclast activity [79]. As well as GJIC, various small molecules also play important roles in communication between osteocytes and osteoblasts, such as PGE2 and nitric oxide NO, as well as larger peptides such as IGFs.

Furthermore, osteocytes can control osteoclast activity through factors which can promote associated processes, such as osteoclast precursor recruitment, angiogenesis, and endothelial activation, including RANKL, GJIC, and vascular endothelial growth factor (VEGF). Glucocorticoids (GCs) also trigger Cx43 degradation [80]. Deficiency of Cx43 can directly stimulate osteoclastogenesis. Meanwhile, deletion of Cx43 can also act indirectly by causing osteocyte apoptosis, and dying osteocytes produce more RANKL which triggers osteoclast precursor recruitment, resulting in bone resorption. Furthermore, dying osteocytes are the main source of the pro-inflammatory cytokine, high-mobility group protein B1 (HMGB1); thus, osteocytes should be considered as a therapeutic target for OP.

There is little information regarding the effects of PEMFs on osteocytes. PEMF stimulation does not influence the proliferation of osteocyte-like cells (MLO-Y4) [57]. PEMF exposure lowers the risk of osteonecrosis and reduces the empty osteocyte lacuna rate [58]. PEMFs can continually upregulate total PGE2 and total TGF-β1 in MLO-Y4 osteocyte-like cells. PEMF-enhanced concentrations of TGF-β1 are mediated by prostaglandin-dependent mechanisms including COX-1 [57]. Additionally, PEMF treatment can induce NO_2 in a time-dependent manner [57]. PGE2 plays a key role in osteoblast differentiation, and extracellular matrix synthesis is stimulated by TGF-β1. Furthermore, NO_2 suppresses osteoblast activity,

stimulates apoptosis, and promotes bone resorption [79]. As we have summarized above, both PGE2 and total TGF-β1 can regulate osteoblast activity positively, suggesting that PEMFs can regulate bone metabolism through osteocyte.

Discussion

Evidence for therapeutic effects

More and more extensive research focused on the physiological effects of PEMFs on OP; the findings are still questionable. The positive effects of PEMFs with different parameters (treatment starting point and its duration, the daily exposure time, the PEMF waveform and subject-related factors) on OP are still controversial. Short-term treatment with PEMFs (1–3 months) [7, 64] reflects positive effects, accompanied with no further beneficial effects on BMD in a long-term follow-up (1–8 years) [65]. Vary reasons could be account for these different results. First, different groups have applied PEMF treatment using different clinical designs and parameters. And, the sample size of these studies was too small for a clinical trial. Moreover, the time using dual-energy X-ray to detect BMD may be responsible for inconsistency. Six to 12 months of at least a significant change of BMD testing should also be calculated to determine the real effects of PEMFs on BMD, initiating the treatment for PMOP [81, 82]. Thus, short-term treatment with PEMFs less than 3 months might not reflect a real change. Detecting BMD in long-term usage of PEMFs should be lasting at least 6 months. Moreover, bone metabolism is a dynamic remodeling activity, which can be reflected by BTMs, within 1–6 months. It has been demonstrated that PEMFs could increase biomarkers associated with bone formation and decrease biomarkers associated with bone resorption, independent changes of BMD [67]. BTMs are recommended to predict bone loss and fracture risk [82]. PEMFs may be potential in preventing bone loss and bone microarchitecture in OP.

Moreover, the effects of PEMFs on osteogenesis remains inconsistent with a window effect, both found in animal and clinical experiments. Different PEMF parameters (field intensity, frequency, exposure time) may result in controversial effects on osteoblast activity.

The different PEMF parameters (including field intensity, frequency, exposure time) contribute to varying effects of PEMFs on proliferation of osteoblasts. Sollazzo et al. found that PEMFs (2 mT, 75 Hz) appeared to induce MG63 cell proliferation [32]. Lin et al. noted that exposure to PEMFs (2.5 mV, 75 Hz), enhanced osteoblasts proliferation [83, 84]. But, Martino et al. [85] detected no significant changes in cellular proliferation after exposed to repetitive pulse burst PEMFs (9 mV/cm, 15 Hz). Lohmann et al. [57] indicated that PEMFs (15 Hz, for 24 h, 48 h, or 72 h) had no effects on proliferation of ROS 17/2.8 cells. Moreover, Zhou et al. [86] noticed that PEMFs (0.9–4.8 mT, 50 Hz, 30 min/day for 3 days) inhibited the osteoblast proliferation.

ALP is regarded to act as an early marker of maturation of extracellular matrix. Differentiation degree of osteoblasts can be reflected by the activity of ALP. The different PEMF parameters account to contradictory results about the effects of PEMFs on differentiation of osteoblasts. Sollazzo et al. [32] reported that PEMFs appeared to induce MG63 cell differentiation. While Lin et al. [83, 84] noted PEMF could inhibit differentiation of osteoblasts through decreasing ALP activity. Exposure to 50 Hz PEMFs [86] significantly promoted differentiation of osteoblasts in an intensity-dependent manner with peak activity shown at 1.8 and 3.6 mT [87, 88]. Moreover, 0.6 mT was demonstrated as the optimal intensity of 50 Hz PEMFs from the 0.6 to 3.6 mT range in stimulating both proliferation and osteogenic differentiation of rat calvarial osteoblasts in vitro [89].

The controversy arising from these studies may be due to differences in cell types. For example, Diniz et al. reported that PEMF (15 Hz) treatment accelerated the proliferation and differentiation of murine MC3T3-E1 cells [28]. However, Chang et al. showed that PEMFs (15 Hz) stimulated proliferation of murine primary cultured osteoblast proliferation but not their differentiation [90].

Several mechanisms of PEMFs on bone metabolism have been elucidated, for example, PEMFs' influence osteoblast lineage cells proliferation and differentiation through Wnt signaling pathway and RANKL/OPG pathway [39, 91]. Furthermore, PEMFs have been demonstrated to increase cell viability and decrease osteoclast number as well as expression of CTSK, NFATC1. Both CTSK and NFATC1 are responsible for osteoclastogenesis [52]. Further investigation is required to detect the specific mechanism in effects of PEMF on bone metabolism.

Limitations and adverse effects

The number of the clinical studies is limited. Most of the trials we summarized were based on small sample sizes. Moreover, clinical experiments are somehow different from the animal experiments. For example, PEMF was demonstrated to improve the fracture healing response in skeletally mature OVX rats [92]. There is no evidence about whether PEMF might improve bone-healing responses in at-risk osteoporotic patients. Moreover, the parameters of PEMFs are varied, resulting in different outcomes. Short term of PEMFs was demonstrated by using a parallel group design. However, it is more reliable to use crossover design for acute treatment effects. This method can avoid the impact of baseline group differences on the results of the study, as each person will then act as his or her own control. Moreover, lack of long-term studies reporting effects after exposure to PEMFs is another

limitation, and it must be corrected using appropriate methods to assume the long-term impact [93]. Moreover, blinding is a basic requirement in studies of PEMF treatment, but it is also a major challenge. There is little information about the details on how to achieve blindness in several articles. Therefore, unblinded methods may occasionally occur to produce false results.

None of the trials reported the issue of adverse effects. However, it is not recommended for patients with cardiac devices [94]. It has been reported that the magnetic field may increase the risk of cancer in children [95, 96]. However, exposure to PEMFs might impair cancer cell viability [97–99]. The controversy results might dependent on different study design. Moreover, using of electric devices like heating blankets, hairdryers, or electric razors cause higher risks of cancer in adults [100, 101]. In all, it has been demonstrated that PEMFs have an effective influence on oogenesis using animal models and cells. However, the role of PEMFs in OP patients are not well explored, and more reliable evidence from high-quality, randomized controlled trials, with large sample sizes and long-term follow-up, is required to validate these findings. Furthermore, it's important to take contraindications of long-term PEMFs into account further studies.

Conclusions

Based on recent studies of PEMFs and their potential role in mediating bone metabolism, PEMFs might be recommended for treatment of OP. While adverse effects of the long-term application of PEMF have not been explored so far and a small number of sample sizes, the evidence for the therapeutic effects of PEMF devices is not sufficient. Therefore, more reliable evidence from high-quality, randomized controlled trials, with large sample sizes and long-term follow-up, is required to validate these findings and regard the possible health benefits or risks of using PEMF. Furthermore, gene-knockout mice should be used to identify the specific target involved in the treatment of OP by PEMFs.

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Compliance with ethical standards

Conflicts of interest None.

Abbreviations *ALP*, alkaline phosphatase; *BMD*, bone mineral density; *BMP-2*, bone morphogenetic protein 2; *BMSCs*, bone marrow mesenchymal stem cells; *BSAP*, bone-specific alkaline phosphatase; *CA II*, carbonic anhydrase II; *CTSK*, cathepsin K; *CTX*, *C-terminal telopeptide*; *DKK1*, dickkopf-related protein 1; *ECM*, extracellular matrix; *ERK*, extracellular regulated protein kinases; *GCs*, glucocorticoids; *GJIC*, gap junction intercellular communication; *HMGB1*, high-mobility group protein B1; *IGF*, insulin-like growth factor; *IL-1β*, interleukin 1 beta; *IL-6*, interleukin 6; *IRS-I*, insulin receptor substrate-I; *MMP*, matrix metalloproteinase; *mMSCs*, mesenchymal marrow stromal/stem cells; *mTOR*, mammalian target of rapamycin; *NFATC1*, nuclear factor of activated T cells 1; *NF-κB*, nuclear factor kappa B; *NO*, nitric oxide; *NOS*, NO synthase; *OC*, osteocalcin; *OP*, osteoporosis; *OPG*, osteoprotegerin; *OVX*, ovariectomized; *PEMF*, pulsed electromagnetic fields; *PGE2*, prostaglandin E2; *PINP*, propeptide type I collagen; *PMOP*, postmenopausal osteoporosis; *PPAR-γ*, peroxisome proliferator-activated receptor gamma; *PTH*, parathyroid hormone; *RANK*, receptor-activator of nuclear factor kappa B; *RANKL*, RANK ligand; *Runx2*, runt-related transcription factor 2; *SCI*, spinal cord injury; *TGF-β*, transforming growth factor; *TNF-α*, tumor necrosis factor-alpha; *TRAcP5b*, tartrate-resistant acid phosphatase 5b; *VEGF*, vascular endothelial growth factor

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