Review

Effects of Pulsed Electromagnetic Fields on Postmenopausal Osteoporosis

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Postmenopausal osteoporosis (PMOP) is considered to be a well-defined subject that has caused high morbidity and mortality. In elderly women diagnosed with PMOP, low bone mass and fragile bone strength have been proven to significantly increase risk of fragility fractures. Currently, various anabolic and anti-resorptive therapies have been employed in an attempt to retain healthy bone mass and strength. Pulsed electromagnetic fields (PEMFs), first applied in treating patients with delayed fracture healing and nonunions, may turn out to be another potential and effective therapy for PMOP. PEMFs can enhance osteoblastogenesis and inhibit osteoclastogenesis, thus contributing to an increase in bone mass and strength. However, accurate mechanisms of the positive effects of PEMFs on PMOP remain to be further elucidated. This review attempts to summarize recent advances of PEMFs in treating PMOP based on clinical trials, and animal and cellular studies. Possible mechanisms are also introduced, and the future possibility of application of PEMFs on PMOP are further explored and discussed. Bioelectromagnetics. 2017;9999:XX–XX.

Keywords: PMOP; PEMFs; bone homeostasis; bone quality; bone cells

INTRODUCTION

Postmenopausal osteoporosis (PMOP) is a silent skeletal disorder characterized by compromised bone strength, which increases risk of fracture [Watts et al., 2010]. In women, increasing bone turnover, continuous bone loss, and consequent fractures are critical manifestations of PMOP, which have impaired quality of life and have led to increased mortality [Baron and Kneissel, 2013]. Thus, osteoporosis is becoming a global public issue and heavy burden on society. In the future, almost half of the population over the age of 60 in developed countries will suffer from osteoporosis, 80% of which are postmenopausal women [Cummings and Melton, 2002; Watts et al., 2010]. It has been conservatively estimated that about 17 billion dollars was directly spent on the care of osteoporotic fractures in the United States in 2005, and the direct cost of osteoporosis could increase to 25 billion dollars by 2025 [Burge et al., 2007].

Recommendations from high-quality evidenced-based guidelines for PMOP [Watts et al., 2010;

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Compston et al., 2013; Kanis et al., 2013] include drugs such as alendronate, risedronate, zoledronic acid, denosumab, raloxifene, calcitonin, and teriparatide, hormone replacement therapy (estrogen and androgen), and supplements such as calcium and vitamin D. They have proven to be effective in preventing bone loss and reducing the incidence of fractures based on evaluations at different stages. In addition to pharmacotherapy, physical therapy with the highest recommendation that is graded by the newly released guidelines for the treatment of PMOP [Camacho et al., 2016] may prevent bone mass loss and improve patients' quality of life.

As a kind of physical therapy, therapeutic time-varying pulsed electromagnetic fields (PEMFs) with specific signal shapes and extremely low frequencies (between 5 and 300 Hz) or characteristic shortwaves are generated by two or more external electromagnetic coils [Hug and Roosli, 2012]. Typical characteristics of PEMFs are summarized in Table 1. PEMFs with specific parameters (e.g., frequency, waveform, magnitude) can result in specific biological responses and therapeutic effects. Thus, PEMFs with non-invasive and athermal characteristics were initially tested by the National Aeronautics and Space Administration (NASA) in the 1970s and were approved by the Food and Drug Administration (FDA) for clinical treatment [Bassett, 1989, 1993]. PEMFs have been successfully employed as an adjunctive therapy for the treatment of nonunion fractures, pain [Liu et al., 2016], inflammatory diseases including arthritis, and osteoporosis. Since then, more clinical trials [Tabrah et al., 1990; Garland et al., 1998; Prevention, 2001; Liu et al., 2013, 2015] have suggested that PEMFs, which are as effective as mechanical stimulation and drugs on retaining bone mass, can prevent bone mass loss, reduce discomfort such as pain, and improve functional outcomes in patients with PMOP. Additionally, results of experimental research [Akca et al., 2007; Sun et al., 2009; Jansen et al., 2010; Shen and Zhao, 2010; Jing et al., 2014; Petecchia et al., 2015; Yan et al., 2015] have shown that in addition to improving bone quality and strength as well as promoting osteogenesis, PEMFs can also inhibit osteoclastic resorption with no stated side effects. However, there is an effective window with specific electromagnetic field parameters and treatment period in the use of PEMFs in PMOP. Furthermore, the possible mechanisms by which PEMFs act on bone remodeling remain to be further elucidated.

Few reviews have been conducted to comprehensively summarize the efficacy of PEMFs on osteoporosis. Therefore, this article comprehensively reviews recent studies regarding the effects of PEMFs on PMOP treatment as well as the possible mechanisms, extending our previous review that summarized the clinical efficacy of PEMFs on osteoporosis [Huang et al., 2008a]. Literature published before January 2017 has been included, featuring clinical trials, animal studies, and cellular studies. PubMed, Embase, Web of Science, and Google Scholar databases were searched to identify all relevant peer-reviewed articles. The following key words and free text words were included: "pulse electromagnetic fields," "pulse electromagnetic field," "pulsed electromagnetic fields," "pulsed electromagnetic field," "PEMFs," "PEMF," "electromagnetic field," "electromagnetic fields," "postmenopausal osteoporosis," "PMOP," "osteoporosis," and their combinations. Articles relevant to the possible effects or mechanisms of PEMFs on osteoporosis as well as bone metabolism were identified by reading the titles and abstracts. The inclusion criteria are as follows: clinical trials that investigated the effects of PEMFs on PMOP or on senile female subjects (age $\geq$ 65) diagnosed with osteopenia or osteoporosis [Camacho et al., 2016]; and experimental studies using different animal models of osteoporosis or bone cells (including bone mesenchymal stem cells [BMSCs], osteoblasts, and osteoclasts) that elucidated the bio-effects and possible mechanisms of PEMFs in regulating bone homeostasis.

CLINICAL ADVANCES IN RESEARCH OF PEMFs ON PMOP

Effects of PEMFs on Bone Mineral Density

Bone mineral density (BMD) is the bone mineral content per volume. Densitometry remains the preferred choice for diagnosing osteoporosis and

TABLE 1. General Characteristics of Pulsed Electromagnetic Fields

Composition	Intensity	Frequency	Waveforms	Other characteristics
A pulsed signal generator; Coils like Helmholz and solenoid.	Confusion exists between some micro tesla (μT) and several tens of milli tesla (mT)	General spectrum: 5–500 Hz; Spectrum for medical conditions: 5–300 Hz.	Basic periodic waves: sinusoidal; Other periodic waves: asymmetric, biphasic, square, triangle, sawtooth.	The rate of field strength change in time; Capacitive and inductive coupling; Therapy time.

monitoring therapeutic intervention, due to limited clinical assessments of bone properties to determine bone strength. Efficacy of PEMFs on BMD is increasingly more certain with increasingly accumulated corresponding knowledge, though there is always controversy. These results are described in Table 2. Tabrah et al. [1990] adopted the following parameters, which were 72 Hz, 2.85 mT PEMFs, and a duration of 10 h per day to treat 20 women with PMOP. BMD of treated radii increased significantly in the sixth week but decreased significantly during the next 36 weeks after an exposure period of 12 weeks. However, PEMFs were found to have no long-term effects on BMD with 8-year follow-ups [Tabrah et al., 1998]. Furthermore, it was found in our latest [Liu et al., 2013] randomized and actively controlled clinical trial that one course of PEMF treatment with specific parameters (8 Hz, 3.82 mT, 40 min/day, 6 times/week, and 30 times as one complete course of treatment) was equally effective when compared to administration of alendronate (70 mg/week) for PMOP within 24 weeks. Specifically, there was no significant difference in the mean percentage change of BMD in lumbar spine and left proximal femur between PEMFs and alendronate groups from baseline to 24 weeks. But a minor decline trend of bone mass during one month of treatment exposure (8 Hz, 3.82 mT) could be observed in our research, with no statistical differences in intragroup comparison or compared with the alendronate group. In contrast to the above-mentioned studies, no significant increase in BMD after 3 months could be found in a single-blind and randomized pilot study [Giordano et al., 2001], which adopted the following parameters: 100 Hz and 10 ± 2 G PEMF exposure. Similarly, another randomized and sham-controlled study [Spadaro et al., 2011] found that the parameters of 8 weeks, 15 Hz, and 2 mT PEMFs did not result in long-term positive changes in BMD in subjects with forearm disuse osteopenia, after adjusting for age, gender, and baseline BMD.

Multiple reasons might be responsible for these controversial results. First, all those trials were conducted under completely different clinical designs with a time- or frequency-dependent window effect of PEMF exposure (frequency range: 8–100 Hz, intensity field range: 0–3.8 mT, exposure times: 10 min-h/day for up to 3 months), and some of the patients did not have PMOP or strictly diagnosed PMOP. Secondly, the sample size of these studies was too small for a clinical trial; besides, there was no high-quality, evidence-based research available to be included in this review. As a result, large heterogeneity of results from current studies might contribute to inconsistency. Moreover, least significant change [Watts et al., 2010; Lewiecki et al., 2016] of BMD testing should also be calculated to determine whether a difference in report was real or was simply within the inherent variability of measurements. Six to 12 months after initiating the treatment for PMOP recommended by clinical practice guidelines [Watts et al., 2010], the response can be monitored accordingly so as to ensure an accurate report of BMD testing. Therefore, either positive results or negative results obtained from the above reviews might not reflect a real change after a period of treatment for 3 months or even less. Thus, BMD changes in PMOP under PEMF exposure should be further monitored in dynamic and long-term studies for at least 6 months.

Effects of PEMFs on Bone Turnover Markers (BTMs)

Although BTMs could not be used for the diagnosis of osteoporosis, they could be applied in assessing dynamic skeletal activity associated with the response to therapeutic intervention within 1–6 months as well as in predicting bone loss and fracture risk in a BMD-independent manner. The International Osteoporosis Foundation (IOF) recommends that clinical trials should use bone resorption products of collagen degradation, such as serum C-terminal telopeptide (S-CTX) and osteoblast-derived products like serum carboxy-terminal propeptide of type I collagen (PINP) to analyze BTMs [Vasikaran et al., 2011]. PEMFs have played a positive role in modulating bone turnover biomarkers for treating PMOP with limited reliable proof. Studies on dynamic BTMs are described in Table 2. Though negative BMD change was reported in a trial [Giordano et al., 2001], the authors found that PEMF exposure with parameters of 100 Hz and 2.85 mT (60 min/day, 3 times a week for 3 months) could increase serum osteocalcin (OC) and PINP levels, which were biomarkers associated with bone formation. Similarly, PEMFs with parameters of 8 weeks, 15 Hz, and 2 mT maintained the expected normal level of serum bone-specific alkaline phosphatase (BSAP) and decreased CTX level, which were independent of BMD change, but were still beyond normal levels in patients with forearm disuse osteopenia [Spadaro et al., 2011].

Effects of PEMFs on Functional Outcomes

PMOP, which was associated with fragile bone strength and high risk of fractures, was strongly linked with deteriorated functional outcomes and disability; however, the functional outcomes were used as indicators in a few studies. Improvement of physical function is described in Table 2. It was

TABLE 2. Effects of PEMF on Osteoporosis in Clinical Studies

Refs.	Type of study	Subjects	Device and treatment parameters	Treatment duration	Main results (PEMF treatment vs. control group)		
					BMD	Biomarkers of bone remodeling	Outcomes of function
Liu et al. [2013]	A randomized, active-controlled clinical trial	41 female patients with PMOP (21 receive alendronate and 20 receive PEMFs treatment); age between 45–70 years; disease duration: at least 1 year.	Whole-body device "XT-2000B"; intensity: 3.82 mT; frequency: 8 Hz.	PEMF: 40 min/d, 6 sessions/week, 30 sessions in total; ALN: 70 mg/w, 24 weeks; All participants receive 600 mg/d calcium and alfacalcidol vitamin D supplement 0.25 μg/bid.	BMD (L1-4): −; BMD (left proximal femur): −	N/A	LE MMT: −; BBS: −
Giusti et al. [2013]	A pilot randomized-controlled trial	41 male or female patients with T-score of femoral neck <-2 (25 intervention, 16 placebo control); age ≥ 70 years; walk with or without aids; fall once or more in the last 3 years and were not cognitively impaired.	THS 280 E device intensity: 1.5 mW	10 min/d, for 1 month.	N/A	N/A	Gait speed: ↑; Stride length: −; Support base: −; Double support phase: −
Spadaro et al. [2011]	A randomized, double-blind, sham-controlled study	82 male or female patients with forearm disuse osteopenia (23 1 h/d PEMF,18 2 h/d PEMF, 21 4 h/d PEMF, 20 sham); age 18–80.	Forearm-focal device Model 202 L; intensity: 2 mT frequency: 15 Hz burst duration: 5 ms	1,2,4 h/d for 8 weeks.	forearm BMD (DXA): −; forearm BMD (QCT): −.	Serum CTX: ↓ Serum ALP: −	N/A
Giordano et al. [2001]	A single-blind, randomized pilot study	40 outpatients with PMOP diagnosed according to the criteria of the fifth Consensus Development Conference (20 intervention, 20 placebo); mean age: 55.9 (placebo), 56.3 (intervention)	Self-designed PEMF device; intensity: 0–12 G; frequency: 100 Hz.	60 min/d; 3 times/week for 3 months.	BMD (L2-4): −; BMD (femur neck): −	Serum P1NP; OC: ↑ Serum Calcium; phosphate; ALP: − Urine (24-h samples) Calcium; phosphate; hydroxyproline: −	N/A
Tabrah et al. [1990]	Observational study	20 female patients with PMOP; age: 57–75 ± 5; treatment history.	Forearm-focal PEMF device, self-designed; intensity: 2.85 mT; frequency: 75 Hz.	10 h/d for 12 weeks	BMD of the treated radii: ↑ (increase during the exposure period and decrease during the following 36 weeks)	N/A	N/A

ALN, alendronate; BMD, bone mineral density; BBS, Berg balance scale score; OC, osteocalcin; PMOP, postmenopausal osteoporosis; PEMF, pulsed electromagnetic fields; P1NP, N-terminal propeptide of type 1 procollagen; LE MMT, lower extremity manual muscle test score; BMD, Biomarkers of bone remodeling.
N/A, not applicable.
Outcomes of function: ↑, significant increase; −, no difference; ↓, significant decrease.

indicated in a pilot randomized-controlled trial [Giusti et al., 2013] that 1.5 mW PEMFs (10 min/day for 1 month) resulted in dramatically improved gait characteristics, self-selected gait speed (cm/s), and stride length (cm) in older adults with low BMD. The total lower extremity manual muscle test (LE MMT) score and Berg Balance Scale (BBS) score were deemed as secondary endpoints in our previous research [Liu et al., 2013], in which enhanced functional outcomes in patients with PMOP after PEMFs treatment had been confirmed.

ADVANCES IN BASIC RESEARCH OF PEMFs ON PMOP

Effects of PEMFs on Bone Metabolism in Animal Studies

PEMFs on bone strength. Bone density and bone quality were two main characteristics of bone strength. Consistent with clinical trials, PEMFs could prevent bone loss and deterioration of bone microstructure in different animal models of osteoporosis. Evidence on improvement of bone quality is described in Table 3. Zati et al. [1993] reported that PEMFs (under the parameters of 50 Hz and 30 Gauss, 1 h/day for 4 months) could slow down ovariectomy-induced bone loss by 10% in rats, though PEMFs with 70 Gauss did not have a significant protective effect. Other studies also suggested that PEMFs led to markedly suppressed trabecular bone loss and improved cortical and trabecular bone structure in ovariectomized rats [Sert et al., 2002; Chang and Chang, 2003]. PEMFs with parameters of 15 Hz and 2.4 mT (exposure time range: 2–8 h/day for up to 12 weeks) were applied in a series of studies to treat different rat models of osteoporosis, and all showed similar beneficial results [Jing et al., 2010, 2011, 2013, 2014]. In addition, they also found that specific parameters of PEMFs could alleviate the deterioration of trabecular and cortical bone microarchitecture as well as the reduction of bone mechanical properties, including maximum load, stiffness, and elastic modulus, evidenced by micro-computed tomography (μCT) scan and three-point bending test. In line with their findings, results of dual energy X-ray absorptiometry (DEXA) revealed that PEMFs could greatly increase BMD in ovariectomized [Zhou et al., 2012, 2013] and hindlimb-suspended [Shen and Zhao, 2010] rats. Moreover, results from our research group [Zhou et al., 2012, 2013] demonstrated that PEMFs with the parameters of 8 Hz and 3.8 mT (40 min/day, 5 days/week for 12 weeks) could mitigate the deterioration of bone microarchitecture and strength, as was evidenced by histological and biomechanical analyses. Meanwhile, other research groups also reported that PEMFs with the same parameters (8 Hz, 3.8 mT, 40 min/day, 5 days/week for 12 weeks) had the same beneficial effect via significantly increasing BMD (DEXA), bone mechanical properties, and parameters of μCT (including trabecular bone volume ratio, trabecular number, trabecular thickness, and separation) in streptozotocin- and ovariectomy-induced bone loss, respectively [Zhou et al., 2015, 2017]. However, it was suggested in a study [van der Jagt et al., 2012] that the efficacy of PEMFs with the parameters of 15 Hz and 1 G (2 h/day for 3–6 weeks) might be a source of controversy since micro-CT scanning could not detect any changes in cancellous or cortical bone relative to the untreated controls. Besides, it was discovered that PEMFs generated by Helmholtz coils (64 cm I.D., 200 turns/coil) with parameters of 15 Hz and 5.6 A peak-to-peak square wave could not significantly increase the calcium content in femur [Takayama et al., 1990]. Accordingly, the window effect might also exist within the scope of certain parameters (frequency range: 7.5–50 Hz, intensity field range: 0.1–3.8 mT, exposure times: 40 min–8 h/day for up to 12 weeks), and varying treatment periods of PEMFs might account for the diverse results. Moreover, variations in animals (genders, age, and models) under different experimental designs and conditions might also affect the consistency of results.

PEMFs on bone turnover biomarkers. Normal structural and functional integrity of bone was maintained by dynamic remodeling activity. Bone remodeling was a homeostasis state governed by equal rates of osteoblasts that formed new bones and osteoclasts that resorbed old bones. PEMFs displayed a marked preventive effect on osteoporosis-induced high bone turnover in rat models. Results of several studies (Table 3) supported that PEMFs could significantly increase levels of biomarkers of osteoblast-associated bone formation, such as serum bone-specific alkaline phosphatase (BALP), OC, and P1NP, but they produced only minor preventive effects on biomarkers of osteoclast-associated bone resorption [Jing et al., 2011, 2014; Zhou et al., 2015], such as serum C-terminal crosslinked telopeptides of type I collagen (CTX-I) and tartrate-resistant acid phosphatase 5b (TRAcP5b). Effects of PEMF on bone loss might be related to significantly improved bone formation when taking the positive change of bone mass into consideration. Interestingly, it was reported [Jing et al., 2010, 2013] that PEMFs could significantly slow down the rate of bone turnover, leading to decreases in serum B-ALP, bone Gla protein (BGP), and TRAcP5b in OVX rats. The inhibitory effects of

TABLE 3. Effects of PEMF on Osteoporosis in Animal Studies

Refs.	Animal model	Parameters of PEMF	Phenotypes	Correlative gene expression	Possible molecular mechanisms
Zhou et al. [2017]	50 3-month-old ovariectomized female Sprague–Dawley rats with combined treatment (IBN + PEMF) or monotherpay	Frequency: 8 HZ; Intensity: 3.8 mT; Exposure time: 40 min/d, 5 d/week,12 weeks	Bone formation markers (BALP): ↑; Bone resorption markers (TRAcP5b): ↓; BMD (DEXA): +; BV/TV; Tb.Th; Tb.N; Tb.Sp (μCT): +; Three-point bending test: +	Gene expression in femur ↑: OPG ↓: RANKL	The favorable effect of monotherapy or the combination treatment on bone strength may be attributable to the regulation of RANK/RANKL/OPG pathway.
Zhou et al. [2015]	30 3-month-old streptozotocin-induced diabetic Sprague–Dawley rats, 15 males and 15 females, respectively	Frequency: 8 HZ; Intensity: 3.8 mT; Exposure time: 40 min/d, 5 d/week,12 weeks	Bone formation markers (BALP): ↑; Bone resorption markers (TRAcP5b): ↑↓; BMD (DEXA): +; BV/TV; Tb.Th; Tb.N; Tb.Sp (μCT): +; Three-point bending test: +	Gene expression in femur ↑: LRP5, β-catenin, Runx2 ↓: DKK1 −: OPG; RNAKL	PEMFs can protect DM-induced the deterioration of bone quality by restoring Runx2 expression through regulation of Wnt/β-catenin signaling
Jing et al. [2014]	30 3-month-old hindlimb-suspended male Sprague–Dawley rats	Frequency: 15 HZ; Intensity: 2.4 mT; Burst width: 5 ms; pulse width: 0.2 ms; Exposure time: 2 h/d, 4 weeks	Bone formation markers (OC and P1NP): ↑; Bone resorption markers (CTX-I and TRAcP5b): ↑↓; BV/TV; Tb.Th; Tb.N; Tb. Sp; Ct.Ar; Ct.Th (μCT): +; Three-point bending test: +; N.Ob/BS; N.Oc/BS; MAR; BFR/BS: +	Gene expression in tibia ↑: Wnt1, LRP5, β-catenin, OPG, OC; −: RANKL, RANK, Sost	Canonical Wnt signaling might be involved in skeletal anabolic effects of PEMF.
Zhou et al. [2013]	30 3-month-old ovariectomized female Sprague–Dawley rats	Frequency: 8 HZ; Intensity: 3.8 mT; Exposure time: 40 min/d, 5 d/week, 12 weeks	Serum 17b-estradiol (E2): ↑; Bone resorption markers (TRAcP5b): ↓; Dual energy X-ray absorptiometry (DEXA): +; Biomechanical compression testing: +; Histological analysis: +	Gene expressions in femur ↑: OPG ↓: RANKL	PEMF may modulate the process of osteoclast activation and subsequent bone resorption,at least partially,through regulation of the RANK/RANKL/OPG pathway
Jing et al. [2013]	30 3-month-old ovariectomized female Sprague–Dawley rats	Frequency: 15 HZ; Intensity: 2.4 mT; Burst width: 5 ms; pulse width: 0.2 ms; Exposure time: 8 h/d,10 weeks	BMD; BV/TV; Tb.Th; Tb.N; Tb.Sp (μCT): +; Three-point bending test: +	Gene expression in tibia ↑: Wnt1, LRP5, β-catenin; −: RANKL, RANK	PEMF modulated bone microarchitecture and strength via Wnt/LRP5/β-catenin signaling.
Zhou et al. [2012]	30 3-month-old female ovariectomized female Sprague–Dawley rats	Frequency: 8 HZ; Intensity: 3.8 mT; Exposure time: 40 min/d, 5 d/week, 12 weeks	Serum 17b-estradiol (E2): ↑; Bone formation markers (BALP): ↑; BMD (DEXA): +; Tb.Ar; Tb.Wi; Tb.N; Tb. Sp (histomorphometric analysis): +; Biomechanical compression testing: +	Gene expression in femur ↑: Wnt3a; LRP5, β-catenin, Runx2; c-myc ↓: DKK1	The activation of Wnt/β-catenin signaling pathway plays an important role in these beneficial effects of PEMFs on OVX rats.
van der Jagt et al. [2012]	20-week-old ovariectomized female Wistar WU rats	Frequency: 15 HZ; 7.5 HZ; 50–150 kHz; non-continuous PEMF stimulus; Intensity: 1 G; Exposure time: 2 h/d, 3 and 6 weeks	BV/TV; CtV; Ct.Th; Conn.D; SMI; Tb.Th (μCT): −	N/A	N/A
Jing et al. [2011]	16 streptozotocin-induced diabetic and 8 non-diabetic	Frequency: 15 HZ; Intensity: 2.4 mT; Burst width: 5 ms;	Bone formation markers (OC): ↑; Bone resorption markers	N/A	N/A

(*Continued*)

TABLE 3. (*Continued*)

Refs.	Animal model	Parameters of PEMF	Phenotypes	Correlative gene expression	Possible molecular mechanisms
	3-month-old male Sprague–Dawley rats	pulse width: 0.2 ms; Exposure time: 8 h/d, 8 weeks	(TRAcP5b): ↑↓; BV/TV; Tb.Th; Tb.N; Tb.Sp; Ct.Ar; Ct.Th (μCT): +; Three-point bending test: +; Bone histomorphometric analysis: ±		
Shen and Zhao [2010]	80 4-month-old disuse-induced female Sprague–Dawley rats	Frequency: 15 HZ; Intensity: 8 G; Pulse duration: 8 ms; Exposure time: 2 h/d, 8 weeks	Serum TGF-β1: ↑; Serum IL-6: ↓; Dual energy X-ray absorptiometry (DEXA): +; Histological observation: +	N/A	N/A
Jing et al. [2010]	32 3-month-old ovariectomized female Sprague–Dawley rats	Frequency: 15 HZ; Intensity: 9.6 G; Burst and pulse width: 5 ms; 0.2 ms; Exposure time: 9–15/d, 0 to 6/d, 12 weeks	Serum ALP and BGP: ↓; Urinary deoxypyridinoline: ↓; Dual energy X-ray absorptiometry (DEXA): +; Tb.Ar; Tb.Wi; Tb.N; Tb.Sp (histomorphometric analysis): +	N/A	N/A
Chang and Chang [2003]	35 3-month-old ovariectomized female Sprague–Dawley rats	Frequency: 7.5 Hz; Intensity: 1 2 mV/cm; Pulse duration: 0.3 ms; Exposure time: 8 h/d, 30 d	Serum PGE2: ↓; Microradiographs of proximal tibia metaphyses: +; Histomorphometrical analysis: +	N/A	N/A
Sert et al. [2002]	18 8-week-old ovariectomized female albino Wistar rats	Frequency: 50 Hz; Intensity: 1 mT; Exposure time: 4 h/d, 6 weeks	Mineral levels of tibial bones: −; Microscopic analysis of tibial bones: +	N/A	N/A

ALP, alkaline phosphatase; BGP, bone Gla protein; BMD, bone mass density; BV/TV, trabecular bone volume ratio; BFR/BS, bone formation rate per bone surface; CTX-I, C-terminal crosslinked telopeptides of type I collagen; Conn.D, connectivity density; Ct.Ar, cortical area; Ct.V, cortical volume; Ct.Th, cortical thickness; DEXA, dual energy X-ray absorptiometry; IBN, Ibandronate; IL-6, interleukin- 6; MAR, mineral apposition rate; N.Ob/BS, number of osteoblasts per millimeter of trabecular bone surface; N.Oc/BS, number of osteoclasts per millimeter of trabecular bone surface; OC, osteocalcin; OPG, osteoprotegerin; PEMF, pulsed electromagnetic fields; P1NP, N-terminal propeptide of type 1 procollagen; PGE2, prostaglandin E2; PCR, polymerase chain reaction; RANKL, receptor activator of NF-κB ligand; SMI, structure model index; TRAcP5b, tartrate-resistant acid phosphatase 5b; TGF-β, transforming growth factor-β; Tb.Ar, trabecular area; Tb.Wi, trabecular width; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation.

N/A, not applicable.

Phenotypes: ↑, significant increase; ↑↓, minor or no difference; ↓, significant decrease; +, positive change; ±, partial or no change; −, negative change.

Correlative gene expression: ↑ upregulation; ↓ downregulation.

PEMFs on high bone turnover and resorption might interact with the promotion of bone formation to prevent bone loss. Furthermore, PEMFs could improve bone anabolism through upregulating serum 17b-estradiol (E2) [Zhou et al., 2012, 2013], transforming growth factor-β (TGF-β) [Shen and Zhao, 2010], and cytokines promoting osteoblastogenesis; and it could prevent bone catabolism through downregulating serum interleukin-6 (IL-6) [Shen and Zhao, 2010], prostaglandin E2 (PGE2) [Chang and Chang, 2003], inflammatory cytokines, and other mediators promoting osteoclastogenesis.

Effects of PEMFs on Bone Cells

PEMFs on osteoblastic cell lines. BMSC-derived osteoblasts were involved in the bone formation process, including synthesis of bone matrix and formation of dense mineralization. As the active markers of osteoblasts, type 1 collagen and alkaline phosphatase (ALP) were critical during the mineralization process. Post-menopausal dysfunction of osteoblasts might contribute to osteoporosis. PEMFs could promote bone formation involving a series of responses from osteoblasts and progenitor cells, which was consistent with results from animal and clinical studies. Numerous studies (Table 4) confirmed that PEMFs could accelerate osteoblast proliferation, differentiation, and mineralization, which was linked with osteoblast-oriented bone formation [Diniz et al., 2002; Chang et al., 2004b; Patterson et al., 2006; Li et al., 2007; Schnoke and Midura, 2007; Sun et al., 2009; Tsai et al., 2009; Jansen et al., 2010; Sollazzo et al., 2010; Lin and Lin, 2011; Zhou et al., 2011; Esmail et al., 2012; Lin et al., 2015; Petecchia et al., 2015; Yan et al., 2015; Zhai et al., 2016]. Specifically, it was demonstrated in a recent study [Zhai et al., 2016] that PEMFs with the parameters of 15.83 Hz at 20 Gs (2 mT) for 2 h/day rendered the optimal efficacy of enhancing proliferation of MC3T3-E1 osteoblasts. Furthermore, it was shown in that study that PEMFs with specific parameters could significantly improve osteoblast functions, which was evidenced by ALP staining and alizarin red staining. Meanwhile, Ehnert et al. [2015] also discovered that extremely low-frequency PEMFs with specific parameters could partly result in remarkably improved mitochondrial activity, total protein content, alkaline phosphatase activity, and formation of mineralized matrix of human osteoblasts with poor initial osteoblast functions.

However, effects of PEMFs on bone formation remained a source of controversy with a window effect, which was similar to findings in animal and clinical studies. Primarily, bone formation induced by PEMFs might depend on the intensity of magnetic fields and the diversity of pulse stimulation [Matsunaga et al., 1996]. Diversified parameter settings (frequency range: 0.2–75 Hz, intensity field range: 0.1 mT–1 T, exposure times: 3 min–24 h/day for up to 25 days) of PEMF devices employed in these studies might also lead to varying results. Zhou et al. [2011] suggested that the proliferation of primary murine osteoblasts could be inhibited by electromagnetic fields at different intensities, but cellular differentiation and mineralization could be enhanced by 50 Hz of electromagnetic fields at intensities of 0.9–1.8 and 3.0–3.6 mT. Moreover, it was found by Yan et al. [2015] that proliferative effects of PEMFs with parameters of 50 Hz and 0.6–3.6 mT on primary murine osteoblasts were significantly positive, with 0.6 mT having the highest proliferative effects among all intensities, which was in contrast to the effects of 50 Hz sinusoidal electromagnetic fields (SEMFs) at 0.9–4.8 mT on osteoblastic maturation [Zhou et al., 2011]. They also reported that PEMF stimulation at 0.6 mT had positive effects on cellular differentiation and mineralization. On the other hand, osteoblastic cell lines derived from multiple species at various differentiated stages might give rise to conflicting findings. Though both Diniz et al. [2002] and Chang et al. [2004b] reported proliferative effects of 15 Hz PEMFs on MC3T3-E1 cell lines and primary murine osteoblasts, the latter group found that PEMF stimulation had no effect on osteoblast differentiation or mineralization. Furthermore, other confounding factors, such as different experimental conditions, cell densities [Tsai et al., 2009; Jansen et al., 2010], culture periods [Diniz et al., 2002; Sun et al., 2009; Jansen et al., 2010], and concentrations of fetal calf serum [Sollazzo et al., 1997] could also contribute to conflicting findings in cellular studies.

PEMFs on cell lines of osteoclasts. Osteoclasts deriving from hematopoietic cells of monocyte/macrophage lineage played a critical role in breaking down the bone hydrated protein–mineral complex by secreting acid and collagenase. The absence of estradiol might excessively activate osteoclasts and their subsequent bone resorption. PEMFs might have an inhibitory effect on osteoclastogenesis (Table 4). Chang et al. [2004a, 2006] found that PEMFs at 7.5 Hz could significantly suppress osteoclast proliferation and promote apoptosis of murine osteoclasts. It was also reported in similar studies [He et al., 2015] that PEMFs with parameters of 3.8 mT and 8 Hz, which were parallel to the effects of osteoprotegerin (OPG) and estradiol, substantially reduced the

TABLE 4. Effects of PEMF in Bone Cells

Refs.	Cell type	Parameters of PEMF	Phenotypes	Changes in gene expression	Possible molecular mechanisms
Zhai et al. [2016]	MC3T3-E1 osteoblasts	Frequency: 15.38 Hz; Intensity: 0.5,1,2 mT; Exposure time: 0.5, 1, 2, or 6 h/d for 2 d, respectively; 2 h/d for 2, 5, 7 d	Proliferation: ↑; Differentiation: ↑; Mineralization: ↑ (2 h/d and 2 mT is the most optimal parameter)	↑: Ccnd 1; Ccne 1. ↓: ALP in proliferation phase.↑: OCN; COL I; ALP in differentiation phase.↑: Runx2; Wnt1; LRP6; β-catenin in both phases	PEMF can promote osteogenesis-associated gene expressions through the canonical Wnt signaling.
Lin et al. [2015]	MC3T3-E1 osteoblasts	Frequency: single pulse (0.2 Hz); Pulse duration: 5 ms; Intensity: 1 T; Exposure time: 3 min/d, 1, 3, 5, 8, 10, 15, 20 d, respectively	Differentiation: ↑; Mineralization: ↑	↑: Wnt1; Wnt3a; Wnt10b; Fzd9; ALP; BMP2, ↓: Sost in short-term exposure (0–5 d). ↑: PTHrP, ↓: Sost in long-term exposure (0–20 d).	SPEMF increased osteogenic differentiation through Wnt signaling pathway and sclerostin downregulation.
Yan et al. [2015]	Calvarial osteoblasts from newborn Wistar rats	Frequency: 50 Hz; Intensity: 0.6–3.6 mT, 0.6 interval; Exposure time: 90 min/d, 3, 6, 9, 12 d, respectively	Proliferation: ↑; Mineralization: ↑ (0.6 mT is the most optimal parameter)	↑: BMP2; COL1; Osx; Runx2	The primary cilia of osteoblasts are critical in mediating the osteogenic effect of PEMF exposure at one optimal parameter.
Petecchia et al. [2015]	BM-hMSCs	Frequency: 75 ± 2 Hz; Intensity: 2 ± 0.2 mT; Pulse duration: 1.3 ms; Exposure time: 10 min/d, 3,9,15,21,27 d, respectively	Proliferation: ↑; Differentiation: ↑	N/A	The expression of L-type VGCC and modulation of the concentration of cytosolic free Ca2+.
He et al. [2015]	Primary osteoclast-like cells differentiated from the bone marrow of 5-week-old female C57BL/6 mice	Frequency: 8 Hz; Intensity: 3.8 mT; Exposure time: 40 min/d, 3 d	N.OC: ↓	↓: NFATc1; CAII; RANK	PEMF might modulate the process of osteoclastogenesis and subsequent bone resorption, at least partially, through NFATc1, CAII and RANK.
Vincenzi et al. [2013]	hFOB 1.19 osteoblasts	Frequency: 75 Hz; Intensity: 2.5 ± 0.2 mT; Pulse duration: 1.3 ms; Exposure time: 24 h for testing	cAMP production: ↑; Osteoprotegerin production: ↑; IL-6; IL-8; PGE2: ↓	↑: A2A and A3AR	PEMF showed anti-inflammatory responses elicited by A2A and A3AR activation.
Esmail et al. [2012]	MC3T3-E1 osteoblasts	Frequency: 15 Hz; Intensity: 4 mT; Burst width: 10 ms; pulse width: 200 μs; Exposure time: 30 min/d, 24, 48 h	Proliferation: ↑	↑: IGF-1; COX-1; COX-2	PEMF promoted the proliferation of MC3T3-E1 osteoblasts through the COX-2-related signal pathway.
Zhou et al. [2011]	Calvarial osteoblasts from neonatal rats	Frequency: 50 Hz; Intensity: 0.9–4.8 mT,0.3 interval; Exposure time:30 min/d, 0,12,24,96 h and 3,6,9,12 d, respectively	Proliferation: ↓; Differentiation: ↑; Mineralization: ↑	↑: Runx2, COL2; BMP2	Calcium/calmodulin signal pathway and NO-cGMP-PKG pathway are involved to initiate the osteogenesis response.
Cheng et al. [2011]	Primary calvaria rat osteoblast (ROB) cells from 1-d-old rats	Frequency: 50 Hz, T = 0.02 s; Intensity: 1.8 mT; Exposure time:30 min/d, 0 –4 h at 0.5 interval, 12 h,	Differentiation: ↑; Mineralization: ↑	↑: NOS; cGMP; PKG; ALP; Osx	NO-cGMP-PKG signal pathway is activated by SEMF treatment.

(*Continued*)

TABLE 4. (*Continued*)

Refs.	Cell type	Parameters of PEMF	Phenotypes	Changes in gene expression	Possible molecular mechanisms
		24 h and 9, 14 d, respectively			
Lin and Lin [2011]	Murine osteoblast cell line (7F2) and co-cultured with macrophage cells (RAW 264.7)	Frequency: 75 Hz; Intensity: 1.5 mT; Pulse duration: 1.3 ms; Exposure time: a total of 9 h	Proliferation: ↑; Cell Viability (MTT Assay): ↑; ALP activity: ↓	↑: COL I; −: OC	N/A
Sollazzo et al. [2010]	Human osteoblastlike cells (MG63)	Frequency: 75 ± 2 Hz; Intensity: 2 ± 0.2 mT; Pulse duration: 1.3 ms; Exposure time:18 h	N/A	↑: HOXA10; AKT1; CALM1; P2RX7; FN1; VCL; COL1A2; SPARC; ↓: MMP-11;DUSP4	PEMFs induce cell proliferation and differentiation. Furthermore, PEMFs promote extracellular matrix production and mineralization while decrease matrix degradation and absorption.
Jansen et al. [2010]	Human bone marrow stromal cells (BMSCs), SV40-immortalized human fetal pre-osteoblasts (SV-HFO)	Frequency: 15 Hz; Intensity: 1 G; Burst and pulse width: 5 ms, 5 μs; Exposure time: continuous exposure, up to 21 d	Proliferation: −; Differentiation: ↑; Mineralization: ↑	↑: BMP2;TGF-β1; MMP1; MMP3;OPG;OC;IBSP; RANKL; −: ERK1/2 phosphorylation	PEMF and mechanical stimuli may act via different ways in mechanotransduction
Chen et al. [2010]	Osteoclast-Like Cell from ovariectomized 3-month-old female SD rats	Frequency: 8 Hz; Intensity: 3.8 mT; Exposure time: 40 min/d, 3 d	N/A	↓: CA II; −: RANK	PEMF might modulate the process of osteoclastogenesis and subsequent bone resorption, at least partially, through RANK and CA II.
Tsai et al. [2009]	Human mesenchymal stem cells (hMSCs)	Frequency: 7.5 Hz; Intensity: 2 mV/cm; Pulse duration: 300 μs; Exposure time:2 h/d, up to 14 d	Proliferation: delayed ↑; Differentiation: ↑; Mineralization: ↑	↑: Runx2/Cbfa1; ALP (7 d); ↓: Runx2/Cbfa1; ALP (10 d)	N/A
Sun et al. [2009]	Human bone marrow mesenchymal stem cells	Frequency: 15 Hz; Intensity: 1.8 mT; Pulse duration: 200 us; Exposure time:8 h/d during culture period (up to 10 d)	Proliferation: ↑; Differentiation: −; Mineralization: −	N/A	N/A
Schnoke and Midura [2007]	UMR106-01 BSP cell line, MC3T3-E1	Frequency: 15 Hz; Intensity: 0.4 mT; Burst width: 67 ms; pulse width: 260 μs; Exposure time: 36 h	N/A	↑: IRS-1; eNOS; S6 (transient)	The anabolic effects of PEMF may be mediated, in part, through the activation of IRS-1, eNOS and S6.
Li et al. [2007]	Osteoblasts from newborn Wistar-rat calvaria	Frequency: 7.5 Hz; Intensity: 1–2.5 mV/cm,0.5 interval; Pulse duration:300 μs; Exposure time: 20 min/d, 1,2,3,4 d	Proliferation: ↑; TGFβ1; ALP: ↑; PGE2: ↓	N/A	N/A
Patterson et al. [2006]	MC3T3-E1 cell line	Frequency: 15 Hz; Intensity: 0.4 mT; Burst width: 67 ms; pulse width: 260 μs;	N/A	↑: mTOR; p70 S6 kinase; ribosomal protein S6	PEMF exposure might function in a manner analogous to soluble growth factors by activating a unique set of

(*Continued*)

TABLE 4. (*Continued*)

Refs.	Cell type	Parameters of PEMF	Phenotypes	Changes in gene expression	Possible molecular mechanisms
		Exposure time: 10 h/d, 0 h to 2 d			signaling pathways, inclusive of the PI-3 kinase/mTOR pathway.
Chang et al. [2006]	Isolated osteoclasts cocultured with primary osteoblastic cells and bone marrow cells	Frequency: 7.5 Hz; Intensity: 3.0 μV/cm; Pulse duration: 300 μs; Exposure time: 1,8,16 h, respectively	Apotosis: ↑ (8 and 16 h of PEMF exposure)	N/A	N/A
Chang et al. [2004a]	Osteoclast induced from six 8 month old female Wistar rats in intact (INT), sham ovariectomized (SHAM), and ovariectomy (OVX) groups	Frequency: 7.5 Hz; Intensity: 4.8 mV/cm; Pulse duration: 0.3 ms; Exposure time: 1 h per day for 9 d	Proliferation: ↓; TNF-α; IL-6; IL-1β: ↓	N/A	N/A
Chang et al. [2004b]	Osteoblast-like cells isolated from calvaria of newborn ICR mice	Frequency: 15 Hz; Intensity: 0.1 mT; Burst width: 5.016 ms; pulse width: 0.2 ms; Exposure time: 8 h/d, 1,3,5,12 d	Proliferation: ↑; Differentiation: −; Mineralization: −	↑: OPG; ↓: RANKL	N/A
Diniz et al. [2002]	MC3T3-E1 cell line	Frequency: 15 Hz; Intensity: 7 mT; Burst width: 5 ms; pulse width: 150 μs; Exposure time: 24 h/d, up to 25 d	Proliferation: ↑ (early stage); Differentiation: ↑	N/A	N/A

AKT1, V-akt murine thymoma viral oncogene homolog 1; AR, adenosine receptors; ALP, alkaline phosphatase; BMP, bone morphogenetic protein; BSP, bone sialoprotein; cGMP, cyclic guanosine monophosphate; CALM, calmodulin; CA, carbonic anhydrase; COL, collagen; COX, cyclooxygenase; DUSP4, dual specificity phosphatase 4; ERK, extracellular signal-regulated kinase; eNOS, endothelial nitric oxide synthase; FN, fibronectin; HOXA10, homeobox A10; IL-6, interleukin-6; IBSP, bone sialoprotein; IRS, insulin receptor substrate; IGF, insulin-like growth factors; mTOR, mammalian Target of Rapamycin; MMP, matrix metalloproteinase; N.OC, number of osteoclasts; OPG, osteoprotegerin; OC, osteocalcin; PKG, cAMP-dependent protein kinase; PTH, parathyroid hormone; P2RX7, purinergic receptor P2X, ligand-gated ion channel, 7; PGE2, prostaglandin E2; PEMF, pulsed electromagnetic fields; RANKL, receptor activator of NF-κB ligand; SPARC, osteonectin; S6, S6 ribosomal subunit kinase; TGF-β, transforming growth factor-β; VCL, vinculin.
N/A, not applicable.
Phenotypes: ↑, significant increase; −, minor or no change; ↓, significant decrease.
Correlative gene expression: ↑, upregulation; −, minor or no change; ↓, downregulation.

number of osteoclast-like cells induced by macrophage colony-stimulating factor (M-CSF) together with receptor activator of the NF-κB ligand (RANKL).

MECHANISMS OF PEMFs IN PMOP

The Regulation Roles of PEMFs in Osteogenesis

Upregulation of osteogenesis-associated gene expression. The efficacy of PEMFs on bone formation indicates that they might regulate genes involved in osteoblast proliferation and differentiation. As was reported by Zhai et al. [2016], PEMFs could significantly upregulate the expression of two markers of cell cycle progression (*Ccnd 1* and *Ccne 1*) at osteoblast proliferation stage and alkaline phosphatase marker of osteoblast differentiation. Stimulation of PEMFs with different parameters were confirmed in several studies to significantly upregulate the expression of two important transcription factors, *Runx2/cbfa1* [Komori, 2006; Tsai et al., 2009; Zhou et al., 2011, 2015; Ehnert et al., 2015; Yan et al., 2015; Zhai et al., 2016] and *osterix* [Cheng et al., 2011; Ehnert et al., 2015; Yan et al., 2015] at both proliferation and differentiation stages, which were involved in the development of the osteoblastic lineage (Table 4). In addition, PEMF exposure also significantly upregulated the expression of genes involved in bone matrix formation, which were comprised of type 1 collagen (*COL1*) [Lin and Lin, 2011; Zhou et al., 2011; Yan et al., 2015; Zhai et al., 2016]; noncollagenous proteins including osteocalcin (*OC*) [Jansen et al., 2010; Zhai et al., 2016] and bone sialoprotein (*BSP*) [Jansen et al., 2010]; as well as growth factors including insulin-like growth factors-1 (*IGF-1*) [Esmail et al., 2012], *TGF-β* [Jansen et al., 2010], and bone morphogenetic proteins (*BMPs*) [Jansen et al., 2010; Zhou et al., 2011; Lin et al., 2015; Yan et al., 2015]. Furthermore, a microarray analysis (Table 4) on human osteoblast-like cells (MG63) exposed to 18 h of PEMFs indicated that stimulations could also inhibit the process of bone catabolism through upregulating *TIMP1*, a degradation inhibition marker of extracellular matrix induced by matrix metalloproteinase (MMP), and downregulating *MMP-11* and dual-specificity phosphatase 4 (*DUSP4*) that are involved in the inhibition of osteoblast proliferation and differentiation [Sollazzo et al., 2010].

The role of canonical Wnt signaling pathway. Canonical Wnt signaling was a major regulator involved in skeletal development and bone homeostasis [Day et al., 2005; Glass et al., 2005; Baron and Kneissel, 2013]. In nature, canonical Wnt signaling is activated by the binding of extracellular Wnt ligands to the frizzled (FZD) and LRP5/6 co-receptors on the cell membrane, thus inhibiting the degradation of β-catenin and increasing the translocation of β-catenin from cytoplasm to cell nucleus to upregulate target gene transcription [Baron and Kneissel, 2013]. Therefore, mutations targeting canonical Wnt signaling have contributed to negatively affecting bone formation via inhibiting osteoblast maturation and promoting osteoblast apoptosis. Accumulating evidence confirmed that knockout of *Wnt10b* [Bennett et al., 2007], *Lrp5/6* [Masaki et al., 2002; Philippe et al., 2005; Kubota et al., 2008], and *β-catenin* [Joeng et al., 2011] genes resulted in bone formation defects and low bone mass in mice. Inversely, mutations of *SOST* [Kramer et al., 2010], *DKK1* [Wang et al., 2007], *Kremen2* [Schulze et al., 2010], and *Sfrp1/4* [Bodine et al., 2004; Nakanishi et al., 2008], which were antagonists or inhibitors of canonical Wnt signaling, could also lead to disordered bone homeostasis in murine.

Therefore, PEMFs might exert a positive effect on osteogenesis via the activation of canonical Wnt signaling. It was reported by a research group [Jing et al., 2013, 2014] that 4- and 10-week PEMF treatments could markedly enhance mRNA expression of *Wnt1*, *β-catenin*, and *Lrp5* in hindlimb-suspended male rats and ovariectomied female rats, respectively. Zhou et al. [2012] found that 4-week PEMF treatment with parameters of 8 Hz at 3.8 mT could activate canonical Wnt signaling by upregulating *Wnt3a*, *LRP5*, and *β-catenin* and downregulating *DKK1* in ovariectomied rats (Table 3). Zhai et al. [2016] also observed that PEMFs could significantly increase gene and protein expression levels of *Wnt1*, *LRP6*, and *β-catenin* in MC3T3-E1 osteoblasts at both proliferation and differentiation stages, which was consistent with studies in vivo. It was further verified in another study [Lin et al., 2015] that short-term single-pulsed electromagnetic field treatment rendered a series of significantly increased gene expression of canonical Wnt signaling, including *Wnt1*, *Wnt3a*, *Wnt10b*, and *Fzd9* in MC3T3-E1 osteoblasts during osteogenic differentiation stage (Table 4). In addition, PEMFs with parameters of 3.8 mT and 8 Hz could significantly upregulate the mRNA expression of *β-catenin* and *Lrp5* while downregulate that of *DKK1* in rats with Diabetes mellitus (DM)-induced fragile bone quality, as was discovered by Zhou et al. [2015].

Other possible signaling pathways triggered by PEMFs. It had been suggested that the application of electromagnetic fields might also activate several

signaling pathways, such as mTOR or NO/cGMP/PKG signaling pathways. mTOR signaling pathway played a key role in osteoblast differentiation, and mTOR inhibitor, rapamycin, could inhibit osteogenesis both in vivo and in vitro; while over-expression of p70S6K, the downstream effector of mTOR, could significantly increase osteoblast differentiation [Singha et al., 2008; Xian et al., 2012]. Meanwhile, suppressing the activation of nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ), which mainly promoted the differentiation of BMSCs into adipocytes [Sun et al., 2013], could notably enhance the mTOR signaling pathway. It was confirmed by Patterson et al. [2006] that PEMF exposure gave rise to markedly increased levels of three major components of the mTOR signaling pathway, which were mTOR as well as its downstream targets p70 S6 kinase and ribosomal protein S6. Conversely, they also found that effects of PEMFs on the activation of the mTOR signaling pathway could be blocked by rapamycin and PI3-kinase inhibitor, an upstream regulator of mTOR (Table 4).

Additionally, the NO/cGMP/PKG signaling pathway might be another key regulator in osteoblast maturation [Francis et al., 2010], which involved the endogenous formation of NO by nitric synthase and the downstream activation of cyclic guanosine monophosphate (cGMP), soluble guanylyl cyclase (sGC), and cAMP-dependent protein kinase (PKG). It has been discovered in studies that SEMFs with the parameters of 50 Hz at 1.8 mT could significantly upregulate the expression of NOS, sGC, and PKG proteins, and effects of SEMFs on the activation of the NO/cGMP/PKG signaling pathway as well as the osteoblast anabolism could be blocked by inhibitors of these proteins (Table 4) [Huang et al., 2008b; Cheng et al., 2011].

On the other hand, transmembrane receptors such as insulin, parathyroid hormones, Ca^{2+}/calcitonin, or transferrin receptors, which had been proven to be modulated by PEMFs [Ciombor and Aaron, 2005; Schnoke and Midura, 2007; Pilla et al., 2011; Lin et al., 2015], might activate the same signaling cascades, namely, the NO/cGMP/PKG signaling pathway (Table 4). Schnoke and Midura [2007] confirmed that PEMF exposure played a role analogous to that of parathyroid hormone (PTH) and insulin by promoting phosphorylation of the key signaling proteins such as insulin receptor substrate-1 (IRS-1), endothelial nitric oxide synthase (eNOS), and S6 ribosomal subunit kinase, which were involved in bone anabolism. Moreover, Zhang et al. [2010] reported that EMF with parameters of 50 Hz at 0.8 mT resulted in exclusively markedly increased intracellular calcium levels of osteoblasts. On the basis of their work, Cheng et al. [2011] further confirmed that nNOS and eNOS, which were the two Ca^{2+}/calmodulin-dependent nitric oxide synthases, were regulated by intracellular Ca^{2+} concentration in osteoblasts.

Regulatory Effects of PEMFs on Osteoclastogenesis

Downregulation of inflammatory cytokines. Maturation and function of osteoclasts were modulated by multiple factors at different cellular and molecular levels. Estrogen deficiency could lead to significantly increased levels of osteoclastic bone resorption-associated cytokines, which were produced by osteoblasts and bone marrow cells [Teitelbaum and Ross, 2003].

As one of the most potent bone-resorbing factors, inflammatory cytokines were responsible for accelerating osteoclastogenesis and bone resorption [Steeve et al., 2004]. It had been indicated in numerous studies (Tables 3 and 4) that stimulation by PEMFs could significantly decrease levels of inflammatory cytokines [Chang and Chang, 2003; Chang et al., 2004a, 2006; Shen and Zhao, 2010; Vincenzi et al., 2013]. Specifically, Chang et al. [2004a] found that 7.5 Hz PEMF treatment significantly decreased levels of tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β), and interleukin 6 (IL-6) in bone marrow cells derived from ovariectomied rats. Furthermore, they confirmed that 7.5 Hz PEMF treatment could accelerate the apoptosis rate of osteoclasts [Chang et al., 2006]. Thus, the anti-inflammatory effect might be another potential efficacy of PEMFs.

However, the mechanism by which PEMFs downregulated the inflammatory cytokines still remains unclear. Adenosine receptors (ARs) have been proven to be involved in the modulation of inflammation-based pathological processes [Kara et al., 2010; Varani et al., 2011]. PEMF stimulation could increase the expression of selective ARs in various cell types [Varani et al., 2003, 2012]. Vincenzi et al. [2013] suggested that PEMFs could activate expression of A2A and A3ARs (Table 4), the subtypes of ARs, in hFOB 1.19 osteoblasts, so as to induce anti-inflammatory effects by decreasing the release of IL-6/8 and the inflammatory mediator PGE_2 [Chang and Chang, 2003; Shen and Zhao, 2010]. Furthermore, several studies reported that ARs might play important roles in the differentiation and function of osteoblasts [Carroll et al., 2012; Takedachi et al., 2012]. Accordingly, it was possible that PEMFs exerted its anti-inflammatory effects through osteoblasts.

The role of OPG/RANK/RANKL signaling. OPG/RANK/RANKL signaling played a critical role in osteoclast maturation and activity. Receptor activator of the NF-κB ligand (RANKL), a cell surface protein that was mainly expressed by osteocytes and osteoblasts in bone [Yasuda et al., 1998], bound to its specific receptor (RANK), an autonomous protein on the membrane of osteoclasts [Li et al., 2000], to activate several important osteoclastogenesis-related signaling pathways. Osteoprotegerin (OPG), which was mainly secreted by osteoblasts, was a soluble "decoy receptor" for RANKL that prevented the maturation of osteoclasts through blocking the activity of the RANKL/RANK signaling pathway [Lacey et al., 1998]. Any alteration in the ratio of OPG/RANKL could lead to either excessive osteogenesis or excessive bone resorption. Thus, the balance of OPG/RANKL influenced bone remodeling rate [Steeve et al., 2004; Theoleyre et al., 2004].

PEMFs might potentially exert their inhibitory effects on the modulation of osteoclastogenesis via the OPG/RANK/RANKL signaling pathway (Tables 3 and 4). As was verified by Chang et al. [2004b], PEMF exposure could significantly increase the ratio of OPG/RANKL in murine osteoblast-like cells at mRNA levels. Meanwhile, accumulating evidence showed that inflammatory cytokines exerted their regulatory effects on osteoclastogenesis and bone resorption by simulating the production of RANKL [Steeve et al., 2004]. It has been proven that PEMFs could not only significantly increase OPG, but could also reduce the activation of IL-1β-induced NF-kB p65 subunit through activating anti-inflammatory effects of ARs, as described above [Vincenzi et al., 2013]. Also, 8 Hz and 3.8 mT PEMFs might prevent ovariectomy-induced bone loss through suppressing the expression of RANKL while improving that of OPG in lumbar, femur, and tibia, respectively [Zhou et al., 2013, 2017]. Furthermore, a study in vitro [He et al., 2015] found that PEMFs inhibited the expression of carbonic anhydrase II (CA II) that regulated the resorptive activity of osteoclasts and nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), which acted as a key transcription factor in OPG/RANK/RANKL signaling and RANK during the induction of osteoclast-like cells. However, it was suggested by some evidence based on negative results that RANK/RANKL signaling might play a minor role in the regulation of bone remodeling. As was indicated by Chen et al. [2010], though PEMFs inhibited the expression of CA II, they had no effect on the expression of RANK in osteoclast-like cells deriving from ovariectomized rats. It was reported in studies both in vivo and in vitro [Jing et al., 2013, 2014] that no alteration in the mRNA expression of RANK/RANKL could be detected by the treatment of PEMFs.

DISCUSSION

Clinical Application of PEMFs

Taken together, our systematic review demonstrates that the clinical application of PEMFs, which have no side effects such as hormonology impairment [Huifang et al., 2013], may become one major preferred option of physical therapy for patients with PMOP. At first, the clinical studies reviewed in this paper indicate that PEMFs within an effective window can prevent bone loss and may reduce BTMs in PMOP. As one of the many potential therapeutic interventions to treat PMOP, the effects of PEMFs on the improvement of physical function are promising. Compared with the positive efficacy of Alendronate, a first-line treatment for PMOP, results from our research group [Liu et al., 2013] implied that PEMFs with specific parameters (8 Hz, 3.82 mT) showed similar effectiveness within 24 weeks. Secondly, the inhibitory effects of PEMFs on the deterioration of bone strength and high bone turnover are further supported by experimental studies reviewed in this paper. The results indicate that PEMFs may promote the quality of bone gain and subsequently reduce risk of post-osteoporosis fracture.

Although extensive research, especially in recent decades, has improved our understanding of the physiological effects of PEMFs on PMOP, controversy among these findings still exist. The beneficial effects of PEMFs with varying exposure parameters on PMOP remain questionable. Though positive effects can be observed during treatment period (1–3 months), long-term follow-up (1–8 years) data suggest no further positive effects on BMD. Different exposure times (3 min–24 h per day for up to 12 weeks in the course of treatment) in animal and cellular studies also have resulted in various kinds of positive or minor effectiveness on osteoporosis. Also, PEMF exposure at varying intensities and frequencies (frequency range: 0.2–75 Hz and intensity field range: 0.1 mT–1 T) may have inconsistent bio-effects on osteoblasts at different differentiation stages. Moreover, PEMFs with non-sinusoidal signal shapes that consist of monopolar or bipolar rectangular magnetic pulses with periodic peaks are used for PMOP treatment [Hug and Roosli, 2012]. But evidence for comparability between specific signal shapes is lacking. Thus, initiation and optimal dosage of PEMF treatment for patients with PMOP are still uncertain because of varying parameters' window effects.

In addition, although potential adverse effects after long-term application of PEMFs have not been found, two studies indicated that the use of PEMFs may be carcinogenic [Kheifets et al., 2010] and is not recommended for patients with cardiac devices [Gwechenberger et al., 2006]. Interestingly, for treatment purposes, PEMFs at specific parameters (frequency range: 50,120 Hz and intensity field range: 2.5–20 mT) have been proven to be effective in retardation of cancer growth [Cameron et al., 2007, 2014; Tatarov et al., 2011]. Thus, contraindications from long-term PEMF exposure should be addressed in future research.

Trends in Elucidating Therapeutic Mechanism

The possible mechanism for the response of PMOP to PEMF treatment indicates that the enhancement of osteoblast-derived bone formation is associated with promotion of osteoblast formation. Also, upregulation of osteoblastic genes induced through several signaling pathways (such as Wnt signaling) may have a major role. Meanwhile, PEMFs may suppress osteoclast-derived bone resorption by inhibiting maturation of osteoclasts together with molecular signaling pathways relevant to osteoclastogenesis, such as NF-κB signaling, requiring further elucidation.

The relationship between low BMD and high fracture risk is a continuum in PMOP. Despite a net growth of bone density with the mechanism of dual regulation in bone turnover accounting for most of the positive effects of PEMFs on PMOP, the fracture risk-benefit ratio for PEMFs within 10 years remains obscure. The therapeutic agents recommended by the FDA all contribute to a 10-year reduction of fracture risk at different levels [Camacho et al., 2016]. One high-quality randomized controlled trial further demonstrated that rational sequential use of anabolic therapy and antiresorptive agents (e.g., teriparatide and denosumab) resulted in a larger decrease in fracture risk than either one alone and a corresponding increase in BMD during a 4-year follow-up [Leder et al., 2015]. However, the efficacy of combined use of PEMFs with other agents has not been investigated. Therefore, it is imperative to further investigate whether PEMF therapy would solely reduce fracture risk in population with PMOP within 10 years or if the combination would do better.

Moreover, the biological mechanism of PEMFs in treating PMOP has not been perfectly proven. Based on our current understandings, multiple initiators and intracellular signaling pathways may be activated by PEMFs; however, pathway is the most important one and remains unclear. In addition, apart from regulating the balance of bone metabolism, these pathways also play important roles in maintaining homeostasis of other tissues such as the brain and intestines via regulating the evolution and development of normal or cancer stem cell proliferation, morphology, and life-cycle [Suzuki et al., 2004]. The activation of Wnt signaling has been proven to be involved in the development of several kinds of carcinomas such as liver and colorectal cancers [Lustig et al., 2002; Takahashi-Yanaga and Kahn, 2010]. The process of autophagy, a natural cell-repair mechanism regulated by mTOR signaling [Kaushik and Cuervo, 2006], also plays a key role in promoting tumors [Guo et al., 2013]. Nonetheless, it is unknown if the activation of Wnt signaling by PEMFs is associated with carcinogenesis. There are no reports regarding whether autophagy-mediated bone repair is a potential mechanism of PEMFs. Both issues need further investigation.

CONCLUSION

Due to current knowledge of PEMFs and their promising role in treating PMOP, their use can only be recommended on the basis of more reliable evidence derived from ongoing high-quality, randomized controlled trials with the addition of larger sample sizes, adverse events, longer term follow-up period, and physical therapy or osteoporosis-related efficacy indicators. Given the gaps between physiological mechanism and heterogeneous therapeutic effects of PEMFs, basic research involving advanced molecular tools such as gene-knockout mice and better-defined exposure conditions are needed. Only after that, the application of PEMFs for PMOP treatment may be recognized as safe and efficient.

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