Advancements in physical therapy for osteoporosis treatment

Lingli Zhang[1#], Yuan Zhang[1#], Ruixi Chi[2,3], Rui Ji[2], Bo Hu[4*], and Bo Gao[2*]

ABSTRACT

Osteoporosis (OP) is a ubiquitous metabolic bone disease characterized by reduced bone mass and the deterioration of bone microarchitecture. One of its most serious complications, fractures, can induce substantial functional disabilities in patients and are associated with chronic health issues, thereby imposing both medical and economic burdens. At present, the predominant therapeutic approaches for OP include pharmacotherapy and physical therapy (PT). While pharmacotherapy has proven effective, it is not without its drawbacks, such as prolonged treatment durations and adverse effects due to medication. PT, also referred to as physiotherapy, stands out as the most cost-effective alternative treatment for OP. PT involves the application of natural or artificial physical agents, such as sound, light, cold, heat, electricity, and mechanical forces (including motion and pressure), to non-invasively and non-pharmacologically treat local or systemic dysfunctions or pathologies. Its objective is to restore the body's inherent physiological functions. PT offers a diverse array of treatment options for patients with OP who are unsuitable for surgery or for whom surgical intervention is not viable. This review investigates the feasibility of identifying appropriate PT methods tailored to the needs of individuals with OP, with the intent of providing a scientific foundation for improved clinical practice.

Keywords:
Osteoporosis; Physical therapy; Physiotherapy; Mechanisms

#Authors contributed equally.

***Corresponding authors:**
Bo Gao,
gaobo1@fmmu.edu.cn;
Bo Hu,
bhu15@smmu.edu.cn

How to cite this article:
Zhang L, Zhang Y, Chi R, Ji R, Hu B, Gao B. Advancements in physical therapy for osteoporosis treatment. *Biomater Transl.* **2025**

doi: 10.12336/bmt.25.00007

1. Introduction

Osteoporosis (OP) is a multifactorial progressive bone disease characterized by decreased bone mass and deterioration of bone microarchitecture, resulting in increased bone fragility and increased risk of fracture (chronic metabolic bone disease). OP has gradually become an invisible killer affecting human health due to its high incidence, high fracture rate, high disability rate, and high mortality rate. Among them, OP in post-menopausal women and senile OP are the most common and significant forms of bone loss in clinical practice.[1]

Physical therapy (PT), also known as physiotherapy, is a non-invasive, non-pharmacological treatment of local or systemic dysfunctions or lesions of the human body by applying natural or artificial physical factors to the human body, including sound, light, cold, heat, electricity, and force (e.g., motion and pressure), to restore the original physiological functions of the body. PT can increase calcium uptake and absorption, improve bone density, directly or indirectly induce vascularization, enhance intramembranous ossification, promote cartilage ossification, facilitate the coupling of osteogenesis and vascularization, enhance bone formation and bone repair, and alleviate the painful symptoms of OP.[2] Compared to other treatments, the most significant advantage of PT is that patients undergo treatment without experiencing trauma or adverse effects, and it is relatively low-cost. It is a treatment for OP with better application prospects.

This paper focuses on the effects of PT on OP, provides insight into the regulatory mechanisms of sound, light, electricity, magnetism, and heat in OP, and presents an outlook on its clinical translation to explore the feasibility of preventing and treating OP.

In the preparation of this review, we conducted searches using appropriate databases including

PubMed, Web of Science, Scopus, and Cochrane Library, employing a range of search terms, such as "osteoporosis," "physical factor therapy," "traditional treatment methods," "fractures," and "mechanisms," along with their synonyms and related terms to broaden the scope of our search. We utilized various combinations of keywords to expand the search parameters and identified additional studies by evaluating reference lists of eligible studies and relevant review articles. The exclusion criteria included studies that did not provide sufficient data, had ambiguous or irrelevant results, were poorly designed, or had significant biases.

2. Epidemiology of OP

OP is a global health issue. A systematic review synthesizing 86 studies across five continents estimated the global prevalence of OP to be approximately 18.3%.[3] The prevalence of OP in Africa is significantly higher than in other countries. In terms of gender differences, the global prevalence of OP in women is 23.1% (95% confidence interval: 19.8 – 26.9%), while in men it is 11.7% (95% confidence interval: 9.6 – 14.1%). In seven middle- and high-income economies in the Asia-Pacific region, epidemiological estimates from specific locations indicate that approximately 5 – 10% of adults are affected by OP. Among individuals aged 50 and above, the prevalence is higher – typically ranging 20 – 40%, and in some cases even higher – with women disproportionately affected.[4] Comparative assessments of spinal or hip OP prevalence among populations aged 50 and above across several industrialized countries reveal substantial variation: Japan exhibits the highest prevalence at 26.3%, while Australia reports the lowest, at just 2%.[5] OP itself does not lead to disability or death. However, fractures, as severe complications of OP, not only cause significant functional impairment and long-term health issues, such as chronic pain and reduced physical activity capability, but are also directly associated with increased mortality rates. Hip fractures, a major health concern, have an accumulated mortality rate of 20 – 40% within 1 year after occurrence, with higher mortality rates in men than women.[6] The mortality rate after hip fractures varies with age and gender. Statistics show that for women, the mortality rate is 2% before the age of 80, and 8% at age 80. For men, the mortality rate is 2.9% for ages 55 – 59, 8% for ages 80 – 84, and 15% after age 94. In addition, the risk of death significantly increases after vertebral fractures, severe osteoporotic fractures, and minor osteoporotic fractures in patients aged 75 or older.[7]

OP typically requires surgical treatment, with significant variations in treatment costs across different regions. Associated expenses include hospitalization and management of complications. In addition to direct medical costs, community care, elderly care facility expenses, and loss of household productivity must also be considered. There are substantial differences in care models across various regions; developing countries rely on family care, while Western countries tend toward institutional care.[8] It is estimated that the direct care costs in the 1st year after a fracture amount to approximately $30,000 per hip fracture patient, $11,300 for other non-vertebral fractures, and $8,380 for vertebral fractures. In 2003, the total management cost of low-trauma fractures in the United States (US) reached $17 billion, projected to increase to over $25 billion by 2025. In the same year, those costs in Europe amounted to €3.6 billion. The average hospital stay for vertebral fractures is 617 days, followed by 512 days of rehabilitation or nursing home care; hip fractures result in an average hospital stay of 1,306 days and 1,650 days of rehabilitation/nursing home care. In comparison, non-hip, non-spine fractures have a longer hospital stay, averaging 3,805 days, with an average rehabilitation/nursing home care time of 5,186 days.[9] OP and its related fractures impose a significant health and economic burden worldwide.

3. Pathogenesis of OP

Bone is a dynamic organ that is constantly remodeling. Bone remodeling involves two distinct processes: osteoblasts forming new bone and osteoclasts removing old or damaged bone. Differentiating from mesenchymal stem cells (MSCs), osteoblasts are responsible for the synthesis of type I collagen and the deposition of mineralized matrix to promote bone formation.[10] Meanwhile, osteoclasts are specialized bone-resorbing cells formed from the fusion of monocyte precursor cells, and their formation is related to the secretion of macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL) by osteoblasts and osteoclasts.[11] Osteoclasts secrete large quantities of protons into the sealed resorption cavity through H^+ pumps in the presence of folded edges, leading to an acidified microenvironment of bone matrix lysis. In addition, osteoclasts contain a large number of lysosomal enzymes (e.g., histone C, β-glycerophosphate, and β-glucuronidase), which help to degrade the bone organic matrix exposed in the resorption lacunae to promote bone resorption.[11,12] Consequently, the balance of bone remodeling homeostasis is disrupted, with a reduction in osteoblasts, a decrease in bone-like tissue formation and calcium and phosphorus accumulation, a reduction in the ratio of bone minerals to organic matter, sparse and narrow bone trabeculae, a decrease in bone strength, and structural damage and fragility of the bones, ultimately leading to OP.[13]

The main factors contributing to the development of OP include general factors associated with the natural process of aging and bone tissue resorption activated by a sex hormone deficiency, as well as a reduction in osteogenesis and microstructural disorders due to various external factors, such as glucocorticoid administration.[14] OP often occurs in the elderly, predominantly in post-menopausal women.

[1]Department of Sports Science Teaching, School of Athletic Performance, Shanghai University of Sport, Shanghai, China; [2]Department of Orthopedic Surgery, Xijing Hospital, Air Force Medical University, Xi'an, Shaanxi, China; [3]Basic Medical Sciences Academy, Air Force Medical University, Xi'an, Shaanxi, China; [4]Section of Spine Surgery, Department of Orthopedics, Changzheng Hospital, Naval Medical University, Shanghai, China

Age-related OP is called primary OP and is mainly due to a negative balance between bone resorption and bone formation caused by aging, resulting in bone loss. In post-menopausal women, due to decreased estrogen secretion and increased release of pro-inflammatory cytokines, the immune system continues to be hypoactivated,[15] with reduced bone mass and increased risk of fracture (**Figure 1**).

3.1. Oxidative stress and OP

Reactive oxygen species (ROS), or oxygen-free radicals, are highly reactive molecules containing oxygen. ROS is an unavoidable by-product of cellular oxygen metabolism and ATP formation, and when over-accumulated, the intracellular redox equilibrium is disrupted, which can cause oxidative stress. Oxidative stress is one of the critical factors in aging and the cause of many neurological, cardiovascular, and metabolic diseases. It is thought to be a disturbance in the balance between free radical formation and antioxidant mechanisms, leading to the development of various diseases.[16,17] ROS production is a crucial regulator of osteoblast function in bone tissue, and oxidative status influences the homeostasis of bone mineralization.[18]

High levels of ROS negatively affect osteogenesis. Oxidative stress induces apoptosis in osteoblasts and osteoclasts, leading to an imbalance in the remodeling process, followed by altered and deficient bone formation with aging, glucocorticoid treatment, OP, and other skeletal diseases associated with oxidative stress.[14,19] Oxidative stress inhibits osteoblast differentiation through the endoplasmic reticulum kinase (ERK)-dependent nuclear factor kappa-B (NF-κB) signaling pathway.[20] Osteoblasts can produce antioxidants, such as glutathione peroxidase, to prevent ROS production[21] and reduce the level of transforming growth factor-beta (TGF-β), involved in bone resorption.[22] In addition, the growth and maintenance of bone require MSCs for osteogenic differentiation, and ROS can induce senescence and apoptosis of MSCs and inhibit the proliferation and differentiation of bone marrow MSCs (BMSCs) through multiple signaling channels. Geissler *et al.*[23] found that BMSCs cultured *in vitro* for an extended period exhibited reduced antioxidant capacity and elevated ROS levels, resulting in reduced or loss of osteogenic differentiation potential. Yang *et al.*[24] found that H_2O_2-induced oxidative stress inhibited osteogenic differentiation of BMSCs through the autophagy pathways, affected Wnt/β-catenin signaling activity, thus inhibiting the osteogenic differentiation of BMSCs. It degraded the cellular self-renewal ability and hindered the osteogenic differentiation of BMSCs while promoting lipogenic differentiation, resulting in increased bone fat and decreased bone mass.[25]

On the other hand, the impact of ROS on osteoclasts differs from its direct effects on osteoblasts. Instead, ROS stimulates

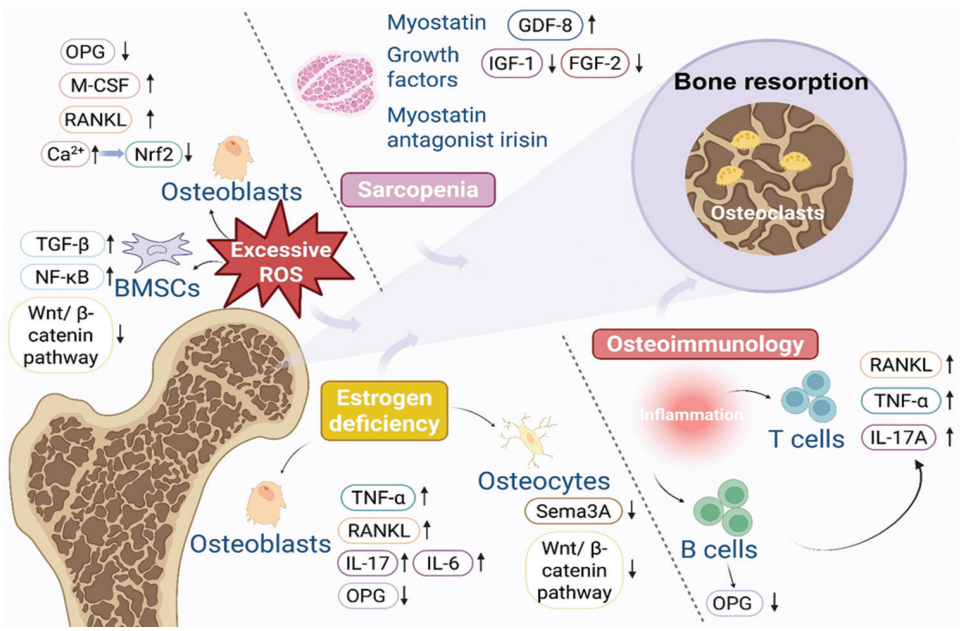

Figure 1. Osteoporosis is associated with oxidative stress, estrogen deficiency, sarcopenia, and bone immunity. Excessive reactive oxygen species reduce the levels of antioxidants, glutathione peroxidase, erythrocyte superoxide dismutase, and erythrocyte nitric oxide through the nuclear factor kappa-B signaling pathway, and promote osteoclast differentiation and bone resorption by stimulating macrophage colony-stimulating factor and receptor activator of NF-κB ligand (RANKL). Estrogen deficiency, on the one hand, reduced semaphorin 3A (sema3A) expression in bone cells, on the other hand, promoted the production of bone resorption factors, such as tumor necrosis factor-alpha (TNF-α), interleukin 17 (IL-17), IL-6, and RANKL, resulting in the imbalance of bone homeostasis. Muscle loss promotes bone resorption by upregulating myostatin (growth/differentiation factor 8), myostatin antagonist irisin, IL-7, and downregulating growth factors (insulin-like growth factor 1 and basic fibroblast growth factor 2). Activated T cells and B cells secrete RANKL, IL-17, IL-10, interferon-gamma, TNF-α, etc. in inflammatory states, stimulating osteoclasts to absorb bone and leading to bone loss. Image created by the authors using BioRender.com. Ruixi, C. (2025) https://BioRender.com/axai6w5.

Abbreviations: BMSC: Bone marrow mesenchymal stem cell; Nrf2: Nuclear factor erythroid 2-related factor 2; OPG: Osteoprotegerin; TGF-β: Transforming growth factor-beta.

bone-forming cells to produce important regulatory factors, such as osteoclastogenesis inhibitory factor (OPG), M-CSF, and RANKL.[26] These factors recognize osteoclast pre-cursor cells and transmit bone resorption signals to osteoclasts, thereby indirectly affecting the differentiation, survival, and activation of osteoclasts. Osteoclasts produce superoxide for bone resorption, and oxidative stress increases osteoclast differentiation and function.[27]

Bai *et al.*[28] used H_2O_2 to co-culture with human-derived bone marrow mononuclear cells. They found that increased intracellular ROS levels from superoxide anion produced by H_2O_2 stimulated *RANKL* mRNA and protein expression in the human osteoblast cell line MG63 and in primary mouse bone marrow stromal cells and cranial osteoblasts to promote osteoblast differentiation and increase osteoclast activity and bone resorption capacity. Similarly, Gong *et al.*[29] also found that H_2O_2 could promote the expression of cytokines related to osteoclast differentiation. In addition, high levels of ROS can mediate the production of RANKL by osteoblasts and BMSCs, indirectly promoting osteoclast differentiation. In a study of post-menopausal women, Jagger *et al.*[30] found that there is a negative correlation between peripheral markers of oxidative stress and antioxidant status and that low levels of antioxidants are responsible for increased bone resorption. Ozgocmen *et al.*[31] compared serum antioxidant enzyme and nitric oxide (NO) levels, oxidative stress markers, between post-menopausal OP women and healthy women. They found that the activity of erythrocyte catalase was significantly reduced in post-menopausal OP women, and erythrocyte superoxide dismutase activity and erythrocyte NO levels were significantly higher in the plasma of OP women. In contrast, glutathione peroxidase activity and NO levels were similar in both groups. Erythrocyte superoxide dismutase, erythrocyte catalase activity, and erythrocyte NO levels were significantly correlated with proximal femur bone mineral density (BMD). Some quality-of-life scores, such as pain, mental, and social functioning, correlated with antioxidant enzyme activity and NO levels. Moreover, there is a significant increase in the levels of malondialdehyde, a final product of lipid peroxidation, in both plasma and erythrocytes of OP patients, suggesting a linear relationship between OP and reduced antioxidant capacity in post-menopausal women.

3.2. Effect of estrogen deficiency on bone reconstruction

Bone remodeling is a process in which bone formation and bone resorption are tightly coupled, maintaining skeletal homeostasis and metabolic balance. Osteoblasts produce new bone, while osteoclasts remove old or damaged bone. This process is known as bone remodeling and is carried out through the basic multicellular unit.[32] The function of osteoblasts and osteoclasts is mainly regulated by systemic hormones and local factors in the body, including sex hormones.[32]

Estrogens (estradiol, estriol, and estrone) are essential hormones that regulate the metabolism and function of bone and skeletal muscle, directly or indirectly, through the estrogen receptor.[33] Estrogens play an essential role in bone remodeling by promoting anti-apoptosis in osteoblasts and osteoclasts as well as pro-apoptosis in osteoclasts, decreasing the rate of activation of bone remodeling, and maintaining the balance between bone conversion.[34-37] Post-menopausal estrogen deficiency increases apoptosis of osteoblasts while decreasing apoptosis and enhancing recruitment of osteoclasts. This imbalance prolongs bone resorption, reduces bone formation, and results in bone resorption outpacing bone formation, thus disrupting the normal bone remodeling process.[38] In addition to affecting osteoblasts and osteoclasts, osteocytes, the most abundant cells in bone, respond to estrogen by producing the signaling protein semaphorin 3A (sema3A). Estrogen increases sema3A bone expression, and sema3A binds to its receptor on osteoblasts to increase cell viability and maintain bone homeostasis;[39] autocrine loop damage caused by estrogen deficiency induces an OP phenotype. Estrogen also regulates osteoblast mechanotransduction by affecting Wnt/β-catenin signalling.[40] Jackson *et al.*[41] found that osteoblasts from ovariectomized mice were significantly attenuated in their ability to activate the Wnt/β-catenin signaling pathway in response to mechanical loading and that ovariectomy-induced loss of estrogen attenuated *in vivo* loading-induced β-catenin signaling in osteoblasts. In particular, a lack of the hormone serum estradiol induced increased apoptosis of osteoblasts in mice and altered the oxidative microenvironment, leading to the loss of osteoblast resistance to oxidative stress.

In addition, estrogen has been implicated in signaling the onset of inflammation and inhibits the production of several pro-inflammatory cytokines.[42] Tumor necrosis factor-alpha (TNF-α), a pro-inflammatory cytokine produced mainly by macrophages and monocytes, promotes osteoclastogenesis and bone resorption. Estrogen deficiency in menopausal women is a critical step in ROS-mediated TNF-α expression.[43] Wu *et al.*[40] found that estrogen deficiency induced the production of TNF-α and interleukin-17 (IL-17) in pro-inflammatory cells by switching memory T cells to effector memory T cells. In ovariectomized mice, estrogen deficiency decreases thiol antioxidant defenses in osteoblasts, which increases the level of ROS and induces the expression of TNF-α. TNF-α indirectly induces osteoclastogenesis while simultaneously inhibiting the release of OPG from osteoblasts, ultimately leading to bone loss.[30] Grassi *et al.*[44] observed in ovariectomized mice that the accumulation of ROS in the bone marrow activates T cells, which in turn promote osteoclastogenesis. This occurs through the upregulation of co-stimulatory molecule cluster of differentiation 80 on dendritic cells, leading to increased production of bone resorption factors, such as TNF-α, IL-17, IL-6, and RANKL.

3.3. Sarcopenia and OP

Sarcopenia is a syndrome of reduced muscle mass and strength associated with aging. The cause of the disease is related to, among other things, long-term reduced activity or impaired mobility and malnutrition.[45] The development of sarcopenia is strongly associated with an increase in body fat. Obesity and sarcopenia reinforce each other and act synergistically to cause physical impairment and metabolic disorders.[46] Obesity is often characterized by a significant increase in body mass, leading to the excessive production of inflammatory response

factors that promote osteoclast differentiation and increase bone resorption. These inflammatory factors also affect the osteogenic differentiation of MSCs, thereby reducing bone formation. For example, in obese patients, TNF-α activates NF-κB by increasing the levels of RANK and RANKL, thereby promoting osteoclastogenesis and contributing to bone loss.

Muscle loading (transmitted from muscle to bone through external forces or internal muscle contraction) is a significant factor in maintaining bone density.[47] Existing studies have shown that a certain amount of muscle load can be transmitted either by deformation (stretching and compression), which is dominant in muscle, or by shear forces exerted by fluids, which respond strongly to the flow of fluids in the tubular network of osteocytes. Cells and tissues respond to these changes by adjusting to mechanical stimuli through the strength and stiffness of the cytoskeleton and extracellular matrix. This process maintains and enhances the structure and number of bone trabeculae, improving the quality of muscle and bone.[48] Therefore, a decline in muscle function and performance increases the risk of both sarcopenia and OP, and is associated with a reduction in BMD.[49]

In addition, skeletal muscle has an endocrine function.[50] Muscle can influence skeletal remodeling through the secretion of various myokines, including myostatin (growth/differentiation factor 8), insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor **2** (FGF-2), and irisin.[50] Elkasrawy *et al.*[51] found that IGF-1 has an important osteogenic role. They also observed that traumatic muscle injury and muscle wasting increase the secretion of skeletal muscle growth inhibitors, which impair cartilage formation and hinder bone healing.

3.4. Bone immunization

Bone immunity is the centralized effect of cells and cytokines between the bone and the immune system.[52] The bone-immune system, which includes all the cells in the bone marrow, emphasizes the bi-directional reaction between the bone and the immune system. It is the crucial link between the immune system and the skeletal system, with the critical immune components acting in the various processes of bone metabolism.[53]

In this system, T cells, B cells, and cytokines are important regulators of bone resorption. Under different circumstances, immune cells may show different responses to the skeletal system.[54] For example, under physiological conditions, B cells may secrete OPG to counteract osteoclast production, inhibiting bone resorption and promoting osteogenesis.[55] Li *et al.*[56] found that B cells are an essential source of OPG, with OPG produced by the B cell lineage accounting for 64% of total OPG in the bone marrow, 45% of which originates from mature B cells. In contrast, mice with a knockout of B cells showed impaired OPG production, decreased BMD, and significantly reduced bone mass. When bone metabolism is disrupted, activated T cells and B cells in an inflammatory state secrete pro-osteoclastogenic factors, such as RANKL, IL-17A, and TNF-α, which stimulate osteoclast activity and promote bone resorption,[57] ultimately leading to bone loss. T-cell-deficient nude mice showed enhanced osteoclast differentiation and reduced bone density due to an immune imbalance of T cells that promotes osteoclast differentiation and bone resorption.[58]

4. PT for OP

Over the past two decades, pharmacological and non-pharmacological treatment options have been extensively developed to improve BMD and reduce the risk of fracture in patients with OP. In general, all available pharmacological treatments for OP reduce the relative risk of new fractures by approximately 50 – 65%.[59] However, in addition to the high cost of treatment, adverse events, such as dyspeptic conditions (nausea, vomiting, and abdominal pain), as well as metabolic and thromboembolic disturbances, are frequently observed in patients receiving pharmacological treatment for OP. These potential clinical limitations, side effects, and adverse reactions limit the adherence to the treatment.[60] In contrast, non-pharmacological treatments usually involve physical exercise and physiotherapy rehabilitation specifically designed for patients with OP. Studies have found that PT techniques are the most cost-effective alternative treatment for OP[61] (**Figure 2**).

4.1. Acoustic therapy: Pulsed ultrasound

Low-intensity pulsed ultrasound (LIPUS) is a high-frequency sound wave that applies micro-stress to bone and surrounding tissues to promote fracture healing and slow down the tendency of bone loss (**Figure 3**). Based on numerous trials, LIPUS was successfully approved by the US Food and Drug Administration (FDA) for the treatment of new fractures and muscle adhesions.[62]

Intensity and frequency are the two basic parameters of LIPUS, which are the key factors affecting bone cells and mechanical stress. Most studies used fixed ultrasound parameters in fracture healing, that is, an intensity of 30 mW/cm^2 (space- and time-averaged intensity), a frequency of 1.5 MHz, a duty cycle of 20%, and a pulse repetition frequency of 1 kHz. LIPUS's intensity strongly correlates with BMD and bone microstructure.[63] Among the LIPUS signals of 15 – 150 mW/cm^2, the intensity of 150 mW/cm^2 was more effective in maintaining bone volume than that of 30 mW/cm^2, which is commonly used in fracture healing, suggesting that ultrasound intensity is positively correlated with therapeutic effects within a specific range.[64] Sun *et al.*[64] demonstrated that LIPUS can be used as a mechanical load to promote muscle function and stimulate bone mass and microarchitecture. Higher-intensity LIPUS was more effective in maintaining bone mass in ovariectomized rats. Meanwhile, Inoue *et al.*[65] found that higher-intensity ultrasound (0.5 and 1.0 W/cm^2) increased cell proliferation and osteoblast activity at the healing site, accelerating fracture healing. However, LIPUS intensities lower than those currently used in clinical practice (30 mW/cm^2) had a positive effect on osteogenic differentiation of bone marrow stromal cells in rats, and LIPUS of 15 mW/cm^2 also had a significant effect on enhancing alkaline phosphatase (ALP) activity and bone mineralization.[66]

The most common application of LIPUS in bone is fracture repair.[67] Growth and differentiation of MSCs play an essential

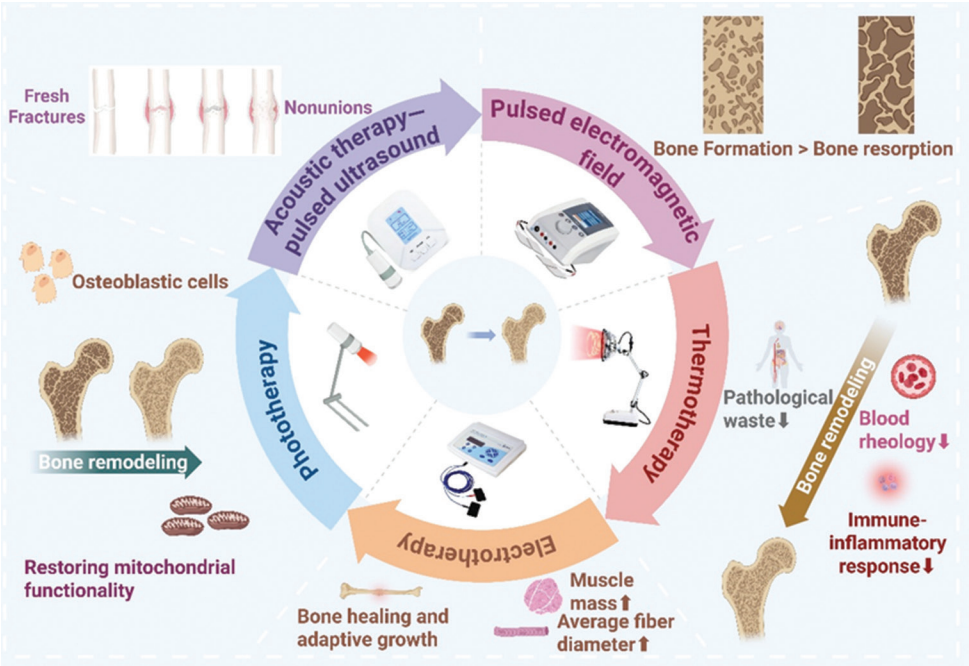

Figure 2. Types of physical therapy for osteoporosis. Image created by the authors using BioRender.com. Ruixi, C. (2025) https://BioRender.com/h0pydi1.

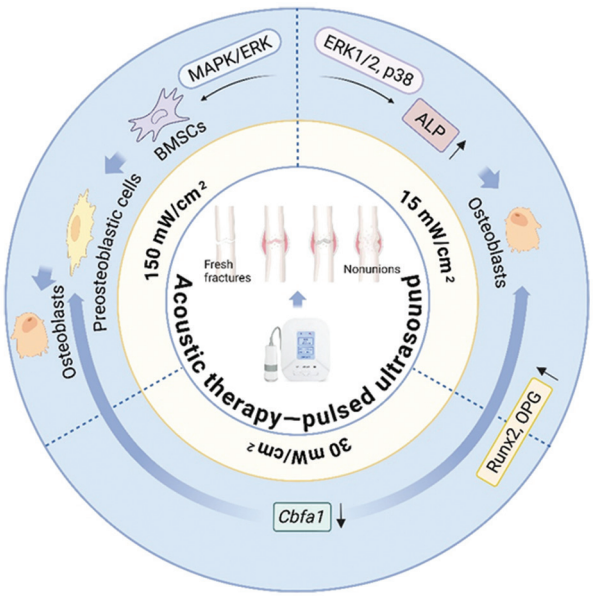

Figure 3. Mechanisms of acoustic therapy in the treatment of osteoporosis. The intensity of 150 mW/cm^2 promotes muscle function, stimulates bone mass and microstructure; 30 mW/cm^2 decreases *Cbfa1* mRNA expression, increases collagen deposition and OPG-positive area, and accelerates osteoblast development. Bone-specific *Cbfa1* gene is a transcription factor crucial for osteoblast differentiation and bone formation. Its main function is to inhibit the differentiation of preosteoblasts into mature osteoblasts. The intensity of 15mW/cm^2 regulates endoplasmic reticulum kinases (ERK1/2) and p38 intracellular signaling pathways, increases alkaline phosphatase (ALP) activity, and enhances bone tissue mineralization. Image created by the authors using BioRender.com. Ruixi, C. (2025) https://BioRender.com/pul0.
Abbreviations: BMSC: Bone marrow mesenchymal stem cell; MAPK: Mitogen-activated protein kinase; OPG: Osteoprotegerin; Runx2: Runt-related transcription factor 2.

role in the repair phase of fracture healing. However, a large proportion of fractured bone healing is delayed, sometimes leading to non-union. In contrast, the mechanical microenvironment of trabecular bone and osteoblasts can be significantly improved by LIPUS irradiation, alleviating the decline of muscle function and bone loss due to the lack of mechanical stimulation.[68] In an animal fracture repair model, LIPUS effectively restored fracture healing in rats with spinal cord injury (SCI) by promoting endochondral ossification, increasing collagen deposition and OPG-positive area, decreasing the area of bone resorption, increasing the density of newly-formed tissues, improving the microstructure, and restoring fracture healing tissues.[69] The bone-specific *Cbfa1* gene is a transcription factor essential for osteoblast differentiation and bone formation, and its primary function is to inhibit the differentiation of pre-osteoblasts to mature osteoblasts.[70,71] Wu *et al.*[72] observed a decline in *Cbfa1* mRNA levels following LIPUS treatment at 3- and 5-week post-irradiation, indicating an accelerated differentiation of osteoblasts. This downregulation of *Cbfa1* expression was associated with enhanced mineralization on the surfaces of the proximal femoral bone matrix and cancellous bone, thereby strengthening the trabecular bone structure. In clinical trials, Zura *et al.*[73] demonstrated that delayed use (90 – 365 days after fracture) of LIPUS was associated with fracture non-union. The fracture healing rate was 96% in elderly patients over 60 who used LIPUS within 90 days of fracture. Moreover, the early use of LIPUS can alleviate sequelae, such as post-fracture pain, to a certain extent.

However, present LIPUS treatments only present an osteogenic effect on healing new bone, with no significant effect on osteogenesis of the distal end of the intact radius.[74] The differences in the physical properties of LIPUS, bone

mineral status, and acoustic and biological properties of bone tissue may explain it. It has been suggested that intact bone may have a significant (25 – 40%) attenuation effect on ultrasound transmission and that LIPUS with a spatially averaged, temporally averaged intensity of 30 mW/cm^2 may be attenuated progressively in the soft tissues and posterior distal radius, especially in cortical bone.[75] However, there are several limitations to the present clinical study, including the sample size, the follow-up time, and the fact that the distal radius site subjected to ultrasound treatment is susceptible. Thus, the potential local osteogenic effect of LIPUS treatment remains to be investigated further.

4.2. Phototherapy

The application of artificial light or daylight radiation to treat disease is known as photobiomodulation therapy (PBMT), which uses wavelengths of 180 nm – 1,000 μm and includes infrared, blue-violet, ultraviolet, and laser therapies. PBMT has been used as a non-invasive alternative, and most studies have demonstrated a beneficial effect on bone formation[76] (**Figure 4**).

PBMT, including low-level laser therapy (LLLT), induces new bone formation. LLLT is a widely applied form of phototherapy. Stein *et al.*[77] demonstrated that the increase in bone formation in the low-level laser irradiation group may be due to the activation of osteoblast activity. Laser therapy improves bone healing by accelerating the development of newly formed bone, activating osteogenic factors, such as Runt-related transcription factor 2 (Runx2) and bone morphogenetic protein 9 (BMP-9), in tibial defects,[78] and increasing the expression of osteocalcin.[79] Low-intensity pulsed laser (LIPL) is a specific type of LLLT, referring specifically to pulsed laser light therapy. Xu *et al.*[80] found that LIPL (650 nm, 2 mW) irradiated at 1.14 J/cm^2 or 2.28 J/cm^2 for 5 min or 10 min, respectively, significantly promoted the proliferation and differentiation of cranial osteoblasts cultured *in vitro*. As a model of osteoblast differentiation *in vitro*, LIPL irradiation at 1.14 J/cm^2 significantly downregulated the expression of *RANKL* mRNA in cranial osteoblasts and upregulated the expression of *OPG* mRNA. It directly promoted osteoblast proliferation and differentiation while indirectly inhibiting osteoclast differentiation by downregulating the *RANKL*/*OPG* ratio. In another study, Pinheiro *et al.*[81] found that four different laser irradiation modes significantly stimulated rat cranial osteoblast proliferation, enhanced bone nodule formation, and upregulated ALP expression. They also observed that low-frequency (8 Hz) pulsed irradiation significantly promoted bone formation *in vitro*. Meanwhile, Kanenari *et al.*[82] suggested that the stimulation of bone formation by LLLT may be based on the *MAP1A* gene, which promotes microtubule assembly and its functional expression. Microtubules play essential roles in cell division, cell morphology and polarity, cell motility, intracellular transport, signal transduction and synthesis, and collagen secretion. Therefore, LLLT irradiation enhanced *MAP1A* expression and regulated microtubule assembly and microtubule functional structure, promoting osteoblast proliferation and differentiation.

OP is partly caused by reduced levels of BMSCs and the preferential differentiation of BMSCs into adipocytes rather

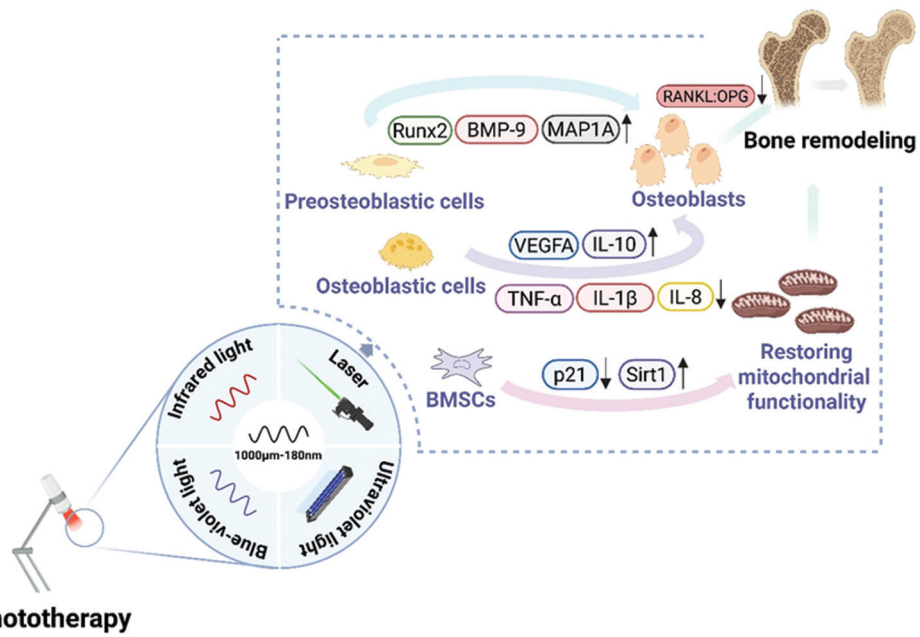

Figure 4. Mechanisms of phototherapy in the treatment of osteoporosis. Laser therapy improves bone healing by activating Runt-related transcription factor 2 and bone morphogenetic protein 9, increasing the expression of osteocalcin and microtubule-associated protein 1A, regulating microtubule assembly and microtubule functional structure, and promoting osteoblast proliferation and differentiation. Consecutive appropriate phototherapy treatments downregulate the senescence marker p21 and upregulate the longevity marker sirtuin 1. Image created by the authors using BioRender.com. Ruixi, C. (2025) https://BioRender.com/m8wdx65.
Abbreviations: BMSC: Bone marrow mesenchymal stem cell; IL-10: Interleukin 10; OPG: Osteoprotegerin; RANKL: Receptor activator of nuclear factor kappa-B ligand; TNF-α: Tumor necrosis factor-alpha; VEGFA: Vascular endothelial growth factor A.

than osteoblasts in aging bones. Thus, age-related declines in the number and function of MSCs may lead to reduced bone formation and impaired bone microarchitecture. Anti-inflammatory effects and differentiation of MSCs toward osteogenesis can be directed by PBMT.[83] Vascular endothelial growth factor (VEGF) is a well-recognized angiogenic factor essential for bone remodeling and repair. Peat *et al.*[84] maintained the viability of equine MSCs after irradiating them with 1064 nm light with an energy density of 9.77 J/cm^2 and a mean output power of 13.0 W for 10 s. The light induced a modest increase in VEGF levels and the release of the anti-inflammatory factor IL-10 during the first 24 h of treatment. In addition, through phototherapy intervention in young and old MSCs, Eroglu *et al.*[85] found that after 3.0 J/cm, 808 nm wavelength treatment, consecutive appropriate PBMT treatments downregulated the senescence marker p21 and upregulated the longevity marker sirtuin (SIRT1) in elderly mice. Moreover, two to three consecutive treatments significantly improved the mitochondrial function and cellular proliferation in both young and aged mice, as well as reversed the signs of aging in aged mice. PBMT also induced actin reorganization, cytoskeletal modification, and morphological changes, as well as increased *Runx2* and *Osx* transcription levels.[86] Furthermore, PBMT could induce bone-directed differentiation of MSCs, inhibit pro-inflammatory molecules, and attenuate local cellular damage. In their study, Yamaura *et al.*[87] added TNF-α to synoviocytes isolated from patients with rheumatoid arthritis and applied PBMT before or after the addition. They found that 25 J/cm^2 PBMT significantly reduced the mRNA and protein levels of TNF-α, IL-1β, and IL-8.

In addition, laser therapy promotes bone healing by stimulating the formation of new bone at the site of injury, improving the biological response of bone tissue. Laser irradiation regulates cellular biochemical reactions, activates mitochondrial respiration, and promotes the consumption of molecular oxygen and the synthesis of ATP within cells.[88] Applying LLLT to de-ovulated rats resulted in a significant increase in serum calcium and inorganic phosphorus levels, as well as a significant decrease in ALP and deoxypyridinoline/creatinine levels. LLLT also increased calcium deposition, ALP levels, osteoclast number, and dense bone thickness in the exposed bone, while decreasing the number of osteoclasts.[89] Zhu *et al.*[83] found that LLLT effectively improved OP in aged rats by increasing BMD, serum bone alkaline phosphatase (BALP), osteocalcin levels, and osteoclasts in the bone marrow, decreasing calcium and phosphorus loss, and improving bone structure and biomechanical properties.

4.3. Electrotherapy

The application of electric current to treat disease is called electrotherapy. It is divided into three main categories: low frequency, medium frequency, and high frequency, depending on the frequency of the current used (**Figure 5**). Electrical stimulation has been used for bone repair for 30 years and approved by the US FDA for prosthetic joints and osteoarthritis.[90]

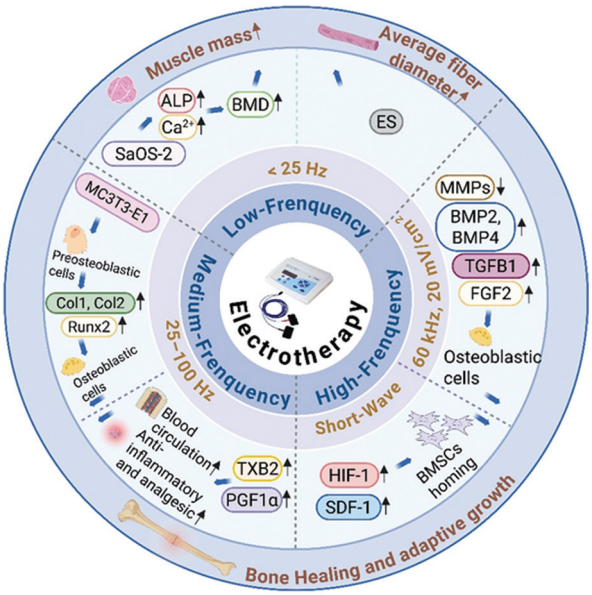

Figure 5. Mechanisms of electrotherapy in the treatment of osteoporosis. Low-frequency electrical stimulation promotes bone regeneration by enhancing alkaline phosphatase (ALP) activity in the human osteosarcoma SaOS-2 cell line, while percutaneous electrical stimulation helps to increase muscle mass and bone mineral density (BMD). The mRNA levels of ossification-related genes, such as Col1, Col2, and Runx2, are upregulated by medium-frequency electrical stimulation to stimulate the osteogenic differentiation of MC3T3-E1 cells. High-frequency electrical stimulation significantly upregulates the mRNA levels of bone morphogenetic protein (BMP)2, BMP4, transforming growth factor-beta 1, and fibroblast growth factor 2; in contrast, it downregulates the matrix metalloproteinases in osteoarthritis cartilage to promote fracture healing. Image created by the authors using BioRender.com. Ruixi, C. (2025) https://BioRender.com/ou283on.
Abbreviations: BMSC: Bone marrow mesenchymal stem cell; ES: Electrical stimulation; HIF-1: Hypoxia-inducible factor-1; PGF1α: Prostaglandin F1α; SDF-1: Stromal cell-derived factor-1α; TXB2: Thromboxane.

4.3.1. Low-frequency electrotherapy

Low-frequency electricity (low-frequency stimulation [LFS]) treats diseases with low-frequency pulsed current with a frequency <25 Hz. It is widely recognized and applied clinically because of its low current, weak electrolysis, high safety, and non-invasiveness. Relatively low-frequency mechanical or electrical stimulation can affect bone formation and resorption *in vitro* and *in vivo*. It is used clinically to inhibit or reverse bone loss.[91]

Low-frequency electrical stimulation promotes bone formation. Maintaining muscle mass is an important factor in maintaining bone mass and bone strength. The larger the muscle volume, the stronger the contraction force, providing a greater pulling stimulus to the bones, thereby increasing bone strength and enhancing the effect of bone remodeling. Muscle contractility induced by low-frequency electrical stimulation attenuates denervation-induced muscle loss and trabecular bone loss, as well as delays muscle atrophy and the deterioration of the mechanical properties of the mid-tibial diaphysis during the early stages in aged rats.[92] In a mouse model of OP caused by

sciatic nerve denervation, Parfitt *et al.*[93] found that muscle fiber cross-sectional area and muscle strength were significantly improved after electrical stimulation intervention, together with increased bone volume fraction and trabecular bone thickness. LFS-induced muscle contraction may promote the production of cytokines; leading to mechanical load on the skeleton through the tendon–bone interface.[94] Mechanical load is one of the main factors affecting bone remodeling. The osteosarcoma cell line SaOS-2 is derived from malignant bone tumors, and it retains some osteogenic differentiation potential (e.g., it can express ALP and mineralized matrix).[95] Bisceglia *et al.*[96] demonstrated that low-frequency electric fields increase the human osteosarcoma cell lines SaOS-2 and liver HepG2 to low-frequency electric fields from a device used in clinical therapy, which increased ALP activity. Similarly, Caputo *et al.*[97] stimulated the human osteosarcoma cell line SaOS-2 with low-frequency electrical stimulation for 4 h and showed a significant increase in ALP activity. Although the osteosarcoma cell line SaOS-2 exhibited increased ALP activity after stimulation, its differentiation process was abnormally regulated, necessitating further investigation to explore if LFS can affect bone formation through this cell line.

Transcutaneous electrical stimulation is commonly used in patients undergoing physical rehabilitation to maintain and restore mass and strength in denervated muscles. Clinical reports have found that direct application of electrical stimulation to denervated muscles in patients with SCI increases muscle mass and mean fiber diameter.[98,99] Experimental animals further support the idea that electrical stimulation can help limit muscle atrophy and improve muscle strength.[100-102] Swift *et al.*[103] and Allen *et al.*[104] found that mechanical stimulation of early wasting osteoporotic mice increased muscle mass and BMD. The extent to which LFS affects bone structure through altering muscle contractile activity depends on the severity of the disease. Duchenne muscular dystrophy is a severe muscular dystrophy caused by mutations or deletions in the dystrophin gene, predisposing patients to skeletal fragility. Chan *et al.*[105] stimulated two types of dystrophic mice by LFS at 10 Hz for 12 h/day for 28 days and found that LFS resulted in thinning of cortical bone and reduction of tibial diaphysis cross-section in myotonic dystrophin-deficient and trophic factor-deficient mice, whereas bone mass was maintained in the intervened healthy mice but with a reduced proportion of high-density bone, an increased amount of low-density bone, and a reduction in bone strength. Thus, muscle activity modulates bone mass, and LFS affects the distribution of BMD in persons with amyotrophic protein deficiency. The extent of the effect depends on the severity of the disease in the organism. On the other hand, 1 – 10 Hz improves calcium deposition in the cellular matrix, which helps to treat fractures and bone non-unions. Hronik-Tupaj *et al.*[106] demonstrated that electrical stimulation (20 mV/cm, 60 kHz) improved the potential for osteogenic differentiation of human MSCs based on calcium deposition due to a twofold increase in the expression of ALP and type 1 collagen.

4.3.2. Medium-frequency electrotherapy

Intermediate-frequency electrotherapy is a method of treating diseases by applying alternating current with a frequency of 25 – 100 Hz. This widely used frequency has moderate physical characteristics, precise curative effects, and minor adverse reactions.

Dynamic electrical stimulation can partially inhibit bone loss and the deterioration of trabecular structure caused by a lack of daily weight-bearing activity. However, the effectiveness of dynamic stimulation depends mainly on the stimulation frequency. Lam and Qin[107] and Qin *et al.*[108] found a significant improvement in the number and structure of bones in tail-suspended mice by applying muscle stimulation at medium-frequencies (50 Hz and 147 Hz). Specifically, the dynamic stimulation at 50 Hz had the most significant preventive effect on bone, resulting in an increase in bone volume fraction and trabecular number, a reduction in trabecular separation, and effectively preventing disuse bone loss. Belanger *et al.*[109] also demonstrated that medium-frequency electrical stimulation reversed bone loss, and after 24 weeks of 25 Hz electrical stimulation, the BMD of the distal femur and proximal tibia had recovered nearly 30% of the bone loss, along with an increase in muscle strength. SCI patients exhibit increased osteoclast activity, suppressed osteoblast activity, and a substantial reduction in lower extremity bone mass.[110] However, treatment with functional electrical stimulation (30 min/day, 3 days/week, for 12 months) reversed the loss of bone mass in the proximal tibia. Exercising 3 times/week was sufficient to sustain this increase.[111] Retrospective studies have concluded that early initiation of medium-frequency electrical stimulation after SCI is beneficial for increasing the number of trabeculae in the distal femur and proximal tibia, as well as enhancing BMD.[112]

Electromagnetic fields (EMFs) stimulate osteoblast and osteoclast activities at the fractured portion of the bone and promote new bone formation by inducing the proliferation of osteoblasts and collagen, suggesting a potential role for electromagnetic stimulation in treating non-healing fractures.[113] Osteoporotic fracture is a common disease.[114] Electrical stimulation is often used as a method of bone repair in clinical trials. Medium-frequency sinusoidal currents positively affect bone healing after osteotomy by increasing bone tissue temperature and calcification activity, as well as decreasing inflammation. The superimposition of 90 Hz and 100 Hz medium-frequency sinusoidal currents *in vivo* accelerates the formation of bone scabs.[115] In the study of Wang *et al.*,[116] ALP activity was examined to promote osteogenic differentiation of MC3T3-E1 cells under different frequencies of electrical stimulation (200 mV/cm, 1 – 100 kHz, 30 min). They found that during the stimulation process, 100 Hz electrical stimulation upregulates the mRNA levels of bone formation-related genes, such as type I collagen, type II collagen, and *RUNX2*. Overall, the functional exertion of EMFs is frequency-dependent, with medium-frequency electrical stimulations at 50 Hz, 90 Hz, and 100 Hz having the potential to modulate bone healing and adaptive growth. In addition, OP causes generalized pain, such as spinal pain, joint pain, and tibial pain.[117] Medium-frequency electrical stimulation combined with electroacupuncture improves local blood circulation. This has anti-inflammatory and analgesic effects. Its mechanisms may be related to the

regulation of the homeostasis between plasma thromboxane B2 and prostacyclin F1-alpha to improve microcirculation,[118] as well as the analgesic effect associated with electrical stimulation. Moreover, compared with the analgesic effect of electrical stimulation, medium-frequency alternating current is more readily accepted and tolerated by patients, making it more valuable for clinical research.[119]

4.3.3. High-frequency electrotherapy

The application of high-frequency electrical currents to the human body for the treatment of disease is known as high-frequency electrotherapy, with short-wave therapy being the most common approach.

Short-wave therapy promotes the migration of MSCs to the injured tissue, thus accelerating the fracture healing process.[120,121] Hypoxia-inducible factor-1 (HIF-1α), a critical transcriptional regulator in osteoblasts, is associated with MSCs migration and differentiation.[122] It can regulate the expression of various cytokines, such as stromal cell-derived factor 1 (SDF-1).[123,124] Short-wave therapy promotes the migration of MSCs, increases local and serum levels of HIF-1 and SDF-1, induces changes in the formation of healing tissues, and improves the microstructure and mechanical properties of healing tissues, thus accelerating the healing process of the fracture site.[125] In addition, fracture healing is associated with increased calcium phosphate mineral salt deposition, usually occurring 2 – 4 weeks after injury. It has been found that high-frequency short-wave irradiation promotes bone healing tissue formation and migration of MSCs at 1 – 3 weeks post-injury.[126] Midura *et al.*[127] demonstrated that high-frequency electrical stimulation delayed bone loss in the tibia of hindlimb-suspended rats, enhanced the mechanical properties of the tibial diaphysis, increased tibial bone density, and showed significantly greater mineral-binding fluorescent dye biomarkers in the osteoclastic luminal and tubular volumes. Similarly, Kostyshyn *et al.*[128] also demonstrated that non-physiological, low-intensity high-frequency electrical stimulation inhibited the side effects of glucocorticoid deficiency on bone structure and metabolism in rats. In addition, whole-body mechanical oscillation resulted in a 30% increase in mineral mass preservation, increased levels of bone remodeling markers osteopontin and tartrat resistant acid phosphatase 5b, and an increase in bone mass. It was shown that electrical stimulation at low intensity and frequencies higher than 1 kHz induced cell differentiation.[129,130] However, high-intensity electrical stimulation above 100 V/cm induced cell membrane electroporation and apoptosis, as well as increased the levels of intracellular Ca^{2+} and ROS.[131] Therefore, when high-intensity stimulation is unavoidable, pulsed electrical stimulation should be used, while continuous high-intensity stimulation should be avoided to minimize potential tissue damage.

Several studies have demonstrated that electrical stimulation preserves or enhances bone mass due to transduction in bone through mechanoreceptors, which open stretch-activated ion channels in osteoblasts. This allows calcium and other ions to enter the cell, triggering a variety of chemical signaling cascades that upregulate several osteogenic factors.[132] Clark *et al.*[132] exposed human cranial osteoblasts to a capacitively coupled electric field with a capacitance of 60 kHz, 20 mV/cm for 2 h and found that mRNA expression of several TGF-β family genes (e.g., *BMP2*, *BMP4*, *TGFB1*, *TGFB2*, and *TGFB3*), as well as *FGF2*, was significantly upregulated. Serum osteocalcin (BGP) and ALP protein levels were also elevated. All the genes studied are closely related to osteogenesis and, therefore, also closely related to fracture healing. Wang *et al.*[133] determined that an optimal capacitive coupling signal (60 kHz, 20 mV/cm, 50% duty cycle for 24 h) specifically and selectively upregulated the expression of multiple osteoinductive BMPs, with mRNA levels of *BMP2*, *BMP4*, *BMP5*, *BMP6*, and *BMP7* increasing several-fold higher than those of the BMP antagonists gremlin and noggin. It has also been shown that the levels of matrix metalloproteinases (MMPs) are elevated in human patients with non-healing fractures. Capacitive coupling has been shown to downregulate MMPs in osteoarthritic cartilage.[134] Therefore, this non-invasive treatment modality may work by upregulating the expression of anabolic proteins and downregulating the expression of catabolic proteins to promote fracture healing.

4.4. Magnetic therapy

A pulsed EMF (PEMF) is a low-frequency magnetic field with a specific amplitude and waveform characterized by a steady change in the amplitude of the field over time.[135] Its primary function is to convert electric current into a magnetic field, which can activate the bioelectric current of living organisms to achieve therapeutic purposes (**Figure 6**). In 1978, Martin first found that PEMF has a therapeutic effect on OP.[136]

Pulsed EMF promotes bone formation by regulating bone's anabolic and catabolic activities, upregulating the expression of genes related to osteogenesis, and downregulating osteoclast-related genes that affect bone resorption.[137] Adenosine A_{2A} receptor is one of the four known adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3). Activation of adenosine A_{2A} receptors exerts a potent anti-inflammatory effect by decreasing the production of pro-inflammatory cytokines (e.g., IL-6, IL-1β, and TNF-α) and promoting the expression of anti-inflammatory cytokines (e.g., IL-10). PEMF shows agonist activity on adenosine A_{2A} and A_3 receptors. In human osteoarthritic fibroblasts, PEMF increases the activation of these receptors and reduces the expression of inflammatory mediators (e.g., prostaglandin E2, IL-6, and IL-8).[138] Lei *et al.*[139] found that serum parathyroid hormone and cyclic adenosine monophosphate (cAMP) levels in mice in the hindlimb suspension (HLS) combined with the PEMF (HLS + PEMF) group remained significantly higher than those of HLS alone. PEMF can maintain bone formation through soluble adenylyl cyclase/cAMP/protein kinase A/cAMP response element-binding protein signaling pathway, thereby reducing bone loss in HLS rats due to a weightless environment. Other studies have also demonstrated that PEMF, by promoting OPG levels[140] and inhibiting IL-6 expression and TGF-β1 secretion, partially affects bone remodeling processes[141,142] and regulates the gene expression of osterix (*OSX*), *OPG*, *TRAP*, and cathepsin K.[143] Therefore, PEMF demonstrates the potential to treat post-menopausal OP by regulating the expression of these genes.

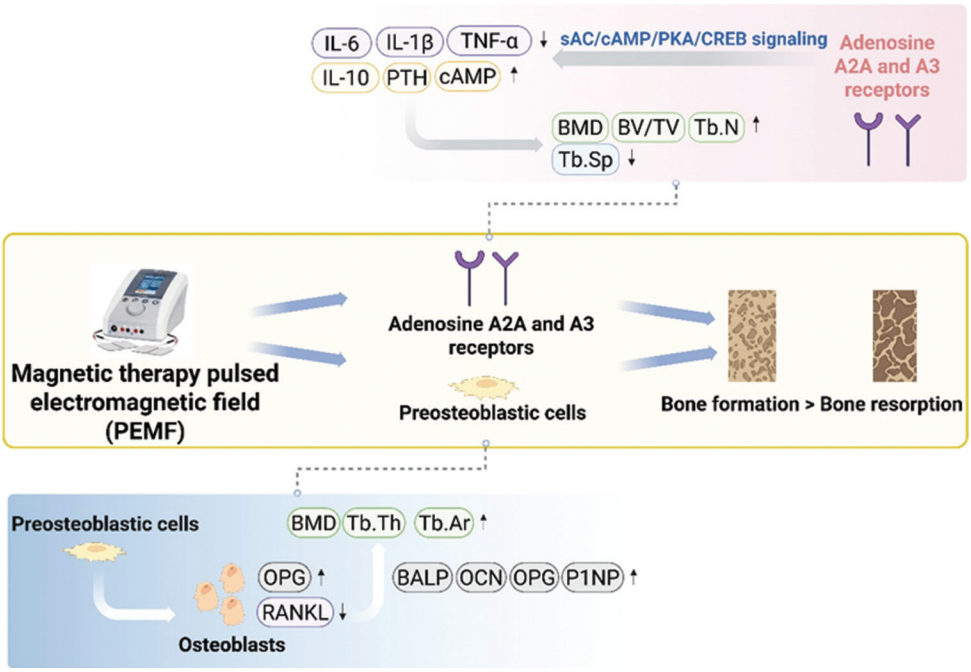

Figure 6. Mechanisms of magnetic therapy in the treatment of osteoporosis. Pulsed electromagnetic fields (PEMFs) can maintain bone formation by activating the soluble adenylyl cyclase/cyclic adenosine monophosphate (cAMP)/protein kinase A/cAMP response element binding protein signaling pathway and adenosine A_{2A} and A_3 receptors. They also reduce inflammatory mediator levels, such as interleukin 6 (IL-6), IL-1β, and tumor necrosis factor-alpha, while increasing the levels of IL-10, parathyroid hormone, and cAMP, thereby improving cancellous and cortical bone microstructures. Additionally, PEMFs increase osteoclastogenesis inhibitory factor (OPG) level and downregulate receptor activator of nuclear factor kappa-B ligand level in osteoblasts; at the same time, upregulate bone alkaline phosphatase, osteocalcin, OPG, and procollagen type 1 overall expression levels. Consequently, these changes reduce osteoclast activation, resulting in greater bone formation than bone resorption. Image created by the authors using BioRender.com. Ruixi, C. (2025) https://BioRender.com/d9r1jo7. Abbreviations: BMD: Bone mineral density; BV/TV: Bone volume fraction; Tb.Ar: Bone trabecular area; Tb.N: Trabecular number; Tb.Sp: Trabecular separation; Tb.Th: Trabecular thickness.

Furthermore, PEMF has been shown to increase BMD in OP patients and prevent bone loss in ovariectomized rats. The most cited mechanism underlying this effect is that PEMF reduces bone loss through the typical Wnt/β-catenin signaling pathway. Typical Wnt signaling is a crucial regulator for bone modeling and remodeling, bone mass, and bone homeostasis.[144,145] Activation of this signaling indirectly inhibits bone resorption and promotes osteoblastogenesis and bone formation.[146] Zhou *et al.*[147] found that PEMF, after 40 min/day, 5 days/week for 12 weeks, significantly inhibited the ovariectomy (OVX)-induced decrease in bone biomechanical properties. Moreover, PEMF increased serum estradiol and bone alkaline phosphatase (BALP) levels, maintained bone mass, inhibited deterioration of bone trabecular microarchitecture and strength, and activated Wnt/β-catenin signaling in OVX rats. The activation of the Wnt/β-catenin signaling pathway promoted the proliferation and differentiation of osteoblast pre-cursor cells and increased osteoclast activity, facilitating the deposition of new bone and increasing BMD.[148] In leptin receptor-deficient mice with type 2 diabetes mellitus, micro-computed tomography assessment revealed a significant improvement in both cancellous and cortical bone microstructure. Furthermore, real-time polymeric chain reaction results showed that PEMF upregulated the tibial gene expression of osteogenesis-related proteins, indicating that activation of Wnt/β-catenin signaling increases bone formation.[149]

In their study, Cai *et al.*[150] reported that femoral *OPG*, *BMP2*, and *RUNX2* mRNA expression levels were increased in diabetic rabbits treated with PEMF and that PEMF prevented and alleviated OP through the RANK/RANKL/OPG system.[139] The RANK/RANKL/OPG signaling pathway is crucial for osteoclastogenesis and osteoblast activity.[151] Osteoblasts express OPG, a "decoy receptor" molecule, and RANKL binds to RANK, which plays an essential role in osteoclast formation, activation, and survival.[152,153] OPG inhibits the RANK/RANKL pathway through competitive binding to RANKL.[154] PEMF can modulate OPG and RANKL expression, thus potentially regulating osteoclast activation and subsequent bone resorption.[142]

4.5. Thermotherapy

Thermotherapy is a therapeutic technique that uses physical energy to raise and maintain tissue temperature, either locally or systemically, at a therapeutic level for a certain period to achieve clinical benefits. In recent years, thermotherapy has been widely used in orthopedic disease treatment, including hot compresses, fumigation, and wax therapy.[155] Studies have shown that moderate thermotherapy (40 – 450r can improve the local blood rheology and hemodynamic properties, accelerate the removal of pathological waste, inhibit the cellular- and humoral-mediated immune-inflammatory response, and

effectively improve chronic pain and other symptoms[156] (**Figure** 7).

Traditional thermotherapy uses physical thermal effects to improve blood circulation and pathological conditions in the affected area. However, traditional single-modality thermotherapy often faces challenges, such as complicated operation, ineffective heat control, and low heat conduction efficiency. Compared to traditional thermotherapy, magnetic thermotherapy offers unique advantages, including high safety, deep tissue penetration, and precise localized treatment of lesions – benefits not typically achievable with traditional thermotherapy methods. Magnetic thermotherapy has been utilized in clinical research related to tumors in the US and some European countries in the past few years. It has also been widely applied in several biomedical fields, including tissue engineering, neural regulation, and immunotherapy.[157] For example, Yu *et al.*[158] developed a magnetic bone repair hydrogel that can drive magnetic hyperthermia, which can undergo liquid–solid phase transitions. The liquid magnetic bone repair hydrogel can effectively fill bone defects and accelerate reconstruction by releasing magnesium ions and enhancing osteogenic differentiation, thereby promoting the regeneration of bone defects.[158]

4.6. Benefits of PT for OP

PT has unique advantages and roles in the management of OP. It promotes bone growth and reconstruction, enhances muscle strength and balance, relieves pain, improves psychological status, and elevates the quality of life. Compared to other treatments, PT differs in its mechanism of action, focus, side effects, and targeted population. The advantages of PT in the management of OP are manifold and can ameliorate the adverse effects of OP at multiple levels. First, PT can improve bone density by stimulating osteoblast activity, promoting bone formation, and inhibiting bone resorption through a variety of mechanisms. For example, LIPUS can promote the proliferation and differentiation of osteoblasts to increase bone density.[63] Second, PT can enhance muscle strength. OP patients often suffer from muscle atrophy and decreased strength, which can increase the risk of falls and fractures. Exercise therapies in PT, such as resistance training and balance training, can enhance muscle strength, increase muscle mass, and improve stability and balance. LFS promotes a significant increase in muscle fiber cross-sectional area and muscle strength in osteoporotic mice.[94] In addition, OP causes generalized pain, such as spinal and joint pain, including tibial pain.[117] Medium-frequency electrical stimulation combined with electroacupuncture can improve local blood circulation with anti-inflammatory and analgesic effects.[118]

Innovative aspects of PT in OP management are reflected in the following three points. First, the application of new equipment and techniques. With the continuous progress of science and technology, an increasing number of new PT equipment and techniques are being introduced for the treatment of OP. For example, low-intensity PEMF therapy can stimulate osteoblast activity and promote bone growth and repair by generating EMFs of specific frequencies and intensities.[137] Second, the development of personalized treatment plans. Each OP patient presents distinct clinical conditions and physical status, making it necessary to develop personalized PT plans. A comprehensive assessment, encompassing bone density, muscle strength, balance, age, gender, and lifestyle habits, enables the formulation of a tailored PT program. Personalized treatment enhances the pertinence and effectiveness of therapy while reducing the risk of complications. Third, the development of a multidisciplinary integrated treatment model. Effective management of OP requires collaboration across multiple disciplines. Combining PT with pharmacological treatment,

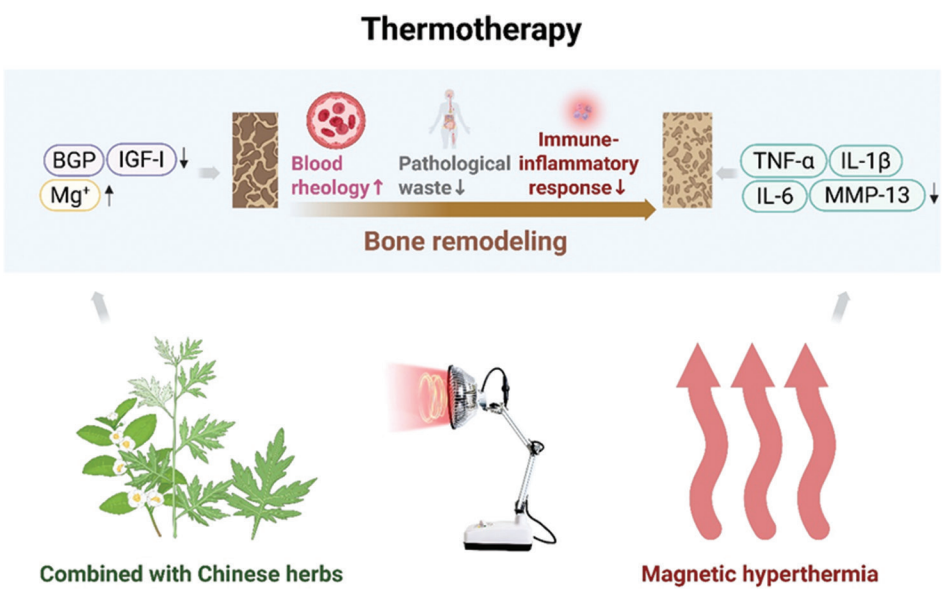

Figure 7. Mechanisms of thermotherapy in the treatment of osteoporosis. Thermotherapy combined with Chinese herbal medicine reduces the levels of immune cytokines, such as serum osteocalcin (BGP) and insulin-like growth factor 1. Magnetic heat treatment reduces the levels of pro-inflammatory cytokines, such as tumor necrosis factor-alpha, interleukin 1beta (IL-1β), IL-6, and matrix metalloproteinases-13, protecting cartilage joints. Image created by the authors using BioRender.com. Ruixi, C. (2025) https://BioRender.com/szps7fu.

nutritional intervention, and psychological therapy can produce synergistic effects and enhance treatment outcomes. For example, PT can enhance the drug absorption and promote the nutrient metabolism, while psychological therapy can alleviate anxiety and depression, thereby increasing patient adherence to treatment.

The potential applications of PT in OP management are extensive. With the development of artificial intelligence, the combination of smart rehabilitation devices and PT will play an important role in the rehabilitation of OP. This innovative therapy can monitor the patients' movement data and physiological indicators in real time, automatically adjust the treatment parameters according to the patients' condition, and provide personalized rehabilitation guidance. At present, PT is mainly used in the treatment of OP. In the future, however, PT holds great potential for the prevention of OP. Across early interventions in high-risk populations, such as appropriate exercise training and PT, bone loss can be delayed, and the incidence of OP can be significantly reduced.

5. Limitations

Due to the limitations of existing studies, this paper has several deficiencies. Although it highlights the potential of PT to improve OP symptoms by promoting bone growth and reducing inflammatory mediators, it does not deeply explore the specific molecular mechanisms underlying each physical modality, largely because many existing studies focus primarily on clinical effects rather than mechanistic pathways. In addition, due to individual differences and the influence of other regulatory factors, it is difficult to propose precise treatment protocols. As a result, personalized treatment plans are not addressed in this paper.

6. Conclusions

PT serves as a non-invasive treatment modality for OP, employing physical stimuli, such as acoustic waves, light, electrotherapy, magnetotherapy, and thermotherapy, to directly target the skeletal system. It promotes bone formation, inhibits bone resorption, alleviates pain, and ameliorates bone microarchitecture, demonstrating significant therapeutic efficacy in enhancing BMD, reducing the production of inflammatory mediators, and accelerating fracture healing. Despite the potential exhibited by PT, further research is required to refine treatment parameters and assess its long-term efficacy and safety. Future animal studies will further substantiate the positive mechanisms of PT in the treatment of OP, including exploring the synergistic effects of PT in conjunction with other therapeutic approaches, thereby providing greater value to clinical research endeavors.

Acknowledgement
None.
Financial support
The work was supported by the National Natural Science Foundation of China (82222046, 82172475), the Key Industry Innovation Chain Project of Key R&D Program in Shaanxi Province (2024SF-ZDCYL-04-03), and Shanghai Key Laboratory of Human Performance (Shanghai University of Sport) (11DZ2261100).

Conflicts of interest statement
All authors declare that there are no conflicts of interest associated with the publication of this manuscript, with the institutions or products mentioned in the text, or with commercial interests that compete with the products described in the text.
Author contributions
Conceptualization: ZL, YZ, RJ, and BH; *Writing – original draft:* RC and BG; *Writing – editing & review*: BG. All authors read and approved the final version of the manuscript.
Ethics approval and consent to participate
Not applicable.
Consent for publication
Not applicable.
Availability of data
Not applicable.

References

1. Foger-Samwald U, Dovjak P, Azizi-Semrad U, Kerschan-Schindl K, Pietschmann P. Osteoporosis: Pathophysiology and therapeutic options. *EXCLI J.* 2020;19:1017-1037. doi: 10.17179/excli2020-2591
2. Whisner CM, Castillo LF. Prebiotics, bone and mineral metabolism. *Calcif Tissue Int.* 2018;102(4):443-479. doi: 10.1007/s00223-017-0339-3
3. Salari N, Ghasemi H, Mohammadi L, *et al.* The global prevalence of osteoporosis in the world: A comprehensive systematic review and meta-analysis. *J Orthop Surg Res.* 2021;16:609. doi: 10.1186/s13018-021-02772-0
4. Chandran M, Brind'Amour K, Fujiwara S, *et al.* Prevalence of osteoporosis and incidence of related fractures in developed economies in the Asia Pacific region: A systematic review. *Osteoporos Int.* 2023;34(6): 1037-1053. doi: 10.1007/s00198-022-06657-8
5. Wade S, Strader C, Fitzpatrick L, Anthony M, O'Malley C. Estimating prevalence of osteoporosis: Examples from industrialized countries. *Arch Osteoporos.* 2014;9:182. doi: 10.1007/s11657-014-0182-3.
6. Guzon-Illescas O, Perez Fernandez E, Crespí Villarias N, *et al.* Mortality after osteoporotic hip fracture: Incidence, trends, and associated factors. *J Orthop Surg Res.* 2019;14:203. doi: 10.1186/s13018-019-1226-6
7. Leboime A, Confavreux CB, Mehsen N, Paccou J, David C, Roux C. Osteoporosis and mortality. *Joint Bone Spine.* 2010;77 Suppl 2: S107-S112. doi: 10.1016/S1297-319X(10)70004-X
8. Tarrant SM, Balogh ZJ. The global burden of surgical management of osteoporotic fractures. *World J Surg.* 2020;44(4):1009-1019. doi: 10.1007/s00268-019-05237-y
9. Cauley JA. Public health impact of osteoporosis. *J Gerontol Ser Biomed Sci Med Sci.* 2013;68(10):1243-1251. doi: 10.1093/gerona/glt093
10. Han Y, You X, Xing W, Zhang Z, Zou W. Paracrine and endocrine actions of bone-the functions of secretory proteins from osteoblasts, osteocytes, and osteoclasts. *Bone Res.* 2018;6:16. doi: 10.1038/s41413-018-0019-6
11. Hakeda Y, Kumegawa M. Osteoclasts in bone metabolism. *Kaibogaku Zasshi.* 1991;66(4):215-225.
12. Vaananen HK, Hentunen T, Lakkakorpi P, Parvinen EK, Sundqvist K, Tuukkanen J. Mechanism of osteoclast mediated bone resorption. *Ann Chir Gynaecol.* 1988;77(5-6):193-196.
13. Zhivodernikov IV, Kirichenko TV, Markina YV, Postnov AY, Markin AM. Molecular and cellular mechanisms of osteoporosis. *Int J Mol Sci.* 2023;24(21):15772.

doi: 10.3390/ijms242115772
14. Liu J, Xu D, Liu L, *et al.* Regular sling core stabilization training improves bone density based on calcium and vitamin D supplementation. *BMC Musculoskelet Disord.* 2023;24(1):815. doi: 10.1186/s12891-023-06896-8
15. Yang F, Su Y, Liang J, *et al.* Casticin suppresses RANK-Linduced osteoclastogenesis and prevents ovariectomyinduced bone loss by regulating the AKT/ERK and NF-kappaB signaling pathways. *Int J Mol Med.* 2023;51(5):43. doi: 10.3892/ijmm.2023.5246
16. Zaric BL, Macvanin MT, Isenovic ER. Free radicals: Relationship to human diseases and potential therapeutic applications. *Int J Biochem Cell Biol.* 2023;154:106346. doi: 10.1016/j.biocel.2022.106346
17. Zhao F, Guo L, Wang X, Zhang Y. Correlation of oxidative stress-related biomarkers with postmenopausal osteoporosis: A systematic review and meta-analysis. *Arch Osteoporos.* 2021;16(1):4. doi: 10.1007/s11657-020-00854-w
18. Wauquier F, Leotoing L, Coxam V, Guicheux J, Wittrant Y. Oxidative stress in bone remodelling and disease. *Trends Mol Med.* 2009;15(10):468-477. doi: 10.1016/j.molmed.2009.08.004
19. Chen Y, Tang W, Li H, Lv J, Chang L, Chen S. Composite dietary antioxidant index negatively correlates with osteoporosis among middle-aged and older US populations. *Am J Transl Res.* 2023;15(2):1300-1308.
20. Bai XC, Lu D, Bai J, *et al.* Oxidative stress inhibits osteoblastic differentiation of bone cells by ERK and NF-κB. *Biochem Biophys Res Commun.* 2004;314(1):197-207. doi: 10.1016/j.bbrc.2003.12.073
21. Chen K, Qiu P, Yuan Y, *et al.* Pseurotin A inhibits osteoclastogenesis and prevents ovariectomized-induced bone loss by suppressing reactive oxygen species. *Theranostics.* 2019;9(6):1634-1650. doi: 10.7150/thno.30206
22. Zou ML, Chen ZH, Teng YY, *et al.* The smad dependent TGF-beta and BMP signaling pathway in bone remodeling and therapies. *Front Mol Biosci.* 2021;8:593310. doi: 10.3389/fmolb.2021.593310
23. Geissler S, Textor M, Kuhnisch J, *et al.* Functional comparison of chronological and *in vitro* aging: Differential role of the cytoskeleton and mitochondria in mesenchymal stromal cells. *PLoS One.* 2012;7(12):e52700. doi: 10.1371/journal.pone.0052700
24. Yang Y, Sun Y, Mao WW, Zhang H, Ni B, Jiang L. Oxidative stress induces downregulation of TP53INP2 and suppresses osteogenic differentiation of BMSCs during osteoporosis through the autophagy degradation pathway. *Free Radic Biol Med.* 2021;166:226-237. doi: 10.1016/j.freeradbiomed.2021.02.025
25. Atashi F, Modarressi A, Pepper MS. The role of reactive oxygen species in mesenchymal stem cell adipogenic and osteogenic differentiation: A review. *Stem Cells Dev.* 2015;24(10):1150-1163. doi: 10.1089/scd.2014.0484
26. Tao H, Ge G, Liang X, *et al.* ROS signaling cascades: Dual regulations for osteoclast and osteoblast. *Acta Biochim Biophys Sin (Shanghai).* 2020;52(10):1055-1062. doi: 10.1093/abbs/gmaa098
27. Agidigbi TS, Kim C. Reactive oxygen species in osteoclast differentiation and possible pharmaceutical targets of ROS-mediated osteoclast diseases. *Int J Mol Sci.* 2019;20(14):3576. doi: 10.3390/ijms20143576
28. Bai XC, Lu D, Liu AL, *et al.* Reactive oxygen species stimulates receptor activator of NF-kappaB ligand expression in osteoblast. *J Biol Chem.* 2005;280(17):17497-17506. doi: 10.1074/jbc.M409332200
29. Gong W, Liu M, Zhang Q, *et al.* Orcinol glucoside improves senile osteoporosis through attenuating oxidative stress and autophagy of osteoclast via activating Nrf2/Keap1 and mTOR signaling pathway. *Oxid Med Cell Longev.* 2022;2022:5410377. doi: 10.1155/2022/5410377
30. Jagger CJ, Lean JM, Davies JT, Chambers TJ. Tumor necrosis factor-alpha mediates osteopenia caused by depletion of antioxidants. *Endocrinology.* 2005;146(1):113-118. doi: 10.1210/en.2004-1058
31. Ozgocmen S, Kaya H, Fadillioglu E, Aydogan R, Yilmaz Z. Role of antioxidant systems, lipid peroxidation, and nitric oxide in postmenopausal osteoporosis. *Mol Cell Biochem.* 2007;295(1-2):45-52. doi: 10.1007/s11010-006-9270-z
32. Bolamperti S, Villa I, Rubinacci A. Bone remodeling: An operational process ensuring survival and bone mechanical competence. *Bone Res.* 2022;10(1):48. doi: 10.1038/s41413-022-00219-8
33. Lu L, Tian L. Postmenopausal osteoporosis coexisting with sarcopenia: The role and mechanisms of estrogen. *J Endocrinol.* 2023;259(1):e230116. doi: 10.1530/JOE-23-0116
34. Almeida M, Iyer S, Martin-Millan M, *et al.* Estrogen receptor-alpha signaling in osteoblast progenitors stimulates cortical bone accrual. *J Clin Invest.* 2013;123(1):394-404. doi: 10.1172/JCI65910
35. Manolagas SC. Birth and death of bone cells: Basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr Rev.* 2000;21(2):115-137. doi: 10.1210/edrv.21.2.0395
36. Manolagas SC. From estrogen-centric to aging and oxidative stress: A revised perspective of the pathogenesis of osteoporosis. *Endocr Rev.* 2010;31(3):266-300. doi: 10.1210/er.2009-0024
37. Riggs BL, Khosla S, Melton LJ 3rd. Sex steroids and the construction and conservation of the adult skeleton. *Endocr Rev.* 2002;23(3):279-302. doi: 10.1210/edrv.23.3.0465
38. Manolagas SC, O'Brien CA, Almeida M. The role of estrogen and androgen receptors in bone health and disease. *Nat Rev Endocrinol.* 2013;9(12):699-712. doi: 10.1038/nrendo.2013.179
39. Hayashi M, Nakashima T, Yoshimura N, Okamoto K, Tanaka S, Takayanagi H. Autoregulation of osteocyte Sema3A orchestrates estrogen action and counteracts bone aging. *Cell Metab.* 2019;29(3):627-637.e5. doi: 10.1016/j.cmet.2018.12.021
40. Wu D, Cline-Smith A, Shashkova E, Perla A, Katyal A, Aurora R. T-Cell mediated inflammation in postmenopausal osteoporosis. *Front Immunol.* 2021;12:687551. doi: 10.3389/fimmu.2021.687551
41. Jackson E, Lara-Castillo N, Akhter MP, *et al.* Osteocyte Wnt/beta-catenin pathway activation upon mechanical loading is altered in ovariectomized mice. *Bone Rep.* 2021;15:101129. doi: 10.1016/j.bonr.2021.101129
42. Vrachnis N, Zygouris D, Vrachnis D, *et al.* Effects of hormone therapy and flavonoids capable on reversal of menopausal immune senescence. *Nutrients.* 2021;13(7):2363. doi: 10.3390/nu13072363
43. Lean JM, Davies JT, Fuller K, *et al.* A crucial role for thiol antioxidants in estrogen-deficiency bone loss. *J Clin Invest.* 2003;112(6):915-923. doi: 10.1172/JCI18859
44. Grassi F, Tell G, Robbie-Ryan M, *et al.* Oxidative stress causes bone loss in estrogen-deficient mice through enhanced bone marrow dendritic cell activation. *Proc Natl Acad Sci U S A.* 2007;104(38):15087-15092. doi: 10.1073/pnas.0703610104
45. Tournadre A, Vial G, Capel F, Soubrier M, Boirie Y. Sarcopenia. *Joint Bone Spine.* 2019;86(3):309-314. doi: 10.1016/j.jbspin.2018.08.001
46. Santilli V, Bernetti A, Mangone M, Paoloni M. Clinical definition of sarcopenia. *Clin Cases Miner Bone Metab.* 2014;11(3):177-180.
47. Kirk B, Zanker J, Duque G. Osteosarcopenia: Epidemiology, diagnosis, and treatment-facts and numbers. *J Cachexia Sarcopenia Muscle.* 2020;11(3):609-618. doi: 10.1002/jcsm.12567
48. Nachury MV, Mick DU. Establishing and regulating the composition of cilia for signal transduction. *Nat Rev Mol Cell Biol.* 2019;20(7):389-405. doi: 10.1038/s41580-019-0116-4
49. Yang YJ, Kim DJ. An overview of the molecular mechanisms contributing to musculoskeletal disorders in chronic liver disease: Osteoporosis, sarcopenia, and osteoporotic sarcopenia. *Int J Mol Sci.* 2021;22(5):2604.

doi: 10.3390/ijms22052604
50. Hamrick MW. The skeletal muscle secretome: An emerging player in muscle-bone crosstalk. *Bonekey Rep.* 2012;1:60. doi: 10.1038/bonekey.2012.60
51. Elkasrawy M, Immel D, Wen X, Liu X, Liang LF, Hamrick MW. Immunolocalization of myostatin (GDF-8) following musculoskeletal injury and the effects of exogenous myostatin on muscle and bone healing. *J Histochem Cytochem.* 2012;60(1):22-30. doi: 10.1369/0022155411425389
52. Weitzmann MN. Bone and the immune system. *Toxicol Pathol.* 2017;45(7):911-924. doi: 10.1177/0192623317735316
53. Arron JR, Choi Y. Bone versus immune system. *Nature.* 2000;408(6812):535-536. doi: 10.1038/35046196
54. Zhang W, Dang K, Huai Y, Qian A. Osteoimmunology: The regulatory roles of T lymphocytes in osteoporosis. *Front Endocrinol (Lausanne).* 2020;11:465. doi: 10.3389/fendo.2020.00465
55. Li S, Liu Q, Wu D, *et al.* PKC-delta deficiency in B cells displays osteopenia accompanied with upregulation of RANKL expression and osteoclast-osteoblast uncoupling. *Cell Death Dis.* 2020;11(9):762. doi: 10.1038/s41419-020-02947-3
56. Li Y, Toraldo G, Li A, *et al.* B cells and T cells are critical for the preservation of bone homeostasis and attainment of peak bone mass *in vivo*. *Blood.* 2007;109(9):3839-3848. doi: 10.1182/blood-2006-07-037994
57. Wang YN, Liu S, Jia T, *et al.* T cell protein tyrosine phosphatase in osteoimmunology. *Front Immunol.* 2021;12:620333. doi: 10.3389/fimmu.2021.620333
58. Harmer D, Falank C, Reagan MR. Interleukin-6 interweaves the bone marrow microenvironment, bone loss, and multiple myeloma. *Front Endocrinol (Lausanne).* 2018;9:788. doi: 10.3389/fendo.2018.00788
59. Ivanova S, Vasileva L, Ivanova S, Peikova L, Obreshkova D. Osteoporosis: Therapeutic options. *Folia Med (Plovdiv).* 2015;57(3-4):181-190. doi: 10.1515/folmed-2015-0037
60. Rozenberg S, Al-Daghri N, Aubertin-Leheudre M, *et al.* Is there a role for menopausal hormone therapy in the management of postmenopausal osteoporosis? *Osteoporos Int.* 2020;31(12):2271-2286. doi: 10.1007/s00198-020-05497-8
61. Uhlemann C, Lange U. Physiotherapy strategies in osteoporosis--recommendations for daily practice. *Z Rheumatol.* 2006;65(5):407-410, 412-416. doi: 10.1007/s00393-006-0084-x
62. Takayama T, Suzuki N, Ikeda K, *et al.* Low-intensity pulsed ultrasound stimulates osteogenic differentiation in ROS 17/2.8 cells. *Life Sci.* 2007;80(10):965-971. doi: 10.1016/j.lfs.2006.11.037
63. Lim D, Ko CY, Seo DH, *et al.* Low-intensity ultrasound stimulation prevents osteoporotic bone loss in young adult ovariectomized mice. *J Orthop Res.* 2011;29(1):116-125. doi: 10.1002/jor.21191
64. Sun S, Sun L, Kang Y, Tang L, Qin YX, Ta D. Therapeutic effects of low-intensity pulsed ultrasound on osteoporosis in ovariectomized rats: Intensity-dependent study. *Ultrasound Med Biol.* 2020;46(1):108-121. doi: 10.1016/j.ultrasmedbio.2019.08.025
65. Inoue S, Hatakeyama J, Aoki H, *et al.* Effects of ultrasound, radial extracorporeal shock waves, and electrical stimulation on rat bone defect healing. *Ann N Y Acad Sci.* 2021;1497(1):3-14. doi: 10.1111/nyas.14581
66. Angle SR, Sena K, Sumner DR, Virdi AS. Osteogenic differentiation of rat bone marrow stromal cells by various intensities of low-intensity pulsed ultrasound. *Ultrasonics.* 2011;51(3):281-288. doi: 10.1016/j.ultras.2010.09.004
67. Hadjiargyrou M, McLeod K, Ryaby JP, Rubin C. Enhancement of fracture healing by low intensity ultrasound. *Clin Orthop Relat Res.* 1998;355 Suppl: S216-S229. doi: 10.1097/00003086-199810001-00022
68. Tian C, Liu H, Zhao C, Zhang C, Wang W. A numerical study on mechanical effects of low-intensity pulsed ultrasound on trabecular bone and osteoblasts. *J Biomech Eng.* 2023;145(5):051010. doi: 10.1115/1.4056658
69. Zamarioli A, Butezloff MM, Ximenez JPB, Volpon JB. Low-intensity pulsed ultrasound partially reversed the deleterious effects of a severe spinal cord injury-induced bone loss and osteoporotic fracture healing in paraplegic rats. *Spinal Cord.* 2023;61(2):145-153. doi: 10.1038/s41393-022-00863-1
70. Xiao G, Jiang D, Ge C, *et al.* Cooperative interactions between activating transcription factor 4 and Runx2/Cbfa1 stimulate osteoblast-specific osteocalcin gene expression. *J Biol Chem.* 2005;280(35):30689-30696. doi: 10.1074/jbc.M500750200
71. Liu W, Toyosawa S, Furuichi T, *et al.* Overexpression of Cbfa1 in osteoblasts inhibits osteoblast maturation and causes osteopenia with multiple fractures. *J Cell Biol.* 2001;155(1):157-166. doi: 10.1083/jcb.200105052
72. Wu S, Kawahara Y, Manabe T, *et al.* Low-intensity pulsed ultrasound accelerates osteoblast differentiation and promotes bone formation in an osteoporosis rat model. *Pathobiology.* 2009;76(3):99-107. doi: 10.1159/000209387
73. Zura R, Mehta S, Della Rocca GJ, Jones J, Steen RG. A cohort study of 4,190 patients treated with low-intensity pulsed ultrasound (LIPUS): Findings in the elderly versus all patients. *BMC Musculoskelet Disord.* 2015;16:45. doi: 10.1186/s12891-015-0498-1
74. Leung KS, Lee WS, Cheung WH, Qin L. Lack of efficacy of low-intensity pulsed ultrasound on prevention of postmenopausal bone loss evaluated at the distal radius in older Chinese women. *Clin Orthop Relat Res.* 2004;(427):234-240. doi: 10.1097/01.blo.0000137557.59228.4d
75. Warden SJ, Bennell KL, Forwood MR, McMeeken JM, Wark JD. Skeletal effects of low-intensity pulsed ultrasound on the ovariectomized rodent. *Ultrasound Med Biol.* 2001;27(7):989-998. doi: 10.1016/s0301-5629(01)00376-3
76. Scalize PH, de Sousa LG, Regalo SC, *et al.* Low-level laser therapy improves bone formation: Stereology findings for osteoporosis in rat model. *Lasers Med Sci.* 2015;30(5):1599-1607. doi: 10.1007/s10103-015-1773-y
77. Stein A, Benayahu D, Maltz L, Oron U. Low-level laser irradiation promotes proliferation and differentiation of human osteoblasts *in vitro*. *Photomed Laser Surg.* 2005;23(2):161-166. doi: 10.1089/pho.2005.23.161
78. Tim CR, Pinto KN, Rossi BR, *et al.* Low-level laser therapy enhances the expression of osteogenic factors during bone repair in rats. *Lasers Med Sci.* 2014;29(1):147-156. doi: 10.1007/s10103-013-1302-9
79. Sella VR, do Bomfim FR, Machado PC, da Silva Morsoleto MJ, Chohfi M, Plapler H. Effect of low-level laser therapy on bone repair: A randomized controlled experimental study. *Lasers Med Sci.* 2015;30(3):1061-1068. doi: 10.1007/s10103-015-1710-0
80. Xu M, Deng T, Mo F, *et al.* Low-intensity pulsed laser irradiation affects RANKL and OPG mRNA expression in rat calvarial cells. *Photomed Laser Surg.* 2009;27(2):309-315. doi: 10.1089/pho.2008.2283
81. Pinheiro AL, Gerbi ME. Photoengineering of bone repair processes. *Photomed Laser Surg.* 2006;24(2):169-178. doi: 10.1089/pho.2006.24.169
82. Kanenari M, Zhao J, Abiko Y. Enhancement of microtubule-associated protein-1 Alpha gene expression in osteoblasts by low level laser irradiation. *Laser Ther.* 2011;20(1):47-51. doi: 10.5978/islsm.20.47
83. Zhu CT, Li T, Zhang P, Zou M, Guo Q, Qu XW. Beneficial effects of low-level laser irradiation on senile osteoporosis in rats. *Eur Rev Med Pharmacol Sci.* 2017;21(22):5230-5238. doi: 10.26355/eurrev_201711_13846
84. Peat FJ, Colbath AC, Bentsen LM, Goodrich LR, King MR. *In vitro* effects of high-intensity laser photobiomodulation on equine bone marrow-derived mesenchymal stem cell viability and cytokine expression. *Photomed Laser Surg.* 2018;36(2):83-91. doi: 10.1089/pho.2017.4344

85. Eroglu B, Genova E, Zhang Q, *et al.* Photobiomodulation has rejuvenating effects on aged bone marrow mesenchymal stem cells. *Sci Rep.* 2021;11(1):13067. doi: 10.1038/s41598-021-92584-3
86. Amaroli A, Sabbieti MG, Marchetti L, *et al.* The effects of 808-nm near-infrared laser light irradiation on actin cytoskeleton reorganization in bone marrow mesenchymal stem cells. *Cell Tissue Res.* 2021;383(3):1003-1016. doi: 10.1007/s00441-020-03306-6
87. Yamaura M, Yao M, Yaroslavsky I, Cohen R, Smotrich M, Kochevar IE. Low level light effects on inflammatory cytokine production by rheumatoid arthritis synoviocytes. *Lasers Surg Med.* 2009;41(4):282-290. doi: 10.1002/lsm.20766
88. Pires Oliveira DA, de Oliveira RF, Zangaro RA, Soares CP. Evaluation of low-level laser therapy of osteoblastic cells. *Photomed Laser Surg.* 2008;26(4):401-404. doi: 10.1089/pho.2007.2101
89. Saad A, El Yamany M, Abbas O, Yehia M. Possible role of low level laser therapy on bone turnover in ovariectomized rats. *Endocr Regul.* 2010;44(4):155-163. doi: 10.4149/endo_2010_04_155
90. Nelson FR, Brighton CT, Ryaby J, *et al.* Use of physical forces in bone healing. *J Am Acad Orthop Surg.* 2003;11(5):344-354. doi: 10.5435/00124635-200309000-00007
91. Lirani-Galvao AP, Bergamaschi CT, Silva OL, Lazaretti-Castro M. Electrical field stimulation improves bone mineral density in ovariectomized rats. *Braz J Med Biol Res.* 2006;39(11):1501-1505. doi: 10.1590/s0100-879x2006001100014
92. Tamaki H, Tomori K, Yotani K, *et al.* Electrical stimulation of denervated rat skeletal muscle retards trabecular bone loss in early stages of disuse musculoskeletal atrophy. *J Musculoskelet Neuronal Interact.* 2014;14(2):220-228.
93. Parfitt AM. Misconceptions (2): Turnover is always higher in cancellous than in cortical bone. *Bone.* 2002;30(6):807-809. doi: 10.1016/s8756-3282(02)00735-4
94. Scheler M, Irmler M, Lehr S, *et al.* Cytokine response of primary human myotubes in an *in vitro* exercise model. *Am J Physiol Cell Physiol.* 2013;305(8):C877-C886. doi: 10.1152/ajpcell.00043.2013
95. Pautke C, Schieker M, Tischer T, *et al.* Characterization of osteosarcoma cell lines MG-63, Saos-2 and U-2 OS in comparison to human osteoblasts. *Anticancer Res.* 2004;24(6):3743-3748.
96. Bisceglia B, Zirpoli H, Caputo M, Chiadini F, Scaglione A, Tecce MF. Induction of alkaline phosphatase activity by exposure of human cell lines to a low-frequency electric field from apparatuses used in clinical therapies. *Bioelectromagnetics.* 2011;32(2):113-119. doi: 10.1002/bem.20630
97. Caputo M, Zirpoli H, De Rosa MC, *et al.* Effect of low frequency (LF) electric fields on gene expression of a bone human cell line. *Electromagn Biol Med.* 2014;33(4):289-295. doi: 10.3109/15368378.2013.822387
98. Kern H, Salmons S, Mayr W, Rossini K, Carraro U. Recovery of long-term denervated human muscles induced by electrical stimulation. *Muscle Nerve.* 2005;31(1):98-101. doi: 10.1002/mus.20149
99. Tamaki H, Yotani K, Ogita F, *et al.* Effect of electrical stimulation-induced muscle force and streptomycin treatment on muscle and trabecular bone mass in early-stage disuse musculoskeletal atrophy. *J Musculoskelet Neuronal Interact.* 2015;15(3):270-278.
100. Guo BS, Cheung KK, Yeung SS, Zhang BT, Yeung EW. Electrical stimulation influences satellite cell proliferation and apoptosis in unloading-induced muscle atrophy in mice. *PLoS One.* 2012;7(1):e30348. doi: 10.1371/journal.pone.0030348
101. Zhang BT, Yeung SS, Liu Y, *et al.* The effects of low frequency electrical stimulation on satellite cell activity in rat skeletal muscle during hindlimb suspension. *BMC Cell Biol.* 2010;11:87. doi: 10.1186/1471-2121-11-87
102. Willand MP, Holmes M, Bain JR, Fahnestock M, De Bruin H. Electrical muscle stimulation after immediate nerve repair reduces muscle atrophy without affecting reinnervation. *Muscle Nerve.* 2013;48(2):219-225. doi: 10.1002/mus.23726
103. Swift JM, Nilsson MI, Hogan HA, Sumner LR, Bloomfield SA. Simulated resistance training during hindlimb unloading abolishes disuse bone loss and maintains muscle strength. *J Bone Miner Res.* 2010;25(3):564-574. doi: 10.1359/jbmr.090811
104. Allen MR, Hogan HA, Bloomfield SA. Differential bone and muscle recovery following hindlimb unloading in skeletally mature male rats. *J Musculoskelet Neuronal Interact.* 2006;6(3):217-225.
105. Chan AS, Hardee JP, Blank M, *et al.* Increasing muscle contractility through low-frequency stimulation alters tibial bone geometry and reduces bone strength in mdx and dko dystrophic mice. *J Appl Physiol (1985).* 2023;135(1):77-87. doi: 10.1152/japplphysiol.00651.2022
106. Hronik-Tupaj M, Rice WL, Cronin-Golomb M, Kaplan DL, Georgakoudi I. Osteoblastic differentiation and stress response of human mesenchymal stem cells exposed to alternating current electric fields. *Biomed Eng Online.* 2011;10:9. doi: 10.1186/1475-925X-10-9
107. Lam H, Qin YX. The effects of frequency-dependent dynamic muscle stimulation on inhibition of trabecular bone loss in a disuse model. *Bone.* 2008;43(6):1093-1100. doi: 10.1016/j.bone.2008.07.253
108. Qin YX, Lam H, Ferreri S, Rubin C. Dynamic skeletal muscle stimulation and its potential in bone adaptation. *J Musculoskelet Neuronal Interact.* 2010;10(1):12-24.
109. Belanger M, Stein RB, Wheeler GD, Gordon T, Leduc B. Electrical stimulation: Can it increase muscle strength and reverse osteopenia in spinal cord injured individuals? *Arch Phys Med Rehabil.* 2000;81(8):1090-1098. doi: 10.1053/apmr.2000.7170
110. Biering-Sorensen F, Bohr HH, Schaadt OP. Longitudinal study of bone mineral content in the lumbar spine, the forearm and the lower extremities after spinal cord injury. *Eur J Clin Invest.* 1990;20(3):330-335. doi: 10.1111/j.1365-2362.1990.tb01865.x
111. Mohr T, Podenphant J, Biering-Sorensen F, Galbo H, Thamsborg G, Kjaer M. Increased bone mineral density after prolonged electrically induced cycle training of paralyzed limbs in spinal cord injured man. *Calcif Tissue Int.* 1997;61(1):22-25. doi: 10.1007/s002239900286
112. Biering-Sorensen F, Hansen B, Lee BS. Non-pharmacological treatment and prevention of bone loss after spinal cord injury: A systematic review. *Spinal Cord.* 2009;47(7):508-518. doi: 10.1038/sc.2008.177
113. Bodamyali T, Kanczler JM, Simon B, Blake DR, Stevens CR. Effect of faradic products on direct current-stimulated calvarial organ culture calcium levels. *Biochem Biophys Res Commun.* 1999;264(3):657-661. doi: 10.1006/bbrc.1999.1355
114. Griffin XL, Costa ML, Parsons N, Smith N. Electromagnetic field stimulation for treating delayed union or non-union of long bone fractures in adults. *Cochrane Database Syst Rev.* 2011;4:CD008471. doi: 10.1002/14651858.CD008471.pub2
115. Laabs WA, May E, Richter KD, *et al.* Bone healing and dynamic interferential current (DIC) (author's transl). *Langenbecks Arch Chir.* 1982;356(3):219-229. doi: 10.1007/BF01261760
116. Wang Y, Cui H, Wu Z, *et al.* Modulation of osteogenesis in MC3T3-E1 cells by different frequency electrical stimulation. *PLoS One.* 2016;11(5):e0154924. doi: 10.1371/journal.pone.0154924
117. Ali A, Arif AW, Bhan C, *et al.* Managing chronic pain in the elderly: An overview of the recent therapeutic advancements. *Cureus.* 2018;10(9):e3293. doi: 10.7759/cureus.3293
118. Zhu F, Ai BW, Gao JY. Experimental study on anti-inflammatory and analgesic effects of electroacupuncture combined with medium frequency therapy in model rats with lumbar nerve root compression. *Zhongguo Zhen Jiu.* 2011;31(8):721-726.
119. Ward AR, Lucas-Toumbourou S, McCarthy B. A comparison of the analgesic efficacy of medium-frequency alternating current and TENS. *Physiotherapy.* 2009;95(4):280-288.

doi: 10.1016/j.physio.2009.06.005
120. Xu H, Feng L, Zeng Z, Xu S. Experimental study on ultrashort wave therapy on the healing of fracture. *Hunan Yi Ke Da Xue Xue Bao.* 1999;24(2):125-127.
121. Wang GJ, Liu J. Clinical randomized controlled trial on ultrashort wave and magnetic therapy for the treatment of early stage distal radius fractures. *Zhongguo Gu Shang.* 2012;25(7):572-575.
122. Ding S, Kingshott P, Thissen H, Pera M, Wang PY. Modulation of human mesenchymal and pluripotent stem cell behavior using biophysical and biochemical cues: A review. *Biotechnol Bioeng.* 2017;114(2):260-280. doi: 10.1002/bit.26075
123. Liao J, Lin Y. Stem cells and cartilage tissue engineering. *Curr Stem Cell Res Ther.* 2018;13(7):489. doi: 10.2174/1574888X1307180803122513
124. Llucia-Valldeperas A, Sanchez B, Soler-Botija C, *et al.* Electrical stimulation of cardiac adipose tissue-derived progenitor cells modulates cell phenotype and genetic machinery. *J Tissue Eng Regen Med.* 2015;9(11):E76-E83. doi: 10.1002/term.1710
125. Ye D, Chen C, Wang Q, Zhang Q, Li S, Liu H. Short-wave enhances mesenchymal stem cell recruitment in fracture healing by increasing HIF-1 in callus. *Stem Cell Res Ther.* 2020;11(1):382. doi: 10.1186/s13287-020-01888-0
126. Schell H, Duda GN, Peters A, Tsitsilonis S, Johnson KA, Schmidt-Bleek K. The haematoma and its role in bone healing. *J Exp Orthop.* 2017;4(1):5. doi: 10.1186/s40634-017-0079-3
127. Midura RJ, Dillman CJ, Grabiner MD. Low amplitude, high frequency strains imposed by electrically stimulated skeletal muscle retards the development of osteopenia in the tibiae of hindlimb suspended rats. *Med Eng Phys.* 2005;27(4):285-293. doi: 10.1016/j.medengphy.2004.12.014
128. Kostyshyn NM, Gzhegotskyi MR, Kostyshyn LP, Mudry SI. Effects of mechanical stimuli on structure and organization of bone nanocomposites in rats with glucocorticoid-induced osteoporosis. *Endocr Regul.* 2021;55(1):42-51. doi: 10.2478/enr-2021-0006
129. Esfandiari E, Roshankhah S, Mardani M, *et al.* The effect of high frequency electric field on enhancement of chondrogenesis in human adipose-derived stem cells. *Iran J Basic Med Sci.* 2014;17(8):571-576.
130. Mardani M, Roshankhah S, Hashemibeni B, Salahshoor M, Naghsh E, Esfandiari E. Induction of chondrogenic differentiation of human adipose-derived stem cells by low frequency electric field. *Adv Biomed Res.* 2016;5:97. doi: 10.4103/2277-9175.183146
131. Nuccitelli R, Lui K, Kreis M, Athos B, Nuccitelli P. Nanosecond pulsed electric field stimulation of reactive oxygen species in human pancreatic cancer cells is Ca(2+)-dependent. *Biochem Biophys Res Commun.* 2013;435(4):580-585. doi: 10.1016/j.bbrc.2013.05.014
132. Clark CC, Wang W, Brighton CT. Up-regulation of expression of selected genes in human bone cells with specific capacitively coupled electric fields. *J Orthop Res.* 2014;32(7):894-903. doi: 10.1002/jor.22595
133. Wang Z, Clark CC, Brighton CT. Up-regulation of bone morphogenetic proteins in cultured murine bone cells with use of specific electric fields. *J Bone Joint Surg Am.* 2006;88(5):1053-1065. doi: 10.2106/JBJS.E.00443
134. Henle P, Zimmermann G, Weiss S. Matrix metalloproteinases and failed fracture healing. *Bone.* 2005;37(6):791-798. doi: 10.1016/j.bone.2005.06.015
135. Zhu S, He H, Zhang C, *et al.* Effects of pulsed electromagnetic fields on postmenopausal osteoporosis. *Bioelectromagnetics.* 2017;38(6):406-424. doi: 10.1002/bem.22065
136. Matsunaga S, Sakou T, Ijiri K. Osteogenesis by pulsing electromagnetic fields (PEMFs): Optimum stimulation setting. *In Vivo.* 1996;10(3):351-356.
137. Wang Q, Zhou J, Wang X, *et al.* Coupling induction of osteogenesis and type H vessels by pulsed electromagnetic fields in ovariectomy-induced osteoporosis in mice. *Bone.* 2022;154:116211. doi: 10.1016/j.bone.2021.116211
138. Adravanti P, Nicoletti S, Setti S, Ampollini A, de Girolamo L. Effect of pulsed electromagnetic field therapy in patients undergoing total knee arthroplasty: A randomised controlled trial. *Int Orthop.* 2014;38(2):397-403. doi: 10.1007/s00264-013-2216-7
139. Lei T, Liang Z, Li F, *et al.* Pulsed electromagnetic fields (PEMF) attenuate changes in vertebral bone mass, architecture and strength in ovariectomized mice. *Bone.* 2018;108:10-19. doi: 10.1016/j.bone.2017.12.008
140. Li B, Bi J, Li W, *et al.* Effects of pulsed electromagnetic fields on histomorphometry and osteocalcin in disuse osteoporosis rats. *Technol Health Care.* 2017;25(S1):13-20. doi: 10.3233/THC-171301
141. Shen WW, Zhao JH. Pulsed electromagnetic fields stimulation affects BMD and local factor production of rats with disuse osteoporosis. *Bioelectromagnetics.* 2010;31(2):113-119. doi: 10.1002/bem.20535
142. Zhou J, Chen S, Guo H, *et al.* Pulsed electromagnetic field stimulates osteoprotegerin and reduces RANKL expression in ovariectomized rats. *Rheumatol Int.* 2013;33(5):1135-1141. doi: 10.1007/s00296-012-2499-9
143. Song ZH, Xie W, Zhu SY, Pan JJ, Zhou LY, He CQ. Effects of PEMFs on Osx, Ocn, TRAP, and CTSK gene expression in postmenopausal osteoporosis model mice. *Int J Clin Exp Pathol.* 2018;11(3):1784-1790.
144. Issack PS, Helfet DL, Lane JM. Role of Wnt signaling in bone remodeling and repair. *HSS J.* 2008;4(1):66-70. doi: 10.1007/s11420-007-9072-1
145. Kubota T, Michigami T, Ozono K. Wnt signaling in bone. *Clin Pediatr Endocrinol.* 2010;19(3):49-56. doi: 10.1297/cpe.19.49
146. Shao X, Yang Y, Tan Z, *et al.* Amelioration of bone fragility by pulsed electromagnetic fields in type 2 diabetic KK-Ay mice involving Wnt/beta-catenin signaling. *Am J Physiol Endocrinol Metab.* 2021;320(5):E951-E966. doi: 10.1152/ajpendo.00655.2020
147. Zhou J, He H, Yang L, *et al.* Effects of pulsed electromagnetic fields on bone mass and Wnt/beta-catenin signaling pathway in ovariectomized rats. *Arch Med Res.* 2012;43(4):274-282. doi: 10.1016/j.arcmed.2012.06.002
148. Bodine PV, Komm BS. Wnt signaling and osteoblastogenesis. *Rev Endocr Metab Disord.* 2006;7(1-2):33-39. doi: 10.1007/s11154-006-9002-4
149. Li J, Zeng Z, Zhao Y, *et al.* Effects of low-intensity pulsed electromagnetic fields on bone microarchitecture, mechanical strength and bone turnover in type 2 diabetic db/db mice. *Sci Rep.* 2017;7(1):10834. doi: 10.1038/s41598-017-11090-7
150. Cai J, Li W, Sun T, Li X, Luo E, Jing D. Pulsed electromagnetic fields preserve bone architecture and mechanical properties and stimulate porous implant osseointegration by promoting bone anabolism in type 1 diabetic rabbits. *Osteoporos Int.* 2018;29(5):1177-1191. doi: 10.1007/s00198-018-4392-1
151. Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys.* 2008;473(2):139-146. doi: 10.1016/j.abb.2008.03.018
152. Lacey DL, Timms E, Tan HL, *et al.* Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell.* 1998;93(2):165-176. doi: 10.1016/s0092-8674(00)81569-x
153. Burgess TL, Qian Y, Kaufman S, *et al.* The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. *J Cell Biol.* 1999;145(3):527-538. doi: 10.1083/jcb.145.3.527
154. Chang K, Chang WH, Huang S, Huang S, Shih C. Pulsed electromagnetic fields stimulation affects osteoclast formation by modulation of osteoprotegerin, RANK ligand and macrophage colony-stimulating factor. *J Orthop Res.* 2005;23(6):1308-1314. doi: 10.1016/j.orthres.2005.03.012.1100230611
155. Antonelli M, Donelli D. Hot sand baths (psammotherapy): A systematic review. *Complement Ther Med.* 2019;42:1-6. doi: 10.1016/j.ctim.2018.10.020
156. Jiang L, Xue W, Wang Y. Retraction notice to "Inhibition of miR-31a-5p

decreases inflammation by down-regulating IL-25 expression in human dermal fibroblast cells (CC-2511 cells) under hyperthermic stress via Wnt/beta-catenin pathway" [Biomed. Pharmacother. 107 (2018) 24-33]. *Biomed Pharmacother.* 2021;142:112129. doi: 10.1016/j.biopha.2021.112129

157. Zheng B, Fan J, Chen B, *et al.* Rare-earth doping in nanostructured inorganic materials. *Chem Rev.* 2022;122(6):5519-5603. doi: 10.1021/acs.chemrev.1c00644
158. Yu K, Zhou H, Xu Y, Cao Y, Zheng Y, Liang B. Engineering a triple-functional magnetic gel driving mutually-synergistic mild hyperthermia-starvation therapy for osteosarcoma treatment and augmented bone regeneration. *J Nanobiotechnology.* 2023;21(1):201. doi: 10.1186/s12951-023-01955-7

Received: February 13, 2025
Revised: April 16, 2025
Accepted: April 30, 2025
Available online: May 28, 2025