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Melatonin-Loaded Nanoparticles: A Preclinical Review for Better Osteoporosis Treatment

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Abstract | **Review Article**

Melatonin-loaded nanoparticles (MLNs) are currently an effective therapeutic approach for osteoporosis due to the superior capabilities of nanotechnology combined with the bone-protective properties of melatonin. When combined into nanosized carriers, drug absorption is enhanced, site-specific targeting is made possible, systemic side effects are decreased, controlled release is provided, and bone mineral density (BMD) is raised. Conventional osteoporosis treatments often result in adverse effects and poor patient compliance, which these properties address. Examples of nanocarriers that offer a range of platforms for efficiently delivering melatonin to bone tissues are Chitosan Nanoparticles (CS-NPs), Poly D, L-lactic-co-Glycolic Acid Nanoparticles (PLGA), Hydroxyapatite Nanoparticles (nHAP), Superparamagnetic Iron Oxide Nanoparticles (SPIONs), Exosomes (Exos), and Lipid Nanoparticles (LNPs). Despite their advantages, MLNs still have a number of challenges before they can be applied in clinical settings. Regulatory approval, toxicity, long-term safety, and scalable production concerns must all be appropriately managed. Furthermore, because osteoporosis is an ongoing problem, long-term animal and clinical trials are necessary to evaluate administration strategies, modify dosage, and determine efficacy. MLNs may serve as adjuvants or the main therapeutic agents, even if their full biological potential is still unclear. Thus, continuing research will be necessary to advance MLNs from experimental models to clinical use. Whenever considered, MLNs are a novel and effective drug delivery technique that could improve the treatment of osteoporosis, reduce systemic issues, and significantly progress the development of drugs based on next-generation nanomedicine.

Keywords: Bone, Melatonin, Minerals, Nanoparticle, Osteoporosis.

1. INTRODUCTION

Melatonin is the principal circadian output signal regulated by the suprachiasmatic nucleus (SCN) in the brain. It maintains an endogenous rhythm essential for physiological homeostasis, hence is the master biological clock (Pevet *et al*., 2011). Circadian rhythm disruptions due to shift work, aging, nocturnal activities or exposure to artificial light has been reported to upregulate osteoporosis (Boivin *et al*., 2014; Hood *et al*., 2017; Munmun *et al*., 2021).

Osteoporosis, a globally prevalent disease, is characterized by reduced bone mass and structural deterioration of bone tissue, leading to increased fragility and susceptibility to fractures (Rawat *et al*.,2015; Shashidhara *et al*., 2025). According to World Health Organization (WHO), it is observed through bone mineral density (BMD) T2-scores, with "<2.5 SD" below the mean for young, healthy women (Salari *et al*., 2021; Lu *et al*., 2025). It is recorded to exhibit significant positive corelation with the age and lifestyle patterns shift, marked by changes in diet, physical activity, and sedentary behavior (Pinheiro, *et al*., 2020; Rozenberg *et al*., 2020; Zhu & Prince, 2015). From future prospectus Between 2030 and 2034, an estimated 263.2 million individuals worldwide are projected to be affected, with 58% women and 41% men (Zhu & Prince, 2014).

New therapies that act on bone metabolism at the microscopic level may help treat osteoporosis (Ali, 2023). Melatonin-loaded nanoparticles (MLNs) may be an appropriate alternative to treat osteoporosis (Dong *et al*., 2025). It has emerged as a multifaceted molecule with potential therapeutic effects on bone metabolism. It can act through melatonin receptors located on mitochondria and plasma membranes or independently as a potent antioxidant. These mechanisms promote osteoblastogenesis, inhibit oseoclastogenesis, and enhance bone density (Munmun *et al*., 2021). A key regulator of osteogenesis is the transcription factor Runx2, whose structural integrity is vital for bone

formation. Mutations in the RUNX2 gene are associated with cleidocranial dysplasia (CCD), a rare skeletal disorder. However, Runx2 must be downregulated for complete osteocyte differentiation and is not essential during the late stages of osteoblast maturation (Thaweesapphithak *et al*., 2024; Liu *et al*., 2015). Hence, the structural integrity of this protein factor makes it a master regulator for osteoporosis (Vimalraj *et al*., 2015). Recent studies have highlighted the role of protein arginine methyltransferase 1 (PRMT1) in RANKL-induced osteogenesis, though its interaction with melatonin in the context of estrogen-deficiency-induced osteoporosis remains unclear (Choi *et al*., 2021). Oxidative stress, driven by excess reactive oxygen and nitrogen species (ROS, RNS) and inducible nitric oxide synthase (iNOS), further exacerbates osteoclast-mediated bone resorption and fracture risk (Munmun & Witt-Enderby, 2021).

Nanotechnology offers promising avenues for enhancing bone health and targeted drug delivery (Zeghoud *et al*., 2024). Novel MLNs would be needed to treat osteoporosis for efficient and targeted drug delivery methods that maximize therapeutic benefits and reduce adverse effects. Polymers such as polylactic acid (PLA) and poly (lactic-co-glycolic acid; PLGA) have demonstrated efficacy in promoting osteogenic differentiation and adapting to bone repair dynamics (Perez *et al*., 2018). In animal models, melatonin-loaded PLA nanoparticles (Mel-PLA-NPs) have shown non-toxic profiles, supporting their suitability for osteoporosis treatment (Ghosh *et al*.,2020). Given the limitations of conventional pharmacological therapies, MLNs may offer a viable alternative for managing osteoporosis (Zhao *et al*., 2022). This review critically evaluates current research to explore the potential of these nanoparticles as a new class of anti-osteoporotic agents. By analyzing the strengths and limitations of existing biomaterials and treatment modalities, it provides insights into the historical context, current advancements, and future directions in osteoporosis therapy.

2. Present Scenario of Osteoporosis

2.1 National Status

The controlled release of therapeutics and active compounds utilizing biodegradable polymer-based nanoparticles has advanced rapidly in recent years (Wei *et al*., 2008). To accomplish the best treatment outcomes, a regulated delivery strategy is required toavoid undesirable side effects and maintain the effectiveness of active molecules and therapies from the physiological environment. Currently, researchers are working on developing biodegradable polymeric nanoparticles using a variety of biodegradable polymers, including “poly (lactic-co-glycolic-acid; PLGA), poly (D, L lactic acid; PLA), poly (ε-Caprolactone; PCL), chitosan gelatin, and poly-alkyl-cyanoacrylates” (Zheng *et al*., 2009; Jain *et al*., 2006; Kumari *et al*., 2010; Pandey *et al*., 2014) for the targeted administration of pharmaceuticals and active compounds linked to malaria, diabetes, cancer, and other dangerous illnesses. Very few groups in India conduct studies on bone deformities (Compston, 2002). The causes of melatonin function in bone abnormalities have never been investigated at the national and international level (Ghosh, 2021). Thus, the work of Pandey *et al*., 2014 is the pioneering information accessible in India regarding the manufacture of melatonin-loaded polymeric nanoparticles and their transport method in comparison to melatonin (Ghosh, 2021).

2.2 International Status

Demographic shifts are predicted to raise the actual number of hip fractures and other fractures in men and woman 50 years of age and beyond by more than 50% between 2005 and 2025, although hip fracture incidence rates are decreasing in the USA(Hughes *et al*., 2009).Melatonin plays an evident role in the execution of several physiological processes, such as immunity (Reiter *et al*., 2000) and reproduction modulation (Haldar *et al*., 1992). Numerous reports are available on how melatonin affects immune regulation in bone marrow (Vishwas *et al*., 2012). However, there is currently limited literature, not even at the international level, about the therapeutic application of melatonin or melatonin-rich compounds in the research of bone abnormalities. Furthermore, there lack many groups researching melatonin-loaded polymeric nanoparticles worldwide. For the transmucosal delivery of melatonin, Hafner *et al*. 2009, synthesized melatonin-loaded lecithin/chitosan nanoparticles and found that the particles improved melatonin transport within the epithelial barrier.

3. Nanoparticle-based drug delivery for bone disorders

3.1 Fundamental advantages of Nanoparticles include biodegradability, controlled release, and targeted delivery mechanism

Nanoparticles are useful in drug delivery because they can target specific areas in the body (Wen *et al*., 2024), release medicine in a controlled way, and break down naturally. These features help improve how well treatments work while lowering side effects, making nanoparticles a promising tool in modern medicine. Biodegradable polymers have been a major breakthrough in medicine, helping improve treatments for over 50 years. Polyesters such as polyglycolic acid (PGA), poly (D, L-lactic acid), and poly (D, L-lactic-co-glycolic acid) (PLGA) were introduced in the 1960s and 1970s as biodegradable suture materials, marking the onset of synthetic biodegradable polymer applications (Kamaly *et al*., 2016). It fosters collaboration among chemists, engineers, biologists, and medical professionals, leading to significant biotechnological advances in drug delivery, biomaterials, tissue engineering, and the development of medical devices (Kamaly *et al*., 2016). Managing bone disorders in clinical settings has remained a persistent challenge over the years. Among

various approaches in bone tissue engineering, significant progress has been made in developing drug delivery systems that utilize functional drugs and suitable carrier materials, driven by recent technological advancements. A wide range of functional nanocarrier-based materials has been designed and applied to improve the complex environment required for bone regeneration. These materials demonstrate antimicrobial properties, inhibit osteoclast activity, and enhance osteogenic processes (Hang *et al*., 2024). Patients at immediate risk of fracture require timely and comprehensive treatment strategies (Shashidhara *et al*., 2025). The effectiveness of current osteoporosis treatments is limited due to adverse side effects and low patient adherence.

3.2 The comparison of traditional melatonin therapy with melatonin loaded nanoparticles

Current treatments for osteoporosis aim to rebalance bone formation and resorption to maintain skeletal health. Anti-resorptive agents such as bisphosphonates (e.g., alendronate, Zoledronate, ibandronate, pamidronate, and risedronate), Anti-RANKL antibody denosumab, as well as anabolic drugs like Teriparatide, a Parathyroid Hormone Analog (PTH), Selective Estrogen Receptor Modulators (SERMs), Hormone Replacement Therapy (HRT), Anti-Sclerostin **(**Romosozumab).While these therapies help increase bone density and reduce fracture risk, they are associated with notable limitations and side effects (Rawat, 2015; Wen *et al*., 2024). Conventional melatonin therapy has limited effectiveness therefore, using MLNs offers promising benefits, such as targeted delivery and reduced toxicity. All these commonly used treatments are summarized in Table 1. However, these highlights the need for further research into their combined use for treating osteoporosis.

(a) Bisphosphonates

Alendronate, zoledronate, ibandronate, pamidronate, and risedronate belong to the drugs that prevent osteoclasts from resorbing bone by adhering to the bones and triggering osteoclast death (Kim *et al*., 2021). Simple bisphosphonates are metabolically integrated into ineffective ATP analogues, preventing intracellular pathways. While nitrogen-containing bisphosphonates (N-BPs) are more potent and inhibit essential enzymes that produce small GTP-binding proteins required for signaling events into osteoclasts (Russell, 2011). The prolonged use of bisphosphonates has been associated with the common but serious risk of aberrant femur fractures (AFFs), despite the fact that they are highly effective in lowering the incidence of fragility fractures (Gedmintas *et al*., 2013). It has been demonstrated that a number of bisphosphonates reduce the risk of fragility fractures in both males with osteoporosis and postmenopausal women (Crandall *et al*., 2014). Before beginning an individual on bisphosphonates, side effects and appropriate medication guidance are crucial. It's crucial to discuss upper gastrointestinal problems and the possibility of jaw osteonecrosis. According to a comprehensive analysis by Khan *et al*., 2014 the prevalence rates of osteonecrosis of the jaw (ONJ), a rare adverse effect of these medicines, range from 1.04 to 69 per 100,000 patient years for oral bisphosphonate therapy and from 0 to 90 per 100,000 for intravenous therapies (Khan *et al*., 2014).

(b) RANK-L Suppressors

Denosumab inhibits the binding of receptor activator of nuclear factor kappa-B ligand (RANKL) to RANK. This prevents full osteoclast activation and osteoclast maturation by disrupting the pathway required to generate transcription factors for osteoclast-associated gene activation (Lu *et al*., 2023). Further supporting denosumab's effectiveness for the long-term therapy of this chronic illness, the 10-year follow-up findings from the freedom extension study showed a sustained reduction in fracture risk with long-term treatment in postmenopausal women with osteoporosis (Bone *et al*., 2017).According to Chen *et al*., 2021 systematic analysis of 11 Randomized Control Trials (RCTs), It lowers the incidence of clinical fractures, non-vertebral, vertebral, and hip fractures while also enhancing BMD in patients (Chen *et al*., 2021). Compared to bisphosphonates, denosumab may result in a radiologically distinct ONJ; subsequently, there are hazards associated with this injectable (Querrer *et al*., 2020).

(c) Selective Estrogen Receptor Modulators (SERMs)

SERMs are drugs that bind with estrogen receptors and trigger tissue-specific actions that, depending on the target tissue, either replicate or prevent estrogen activity. In addition to their selectivity, SERMs can increase bone density without activating tissues like the breast or endometrium, which lowers the risk of malignancies linked to estrogen. Raloxifene is the most widely used SERM for osteoporosis. It activates by adhering to bone estrogen receptors and triggering mechanisms that reduce bone resorption and increase BMD (McClung, 2015; Shashidhara *et al*., 2025).Some side effects are caused by SERMs includes “Menstrual abnormalities, Hot flushes, Vomiting, and vaginal bleeding and discharge”. Raloxifene does not have the same hazards to ONJ or AFF as antiresorptive therapies like bisphosphonates or denosumab (Xiao *et al*., 2023).

(d) Hormone Replacement Therapy (HRT)

HRT is still recognized as an option in many current guidelines, especially for younger postmenopausal women with associated menopausal symptoms, even though it may not be the first-line treatment for osteoporosis in all postmenopausal women, especially those at higher risk of breast cancer or cardiovascular events (Gregson *et al*., 2022; Camacho *et al*., 2020). A major factor in postmenopausal osteoporosis in woman is the loss of estrogen, a primary sex hormone that plays a crucial role in osteoporosis by causing osteoclast death through the fas ligand(Kim *et al*., 2021). Since the early 1990s, there has been

considerable RCT evidence supporting the use of hormone replacement therapy (HRT) in postmenopausal osteoporosis. It has been demonstrated to improve Bone Mineral Density (BMD) and lower the risk of hip and spine osteoporotic fractures. More recent meta-analyses have confirmed this (Lin *et al*., 2021). Breast cancer, VTE, cardiovascular disease, and stroke are among the dangers associated with HRT; these risks are frequently formulation-dependent (Mayor, 2002).

(e) PTH Analogous

Teriparatide functions by stimulating osteoblasts and decreasing their apoptosis. It is a synthesized form of biosynthetic parathyroid hormone isomers 1-34 (PTH1-34). In postmenopausal woman, this activity lowers fracture rates and increase bone mineral density (Neer *et al*., 2001). These results have been supported by systematic review, which show that when treating postmenopausal osteoporosis, teriparatide has a greater effect on lowering fracture rates and enhance bone mineral density (Yuan *et al*., 2019). Patients may find the daily subcutaneous injection schedule for teriparatide to be uncomfortable. Side symptoms such as nausea, dizziness, headaches, and leg cramps might make adherence even more difficult. It also has similar effects as PTH, so it is related to hypercalcemia (Brixen *et al*., 2004). Following PTH analog treatment, sequential therapy with a bisphosphonate or denosumab may maintain and even increase bone density (Niimi *et al*., 2018).

(f) Anti-Sclerostin

Romosozumab is a human monoclonal antibody that targets sclerostin. It is a direct inhibitor of bone formation and an indirect promoter of bone resorption, two of the several mechanisms *via* which its effects in osteoporosis are mediated (Rossini *et al*., 2013). Therefore, this monoclonal antibody can both promote bone formation and decrease bone resorption (Bandeira *et al*., 2017). A monthly subcutaneous injection of romosozumab is administered. The mechanism of action of romosozumab is defined by the simultaneous stimulation of bone formation and repression of bone resorption, which results in an immediate increase in the demand for calcium for matrix mineralization while simultaneously decreasing the release of calcium from bone. Serum calcium levels may drop promptly and possibly drastically as a result. Patients with earlier vitamin D deficiency are more at risk, which emphasizes the therapeutic significance of determining vitamin D status prior to starting romosozumab medication (Gregson *et al*., 2022; Camacho *et al*., 2020; Cosman *et al*., 2016). Significant BMD losses occur after stopping romosozumab, frequently returning to pre-treatment levels. However, BMD improvements can be maintained by starting denosumab or bisphosphonate after this (McClung *et al*., 2018).

Table 1: Characteristics and Side Effects of Different Drugs for the Treatment of Osteoporosis

Drug Class	Drug example	Action Pathway	Side Effects	Reference
Bisphosphonates	Alendronate, zoledronate, ibandronate, pamidronate, risedronate	Prevent osteoclast and bone reabsorption	Gastroesophageal irritation, Osteonecrosis of jaw (ONJ), Suppression of bone turnover, Erosive esophagitis, Severe Musculoskeletal pain, Hypocalamia, Esophageal cancer, Ocular inflammation, Atrial fibrillation, Subtrochanteric, Femoral Fractures	(Kennel & Drake, 2009; Russell, 2007)
RANK-L Suppressors	Denosumab	Block binding to RANK, Prevent osteoclast development and maturation	Cellulitis, Eczema, Necrosis of jaw bones	(Wen *et al*., 2024)
SERMs	Raloxifene, bazedoxifene, lpriflavone, tamoxifen	Reduce bone resorption activity and osteoclast differentiation	Menstrual abnormalities, Hot flushes, Vomiting, and vaginal bleeding and discharge	(Bandolia & Khan, 2019)
HRT	Various combinations of Estrogen/ progesterone	Promotes osteoclast apoptosis and suppresses osteoclastogenesis	Increased risk of breast cancer, VTE, stroke, Uncoupling of bone formation and resorption	(Shashidhara *et al*., 2025; Wang *et al*., 2023)
PTH Analogous	Teriparatide, Abaloparatide	Primarily Stimulate the osteoblast activity, For the treatment of postmenopausal osteoporosis in women	Rare cases of osteosarcoma with teriparatide and hypercalcemia	(Kraenzlin & Meier, 2011; Shashidhara *et al*., 2025; Wen *et al*., 2024)
Anti-Sclerostin	Romosozumab, Blosozumab	Enhances bone formation & decreases resorption	Loss of BMD during discontinuation, Cardiovascular incidents (possible hazard)	(Aditya & Rattan, 2021; Rauner *et al*., 2021; Shashidhara *et al*., 2025)

4. Formulation and Preparation of Melatonin-Loaded Nanoparticles Using Emulsification, Nanoprecipitation, and Solvent Evaporation Techniques

Melatonin-loaded nanoparticles (MLNPs) are developed using techniques such as solvent evaporation, nanoprecipitation, and emulsification. These methods facilitate the creation of biodegradable and biocompatible carriers that enhance the delivery of melatonin- a compound with potential therapeutic effects on bone health. This approach supports the effective treatment of osteoporosis by improving melatonin stability and targeted release.

Among the polymers used for preparing polymeric nanoparticles (PNPs) poly-DL- lactic-co-glycolic acid (PLGA) is the most widely utilized. Its popularity in drug delivery research stems from its excellent biodegradability and low systemic toxicity. Moreover, PLGA has been approved by the U.S. Food and Drug Administration (FDA) for various medical applications, reinforcing its safety and effectiveness (Dinić *et al*., 2018; Hernández-Giottonini *et al*., 2020; Puricelli *et al*., 2023).

4.1 Emulsification

Emulsification techniques typically involve mixing a volatile, water-immiscible organic solvent with an aqueous phase, followed by the application of high shear force to create a stable emulsion. As the organic solvent evaporates, polymeric nanoparticles (PNPs) are formed, as shown in figure 1. This approach is valued for its non-toxic nature, rapid processing, and ability to produce nanoparticles with small and uniform sizes (Mallakpour *et al*., 2016; Hernández-Giottonini *et al*., 2020). In one variation of this method, melatonin is combined with a polymer such as Polylactic Acid (PLA) and dissolved in an organic solvent like Dichloromethane (DCM). This solution is then dispersed into the aqueous phase to form an emulsion, which is subsequently processed to generate nanoparticles. These nanoparticles can be characterized using imaging techniques such as Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM; Ghosh, 2020).

Despite of its advantages, this technique requires careful optimization for each drug formulation. The high energy input during emulsification may affect the stability of sensitive compounds, posing a challenge for certain therapeutic agents (Masood, 2015; Yang *et al*., 2015; Hernández-Giottonini *et al*., 2020).

4.2 Solvent Evaporations

The solvent evaporation method involves dissolving both the polymer and melatonin in a volatile organic solvent (Figure 1). As the solvent evaporates, solid nanoparticles are formed. By adjusting formulation parameters, this process can be optimized to achieve sustained release profiles, thereby improving the therapeutic effectiveness of melatonin in treating osteoporosis (Chuffa *et al*., 2021).

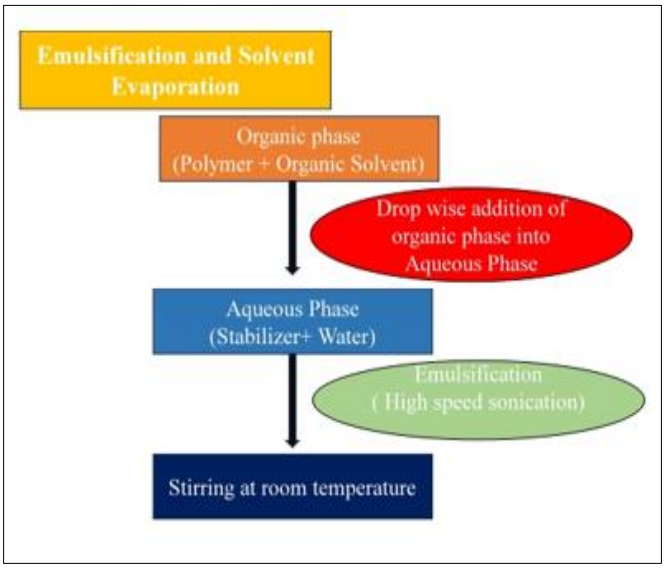

Figure 1: A general overview of Design and Manufacturing of Melatonin-Loaded Nanoparticles by Emulsification and solvent evaporation, used for synthesis of polymeric nanoparticle (PNPs)

4.3 Nanoprecipitation

Nanoprecipitation is a straightforward and efficient technique that employs miscible solvents to produce nanoparticles. It offers several advantages, including operational simplicity, reliable reproducibility, and low energy requirements, making it a widely adopted method in nanoparticle synthesis (Almoustafa *et al*., 2017; Hernández-Giottonini *et al*., 2020). In this method, a solution of melatonin and polymer is rapidly mixed with a non-solvent, causing the polymer to precipitate and encapsulate the melatonin in figure 2.

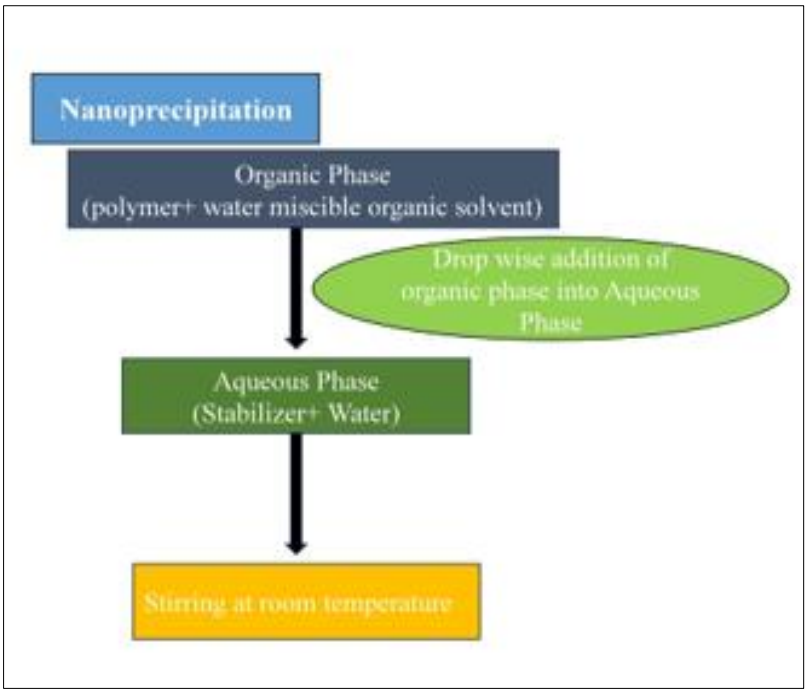

Figure 2: A general Overview of Design and Manufacturing of Melatonin-Loaded Nanoparticles by precipitations used for synthesis of polymeric nanoparticles (PNPs)

While these techniques show promise in improving drug delivery and therapeutic outcomes, challenges remain in achieving optimal targeting and controlled release, which are critical for effective osteoporosis management (Bastola, 2025).

5. Preclinical Evaluation of Melatonin-Loaded Nanoparticles in Experimental Models of Osteoporosis

In vivo animal studies play a crucial role in assessing the systemic safety and immune modulating properties of MLNs. It highlights recent findings about their potential toxicity and effects on cytokine regulation. Encapsulating melatonin in biocompatible polymeric systems protects it from oxidation, reduces toxicity, and prolongs its half-life. These effects collectively improve its pharmacokinetic profile, leading to better therapeutic outcomes and greater patient compliance (Cheaburu-Yilmaz *et al*., 2024).

5.1 Toxicity Studies Targeting the Liver, Kidney, and Other Organ System

The systemic toxicity of the MLNPs was evaluated *in vivo*. Histopathology and blood biochemistry revealed mild hepatotoxicity in treated mice. Histopathology also indicated mild nephrotoxicity; however, this was not confirmed by kidney biochemical analysis (Cheaburu-Yilmaz *et al*., 2024). Biochemical analysis of blood samples from mice in the control and treatment groups (polymer alone and polymer loaded with melatonin, MLT) revealed mild liver toxicity, as indicated by changes in Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) levels. The polymer alone did not alter ALP activity, whereas the drug-loaded polymer caused a marked reduction in ALP, likely due to zinc depletion, since zinc is an essential cofactor for ALP. This suggests that the formulation may induce an acute loss of zinc in the liver and bone marrow, which is accompanied by significant elevations in ALT and AST. The effect of the polymer itself on ALP appears negligible. Such transient adverse effects are commonly observed with several approved drugs and are generally considered tolerable (Meunier & Larrey, 2019). Histopathological examination of liver samples was consistent with the biochemical findings, showing only minor adverse changes at the hepatocellular level.

Kidney findings showed some inconsistency between clinical biochemistry and histopathology. Mild adverse changes were observed in kidney histopathology, whereas Blood Urea Nitrogen (BUN) and creatinine levels remained unchanged, with no significant differences between the control and treatment groups (Meunier & Larrey, 2019; Cheaburu-Yilmaz *et al*., 2024). Based on the preliminary findings, the successfully synthesized Mel-PLA nanoparticles showed no toxic effects in animal models (Ghosh, 2020). Importantly, no study has reported serious or harmful effects in animals, even at high doses of melatonin, suggesting that melatonin is relatively safe (Amstrup *et al*., 2013). It will be expected that demand in melatonin receptors and melatonin-based treatments will continue to rise, this shows in Table 2 (Emet *et al*., 2016).

Table 2: Modified melatonin metabolism in multiple disorders and systems

Systems	Effect
Sleep Inflection	Prevention of Jet Lag and Phase Shift
Mental Health	Anxiolytic, anxiety-reducing, antidepressant, and drug addiction treatment
Central Nervous System	Anti-inflammatory, neuroprotective, and pain modulating; helps control the development of memories; and serves as therapy for brain edema. Children with antiepilepsy
Endocrine System	Ovarian physiology, seasonal reproduction, supressing the release of hormones that regulate reproduction. Type 2 diabetes and osteoblast differentiation
Autoimmune disorders	Types 1 diabetes, rheumatoid arthritis, autoimmune liver disease, multiple sclerosis, and SLE in females
Cardiovascular system	Cardiac Syndrome X and hypertension
Locomotory System	Antinociceptive, modulating locomotor activity
Oncology	Anti-tumor
Others	Hepatoma, pineal calcification, antioxidant, retinal, and infection

(Emet *et al*., 2016).

5.2 Modulation of Immune Responses Involving Cytokines in Osteoporosis treatment

Cytokines are chemicals produced or expressed on cell membranes that control a number of biological processes, such as cell development, differentiation, proliferation, and survival (Lin, 2023). Melatonin, a hormone with demonstrated immunomodulatory qualities that include immunological stimulation and anti-inflammatory effects, is secreted by the pineal gland. It can be added to a nanoparticle-based delivery system to boost its therapeutic efficacy by improving cellular targeting and lowering systemic adverse effects. It has long been known for controlling neuroendocrine processes, although new studies show that it also plays a complex role in immunological regulation. Melatonin's complex relationship with the immune system raises the possibility of therapeutic uses for immunologically related conditions (Koul *et al*., 2024).

A cytokine involved in immune modulation is Interleukin-2 (IL-2), which is particularly important for T cell survival and proliferation. IL-2 may have a therapeutic effect for osteoporosis, a disorder characterized by poor bone density and an increased risk of fracture, as recent developments in osteoimmunology indicate that it also contributes to bone remodeling.

Resorption of bone is significantly facilitated by Tumor Necrosis Factor (TNF), which comprises TNF-α and TNF-β. By increasing osteoclast production and inhibiting osteoblast activity, it causes bone loss. Several paths, including ones independent of the RANKL/RANK signaling axis, can be used by TNF to promote osteoclast development (Suda *et al*., 2001; Kobayashi *et al*., 2000). TNF-α increases the expression of RANKL and Macrophage-Colony-Stimulating Factor (M-CSF) by activating stromal cells, osteoblasts, and T cells. Through M-CSF-mediated pathways, these signals indirectly increase RANK expression in osteoclast precursors, accelerating their development into mature osteoclasts (Kitaura *et al*., 2013). It is closely associated with alterations in RANK and estrogen levels in postmenopausal women with osteoporosis. It increases RANKL-driven osteoclast production *in vitrovia* activating the NF-κB and PI3K/Akt signaling pathways. After menopause, osteoporosis may occur as a result of this synergistic effect (Zha *et al*., 2018).

Cytokines in the IL-6 family interact *via* a common receptor subunit known as glycoprotein 130 (gp130). “IL-6, IL-11, Oncostatin M (OSM), Leukemia Inhibitory Factor (LIF), Cardiotrophin 1 (CT-1), Ciliary Neurotrophic Factor (CNTF), Cardiotrophin-Like Cytokine Factor 1 (CLCF1), Neuropoietin (NP), IL-27, and Humanin” are members of this family (Scheller *et al*., 2013; Rose-John, 2017). Essentially two types of the IL-6 receptor (IL-6R): soluble (sIL-6R) and membrane-bound (mIL-6R). Some reports suggest that, both isoforms have different functions in immune control and IL-6 signaling (Xie *et al*., 2018). The balance of bone formation and resorption between osteoclasts and osteoblasts is maintained in large part by the cytokine network. Cytokine dysregulation may lead to bone disorders such as osteoporosis. Among these cytokines, TNF-α, IL-1, IL-6, IL-7, IL-8, IL-11, IL-15, IL-17, and IL-20 are osteoclastogenesis(Xu *et al*., 2023). In contrast, anti-inflammatory cytokines that inhibit osteoclastogenesis include IL-3, IL-4, IL-10, IL-13, IL-18, IL-19, IL-27, IL-29, IL-32, IL-33, IL-37, and IFNs (Xu *et al*., 2023). The adverse effect of proinflammatory cytokines on osteoporosis bone remodeling has been extensively studied, but the intricate relationship between the immune system and bone makes it challenging to translate this understanding into clinical practice. However, through the work of numerous scientists, some molecularly targeted medications are currently undergoing clinical trials and have shown promising results. For instance, by suppressing the expression of IL-1β, the non-steroidal anti-inflammatory medication benzydamine can prevent osteoclast differentiation and bone resorption (Son *et al*., 2019). The specific effects of different cytokines on osteoblasts and osteoclasts during bone remodeling in osteoporosis were covered in figure3.

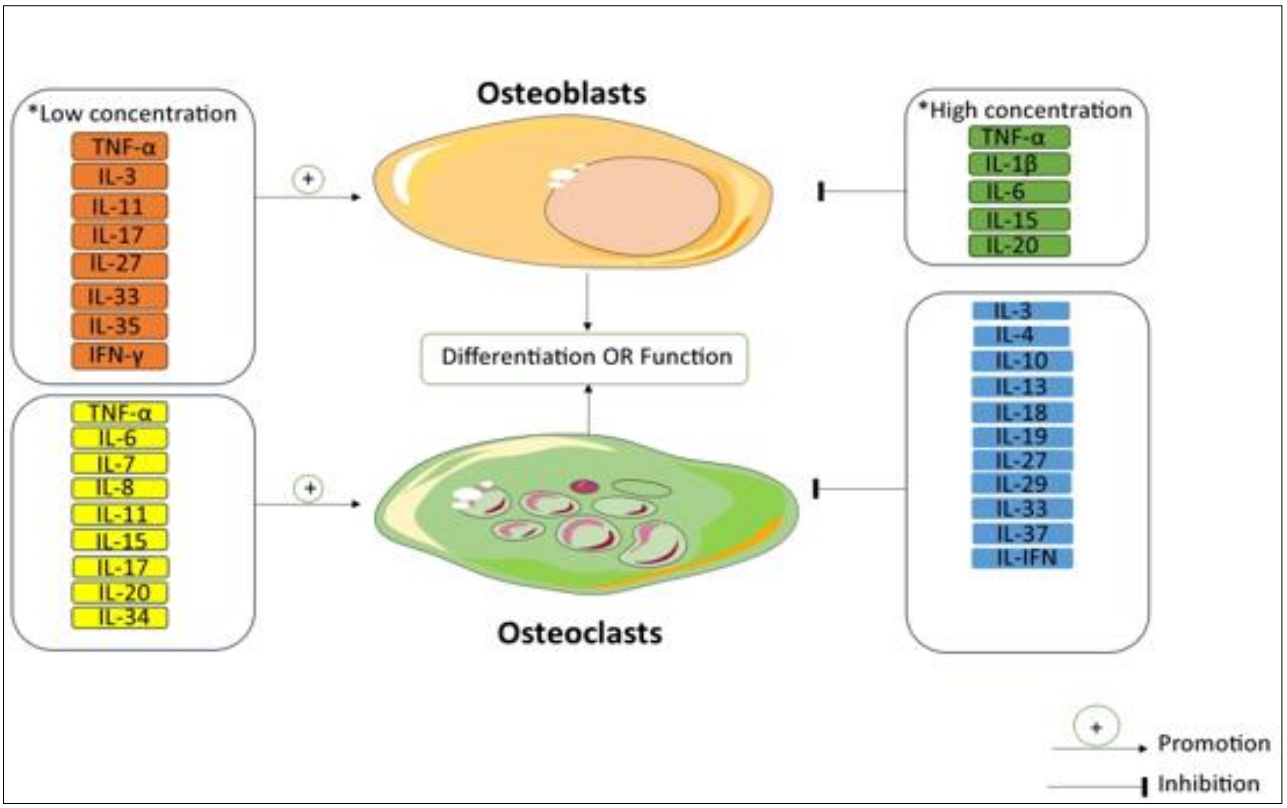

Figure 3: The ways in which various cytokines affect the differentiation or function of osteoblasts and osteoclasts by promoting and suppressing them (Xu *et al*., 2023)

6. Essential Role of Signaling Pathways in Osteoporosis (RANKL/OPG, Wnt/β-catenin, NF-κB)

6.1 RANKL/OPG signaling pathway

Runx2 Primarily produced by progenitor cells, and it is an essential transcription factor for osteoblast synthesis that produces preosteoblast (Udagawa *et al*., 2020). In preosteoblasts, Runx2 triggers Sp7 (Osterix), which initiates mineralization and the formation of an extracellular matrix (Komori, 2020). As a result, high levels of bone gamma carboxyglutamate protein, including osteocalcin and Bone Gamma-Carboxyglutamate Protein (Bglap), are frequently expressed by mature osteoblasts. When mature osteoblasts are encircled by mineralized bone, they develop the osteocyte phenotype, which can be determined by the expression of Sclerostin (Sost), Fibroblast Growth Factor 23 (FGF23), and Dentin Matrix Protein 1 (Dmp1; Komori, 2020). Osteocytes, which make up 90% of all bone cells, are the primary source of the cytokine receptor activator RANKL and Tumor Necrosis Factor (Ligand) Superfamily, Member 11 (Tnfsf11), initiating osteoclastogenesis on osteoclast progenitors and contributing to the maintenance of bone homeostasis (Han *et al*., 2018). Moreover, osteoblast lineage cells express RANKL (Han *et al*., 2018). For it to activate its osteoresorptive effects, RANKL attaches to the osteoclast surface through the RANK receptor.Particularly, osteoblastic stromal cells contain osteoprotegerin (OPG), which inhibits the growth and maturation of the osteoclasts by preventing RANKL from binding to RANK. Consequently, activated osteoclasts adhere to the bone surface, release protons and proteinase, dissolve the minerals in the bone, and disintegrate the matrix (Glasnović *et al*., 2018). The cysteine proteinase and matrix metalloproteinase (MMP) families are the two main proteinases known to be involved in the solubilization of collagenous matrix. The most prevalent gelatinolytic MMP in osteoclasts is MMP-9, which is not rate-restricted, but the primary MMPs that break down bone collagen still exist. On the one hand, Tissue inhibitor of Metalloproteinases-1 (TIMP-1) and Tissue inhibitor of Metalloproteinases-2 (TIMP-2) use zinc-dependent endopeptidase activities to reduce the effects of MMP-9 and MMP-2, respectively (Łukaszewicz-Zając *et al*., 2021). However, cathepsin K, which is rate-restricted, enhances the effect of cysteine proteinase on matrix solubilization (Dai *et al*., 2020). It is interesting to consider that cathepsin K can disrupt the collagen triple helix in many different places, making it more susceptible to any proteinase solubilization. Among all the cysteine proteinases, cathepsin K exhibits the greatest gelatinolytic activity (Dai *et al*., 2020). Consequently, MMPs and cathepsin K serve as essential proteinases in this process shown in figure 4 (Łukaszewicz-Zając *et al*., 2021; Dai *et al*., 2020).

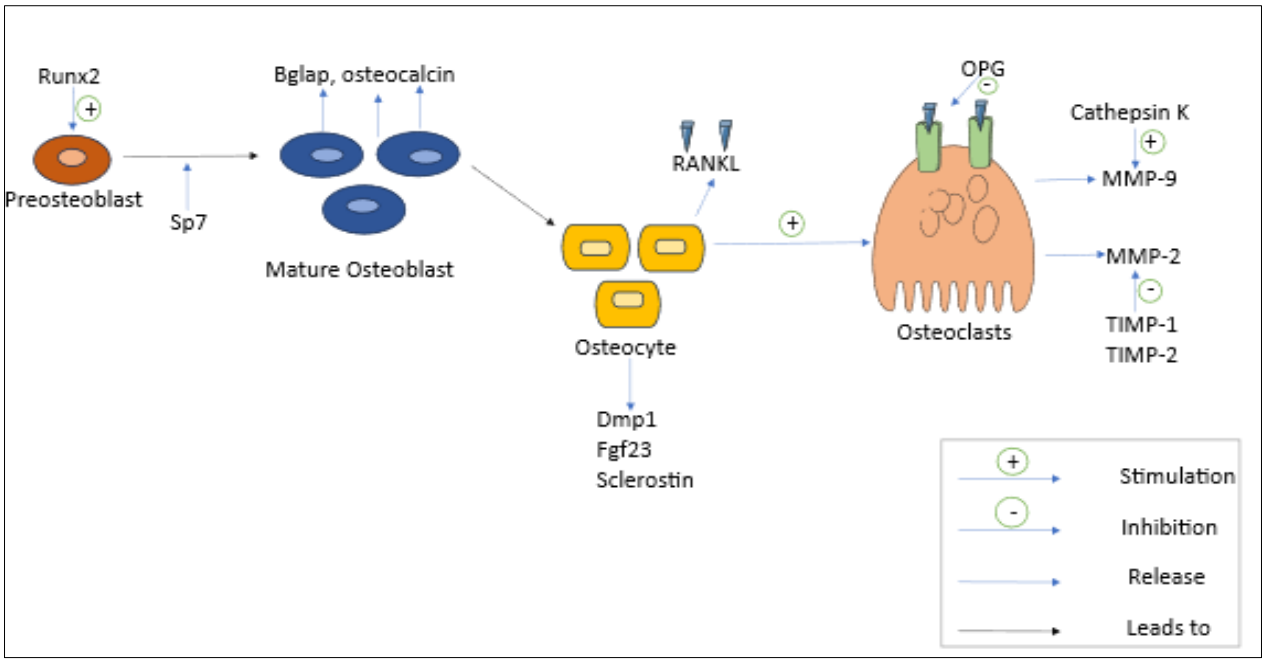

Figure 4: RANKL/OPG signaling pathway. Runx2 is a crucial transcription factor for osteoblast synthesis, which forms preosteoblasts, and is mostly produced by progenitor cells. It activates Sp7 in preosteoblasts (Osterix). Mature osteoblasts produce certain proteins, such as osteocalcin and Bglap. The expression of Sost, Fgf23, and Dmp1 will determine it. OPG is present in osteoblastic stromal cells, and RANKL binds to the surface of osteoclasts via the RANK receptor. Zinc-dependent endopeptidase activities are used by TIMP-1 and TIMP-2 to reduce the effects of MMP-9 and MMP-2, respectively. MMPs and cathepsin K are therefore crucial proteinases in this signaling cascade

6.2 Wnt/β-catenin signaling pathway

The Wnt signaling pathway play a major role in regulating a number of different cellular distinguishing characteristics (Zhao *et al*., 2018). The various stages of skeletogenesis, including the patterning of the embryonic skeletal system, the development of the fetal skeleton, and the remodeling of adult bone, are significantly influenced by the Wnt pathway (Zhao *et al*., 2018). Frizzled-2, Runx2, Axin2, and β-catenin in osteoblasts are downstream factors of Wnt/β-catenin signal transduction, and they specifically promote osteoblast development and mineralization. Several Wnt proteins have been found in humans. Wnt/β-catenin signaling is triggered when Wnt glycoproteins bind to the Frizzled receptor (Fz) and co-receptor known as low-density Lipoprotein Receptor-related Protein LRP5 or 6 (Hua *et al*., 2018). Axin2 is then stimulated by Glycogen Synthase Kinase 3β (GSK3β); this stops β-catenin from being phosphorylated (Feehan *et al*., 2019). Consequently, β-catenin accumulates, is transported into the nucleus and regulates the expression of many target genes by interacting with several transcription factors, such as TCF/LEF, and HIF-1α23. By enhancing OPG synthesis on osteoblasts and regulating RANK/RANKL/OPG signaling, β-catenin inhibits osteoclastogenesis (Figure 5). Hence, promoting canonical Wnt signaling increases bone formation while reducing bone resorption. Consequently, osteoblast proliferation and differentiation are induced by Wnt/β-catenin signals in either an independent or β-catenin-dependent manner (Elahmer *et al*., 2024). However, in the absence of a Wnt signal, cytoplasmic β-catenin is broken down to maintain low levels of β-catenin. A multiprotein complex composed of the scaffolding protein axin, GSK3β, and Adenomatosis Polyposis Coli (APC) increases the degradation of β-catenin by phosphorylating certain amino acid residues in the protein (Tortelote *et al*., 2017). Wnt proteins interact with several factors, including Secreted Frizzled-Related Proteins (SFRPs) and Wnt Inhibitory Factor (WIF), to prevent the activation of the Fz receptor and co-receptor LRP5 OR 6. Additionally, LRP5/6 is competitively bound by intrinsic factors such as sclerostin and proteins of the DKK1 family, which inhibits Wnt signaling and supresses bone growth (Herreros, 2019).

6.3 NF-κB signalling Pathway

The signaling of NF- κB in bone maintenance was first unintentionally revealed following simultaneous deletion of NF-κB1/p50 and NF-κB2/p52 subunits (Iotsova *et al*., 1997; Franzoso *et al*., 1997). These subunits are essential for osteoclast precursor differentiation into osteoclast rather than their survival, which is a feature of NF-κB activity, based on further studies of these double knockout mice (Boyce *et al*., 1999). A signaling cluster is recruited and synthesized at the distal end of the receptor when ligand (L) binds to its cell membrane (CM) receptor (R). Multiple proteins, such as TNF Receptor-Associated Factors (TRAFs), the tyrosine kinase c-Src, p62, cellular inhibitors of apoptosis (c-IAP), and TNF receptor-interacting protein (RIP), are present in signaling complexes. This cluster recruits and activates the MAP kinases, TGF-β activated kinase (TAK1) and NF-κB inducing kinase (NIK) using

lysine 63-linked polyubiquitination chains (K63- pUB). These kinases then activate the classical and alternative IKK complexes, respectively shown in figure 6. The p100/ NF- κB and IkB targets of activated IKK1 and IKK2 are phosphorylated (pp) and then broken down. RelB, released p50/P65 dimers, and processed p52 all go to nucleus, attach to DNA sequences, and initiate transcription (Abu-Amer, 2013). Osteoclastogenesis occurs when NF-κB activity is reduced, and animals develop osteopetrosis when RANKL, RANK, or RANKL-RANKL signaling is inhibited (Iotsova *et al*., 1997; Franzoso *et al*., 1997; Boyce *et al*., 1999).

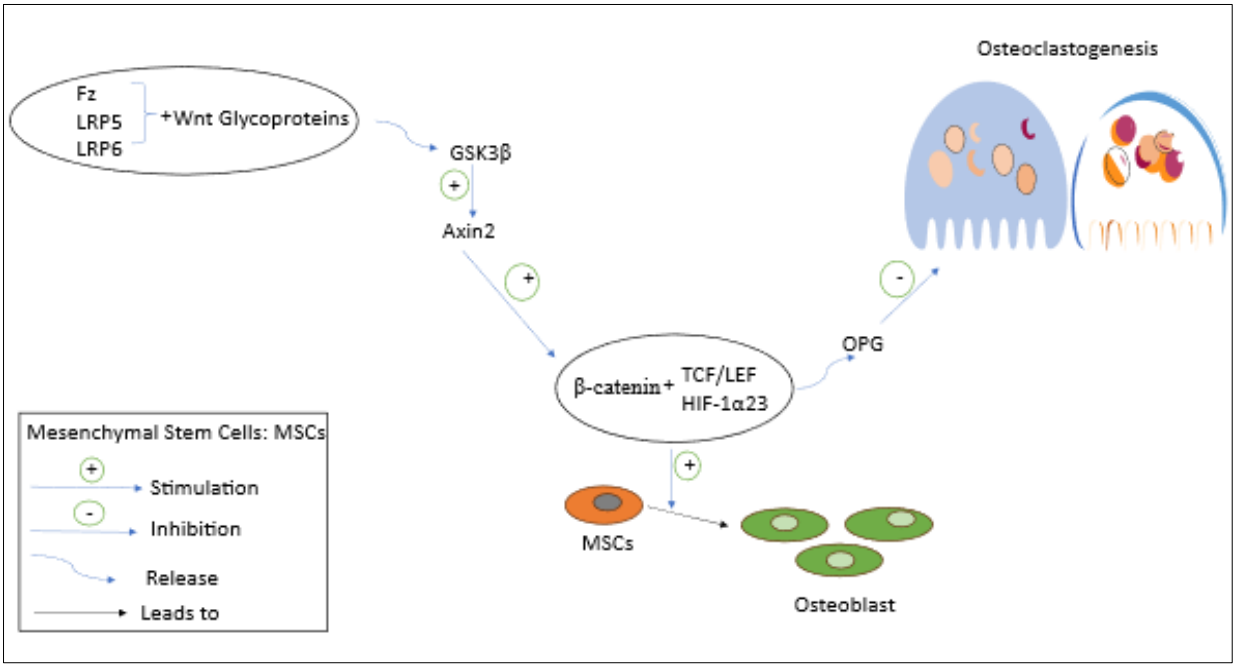

Figure 5: Wnt/β-catenin signaling pathway. In osteoblasts, Frizzled-2, Runx2, Axin2, and β-catenin are downstream factors of Wnt/β-catenin signal transduction that specifically support the growth and mineralization of osteoblasts. When Wnt glycoproteins attach to the Frizzled receptor (Fz) and co-receptor LRP5 or 6, Wnt/β-catenin signaling is initiated. GSK3β then stimulates Axin2, preventing the phosphorylation of β-catenin. As a result, β-catenin builds up, enters the nucleus, and interacts with many transcription factors, including, TCF/LEF, and HIF-1α23, to control the expression of numerous target genes. β-catenin suppresses osteoclastogenesis by increasing OPG production on osteoblasts and controlling RANK/RANKL/OPG signaling

7. Nanocarrier systems and their therapeutic applications in osteoporosis treatment

Melatonin loaded nanoparticles (MLNs), which increase melatonin therapeutic efficacy while reducing its disadvantages, are a major development in drug delivery systems. "Lipid-polymer hybrid nanoparticles, PLGA-based nanoparticles, Lecithin/Chitosan Nanoparticles (CS-NPs), Hydroxyapatite Nanoparticles (HANPs), Superparamagnetic Iron Oxide Nanoparticles (SPIONs), and Exosomes" are few of the nanocarriers that have been developed to enhance the stability, bioavailability, and targeted delivery of melatonin in many different clinical settings. (a)Chitosan Nanoparticles (CS-NPs)

Chitosan is a natural polysaccharide obtained from chitin in crustaceans, insects, and fungi, characterized by its hydrophilicity, biocompatibility, and biodegradability (Ways *et al*., 2018; Sivanesan *et al*., 2021). CS-NPs are effective drug carriers because of their small size, high encapsulation efficiency, and strong loading capacity (Ahmed, 2017; Zhuo *et al*., 2018; Pandey *et al*., 2018). They can also interact with a wide range of molecules, including plant compounds, nanomaterials, hormones, and proteins (Quiñones *et al*., 2018; Zhao *et al*., 2018). Saini *et al*., 2014 used CS-NPs to deliver RLX, which markedly enhanced its oral bioavailability (Saini *et al*., 2014). Similarly, PEGylated, CS-NPs were applied for PTH delivery, producing comparable effects (Narayanan *et al*., 2013). CS-NPs were used to deliver bisphosphonates, which significantly improved bone density and microstructure in osteoporotic rats while reducing cortical porosity on bone surfaces (Santhosh *et al*., 2019). It was used to encapsulate Human parathyroid Hormone 1-34 (PTH1-34) and the results demonstrated their biocompatibility and high encapsulation efficiency. Furthermore, the study confirmed the effectiveness of oral CS-NPs in delivering PTH1-34, highlighting this approach as a promising strategy for future osteoporosis treatment (OP) (Narayanan *et al*., 2012). In a study, researchers encapsulated Simulated Wound Exudate (SWE) in CS-NPs and tested their efficacy in osteoporotic rats. The findings indicated that CS-NPs enhanced the anti-osteoporotic effects of SWE suggesting that oral delivery of SWE *via* CS-NPs may serve as a promising strategy for OP treatment (Alshubaily & Jambi, 2022). In another study, researchers co-delivered Registered Dietitian Nutritionist (RDN) and Teriparatide (TPD) using hyaluronic acid modified CS-NPs. This dual loaded

system remained stable at low temperatures and promoted enhanced bone regeneration, indicating a promising approach for OP therapy (Abourehab, 2019).

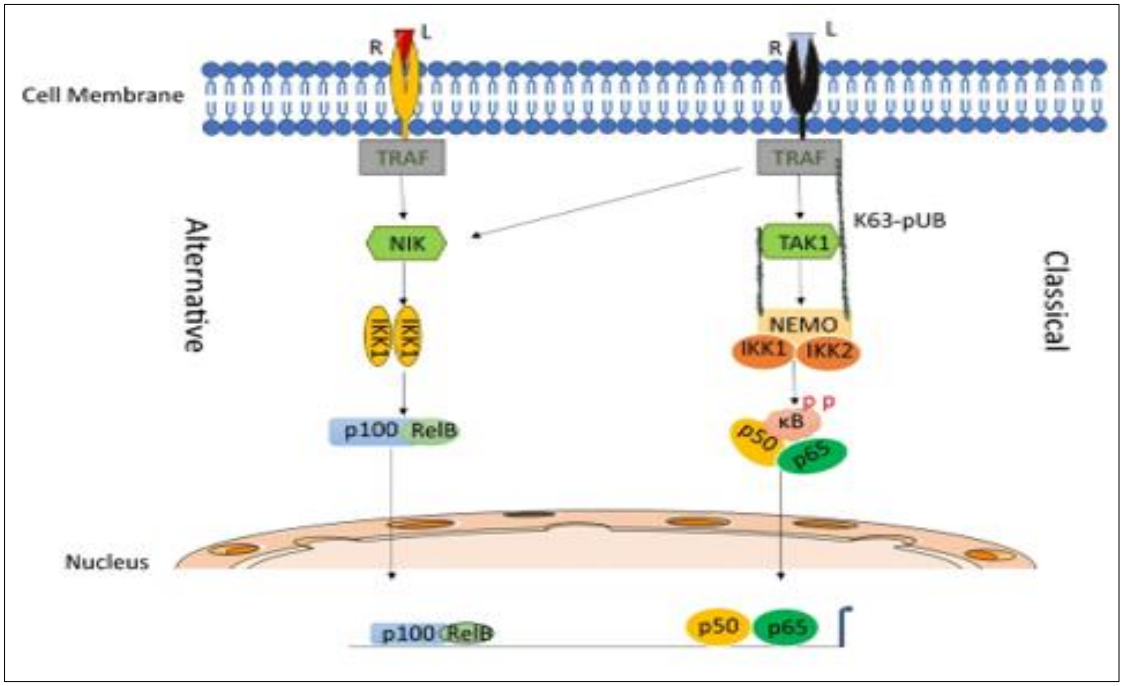

Figure 6: The classical and alternative arms of the NF- κB signaling pathway. When a ligand (L) binds to its cell membrane (CM) receptor (R), a signaling complex is recruited. Numerous proteins, such as TNF receptor-associated factors (TRAFs), the tyrosine kinase c-Src, p62, cellular inhibitors of apoptosis (c-IAP), and TNF receptor-interacting protein (RIP), are found in signaling complexes. The canonical and alternative IKK complexes are activated by the MAP kinases TGF-β-activated kinase (TAK1) and NF-κB-inducing kinase (NIK), which are recruited and activated by this cluster using lysine 63-linked polyubiquitination chains (K63-pUB). The p100/NF-kB and IkB targets that are phosphorylated (pp) by activated IKK1 and IKK2 consequently break down. RelB, released p50/p65 dimers, and processed p52 all go to the nucleus (Nuc), attach to DNA sequences, and trigger transcription (Abu-Amer, 2013)

(b) PLGA Nanoparticles

Poly (DL-lactic-co-glycolide) (PLGA) is a biocompatible material that was employed as a growth factor carrier in the 1990s. It can be readily synthesized and tailored to control polymer degradation and drug release kinetics (Walmsley *et al*., 2015). Advances in nanotechnology have led to extensive investigation of PLGA nanoparticles (PLGA-NPs) as drug delivery carriers (Lo *et al*., 2012). For example, to enhance the bioavailability of hydrophobic drugs, Xi *et al*., 2022 encapsulated Astragaloside (AS) within the hydrophobic core of PLGA nanoparticles. Polyethylene Glycol (PEG) conjugated the PLGA nanocarrier with Alendronate (AL) sodium to achieve bone targeting properties, resulting in an effective delivery system (Xi *et al*., 2022). *In vivo* and *in vitro* studies demonstrated that this nanocarrier improved oral bioavailability and exerted anti-osteoporotic effects, while the addition of AL further enhanced its bone targeting capacity. PLGA-NPs are being investigated as carriers for simvastatin, a drug known to promote bone formation and increase bone density but limited in clinical use due to its hydrophobicity and lack of targeting (Naito *et al*., 2013; Jia *et al*., 2014). To address this limitation, researchers modified PLGA-NPs with tetracycline to confer bone targeting properties. *In vivo* studies demonstrated that simvastatin-loaded, tetracycline modified PLGA-NPs significantly increased bone density in osteoporotic rats compared with free simvastatin and non-targeted nanoparticles (Yuan *et al*., 2015). Similarly, researchers used PLGA-NPs as carriers to deliver estradiol to osteoporotic rats, administering the drug through iontophoresis. The study showed that the negative surface charge of PLGA-NPs, combined with this delivery method, enhanced blood estradiol levels and improved treatment efficacy in osteoporotic rats (Takeuchi *et al*., 2016). Zhang *et al*., 2022 encapsulated Mesenchymal Stem Cells (MSC) Secretome (Sec) into PLGA-NPs and endowed the carrier with bone targeting ability through CXCR4 modification. In an osteoporotic rat model, these nanoparticles accumulated in bone, inhibited osteoclast differentiation, and promote osteoblast proliferation, thereby reducing bone loss induced by surgery (Zhang *et al*., 2022). In another study, PLGA/HAP composite nanocarriers were implanted subcutaneously in mice to deliver BMPs, which led to enhanced bone formation (Kang *et al*., 2009). The PLGA/HAP nanofiber showed favorable morphology and mechanical strength, and when used as a carrier, it enabled sustained BMP release while preserving biological activity *in vivo* (Fu *et al*., 2007).

(c) Hydroxyapatite Nanoparticles (HAPNPs)

Hydroxyapatite, (HAP) with the formula $Ca_{10}(OH)_2(PO_4)_6$, has a structure closely resembling natural bone, making it a promising material for biomedical use in bone diseases. As proposed by Ginebra *et al*.,1999, one potential application of HAP is as bone cement or graft, owing to its osteoconductive and injectable properties. Several studies have investigated the application of bone cement to reinforce osteoporotic bones (Zhou & Lee, 2011; Bai *et al*., 1999; Schildhauer *et al*., 1999; Maestretti *et al*., 2006; Libicher *et al*., 2006). HAP- based bone cement provides key advantages, including low temperature solidification and inherent porosity, which allow it to deliver drugs or active agents for combined therapeutics effects (Ginebra *et al*., 2012). Panzavolta *et al*., 2008 successfully combined bisphosphonates with hydroxyapatite (HAP), creating drug-loaded bone cement with favorable mechanical properties. This drug–HAP combination showed potential to inhibit bone resorption, offering therapeutic benefit in alleviating osteoporosis (Panzavolta *et al*., 2008; Panzavolta *et al*., 2010). Dave *et al*., 2018 synthesized nHAP nanoparticles loaded with Parathyroid Hormone (PTH), enabling targeted delivery of PTH to osteoporotic bone (Dave *et al*., 2018). By dissolving within the bone tissue, these nanocarriers boost local PTH synthesis and metabolism while reinforcing the matrix constitutes parts. In different study, nHAP was inserted into rabbit radial bone defects along with Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2). The usefulness of nHAP as a growth factor carrier was highlighted by the results, which demonstrated that carrying growth factors, considerably increased the growth of bones (Zhu *et al*., 2010). Additionally, nHAP carriers have been employed to carry bisphosphonates, which are often used in OP therapy. Positive anti-osteoporotic effects were shown by this method. According to *in vitro* studies, nHAP increased the inhibitory effects of bisphosphonates on the development of osteoclasts, and its illusion into hydrogels further promoted mineralization, suggesting potential as a novel bone repair material (Kettenberger *et al*., 2015). Salmon Calcitonin (SCT) has been administered using surface stabilized nHAP made by aqueous precipitation. These carriers demonstrated excellent stability, permeability, and loading efficiency. nHAP mediated SCT administration demonstrated significant bone healing in osteoporotic rat models, indicating its promise as an injectable treatment for OP (Kotak & Devarajan, 2020). Similarly, tradition adsorption was used to load Zoledronic Acid (ZOL) into nHAP (ZOL-nHAP). Three months of ZOL- nHAP treatment reversed bone loss, maintained trabecular architecture, and mechanical strength in osteoporotic rats, improving on ZOL alone (Khajuria *et al*., 2014).

(d) Superparamagnetic Iron Oxide Nanoparticles (SPIONs)

Extensively study on magnetic nanoparticles (NPs) emerged in the 1970s when freeman *et al*., 1960 first presented the use of magnetism in medical applications (Freeman *et al*., 1960). Due to their unique properties, SPIONs have become one of the most extensively researched targeted nanomaterials. They have good chemical stability and may be produced from a single source material using comparatively easy technique (Li *et al*., 2018). SPIONs are less harmful than manganese or gadolinium NPs and exhibit good biocompatibility and biological safety (Dadfar *et al*., 2019; Zhi *et al*., 2019). Beyond acting as carriers, SPIONs are essential to the treatment of OP. *In vitro* research, they inhibit the production of osteoclasts while promoting osteoblast differentiation. Further *in vivo* studies showed that it helps stop bone loss and accelerate the healing of bone abnormalities (Li *et al*., 2018; Yang *et al*., 2022; Liu *et al*., 2019). A dual-target scaffold carrier doped with SPIONs and nHAP had been studied by Marycz *et al*., 2021 to deliver miR-21 and miR-124 (Marycz *et al*., 2021). These miRNA molecules were acquired and then released under the scaffolds and the applied magnetic field effect. This increased osteoblast activity and decreased osteoclast activity encouraged the regeneration of osteoporotic bone. In a different study, Bone Marrow Stromal Cells (BMSCs) were implanted into the backs of nude mice after SPIONs were integrated into silk fibroin/hydroxyapatite scaffolds. The finding showed that BMSCs attached, multiplied, and added in the production of new bone. Significantly, by adding magnetic particles, SPIONs increased the stability of silk fibroin/hydroxyapatite scaffolds as well as their capacity for forming bone (Liu *et al*., 2020).

(e) Exosomes

Exosomes are tiny, 40-160 nm diameter vesicles enclosed in a lipid bilayer (Kalluri & LeBleu, 2020). Researchers suggest that cells encapsulate proteins, mRNA, lipids, and microRNAs (miRNAs) in exosomes to facilitate effective intracellular communication (Liang *et al*., 2021; Xu *et al*., 2023). Due to their inherent role as communication carriers, researchers are exploring exosomes as potential drug delivery system. As a potentially effective method of treating OP, researchers are investigating modified exos loaded with therapeutics substances. To treat OP, researchers developed an exos based delivery method using human Induced Pluripotent Stem Cells (iPSCs). These exos generated from Mesenchymal Stem Cells (MSCs) were designed to transport siRNA that target the Shn3 gene (siShn3) to osteoblasts. This strategy inhibited osteoclast development and produced anti-osteoporotic effects by suppressing Shn3 expression, promoting osteoblast differentiation, and lowering the expression of Receptor Activator of Nuclear Factor-kB Ligand (RANKL; Cui *et al*., 2021). Lu *et al*., 2020 also studied stem cell derived exos, demonstrating that BMSC-derived exosomes loaded with miR-29a had high osteogenic activity and emphasized their potential as an OP treatment (Lu *et al*., 2020). Furthermore, a recent study used extracellular vesicles produced from red blood cells as delivery vehicles to deliver anti-miR-214

to osteoclasts *via* a bifunctional peptide. The findings showed that these vesicles had the ability to target bone, successfully supress osteoclast activity, and promote osteogenesis. Consequently, these carriers are a viable approach to treating OP (Xu *et al*., 2023). Hu *et al*., 2021 fused exos with liposomes to create hybrid exosomes and used them to deliver antagomir-188 to the skeleton *via* the C-X-C motif Chemokine Receptor 4 (CXCR4). This targeted deliver promoted osteoblastic differentiation of BMSCsand effectively reversed age-related trabecular bone loss (Hu *et al*., 2021).

(f) Lipid Nanocarriers

Researchers are developing lipid nanocarriers that target bone tissue selectively in order to treat bone disorders. Song *et al*., 2015 developed pamidronate-conjugated liposomes, while Ferreira *et al*., 2020 created alendronate salt-conjugated PEGylated liposomes. The latter revealed efficient bone targeting capacity, while the former exhibited increased affinity for bone tissue (Song *et al*., 2015; Oliveira *et al*., 2015). In a recent study, antagomir-148a (a miRNA inhibitor of osteoclastogenic miR-148a) was delivered using modified liposomes. In osteoporotic animals, these liposomes preferentially aggregated in bone, inhibited the expression of miR-148a in osteoclasts, and subsequently decreased bone resorption (Liu, *et al*., 2015). Researchers employed Lipid Nanoparticles (LNPs) to deliver siRNA that targets the GNAS gene in MSCsin another study on LNPs mediated nucleic acid delivery. Experiments conducted both *in vivo* and *ex vivo* demonstrated that this method successfully reduced GNAS expression and increased MSCs capacity for osteogenic differentiation. This strategy shows the potential of LNPs siRNA delivery as an OP treatment method (Basha *et al*., 2022). A novel ionizable lipid with a C18 tail and an ionizable head group was developed to deliver the Bone Morphogenic Protein-9 (BMP-9) gene for OP therapy. In this study, the ionizable LNPs demonstrated high delivery efficiency, and both *ex vivo* and *in vivo* experiments confirmed the effective transfection and safety of BMP-9, leading to the reversal of OP (Vhora *et al*., 2019). To address the limited oral bioavailability of vitamin D and raloxifene (RLX) hydrochloride, researchers, created a lipid nanocarriers that can carry both substances at the same time. Pharmacokinetic investigations in healthy individuals revealed that these nanocarriers enhanced the average level of vitamin D metabolites from 91 ± 29 nmol/L to 174 ± 36 nmol/L and increased RLX bioavailability by 385.6% when compared to traditional commercial formulations(Hosny *et al*., 2020).The interaction between the lipid carriers and bile salts after enzymatic breakdown in the intestines, which shields the medications from premature metabolism, is probably responsible for the increased bioavailability (Hosny *et al*., 2020). Another LNPs composed of D-alpha-Tocopheryl Polyethylene Glycol Succinate (TGPS), glyceryl distearate, and Carbopol 940 was created as a Bioadhesive system. This formulation successfully encapsulated Raloxifene (RLX) and showed enhanced bioavailability in rat models, suggesting its potential as an effective drug delivery system for the treatment of OP (Du *et al*., 2021). Although it has been demonstrated that Simvastatin (SIM) enhances osteoblast development and mineralization, its low bioavailability and poor bone targeting restrict its therapeutic application. In order to get over these restrictions, SIM was delivered directly to bone tissue using LNPs and a targeting peptide, which increased its osteogenic impact. This method proved that LNPs are efficient OP treatment carriers (Tao *et al*., 2020).

Table 3: Nanocarrier systems and their therapeutic applications in Osteoporosis Treatment

Nanocarriers	Properties	Carrier	Therapeutic Agent	Results	Reference
CS-NPs	Naturally abundant and stable during storage	CS-NPs	RLX, PTH, RDN	Enhancing oral drug bioavailability while preventing bone loss	(Saini *et al*., 2014; Narayanan *et al*., 2013; Santhosh *et al*., 2019)
		CS-NPs	PTH-134	Good biocompatibility with efficient embedding and effective delivery	(Narayanan *et al*., 2012)
		CS-NPs Hyaluronic acid-CSNPs	SWE RDN and TPD	Strengthen the ability of SWE to counteract osteoporosis, Promoting bone regeneration through combined actions	(Alshubaily & Jambi, 2022) (Abourehab, 2019)
PLGANPs	Biodegradable with favorable drug release profile	AL sodium-mPEG-PLGA	Astragalosie	Enhancing the oral bioavailability and anti-osteoporotic efficacy of AS	(Xi *et al*., 2022)
		Tetracycline decorated	Simvastatin	Enhancing bone density in osteoporotic rats	(Yuan *et al*., 2015)

		PLGA NPs	Estradiol	Increasing blood estradiol levels to improve osteoporosis treatment efficacy	(Takeuchi *et al*., 2016)
		PLGA NPs	MSC-Sec	Suppressing osteoclast differentiation while stimulating osteoblast proliferation	(Zhang *et al*., 2022)
		PLGA/HAP	BMP	Enhancing bone formation in mice	(Kang *et al*., 2009)
nHAP	Structurally resembling bone tissue and exhibiting a natural bone-cement effect	nHAP	PTH	Synergistically enhancing bone matrix formation	(Dave *et al*., 2018)
		nHAP	RhBMP-2	Synergistically stimulating bone formation	(W. Zhu *et al*., 2010)
		nHAP	bisphosphonate	Enhancing bisphosphonate-mediated inhibition of bone resorption	(Kettenberger *et al*., 2015)
		nHAP	SCT	Effective bone repair observed in vivo	(Kotak & Devarajan, 2020)
		nHAP	ZOL	Inhibiting bone loss, preserving trabecular structure, and strengthening bone	(Khajuria *et al*., 2014)
		Zinc-nHAP	RDN	Preserving the structure of cortical and trabecular bone	(Khajuria *et al*., 2016)
	Superparamagnetic properties that promote bone regeneration and suppress bone loss	nHAP-based composite co-doped with SPIONs	MiR-21, miR-124	Enhancing osteoblast activity while suppressing osteoclast activity	(Marycz *et al*., 2021)
		silk Fibroin/ hydroxyapatite scaffolds incorporate with SPIONs	BMSCs	Promoting BMSC adhesion and proliferation while enhancing osteogenic activity	(Liu *et al*., 2020)
Exos	Abundant source suitable for autologous transplantation	iPSC-Exo	SiRNA-Shn3	Silencing the Shn3 gene reduces autologous RANKL expression and inhibits osteoclast formation	(Cui *et al*., 2021)
		Hybrid Exo (Exo with liposome)	Antagomir-188	Promoting osteogenic differentiation of BMSCs while preventing bone loss	(Hu *et al*., 2021)
		BMSC-derived exosomal	MiR-29a	Enhancing osteogenesis	(Lu *et al*., 2020)
		Red Blood cell extracellular vesicles (RBCEVs)	Anti-miR-214	Suppressing osteoclast activity while enhancing osteogenesis	(Xu *et al*., 2023)
Lipid nanocarriers	Easily modifiable with high loading efficiency	Liposome	Antagomir-148a	Suppressing osteoclast-mediated bone resorption	(Liu, Dang, *et al*., 2015)
		Lipid nanoparticle (LNP)	SiRNA-GNAS	Enhancing the differentiation MSCs into osteoblasts	(Basha *et al*., 2022)
		Ionizable LNP	BMP-9 gene	Demonstrated safety and effective bone regeneration	(Vhora *et al*., 2019)
		Nanostructure lipid carrier	RLX hydrochloride, Vitamin D	Enhancing drug permeability	(Hosny *et al*., 2020)
		Solid LNP (SLNP)	RLX hydrochloride	Enhanced pharmacological effects	(Nabi-Meibodi *et al*., 2013)
		Bioadhesive nanoparticle	RLX	Enhancing the oral bioavailability of drugs	(Du *et al*., 2021)
		LNP	SIM	Enhancing SIM-induced bone formation	(Tao *et al*., 2020)
		Bilosome	Risedronate (RDN)	Enhancing drug permeability while minimizing toxicity	(Elnaggar *et al*., 2019)

8. Obstacles and future directions of melatonin loaded nanomedicines in Osteoporosis Treatment

8.1 Personalized nanomedicine for osteoporosis treatment

A possible type of personalized nanomedicine for OP is MLNPs. These nanoparticles exploit melatonin dual function of boosting osteoblast activity and suppressing osteoclast growth by enhancing medication delivery and targeting treatment to certain bone locations. They provide a more successful therapy approach by directly addressing the major mechanism causing osteoporosis. Melatonin may work in association with treatments like bisphosphonates to strengthen bones and promote healing (Zheng *et al*., 2023). Drug transport efficiency can be increased by creating functionalized nanoparticles that bind to bone tissue more successfully. The prolonged release of melatonin enabled by these nanoparticles prolongs its therapeutic effect while lowering undesirable systemic side effects (Mishra & Shukla, 2024; Cai *et al*., 2025). In modern medicine, melatonin and associated treatments offer a potential path. It is essential for controlling circadian rhythm because it affects clock genes. The hypothalamic suprachiasmatic nucleus (SCN) expresses these genes in rhythmic manner. For example, melatonin release and pineal gland activity are tightly associated with the diurnal change of the clock gene per1. Turkish scientist Aziz Sancar, who was awarded the Nobel prize (2015), significantly advanced our knowledge of these molecular pathways. Building on the understanding, MLNPs can alter these pathways and gene expressions, providing novel therapeutic options for osteoporosis (Manev & Uz, 2006). A multifaceted approach is required due to the complicated nature of osteoporosis, which is influenced by numerous genetic, environmental, and lifestyle factors. But new developments in omics technologies, such as proteomics, metabolomics, transcriptomics, genomics, and epigenomics, provide effective tools for analyzing these complex relationships. By combining these omics techniques, we hope to improve our knowledge of osteoporosis susceptibility and develop personalized risk prediction models. This will make it possible to identify high-risk individuals earlier, enabling immediate action and possibly resulting in more efficient, focused treatments (Enitan *et al*., 2023; Shashidhara *et al*., 2025).

8.2 Need for long-term animal and clinical trials

Melatonin-loaded nanoparticles (MLNs) are a unique way to treat osteoporosis because nanoparticles-based systems for drug delivery provide greater bioavailability, controlled release, and targeted management.

8.2.1 Reasons for prolonged Animal studies:

(a) Bone Remodelling Dynamics: Since osteoporosis takes years to develop, short term studies lack the ability to accurately measures the long-term effects of MLNs on the turnover (Bonucci & Ballanti, 2013).

(b) Biodistribution and pharmaceutical kinetics: Drug metabolism and tissue targeting are modified by nanoparticle compositions. To evaluated prolonged release, storage in bone, and removal from non-target organs, long-term animal investigations are crucial (Chuffa *et al*., 2021).

(c) Toxicology and safety: Nanoparticle accumulation in the liver, kidney, or spleen may result from long-term dosage. Subtle toxicities that are not visible in short-term studies can be found in long-term animal testing (Attari *et al*., 2022).

(d) Comparative effectiveness: Experiments conducted on various animal models, such as rats, rabbits and non-human primates, may enhance translational relevance and provide insight into species-specific responses.

8.2.2 Significance of Clinical Studies:

(a) Stats for quality of life: Trails should evaluate patient-reported outcomes, including pain relief, mobility, and tolerance, in addition to BMD.

(b) In various kinds of demographics: Individuals with complications, postmenopausal women, and elderly patients may react differently. Clinical trials identify rare adverse effects and provide widespread use (Chuffa *et al*., 2021).

(c) Long-term effectiveness: Treatments for osteoporosis must show an ongoing decline in fracture risk. Long-term studies can verify if MLNs sustain gains in BMD over time.

(d) Optimization of dosage: Melatonin nanoparticles kinetics in humans may be distinct from those of free melatonin. To determine safe and efficient dosage plans, clinical trials are necessary (Jazi *et al*., 2023).

9. CONCLUSION

Melatonin-loaded nanoparticles (MLNs) integrate the preventive properties of melatonin with the accuracy of nanotechnology to offer an effective therapy for osteoporosis. Their nanosized carriers offer regulated release, increased bone mineral density, improved absorption, targeted distribution, and fewer systemic side effects. These advantages exceed the drawbacks of existing treatments, although issues including toxicity, long-term safety, regulatory and statutory approval, and scalable production still exist. Long-term animal and clinical trials are essential for understanding the chronic nature of osteoporosis. The establishment of safe, efficient, and site-specific osteoporosis therapeutics, using MLNs, requires ongoing studies into nanocarrier systems.

Abbreviations:

MLNs	Melatonin loaded nanoparticles
BMD	Bone Mineral Density
CS-NPs	Chitosan Nanoparticles

PLGA	Poly D, L-lactic-co-Glycolic Acid
HAPs	Hydroxyapatite Nanoparticles
SPIONs	Superparamagnetic Iron Oxide Nanoparticles
Exos	Exosome
LNPs	Lipid Nanoparticles
SCN	Suprachiasmatic nucleus
WHO	World Health Organization
CCD	Cleidocranial Dysplasia
PRMT1	Protein Arginine Methyltransferase 1Reactive
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
iNOS	Inducible Nitric Oxide Synthase (iNOS)
PTH	Parathyroid Hormone
SERMs	Selective Estrogen Receptor Modulators
HRT	Hormone Replacement Therapy
ONJ	Osteonecrosis of the Jaw
FDA	Food and Drug Administration
DCM	Dichloromethane
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ALP	Alkaline Phosphatase
BUN	Blood Urea Nitrogen
LIF	Leukemia Inhibitory Factor
CT	Cardiotrophin
CNTF	Ciliary Neurotrophic Factor
CLCF	Cardiotrophin-Like Cytokine Factor
NP	Neuropoietin
MMP	Matrix Metalloproteinase
DMP	Dentin Matrix Protein
TIMP	Tissue Inhibitor of Metalloproteinases
LRP	Lipoprotein Receptor-Related Protein
GSK3β	Glycogen Synthase Kinase 3β
SFRPs	Secreted Frizzled-Related Proteins
WIF	Wnt Inhibitory Factor
TRAFs	TNF Receptor-Associated Factors
c-IAP	Cellular Inhibitors of Apoptosis
RIP	Receptor-Interacting Protein
TAKTGF-β	Activated Kinase
NIKNF-κB	Inducing Kinase
RDN	Registered Dietitian Nutritionist
TPD	Teriparatide
rhBMP	Recombinant Human Bone Morphogenetic Protein
MSC	Mesenchymal Stem Cell
iPSCs	Induced Pluripotent Stem Cells
RANKL	Receptor Activator of Nuclear Factor-kB Ligand

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