Abstract. Bone-related diseases comprise a large group of common diseases, including fractures, osteoporosis and osteoarthritis (OA), which affect a large number of individuals, particularly the elderly. The progressive destruction and loss of alveolar bone caused by periodontitis is a specific type of bone loss, which has a high incidence and markedly reduces the quality of life of patients. With the existing methods of prevention and treatment, the incidence and mortality of bone-related diseases are still gradually increasing, creating a significant financial burden to societies worldwide. To prevent the occurrence of bone-related diseases, delay their progression or reverse the injuries they cause, new alternative or complementary treatments need to be developed. Melatonin exerts numerous physiological effects, including inducing anti-inflammatory and antioxidative functions, resetting circadian rhythms and promoting wound healing and tissue regeneration. Melatonin also participates in the health management of bone and cartilage. In the present review, the potential roles of melatonin in the pathogenesis and progression of bone injury, osteoporosis, OA and periodontitis are summarized. Furthermore, the high efficiency and diversity of the physiological regulatory effects of melatonin are highlighted and the potential benefits of the use of melatonin for the clinical prevention and treatment of bone-related diseases are discussed.

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1. Introduction

Bone-related diseases, such as fractures, osteoporosis and osteoarthritis (OA), severely affect the quality of life of patients due to their high incidence, slow recovery, associated pain and negative effects on patient behavior. Due to the aging of the population in several countries, the prevalence of bone-related diseases is projected to further increase in the following few decades. Data from 2013 indicated that ~57 million Americans aged >50 years suffered from bone diseases, among which 48 million had osteopenia and 9 million had osteoporosis. The diseases were accompanied by a risk of fracture in these individuals. Without intervention, the prevalence of osteopenia is projected to increase to 64.3 million American individuals and that of osteoporosis to 11.9 million by the year 2030 (1). Bone disease is the focus of a large number of clinical studies, due to the accompanying high combined lifetime risk of forearm, hip and vertebral fractures (40%), which is comparable to the rate of cardiovascular diseases (2). In addition to the high morbidity...
and mortality rates, fractures related to osteoporosis have also created a significant financial burden to societies worldwide. For instance, osteoporosis-related costs in the European Union amounted to ~€37 billion in 2010; the majority of that amount was spent on fracture therapy and long-term fracture care, accounting for 66 and 29%, respectively, of the budget (3).

Melatonin, a common molecule with a simple structure known as N-acetyl-5-methoxytryptamine, exists in almost all living organisms (4). Melatonin is secreted by pinealocytes in the pineal gland, while certain tissues can also produce a small amount of melatonin locally (5). There is a synchronization between melatonin production and the light/dark (L/D) cycle. Melatonin is synthesized and secreted in dark environments. When retinal photoreceptive ganglion cells are stimulated by light (mainly in the blue range), the synthesis and secretion of melatonin reduces until no more is secreted (6,7). Generally, levels of melatonin begin to increase early in the evening and peak at 12-2 a.m., followed by a progressive decrease thereafter (8). As the organism ages, melatonin production gradually declines. Melatonin levels continuously decrease from the age of 40-45 years (6).

Currently, melatonin is considered a potent cytoprotective agent, rather than a hormone in the classical sense (9). Melatonin has excellent lipophilic properties and can easily enter the cell membrane and subcellular compartment (10). Melatonin can synchronize the circadian clock in peripheral tissues, maintain the synchronization of bone metabolism with L/D cycles and participate in numerous important physiological processes, such as anti-inflammatory, antitumor and antioxidation processes, as well as regulating circadian and endocrine rhythms, regulating immunity, and promoting wound healing and tissue regeneration (7,11). Melatonin plays a positive role in bone-related diseases by exerting multiple effects. Although there are several physical and drug treatments for bone-related diseases, melatonin has the advantage over other drugs of being inexpensive, and having a wide safety margin, a wide impact on tissues and almost no side effect, suggesting its potential as a main or complementary treatment strategy for a large range of bone diseases.

2. Bone injury

Basic study of bone injury. Bone injury is very common in clinical practice. A variety of pathologies, such as tumors, trauma and surgery, as well as other factors, are likely to cause varying degrees of bone injury. This is a major issue for clinical treatment at present, and an important challenge that will threaten human health in the ensuing 50 years. The bone defect size largely determines the amount of bone repair. The larger the defect, the more difficult to obtain a satisfactory repair (12). Bone repair is often affected by a variety of negative effects, such as possible infection and ischemia of the bone injury site or adjacent tissues, and systemic diseases. Therefore, clinical intervention is required for bone repair.

Possible effects of melatonin on bone injury repair. As shown in Fig. 1, considerable evidence has demonstrated that melatonin contributes to bone repair. There are different claims about the effects of melatonin on osteogenic and osteoclastic activities. Melatonin has been shown to enhance the vertical bone augmentation of rat calvaria by increasing new bone regeneration, neovascularization and the number of osteoblast-like cells (13). It has also been shown to increase the cartilage and callus at the fracture site (14). At pharmacological doses, melatonin has been shown to stimulate osteoblast proliferation and alkaline phosphatase (ALP) activity in a dose-dependent manner. In vitro, melatonin increases the expression of collagen type I (Col1), bone sialoprotein, osteopontin and osteocalcin (OCN), and promotes the production of mineralized extracellular matrix (ECM). At the same time, femoral neocortical bone is enhanced in mice administered an intraperitoneal (i.p.) injection of melatonin (15). However, Histing et al (14) reported that melatonin delayed bone healing in a mouse model of femoral fracture. They explained that melatonin exerted a positive effect on bone repair by inhibiting bone resorption instead of promoting bone regeneration. The levels of receptor activator of NF-κB ligand (RANKL) were reduced in the mice with fractures that received a daily dose of 50 mg/kg melatonin, which may inhibit bone resorption by damaging the balance between osteoprotegerin (OPG) and RANKL.

In addition to exhibiting osteogenic and osteoclast activity, the role of osteoblast and osteoclast differentiation during the process of bone repair is also of interest. Sethi et al (16) noted that osteoblast differentiation requires chronic and uninterrupted melatonin exposure. Melatonin may promote mesenchymal stem cell (MSC) proliferation and migration, at least partly by upregulating neurotropic Y (NPY) and NPY receptor Y1 (NPY1R), accelerating osteogenic differentiation and promoting fracture healing in the rat femur. Concurrently, NPY/NPY1R expression has been shown to be increased in the fracture zone and serum (17). Osteogenic and chondrogenic differentiation can be promoted, and adipogenesis can be inhibited through the enhancement of runt-related transcription factor 2 expression and the Wnt/β-catenin signaling pathway, as well as the inhibition of peroxisome proliferator-activated receptor (PPAR)-γ (18). The upregulation of the platelet-derived growth factor/protein kinase B pathway partly contributes to the enhanced osteogenic potential and weakened osteoclastic differentiation of MC3T3-E1 cells, and promotes fracture healing in mice with femoral fracture following melatonin treatment (19). In addition, the BMP, extracellular regulated kinase (ERK) and Wnt signaling pathways also participate in the process, while melatonin can improve wound healing and trigger osteogenesis markers in a dose-dependent manner (20). In addition, endochondral bone formation is an important form of osteogenesis. Melatonin increases the expression of chondrogenic differentiation genes in MSCs, which could be partly blocked by luzindole (21).

Inflammation and oxidative stress are inevitable during bone injury. Melatonin is known for its potent antioxidative and anti-inflammatory properties. Oxidative stress produces reactive oxygen species (ROS) and is usually promoted by aging (22), which can lead to excessive bone resorption (23,24). Melatonin is considered a potent natural antioxidant, not only due to the direct inhibition of ROS, but also due to the mobilization of the intracellular antioxidative enzyme system. Melatonin can protect MSCs against oxidation-induced apoptosis by reducing ROS production, enhancing cell viability and promoting continued differentiation (25). Melatonin can
increase solute carrier family 39 member 1 expression, activate the mitogen-activated protein kinase (MAPK)/ERK pathway, increase phosphorylated-ERK1/2/5 levels and significantly inhibit the production of ROS; moreover, zinc uptake in cells is increased (26). All the above-mentioned processes can inhibit cell apoptosis (27). In addition, melatonin is beneficial for the inhibition of oxygen free radical activity during fracture healing and the regulation of antioxidant enzyme activity, which promotes fracture healing. In a previous study, it was observed that in contrast to the fracture group, more bone binding was observed in the melatonin treatment fracture group at the same healing time (28 days after the fracture) (28).

Vascular injury often exists simultaneously in bone injuries, such as fractures, which inevitably leads to ischemia and/or hypoxia at the injury site and is extremely unfavorable for the repair of defects. Ischemia/reperfusion injury can cause excessive ROS production in tissues and lead to cell damage (29,30). Melatonin can eliminate these adverse effects and can be used in fractures with vascular injury and compartment syndrome (31). In hypoxic environments, MC3T3-E1 cells stimulated by melatonin prefer to differentiate towards osteoblasts and promote mineralization through the p38 mitogen-activated protein kinase and protein kinase D1 pathways (32). The effect of melatonin on angiogenesis mediators also plays an important role in bone regeneration (33). Growth factors are considered potential modulators of angiogenesis. For instance, vascular endothelial growth factor (VEGF) contributes to angiogenesis (34). Melatonin treatment has been found to elevate the level of VEGF during granulation tissue formation and accelerate the angiogenic process (35), indicating that melatonin can provide beneficial effects to bone defect repair with vascular injury.

Moreover, diabetes mellitus (DM) induces high levels of ROS production. In DM model rats with fractures, the process of fiber formation and trabecular mineralization was more rapid in the melatonin treatment group compared with the control group (36).

As previously demonstrated, the use of melatonin pre-operatively helped improve the quality sleep of patients and reduced the use of opioid drugs during surgery. The normal circadian rhythm of melatonin secretion can be altered by anesthesia (37). The pre-operative use of melatonin may also have the potential to reduce the incidence of delirium; however, certain existing studies have reached varying conclusions. Al-Aama et al (38) thought exogenous melatonin administered nightly may decrease delirium occurrence in elderly medical in-patients. Sultan (39) pointed out that melatonin was successful in decreasing post-operative delirium. However, the study by de Jonghe et al (40) came to the conclusion that treatment with melatonin did not reduce the incidence of delirium in older-aged patients with hip fracture surgery. Melatonin can be used in combination with various bone graft materials to stimulate bone regeneration in large or comminuted bone injuries (41). In addition, melatonin can significantly protect bones from radiation injury and prevent epiphyseal growth plate damage (42).

The time and dose of melatonin treatment is worthy of consideration, since high doses, such as 50 mg/kg, can cause decreased bone remodeling in mice, thus delaying fracture healing (14). Melatonin supplementation should also be carried out at moderate doses to avoid adverse effects.
out at night as far as possible, to adhere to its natural secretion law, to avoid breaking the normal secretory circadian rhythm, which can have adverse consequences. Evidence from the relevant animal studies is presented in Table I.

3. Osteoporosis

Study basics of osteoporosis. Osteoporosis is considered one of the most common diseases, and is becoming increasingly prevalent with the aging of the global population. Millions of individuals worldwide suffer from osteoporosis, particularly postmenopausal women (43-45) and the elderly (46,47). The reduced bone density and damaged bone architecture caused by osteoporosis can increase the risk of fragility fractures, and lead to a higher morbidity and mortality. A variety of drugs with varying degrees of efficacy and side-effects have been used for the treatment of osteoporosis (48). Even with the existing osteoporosis treatments, the prevalence of osteoporosis is steadily increasing (49), which has created a huge economic burden to societies worldwide (50-52). Thus, novel strategies need to be developed to prevent or combat bone loss for the treatment of osteoporosis and its complications.

Potential effects of melatonin on osteoporosis. Melatonin is involved in the regulation of bone mass accumulation and loss (Fig. 2). Egermann et al (53) confirmed that the bone mass significantly decreases following pinealectomy. The decrease of melatonin secretion is associated with menopause and is one of the most important causes of osteoporosis (54). The production of melatonin decreases with age (55), which may lead to a higher bone loss among the elderly. In addition, the expression of melatonin receptor 1A (MTNR1A) on the surface of human osteoblasts decreases with age, which is more pronounced in women (15).

It has been demonstrated that melatonin supplementation can improve perimenopausal- and age-related osteoporosis. Melatonin supplementation is well-tolerated and can attenuate perimenopausal symptoms, as well as restore the balance of bone remodeling to avoid bone loss and osteoporosis (56). The daily oral melatonin administration (100 mg/kg body weight) has been reported to increase bone formation to prevent ovariectomy-induced bone degeneration in mice (57). Although its efficacy requires further confirmation, melatonin is considered a safe nutritional supplement for peri- and postmenopausal women to improve bone density (58). Moreover, a dietary melatonin supplement has been shown to improve the microstructure and biomechanical properties of the bones of aged rats (59).

Estrogen deficiency, the major characteristic of menopause, contributes to osteoporosis. Increased levels of nucleotide-binding domain and the leucine-rich repeat pyrin 3 domain (NLRP3) inflammasomes have been observed in the hippocampus of female mice with estrogen deficiency (60). Melatonin can attenuate osteoporosis induced by estrogen deficiency and can improve the osteoblastic differentiation potential by inhibiting NOD-, LRR- and pyrin domain-containing protein 3 inflammasome activation. The modulation of the Wnt/β-catenin pathway is involved in this process (61).
Table I. Evidence of the effects of melatonin on bone injury in animal studies.

| Refs. | Objectives | Model | Route of administration | Time of administration | Frequency | Doses | Duration | Outcomes |
|-------|------------|-------|--------------------------|------------------------|-----------|-------|---------|----------|
| 13    | Fischer rats | Calvarium holes | Powder local implantation | - | Only once | 10 mg | - | After 12 weeks: Increased new bone regeneration, neovascularization and osteoblast-like cells |
| 14    | CD-1 mice | Femur fracture | i.p. | - | Daily | 50 mg/kg | 2 and 5 weeks | 2 weeks: Delayed healing 5 weeks: Increased cartilage and callus at the fracture site |
| 15    | Mice | - | i.p. | - | Daily | 100 mg/kg | 21 days | More new femoral neocortical bone formation |
| 17    | Sprague-Dawley rats | Femoral fracture | i.p. | Morning | Daily | 30 mg/kg | 8 weeks | Promotive fracture healing and ALP activity, inhibited osteoclasts differentiation |
| 19    | C57BL/6J mice | Femoral fracture | i.p. | - | Daily | 50 mg/kg | 5 weeks | Promotive fracture healing and ALP activity, inhibited osteoclasts differentiation and obviously bridging callus formation |
| 28    | Sprague-Dawley rats | Femoral fracture | i.p. | - | Daily | 30 mg/kg | 28 days | More bony union |
| 31    | Wistar-albino rats | Tibia fracture | i.p. | - | Daily | 25 mg/kg | 14 days | Melatonin eradicated adverse effects of ischemia on fracture healing |
| 33    | Albino New Zealand rabbits | Tibial defect | Powder local implantation | - | Only once | 1.2 mg | - | After 1 week and 2 weeks: Longer cortical bone and more blood vessels formation |
| 36    | Sprague-Dawley rats | Tibial defect and DM | i.p. | 19:00-20:00 | Daily | 250 µg | 10 and 30 days | 10 days: Higher number of osteoblasts and blood vessels as well as larger new mineralized surface 30 days: Lower level of advanced oxidation protein products and malondialdehyde |
| 42    | Sprague-Dawley rats | Radiation | i.p. | 30 min prior to radiation | Daily | 15 mg/kg | 3 days | 6 weeks later: A superior radioprotective function of melatonin over amifostine in preventing radiation-induced epiphysial growth plate injury |

i.p., intraperitoneal; DM, diabetes mellitus.
Type 2 DM and osteoporosis are both negatively affected by aging and lifestyle changes and quite often coexist. A high risk of fracture has been identified in patients with type 2 DM, particularly those with long periods of DM, poor glycemic control and diabetic complications (62,63). Reduced bone remodeling is one of the characteristics of DM, and autophagy is considered to be a potential target for the management of diabetic osteoporosis (64,65). The level of autophagy in osteoblasts may be reduced, and the process of DM-induced osteoporosis may be delayed by melatonin by inhibiting the ERK signaling pathway (66). Patients with multiple sclerosis (MS) have decreased serum melatonin levels and are also at risk of osteoporosis, while melatonin therapy can reduce the risk and normalize bone metabolites in MS (67). In addition to the modulation of bone formation and resorption, there are other effects of melatonin on bone metabolism, such as modulating calcium metabolism to prevent osteoporosis and hypocalcemia (68). Related evidence from relevant human and animal studies is presented in Table II.

4. Osteoarthritis

**Basic study of OA.** OA accompanied by chronic joint degeneration is one of the most common joint diseases, affecting ~3.8% of the world population (69). Aging, obesity, sex, genetics, diet-related factors, specific bone/joint shapes and numerous other factors may cause the degenerative injury of articular cartilage, and reactive hyperplasia of the articular margin and subchondral bone, which may lead to the occurrence of OA (70-72).

The functions of articular cartilage depend on cartilage ECM, which primarily comprises proteoglycan and Col2α1 (73). OA is characterized by the imbalance between cartilage ECM anabolism and catabolism (74). The development of OA occurs due to the presence of oxidative stress and inflammation (75). For instance, interleukin (IL)-1β is considered a primary inflammatory mediator in joints with local OA, and is involved in the early inflammatory process of OA, inducing chondrocyte metabolic disorders and cartilage dysfunction, ultimately resulting in joint dysfunction (76,77).

The current therapies for OA mainly focus on reducing joint load and large motion, with the aim of relieving symptoms, delaying the pathological process and improving the quality of life of patients with OA (78). Appropriate physical therapy, proper exercise, drug therapy and joint replacement surgery, among others, can be considered as treatment methods. Anti-inflammatory and analgesic drugs can be used to alleviate the symptoms of patients. However, drugs currently used in the treatment of OA, such as glucocorticoids and analgesics, have certain side-effects (79). Therefore, it is necessary to evaluate the risks of drugs and to identify novel types of low-risk drugs.

**Potential effects of melatonin on OA.** Inflammation plays a crucial role in the pathogenesis of OA, since mild and chronic inflammation have been shown to contribute to the symptoms and progression of OA (80,81). The self-repair ability of cartilage is limited, with the cell-based articular cartilage repair ability in inflamed joints being even lower. Melatonin intervention can partly restore the chondrogenic differentiation ability of MSCs affected by IL-1β-induced inflammation (21,77). In addition, the accumulation of ECM increases due the enhancement of ECM synthesis and the reduction of the degeneration enzyme expression induced by IL-1β (82). The effect of long-term intervention (21 days) is significant. Melatonin can also reduce the phosphorylation of p65 and IκBα, thereby inhibiting downstream NF-κB signaling pathway activation, which plays a key role in metabolism, inflammation and apoptosis (83).

ROS can be detected in the joints of OA model rats, which can cause hyaluronic acid depolymerization and molecular configuration changes, resulting in a decrease in the viscosity of synovial fluid (84). The age-related imbalance in ROS production is responsible for cartilage degradation and chondrocyte death (85). Pro-inflammatory cytokines mediate intracellular ROS production during inflammation, impairing the viability of cells and leading to apoptosis and senescence in various cell types (86). As part of the anti-inflammatory properties of melatonin, the dynamic action of the sirtuin 1 (SIRT1) pathway is notable. Oxidative stress upregulates SIRT1 in chondrocytes, while melatonin can reduce the production of nitric oxide, cyclooxygenase-2, inducible nitric oxide synthase and prostaglandin E2 by decreasing the expression and activity of SIRT1 (87). The expression of SIRT1-dependent nuclear factor of activated T cells 5 and nicotinamide phosphoribosyltransferase in IL-1β-stimulated chondrocytes can be suppressed by melatonin to alleviate OA (88). At the same time, melatonin also functions by activating antioxidant enzymes. Excessive ROS production reduces antioxidant enzyme expression in the progression of OA (89), while melatonin can induce the production of antioxidant enzymes, such as superoxide dismutase (90), while inhibiting ROS production (25), further suppressing oxidative stress.

The improper production of circadian clock-regulated hormones may also be involved in the occurrence of OA (91). The expression of circadian clock genes in chondrocytes is altered during the inflammatory process of OA (92), as the expression peak of brain and muscle ARNT-like 1 (Bmal1) is decreased, while that of period circadian regulator 2 (Per2) is increased (93). Per2 knockdown can reduce the expression of major cartilage degenerative enzymes, suggesting that the high expression of Per2 is one of the reasons for the progression of OA. The decline in Bmal1 expression is also associated with the mechanisms of OA (94), and can be restored by melatonin (95). Another study demonstrated that clock-related gene expression decreased in abnormal cartilage samples, and a nano-molar dose of melatonin restored clock-related gene expression and corrected the abnormal chondrocyte phenotype (95).

Multiple microRNAs (miRNAs/miRs) are involved in OA (96); among these, miR-140-5p has been shown to be expressed in cartilage and plays an important role in the differentiation of chondrocytes and the degeneration of cartilage (97). OA-associated cartilage changes occur in mice that lack miR-140 (98), while the overexpression of miR-140 has been shown to inhibit the synthesis of matrix catabolic enzyme (99). Elevated levels of pro-inflammatory cytokines in cartilage may reduce miR-140 expression (100). The protective roles of melatonin in OA-induced cartilage degradation are partly associated with the upregulation of miR-140 and the activation of the SMAD signaling pathways (82), which can
| Refs. | Objectives | Model | Route of administration | Time of administration | Frequency | Doses | Duration | Outcomes |
|-------|------------|-------|--------------------------|------------------------|-----------|-------|---------|----------|
| 56    | Perimenopausal women | -     | Oral                     | Night                  | Daily     | 3 mg  | 6 months | The ratio of type-I collagen cross-linked N-telopeptide (NTX): OCN trended downward to 1:1. Improved physical domain scores |
| 57    | C57BL/6 mice (female) Ovariectomy | Oral gavage | 6 weeks after surgery | Daily                  | 100 mg/kg | 6 weeks | Increased bone formation |
| 59    | Wistar rats | -     | Oral diluted in drinking water | -                     | -         | 10 mg/kg/day | 10 weeks | Higher bone volume, bone trabecular number, trabecular thickness, cortical thickness, bone stiffness, flexural modulus and ultimate load |
| 61    | C57BL/6J mice (female) Ovariectomy | i.p.  | -                        | Daily                  | 10, 50 mg/kg | 8 weeks | Melatonin alleviated bone loss in a dose-dependent manner |
| 66    | Sprague-Dawley rats Type 2 DM | i.p.  | -                        | Daily                  | 50, 100 mg/kg | 4, 8, 12 weeks | Melatonin improved the bone microstructure and reduced the level of autophagy (50 mg/kg was better than 100 mg/kg) |
| 67    | C57BL/6 mice (female) Experimental autoimmune encephalomyelitis | i.p.  | -                        | Daily                  | 10 mg/kg | 13 days | Increased 25-hydroxyvitamin D, calcium and OCN |

DM, diabetes mellitus; OCN, osteocalcin.
inhibit NF-κB pathways in articular cartilage (101). In addition, other miRNAs participating in the protection of the cartilage, such as miR-526b-3p and miR-590-5p, can be upregulated by melatonin, improving the chondrogenic differentiation of MSCs (102).

An intra-articular glucocorticoid (GC) injection is a method used to alleviate inflammation and chronic pain in patients with OA. However, evidence has suggested that treatment with GCs may only be effective in the short-term, while the long-term use of GCs may not be effective, or may even aggravate cartilage degradation (103,104). It has been reported that dexamethasone-induced ECM degradation in chondrocytes in a dose-dependent manner and reduced the intracellular proportion of nicotinamide adenine dinucleotide (NAd)+/NAd + hydrogen (NA dH) and the reduced intracellular concentration of NADP/NADPH. Melatonin pre-treatment can reverse these negative effects, possibly via the NAD+‑dependent activation of SIRT1 (105), which can promote chondrocyte survival and ECM synthesis to prevent dexamethasone‑induced damage to chondrocytes. On the other hand, NADPH oxidase has been shown to mediate the proliferation of multiple cell types, including stem cells; however, it is a major source of ROS (106). Melatonin can effectively regulate the ROS production levels of NADPH oxidase. In general, NADPH oxidase produces ROS at non-cytotoxic levels; when ROS production increases to a harmful level, excess ROS are removed by melatonin (25).

Transforming growth factor (TGF‑β) is considered to be one of the synthetic factors engaged in cartilage maintenance (107). A large amount of TGF‑β is present in healthy cartilage; however, the level of TGF‑β is decreased in OA‑affected cartilage (108). Although TGF‑β exerts protective effects on cartilage, it has been proven that it can also be a destructive factor (109). Following the exogenous melatonin (1-10 ng/ml) treatment of porcine articular chondrocytes, the level of intracellular TGF‑β1 has been shown to increase. This suggests that melatonin can promote porcine chondrocyte ECM synthesis, possibly through the TGF‑β signaling pathway, and the use of melatonin instead of TGF‑β in the treatment of OA may provide a suitable amount of TGF‑β and reduce adverse reactions (110).

Melatonin can be detected in synovial fluid (111), indicating that melatonin secreted in its natural state can reach the articular cavity. The nutrition of chondrocytes in articular cartilage mainly originates from the synovial fluid, and therefore an intra-articular melatonin injection may be a potentially effective method of melatonin therapy for OA. However, melatonin treatment may only be effective in the short-term and at low concentrations. When melatonin stimulation persists, the proteolytic cleavage of RANKL proteins in the synovium is promoted, causing severe subchondral bone erosion (95). Low concentrations of melatonin, such as 1 nM, can restore Col2α1 expression by inhibiting matrix metalloproteinase (MMP)-13; on the contrary, high concentrations, such as 1 mM, cannot rescue the reduced expression of Col2α1 caused by tumor necrosis factor (TNF)‑α exposure (112). ROS generation is promoted if the concentration of melatonin is too high or the incubation time is too long (113). It has been reported that pharmacological concentrations (µM‑mM) of melatonin promote the production of ROS and pro-inflammatory cytokines (114).

The results of the study by Liu et al (25) demonstrated that short-term melatonin treatment (5 days) promoted MSC proliferation, while long-term culture had no significant effect. These results suggested extra attention should be paid to the dose and duration of melatonin therapy, in order to avoid severe side-effects. Mild to moderate exercise can inhibit the inflammatory processes through joint compression-mediated biomechanical stimulation, helping to reduce the negative effects of high-dose and/or long-term melatonin use. There may be a synergistic effect on cartilage protection between melatonin therapy and treadmill exercise, which appears to be more effective in the early stage of the disease (112). Moreover, melatonin‑combined exercise can not only restore disordered molecular clocks and correct cartilage abnormalities, but also reduce periarticular bone defects and maintain articular bone homeostasis in late-stage OA (95). However, only mild to moderate exercise is beneficial, as strenuous daily exercise aggravates OA (Fig. 3) (115). Evidence on this matter from relevant animal studies is presented in Table III.

5. Periodontitis

**Basic study of periodontitis.** Periodontitis, an inflammatory and destructive disease of the periodontal tissues, is characterized by the loss of periodontal attachment. Periodontitis is also considered as inflammation of the alveolar bone, since marginal alveolar bone loss is a key secondary feature of periodontitis, with teeth loosening and complete loss occurring at the terminal stage. The common characteristics of periodontitis usually include redness in the periodontal tissue, pain, pus overflow, halitosis and dental stone formation, which progresses to alveolar bone resorption. Dental plaque is the primary initiating factor of periodontitis, creating and maintaining an inflammatory environment in the periodontal area. Gingival inflammation (gingivitis) induced by dental plaque is the most common and mildest type of periodontal disease, with the potential to develop into periodontitis in the case of no intervention. ROS generation is another important feature of periodontitis. Oral bacteria, as well as the inflammatory and immune reaction, lead to the generation of ROS, contributing to the progression of the disease (116,117).

Scaling and root planning (SRP) is considered an extremely effective basic treatment method for periodontitis. Generally, adherence to basic periodontal therapy can improve periodontal status in most patients with chronic periodontitis. However, in certain patients, progressive attachment loss cannot be terminated by SRP alone (118). For this reason, adjuvant treatment with SRP should be considered.

**Potential effects of melatonin on periodontitis.** Saliva and plasma melatonin levels are significantly lower in individuals with periodontal disease compared to clinically healthy subjects (119); however, the ratio of saliva and plasma melatonin levels is similar to that in healthy subjects (120). In a previous study, serum melatonin was introduced into the oral cavity through the salivary glands at a stable proportion (~33%, the ratio of salivary and serum melatonin) (121). Moreover, the gingiva is one of the external sites of melatonin synthesis, and melatonin receptor 1 has been found in human gingiva, indicating that melatonin may play a receptor‑mediating role.
in the oral cavity (122). Certain studies have suggested that melatonin may serve as a potential supplementary therapy and a biomarker detecting the dynamics of periodontal disease (123,124).

Melatonin levels in saliva and gingival crevicular fluid vary inversely with the severity of periodontitis (125,126), suggesting that melatonin may provide protective effects against the destruction of periodontal tissues. Srinath et al (119) suggested that melatonin had antibacterial properties, since Prevotella intermedia, Streptococcus mutans and Porphyromonas gingivalis, the primary bacteria in the occurrence and progression of periodontitis, were sensitive to melatonin.

In a previous study, patients with severe periodontitis received nonsurgical periodontal therapy (NSPT) following the oral administration of melatonin at 1 mg/day (a dietary supplement dosage advised by the Italian Ministry of Health) for 1 month (127). All patients were able to tolerate melatonin well, demonstrating a significant reduction in probing depth (Pd) within 6 months, suggesting that melatonin supplementation is likely to promote the healing process of periodontal pockets following long-term treatment (127). A small number of mild adverse reactions were observed at the initial stage of oral administration, which disappeared within a few days without affecting the compliance of the patients (127). Another study reported that, when combined with NSPT, the oral administration of 2 mg melatonin daily for 30 days improved the clinical attachment level (CAL) and PD significantly following long-term treatment (3 and 6 months) (128). Bazyar et al (129) obtained similar results with 6 mg melatonin.
Table III. Evidence of the effects of melatonin on osteoarthritis in animal studies.

| Refs. | Objectives | Model | Route of administration | Time of administration | Frequency | Doses | Duration | Outcomes |
|-------|------------|-------|--------------------------|------------------------|-----------|-------|----------|----------|
| 82    | C57BL/6J mice | Surgically-induced osteoarthritis | Intra-articular injection | After surgery | Twice a week | 10 mg/ml (10 µl) | 4 weeks | Attenuated OA progression |
| 87    | New Zealand white rabbits (female) | Surgically-induced osteoarthritis | Intra-articular injection | Beginning on the day of surgery | Weekly | 20 mg/kg | 4 weeks | Reduced cartilage degradation |
| 88    | Lewis rats | Surgically-induced osteoarthritis | Intra-articular injection | On day 3 following the surgery | Once | 10 mg/ml (20 µl) | - | Repressed expression of relevant genes in rat OA pathogenesis after 3 weeks |
| 95    | Sprague-Dawley rats | Intra-articular collagenase injection-induced osteoarthritis | Subcutaneous injection | - | Twice daily | 10 mg/kg | 4 weeks | Melatonin prevented periarticular muscle damage and cartilage degeneration. But prolonged melatonin administration leaded to subchondral bone erosion |
| 112   | Sprague Dawley rats | Intra-articular collagenase injection-induced osteoarthritis | Subcutaneous injection | 07:00 and 19:00 | Twice daily | 10 mg/kg | 1, 4 weeks | Melatonin with treadmill exercise may have both preventive and synergistic effects on rescue from cartilage degeneration and is more effective in the initial phase (1 week) |
daily administration daily with NPST. In a rat model of periapical periodontitis, following an i.p. injection of melatonin (10 mg/kg) for 21 days, radiological periapical bone loss and osteoclasts were decreased, the OPG level was increased, and the IL-1β, RANK and RANKL levels were decreased, as compared with the positive control. In addition, the bacteria localization score has been shown to be significantly lower following melatonin treatment (130). Renn et al reported that preventive melatonin supplementation suppressed the Toll-like receptor 4/myeloid differentiation factor 88 pathway to inhibit the activation of pro-inflammatory cytokines and normalize the balance between RANKL and OPG, thus suppressing the progression of periodontitis.

There may be a bidirectional association between DM and periodontitis, both of which are common chronic diseases. According to previous studies, DM is considered to be a risk factor of periodontitis development, increasing the prevalence and severity and promoting the progression of periodontitis (132,133). On the other hand, periodontitis may also increase the complications of DM (134). Patients with DM or periodontal disease have reduced melatonin levels in serum and saliva, and when the two diseases coexist, these levels are further reduced (135). In a previous study, in patients with mild to moderate periodontitis with DM, melatonin supplementation at 6 mg once a day for 8 weeks clearly increased the serum melatonin levels; moreover, PD, CAL loss and the high sensitivity-C reactive protein and IL-6 levels were reduced during NSPT (129). Balci Yuce et al reported that melatonin decreased osteoclasts and inhibited alveolar bone resorption in rats suffering from both DM and periodontitis; however, no decrease in bone loss was observed in rats with periodontitis alone. Oral local melatonin application was found to delay bone loss during periodontitis in patients with DM by down-regulating pro-inflammatory factors (137,138).

There is a clear association between periodontitis and obesity. The prevalence of periodontitis and degree of inflammation in obese or overweight patients seem to be higher compared to individuals with normal weight (139,140). An experimental study reported that, when the two diseases coexisted, a significant elevation was observed in periodontal destruction, lipid dysbolism, glucose levels and hepatic damage parameters, thus revealing comorbidity effects (141). These comorbidity effects may be associated with the circadian clock (142). Considered as an important modulator of the circadian clock, melatonin may be a key mechanism in this comorbidity effect (11,143).

Reduced levels of melatonin have been found to be associated with obesity (144). On the other hand, patients with periodontitis, and aggressive periodontitis in particular, are likely to have significantly lower levels of melatonin in saliva and gingival crevicular fluid (120,123). Melatonin supplementation helps restore lipid and glucose metabolism, reduce pro-inflammatory factor expression, improve body weight control and avoid obesity-related complications in obese patients (144-146). Rats with obesity or periodontitis have been shown to exhibited significantly lower circulating melatonin levels, although these levels are further reduced in rats with both obesity and periodontitis (147). In addition, a markedly increased destruction of periodontal tissue was observed in rats suffering from both obesity and periodontitis, with evident inflammatory infiltration and osteoclastic activity (147). There were almost significant negative associations between circulating melatonin levels and periodontal pocket depth, dental plaque index and modified gingival index (147). Combined therapy with SRP and melatonin supplementation significantly reduced alveolar bone destruction and pro-inflammatory cytokines in rats with comorbidities of obesity and periodontitis, providing a protective effect (148). When periodontitis, pinealectomy, or a combination of both are present, the TNF and insulin concentration, as well as the homeostasis model assessment of insulin resistance index, are increased, indicating insulin resistance (149). Pineal excision can also lead to lipid profile dysregulation, which may be improved to a similar degree to the control group by melatonin alternative therapy, indicating that alternative melatonin therapy provides a therapeutic effect against dyslipidemia (149).

Periodontal ligament cells can secrete various cytokines to modulate and maintain the homeostasis of periodontal tissues, thereby playing an arrestive role in alveolar bone metabolism (150). Periodontal tissue regeneration can be enhanced by conditioned medium from periodontal ligament stem cells in a concentration-dependent manner by suppressing TNF-α production (151). There is an important balance between cementum formation and bone loss during the maintenance of periodontal health. Melatonin inhibited ethanol-induced ROS production and senescence-like phenotypes in human periodontal ligament stem cells and cementoblasts. In addition, it restored the decreased osteoblastic/cementoblastic differentiation, and increased osteoclastic differentiation through the protein never in mitosis gene A interacting-1 pathway. Furthermore, the downregulation of certain pathways, such as the MAPK, AMP-activated protein kinase, mammalian target of rapamycin (mTOR) and nuclear factor of activated T-cells c-1 pathways, has been suggested to exert protective effects against ethanol-induced senescence (152).

El-Sharkawy et al reported that a daily dietary supplement of 10 mg melatonin may be an effective complementary treatment for patients with insomnia with generalized chronic periodontitis, resulting in an improved CAL and sleep quality, as well as lower PD and salivary TNF-α levels. During the entire study period, the improvement in insomnia was maintained for up to 6 months without any rebound, even though the daily dietary melatonin supplement was administered for only 2 months. As shown by previous data, the level of systemic inflammatory markers in sleep disorders increased significantly (154). There may be a certain degree of bidirectional association between sleep disorders and periodontal disease. Therefore, improving sleep quality may itself improve the response to periodontal therapy (Fig. 4). Evidence from relevant human and animal studies on this matter are presented in Table IV.

6. Differential effects of melatonin administered at various concentrations and times

In the majority of previous studies, melatonin has been found to play a positive role in bone tissue and bone-related diseases. Melatonin supplementation in humans has a generally favorable safety profile. Clinical studies have demonstrated that the use of melatonin in the short-(days) and medium-term (weeks
to months) is safe, with only minor, transient adverse reactions reported (155). In addition to the most commonly reported adverse reactions, which are related to fatigue, mood and psychomotor or neurocognitive performance, a few studies have reported adverse events associated with endocrine and cardiovascular function, as summarized in a critical systematic review of clinical evidence (156). The safety of melatonin application in pregnant and lactating women is unknown, due to the lack of relevant research.

Although there is considerable evidence to suggest that melatonin exerts a positive effect on bone health, certain studies have reached different conclusions. Frisher et al (157) reported an association between melatonin and a significantly increased risk of fracture. In addition, in that study, it was shown that the concentration and administration time may impact the effects of melatonin treatment. As shown in Tables I-IV, the usual strategy for animal experiments is i.p. injection at doses between 10-50 mg/kg. A small number of studies have used higher doses, such as 100 mg/kg (15,66,131). The intra-articular injection is a method commonly used in studies on OA, where the doses are lower (0.1-0.2 µg) (82,88). In addition, the normal dose used for subcutaneous injection is 10 mg/kg (95,112). A few studies have used other methods, such as oral gavage (57), the addition of melatonin to drinking water (59,148,149) and melatonin powder implantation (13,33). Apart from one-off administration (13,33), the shortest duration of melatonin treatment was 3 days (42), and the longest being up to 12 weeks (66). The majority of experiments used melatonin for 2-4 weeks. For clinical research, the most common route is by oral administration, with doses fluctuating between 1-10 mg/day (56,127-129,153). In addition, melatonin can be used locally in the form of 1% orabase cream for the treatment of periodontitis (137,138). The drug treatment durations were ~1-2 months, and 6 months in one study (56).

To date, there is no consensus on the optimal route, dosage and time of melatonin administration. Further research is therefore required to explore the optimal route, dosage and administration time of melatonin, and determine whether long-term melatonin supplementation has any adverse effects, as well as whether melatonin can be used as a daily adjuvant in elderly, perimenopausal and postmenopausal women, and in patients with periodontitis.

7. Conclusion and future prospects

Melatonin is a common molecule mainly produced by the pineal gland. As an important regulatory factor of circadian rhythm, melatonin has the ability to synchronize and maintain the circadian clock in peripheral tissues with L/D cycles. In addition, melatonin is also considered cytoprotective, due to its anti-inflammatory, antitumor and antioxidant effects, and its ability to regulate hormones, the immune system and tissue regeneration.

Bone-related diseases have a high incidence, and are associated with severe and persistent symptoms, a slow recovery and a high impact on the lives of patients, as well as a heavy economic burden. The most common treatment usually exerts a curative...
Table IV. Evidence of the effects of melatonin on periodontitis in animal and clinical studies.

| Refs. | Objectives | Model | Route of administration | Time of administration | Frequency | Doses | Duration | Outcomes |
|-------|------------|-------|--------------------------|------------------------|-----------|-------|----------|----------|
| 127   | Patients with untreated severe periodontitis | With NSPT | Oral | After NSPT | Daily | 1 mg | 1 month | PD reduced even at 6 months |
| 128   | Patients with chronic periodontitis | With NSPT | Oral | After NSPT | Daily | 2 mg | 4 weeks | Improved CAL and lower PD in a long time as 3 and even 6 months |
| 129   | Patients with chronic periodontitis and type 2 DM | With NSPT | Oral | 1 h before bed time | Daily | 6 mg | 8 weeks | Improved CAL and lower PD |
| 130   | Sprague-Dawley rats | Periapical lesions | i.p. | - | Daily | 10 mg/kg | 21 days | Reduced radiological periapical bone loss |
| 131   | Wistar rats | Experimental periodontitis | i.p. | 10:00-10:30 | Daily | 10, 50, 100 mg/kg | 14 and 28 days | Reduced pro-inflammatory cytokine level and suppressed progression of periodontitis |
| 136   | Wistar rats | Experimental DM and periodontitis | i.p. | - | Daily | 10 mg/kg | 4 weeks | Inhibited resorption of alveolar bone |
| 137   | Patients with periodontal disease and DM | - | Topical application | - | Daily | 1% orabase cream | 20 days | Significant decrease of the gingival index, PD and salivary levels of RANKL, and significant rise of salivary OPG |
| 138   | Patients with periodontal disease and DM | - | Topical application | - | Daily | 1% orabase cream | 21 days | Statistically significant decrease of the gingival index, PD, and IL-1β, IL-6 and prostaglandin E2 in gingival crevicular fluid |
| 148   | Wistar rats | Obesity and periodontitis | Dissolved in drinking water | - | Daily | 25 µg/ml | 4 weeks | Combined therapy of SRP and melatonin supplement significantly reduced alveolar bone destruction and proinflammatory cytokines |
| 149   | Wistar albino rats | Pinealectomy and periodontitis | Dissolved in drinking water | 7:00 p.m. - 7:00 a.m. | Daily | 5 mg/kg | 28 days | Melatonin efficiently prevented insulinresistance, improved lipid profile, and increased plasma levels of insulin and TNF |
| 153   | Patients with generalized chronic periodontitis and primary insomnia | With SRP | Oral | 1 h before bed time | Daily | 10 mg | 2 months | Greater CAL and sleep quality, lower PD and salivary TNF-α levels |

NSPT, nonsurgical periodontal therapy; PD, probing depth; CAL, clinical attachment level; DM, diabetes mellitus; OPG, osteoprotegerin; IL, interleukin; SRP, scaling and root planning; TNF, tumor necrosis factor.
effect and some side-effects. Since melatonin is inexpensive, with a wide safety margin, has a wide impact on tissues and almost no side-effects, the use of melatonin as a supplementary treatment may be a potential therapeutic option for bone disease (Fig. 5). As a widely available and versatile molecule in vivo, melatonin has potent antioxidant and anti-inflammatory properties in a variety of bone diseases. In addition, melatonin also plays a vital role in promoting osteogenesis and inhibiting osteoclastogenesis (13,14). The promotion of vascularization will also provide a good boost for bone repair (31). A close association has been identified between cartilage and bone. Subchondral osteogenesis is an important type of osteogenesis (21), and cartilage and bone metabolic disorder is a common challenge in OA (72). Melatonin also protects and promotes the differentiation of cartilage (77), which is important for bone regeneration and bone development. Melatonin always drives MSCs to differentiate toward osteoblasts (17-19) and chondroblasts (18,21,77) and protects MSCs from disease-induced apoptosis (25,27,83). Patients with bone disease may also have other chronic metabolic diseases, such as diabetes (62,63,132-134), which have adverse effects on bone health and lead to comorbidity effects. Despite these comorbidity effects, melatonin can still have beneficial effects (66,129,136), which provides a novel insight for future multi-disease combination therapy.

Stomatologists pay great attention to the protection of periodontal tissue. The protective effects of melatonin on periodontal tissue are significant, helping patients with periodontitis retain more alveolar bone, providing more possibilities for natural tooth retention and subsequent tooth defect repair. In addition, the protective effects of melatonin on alveolar bone can also accelerate the process of osteointegration during implant restoration, to obtain a better implantation effect (158). Melatonin can be used to inhibit the enhancement of oxidative stress in oral tissues in the period immediately following tooth extraction to avoid excessive alveolar bone loss (159).

Human tissues are regulated by a circadian rhythm. In particular, bone metabolism is closely associated with the circadian rhythm, including the development, repair and remodeling of bone tissue; cartilage metabolism is also regulated in a similar manner (160-162). The disruption of the circadian rhythm leads to a series of adverse effects. As an important hormone regulating the circadian rhythm, melatonin helps restore the circadian rhythm and this also exerts a positive effect on bone tissue and bone disease. MSCs, mesenchymal stem cells; ROS, reactive oxygen species; NLRP3, nucleotide-binding domain and the leucine-rich repeat pyrin 3 domain; VEGF, vascular endothelial growth factor; DM, diabetes mellitus; OA, osteoarthritis; GC, glucocorticoid.
elderly (163) and perimenopausal women (164). Melatonin can improve the sleep quality and mental health of patients, thus improving patient compliance; thus, the efficacy of melatonin treatment can be further consolidated.

In conclusion, melatonin functions as a protector in bone injury, osteoporosis, OA and periodontitis by exerting multiple effects. Melatonin supplementation in humans has a generally favorable safety profile. Due to the protective effects of melatonin, as well as its low price and high safety, exploring melatonin as a supplement to bone tissue and bone-related disease therapy is worthwhile. However, since a few studies have reported adverse effects, and there is no consensus on the optimal program of melatonin administration, further research is required to explore the optimal administration conditions and safety of long-term melatonin supplementation.

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Authors’ contributions

XL was involved in data curation, investigation and visualization, and in the writing of the original draft. SY was involved in the conceptualization of the study, as well as in the preparation of the figures, writing of the original draft, and in the writing, reviewing and editing of the manuscript. GC, WZ, JP and XH were involved in the writing, reviewing and editing of the present review article. LC was involved in the conceptualization of the study, as well as in funding acquisition, project administration, study supervision, and in the writing, reviewing and editing of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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