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Research Article

Melatonin Reduces Microgravity-Induced Bone Loss: Implications for the Challenges of Long-Term Spaceflight

Mu-Tai Liu[1]* and Russel J Reiter[2]

[1]*Department of Radiology, Yuanpei University of Science and Technology, Taiwan*

[2]*Department of Cell Systems and Anatomy, The University of Texas Health Science Center, San Antonio, Texas, USA*

***Corresponding Author:** Mu-Tai Liu, Department of Radiology, Yuanpei University of Science and Technology, Taiwan.

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Abstract

Spaceflight, especially because of microgravity, critically affects the health of astronauts who participate in a long-term space mission. Microgravity exposure causes osteoporosis-like bone loss and muscle atrophy in astronauts. Bone mineral density (BMD) reduced at the rate of 1% per month at the lumbar spine and 1 - 1.6% per month at the hip in astronauts of the Russian/MIR space station and International Space Station (ISS). Bone remodeling is regulated primarily by the activity of bone-resorbing osteoclasts and bone-forming osteoblasts. Microgravity with reduced mechanical load on the weight-bearing bones leads to an increase in bone resorption and a reduction in bone formation. Microgravity inhibits bone formation through effects on the Wnt/β-catenin signaling pathway. Melatonin induces human mesenchymal stem cells (hMSC) differentiation into mature osteoblasts via multiple signal transduction mechanisms which include MEK/ERK1/2, runt-related transcription factor 2 (RUNX-2), osteocalcin, BMP-2 and Wnt/β-catenin. It promotes the synthesis and mineralization of new bone. It significantly inhibits the increase in the expression of genes for osteoclastogenesis, including *Mmp-9*, *cathepsin K*, *Trap and Rankl*, in microgravity conditions compared to that on the ground. Conversely, it enhances the expression levels of osteoclast inhibitory genes, *Opg* and *Calcitonin*. Treatment with melatonin is a potential approach for inhibiting osteoclast activity in microgravity. Melatonin is recommended as a promising agent for the prevention of bone loss during space exploration. With the technological advancements in stem cell-based therapy, mesenchymal stem cells (MSCs) with the capacity to differentiate into osteoblasts may serve as a novel approach for the treatment of osteoporosis. Bone marrow mesenchymal stem cells (BM-MSCs) increase trabecular bone, inhibit the loss of BMD and prevents osteoporosis. The addition of strontium-containing hydroxyapatite nanoparticles (nSr-HAP) to human bone marrow mesenchymal stem cells (hBM-MSCs) may be a potential strategy to promote bone regeneration for microgravity-induced osteoporosis. Furthermore, combined melatonin and bone marrow mesenchymal stem cell therapy may serve as a promising regimen for the treatment of osteoporosis. The study suggests the possible prospective strategy for both preclinical and clinical studies of combined melatonin and mesenchymal stem cells in the treatment of microgravity-induced osteoporosis-like bone loss in order to provide advanced health care for astronauts on long-term space exploration missions.

Keywords: *Spaceflight; Astronauts; Microgravity; Melatonin; Osteoclasts; Osteoblasts; Osteocytes; RANKL; Bone; Mesenchymal Stem Cells*

Introduction

Space is an extreme and hazardous environment characterized by various stress factors that might seriously impact the health of astronauts [1-3]. Currently, astronauts live and operate on board orbiting space stations in Low Earth Orbit (LEO). In the near future, newly-operated space missions to explore the Moon and Mars will involve long-term stay for crew members in outer space outposts [1]. Spaceflight, especially because of microgravity, critically affects the health of astronauts who participate in a long-term space mission. Microgravity exposure causes osteoporosis-like bone loss and muscle atrophy in astronauts [3,4]. Bone mineral density (BMD) reduces at the rate of 1% per month at the lumbar spine and 1 - 1.6% per month at the hip in astronauts of the Russian MIR space station and International Space Station (ISS) [4]. The rapid bone loss is one of the main challenges that remain to be resolved in order to preserve health in outer space [5].

Bisphosphonates, anti-resorptive agents, are characterized by a strong affinity for hydroxyapatite in bone, a long skeletal half-life, and inhibition of bone resorption [6]. These agents may be effective in preventing bone loss on long-term space mission [7]. Nevertheless, these agents might increase the probability of atypical femoral fractures and lead to critical side effects in some cases including osteonecrosis of the jaw and atrial fibrillation [5,8]. Consequently, safe and effective agents are urgently needed to prevent bone loss during spaceflight [5].

Melatonin (N-acetyl-5-methoxytryptamine), an indole compound synthesized by the pineal gland and many other tissues, is involved in various essential physiological processes [9-12]. Osteoporosis is a bone disorder associated with impaired bone formation and excessive bone resorption [13]. Melatonin prevents bone resorption and enhance bone formation through mechanisms involving both melatonin receptor-mediated and receptor-independent actions [14]. The three essential mechanisms of melatonin effects on bone function are as follows: (i) the enhancement of the osteoblast differentiation and activity; (ii) an increase in the osteoprotegerin expression by osteoblasts, thus preventing the differentiation of osteoclasts; (iii) and scavenging of free radicals produced by osteoclast activity which is responsible for bone resorption [14]. A study by Koyama., *et al*. showed that treatment with 5 mg/kg per day or 50 mg/kg per day of melatonin in mice significantly increased bone mineral density (BMD) and bone mass (bone volume per tissue volume). The treatment significantly reduced bone resorption parameters (i.e. osteoclast surface and osteoclast number). These findings revealed that melatonin induced an inhibition of bone resorption and an increase in bone mass [15]. Similiarly, Zhou., *et al*. showed that the number of tartrate-resistant acid phosphatase (TRAP)-positive cells and the gene expression of osteoclast-specific markers were significantly downregulated in melatonin-treated bone marrow monocytes. Moreover, melatonin markedly inhibited osteoclast formation in a dose-dependent manner [13]. Ping., *et al*. demonstrated that melatonin activated Wnt/β-catenin signaling pathway and enhanced bone regeneration at osteolytic sites [16]. Melatonin inhibited receptor activator of nuclear factor κ-B ligand (RANKL) induced osteoclast formation and osteoclastic bone resorption [17]. In addition, melatonin mediated its anti-resorption effects by abolishing nuclear factor κ-B activation. These findings further indicated the protective effects of melatonin on bone loss [17].

In this review, we summarize the molecular and cellular mechanisms of melatonin protection against microgravity-induced bone loss. In addition, we discuss recent advances related to melatonin as a potential agent for the prevention of bone loss during spaceflight.

Bone remodeling

Bone is a heterogeneous composite material consisting of a mineral phase (hydroxyapatite), an organic phase (90% type I collagen, 5% non-collagenous proteins (NCPs), 2% lipids) and water [18]. The relative proportions of these constituents vary according to age, site, gender, disease and treatment [18]. There are two types of bone tissue, trabecular bone and cortical bone [19]. Cortical bone constitutes 80% of bone volume, while trabecular bone constitutes the remainder [20]. Trabecular or cancellous bone is the internal compartment of bone tissue that includes approximately 25% bone and 75% marrow by volume. Cortical or compact bone constitutes the dense outer shell of bone tissue, which is approximately 90% bone and 10% pore space by volume [19].

Bone remodeling is regulated primarily by the activity of bone-resorbing osteoclasts and bone-forming osteoblasts. Increased bone resorption and/or decreased bone formation may lead to reduced bone mass and quality, resulting in osteoporosis with increased risk of fracture [21]. Some pharmacological interventions have the ability to decrease bone resorption and/or increase bone formation. Concurrent inhibition of bone resorption and enhancement of bone formation are regarded as a consummate therapeutic strategy against osteoporosis [21].

The bone remodeling process consists of six sequential phases, specifically, activation, resorption, reversal, formation, mineralization, and quiescence (Figure 1). The process is regulated by various local and systemic factors [22]. Calcitonin (CT), parathyroid hormone (PTH), and estrogen are the principal hormonal regulators of osteoclastic bone resorption. Wnt, bone morphogenetic proteins (BMPs), PTH and $1,25(OH)_2$ vitamin D3 are the major regulators of osteoblastic bone formation [22]. Additionally, growth factors such as insulin-like growth factor-1 (IGF-1), transforming growth factor-β (TGF-β), fibroblast growth factors (FGFs) and epidermal growth factor (EGF) play important roles in the regulation of bone remodeling [22].

Osteoblasts are bone-forming cells derived from mesenchymal stem cells in the bone marrow stroma which are responsible for bone matrix synthesis and its mineralization [22,23]. Osteoclasts are multinucleated giant cells formed from the fusion of mononuclear progenitors of the monocyte/macrophage line [22]. Osteocytes, the most plentiful bone cells, establish a three-dimensional network in every part of the osseous tissue. They function as mechanosensors that monitor mechanical stress within bone tissues and react to alterations in both the amount and the direction of loading exerted on bones [24]. A fundamental event that initiates bone remodeling is osteocyte cell death (apoptosis) which occurs in relatively short periods at focal areas of bone microdamage resulting from uncommon mechanical load or normal daily activity [24].

As a consequence of osteocyte apoptosis in a given microscopic region (bone remodeling compartment or BRC), bone multicellular units (BMUs) are recruited. Each BMU is composed of various morphologically and functionally different cell types, principally osteocytes, osteoblasts and osteoclasts. These cells act synergistically on the BRC to replace old bone with new bone [24]. Osteocyte apoptosis in the BRC induces osteoblast activation and the recruitment of osteoclast precursors derived from the bone marrow. Such precursors differentiate into mature osteoclasts, which initiate the resorption of adjacent bone, resulting in the appearance of cutting cones, or lacunae [24]. Activated osteoblasts subsequently proceed in the vestige of bone-destroying cutting cones to fill the cavity by secreting an osteoid matrix. A number of active osteoblasts are trapped in the matrix and ultimately differentiate into osteocytes when the osteoid matrix becomes mineralized; these processes contribute to local bone remodeling events [24].

The effects of microgravity on bone remodeling

Microgravity with reduced mechanical load on the weight-bearing bones leads to an increase in bone resorption and a reduction in bone formation [25,26]. The longer the spaceflight, the more bone minerals are lost [27]. Smith., *et al*. indicated that calcium intake and absorption declined up to 50% and bone resorption increased by greater than 50% during a long-term spaceflight [28]. As a reaction to microgravity, calcium is released from bone leading to inhibition of parathyroid hormone. This induces a reduction in circulating 1,25-dihydroxyvitamin D3 resulting in a decline in calcium absorption [29].

Microgravity inhibits bone formation through effects on the Wnt/β-catenin signaling pathway [29]. This pathway is an essential regulator of osteoblastogenesis [30,31]. Canonical Wnt signaling induces the differentiation of osteoblast precursors into mature osteoblasts [30]. Sclerostin and Dkk-1 suppress the canonical Wnt signaling by binding to Wnt co-receptors, low-density lipoprotein receptor-related protein (LRP) 5 and 6 [32,33]. Microgravity enhances the expression of sclerostin and Dkk-1 [29]. The Wnt-signaling pathway enhances osteoblastic cell differentiation and bone formation. Additionally, this pathway suppresses bone resorption through inhibition of the receptor activator of nuclear factor-κB-ligand (RANKL)/RANK/OPG interaction pathway [34,35].

As higher strains are applied to bone, minor cracks develop, followed by bone resorption that eliminates the damaged bone. Sclerostin plays an essential role in skeletal adaptation to mechanical forces [29]. Nabavi., *et al*. evaluated the effects of microgravity on osteoblasts and osteoclasts during a five-day spaceflight and found an increase in bone resorption by osteoclasts and a decrease in osteoblast cellular integrity [25]. Spatz., *et al*. assessed the expression of sclerostin in osteocytes and reported it is upregulated by mechanical unloading, implying that the mechanical load determines the intrinsic osteocyte response [36]. These findings indicate that the Wnt/β-catenin pathway is a potential target for the strategy to prevent bone loss in microgravity conditions [29].

The effects of melatonin on bone metabolism

Melatonin's actions on osteoblasts

Melatonin induces human mesenchymal stem cells (hMSCs) differentiation into mature osteoblasts [37,38] via multiple signal transduction mechanisms which include MEK/ERK1/2, runt-related transcription factor 2 (RUNX-2), osteocalcin, BMP-2 and Wnt/β-catenin [37-39]. Activation of MEK1/2/ERK1/2 is dependent on the activation of MT2 melatonin receptor and MT2R/Gi/β-arrestin/MEK/ERK1/2 complexes [38,40].

Activation of MAPKs (including ERK1/2, p-38 and JNK) enhances osteogenic gene expression, including Runx2, bone morphogenetic protein 2 (BMP2) and osterix (39). BMPs are included in the transforming growth factor (TGF) superfamily which is categorized into at least 15 subtypes [41]. The binding of BMPs to membrane-bound bone morphogenetic receptors (BMPRs) and phosphorylation of Smad proteins lead to the interaction of Smads with common-partner Smads (Co-Smads), resulting in their translocation to the nucleus and activation of osteogenic genes, Runx2, BMP2 and osterix [39]. Melatonin also activates the Wnt/β-catenin pathway in osteoblasts resulting in enhancement of osteogenic genes, Runx2, BMP2 and osterix (Figure 1). These data document that melatonin promotes the synthesis and mineralization of new bone.

Melatonin's actions on osteoclasts

Osteoblast-mediated induction of osteoclastogenesis occurs by means of the release of macrophage colony-stimulating factor (M-CSF) and RANKL from osteoblasts [39]. Activation of RANK on mononuclear cells by RANKL in company with M-CSF enhances osteoclastogenesis by inducing mononuclear cells to fuse thereby generating multinucleated osteoclasts which dissolve bone matrix by secreting cathepsin K and tartrate-resistant acid phosphatase (TRAP) onto the bone matrix [39]. RANKL exerts its effects by binding RANK on the surface of osteoclast precursors and recruits the adaptor protein, tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6). TRAF6 binding to RANK activates multiple signaling proteins, which include NFκB, Akt/PKB, mTOR and the MAPKs, JNK, ERK and p-38 are involved in osteoclast differentiation as well as determining their activity and survival (Figure 1). Osteoblasts also secrete osteoprotegerin (OPG), which functions as a decoy receptor for RANKL inhibiting osteoclastogenesis and osteoclast activity and inducing osteoclast apoptosis [42,43]. The relative expression of RANKL and *OPG* is essential in regulating osteoclast activity and bone remodeling. Various studies indicate that levels of RANKL and osteoprotegerin are inversely related [44,45].

A reduction in the level of melatonin is associated with an elevated bone resorption, indicating that melatonin is probably acting as an endogenous osteoclast inhibitor [39]. Koyama., *et al*. reported that melatonin-mediated inhibition of bone resorption co-existed with a reduction in RANKL-mediated osteoclastogenesis [15].

Melatonin's ability to inhibit RANKL-mediated osteoclastogenesis is considered to occur via melatonin's action on osteoblasts to induce osteoprotegerin [46]. Zhou., *et al*. reported that melatonin significantly inhibited osteoclast formation of bone marrow monocytes (BMMs) in a dose-dependent manner. The number of TRAP-positive cells and the gene expression of osteoclast-specific markers, includ-

ing TRAP, cathepsin K and osteoclast-associated receptor, were significantly downregulated in melatonin-treated BMMs. Nuclear translocation of p65, a marker of NF-κB activation, in RANKL-treated BMMs was inhibited in the presence of 100 μM melatonin, indicating that the NF-κB signaling pathway was blocked by melatonin [13].

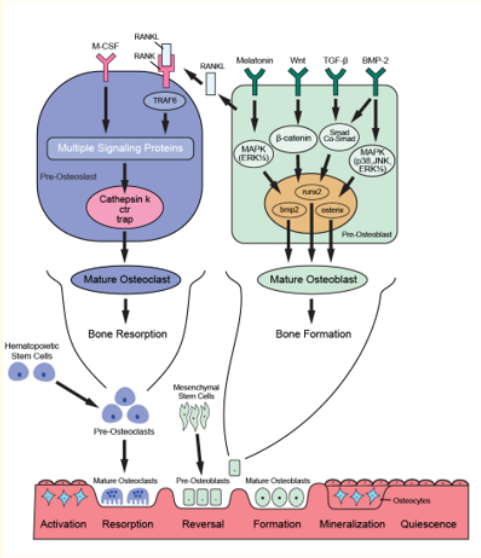

Figure 1: *Melatonin-mediated osteoblast differentiation and osteoclastogenesis. Upper panel: Melatonin-mediated differentiation of mesenchymal stem cells into osteoblasts occurs via several signaling pathways. Activation of MAPKs (ERK1/2, p-38, JNK) by melatonin enhances osteogenic gene expression. Melatonin-mediated osteoblast differentiation also occurs via the Wnt/β-catenin pathway. Translocation of β-catenin to the nucleus induces osteogenic gene expression, including Runx2, Bmp2, osterix. Osteoblast-mediated induction of osteoclastogenesis is due to the release of M-CSF and RANKL from osteoblasts. Activation of RANK on pre-osteoclasts by RANKL promotes osteoclastogenesis by causing pre-osteoclasts to fuse and form multinucleated osteoclasts. RANKL exerts its effects by binding RANK on the surface of pre-osteoclasts and recruits the adaptor protein, TRAF6. TRAF6 binding to RANK activates multiple signaling proteins. Lower panel: The bone remodeling process consists of six sequential phases, specifically, activation, resorption, reversal, formation, mineralization, and quiescence. TGF-β (transforming growth factor-β); Runx2 (runt-related transcription factor 2); BMP-2 (bone morphogenetic protein 2); wnt (wingless type); M-CSF (macrophage colony-stimulating factor); RANKL (receptor activator of nuclear factor κB ligand); RANK (receptor activator of nuclear factor κB); TRAF6 (TNF receptor-associated factor); TRAP (tartrate-resistant acid phosphatase); CTR (Calcitonin receptor); Co-Smad (common mediator Smad).*

The effects of melatonin on bone loss during spaceflight

Astronauts suffer osteoporosis-like loss of bone mass due to exposure to microgravity. Differentiation and activation of osteoclasts are mainly affected by interactions with osteoblastic lineage cells [5]. Ikegama., *et al*. employed goldfish (*Carassius auratus*) scales as a bone model with co-existing osteoclasts and osteoblasts in a calcified matrix. Fish scales and mammalian mineralized skeleton share numerous common features concerning matrix components, cellular morphology, and responses to hormones and mechanical stress [5].

Comparison of melatonin-related enzyme expression in goldfish scales cultured in space with those on the ground

The mRNA expression levels of *acetylserotonin O-methyltransferase* (*Amst*), an enzyme indispensable for melatonin synthesis, was reduced significantly in microgravity conditions compared to those in natural gravity on the earth's surface [5].

Comparison of osteoclast multinucleation of goldfish scales in space with those on the ground

The number of nuclei in multinucleated osteoclasts was significantly greater in microgravity conditions compared to that on the ground. Likewise, the percentage of actin ring-positive multinucleated osteoclasts and the actin ring size were significantly greater under microgravity conditions [5].

Effects of melatonin and microgravity on bone-related gene expression in goldfish scales

Expression level of *matrix metalloproteinase-9* (*Mmp-9*) significantly increased in microgravity. The levels of *cathepsin K* and *Trap* revealed an upward trend in microgravity conditions compared to those on the ground. Microgravity significantly enhanced the expression of *Rankl* but significantly reduced the expression of *Opg* (Figure 2), which resulted in a marked rise in the ratio of *Rankl:Opg*, a crucial indicator of bone resorption [5]. The expression of *Calcitonin* dropped significantly under microgravity conditions compared to that on the ground (Figure 2).

Melatonin significantly inhibited the rise in the expression of genes required for osteoclastogenesis, including *Mmp-9*, *cathepsin K*, *Trap and Rankl*, in microgravity compared to those subjected to earth-strength gravity. Conversely, melatonin enhanced the expression levels of osteoclast inhibitory genes, *Opg* and *Calcitonin* (Figure 2). Melatonin also significantly inhibited the expression level of the osteoclast marker Rank and substantially reduced the expression ratio of *Rankl:Opg* [5]. Additionally, microgravity-dependent induction of osteoblastic markers, *osterix* (*Osx*) and *type I collagen 1a* (*Col1a*), was maintained by melatonin, indicating that it also targets specific genes of osteoblasts in microgravity conditions [5].

A randomized, controlled trial revealed a significant rise in bone mineral density (BMD) at the femoral neck of postmenopausal women with osteopenia in response to melatonin when compared to placebo [47]. Results from the Melatonin Osteoporosis Prevention Study (MOPS) reported that melatonin treatment exhibited a downward trend of the ratio of N-telopeptide (NTX, bone resorption marker): osteocalcin (OC, bone formation marker), improved physical symptoms related to perimenopause, and restored imbalances in bone remodeling to prevent bone loss in perimenopausal women [48]. These investigations suggested that melatonin may act as an anti-osteoclastic agent in humans. The results indicate that treatment with melatonin is a potential approach for inhibiting osteoclast activity in microgravity [5]. On the basis of the findings summarized in this report, melatonin should be seriously considered as a non-toxic agent to reduce bone loss during space exploration.

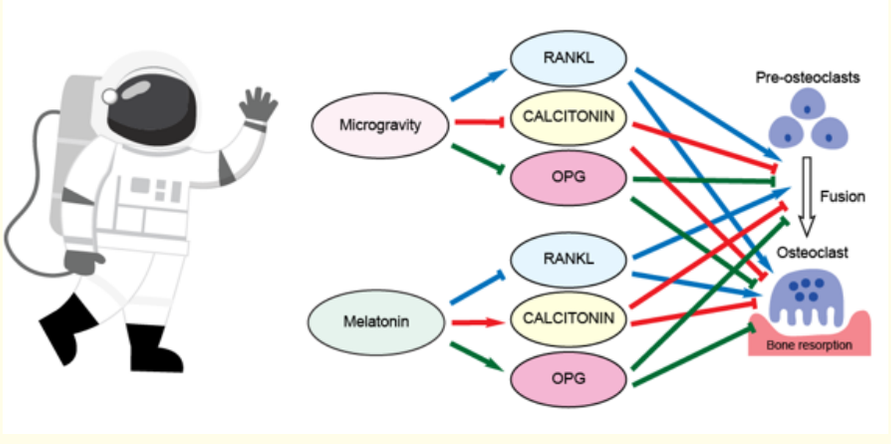

Figure 2: *Microgravity during spaceflight promotes the multinucleation and resorptive activity of osteoclasts, enhances the gene expression of Rankl (a major gene for osteoclastogenesis) and inhibits gene expression of Calcitonin and Opg. OPG (osteoprotegerin) plays an essential role in bone remodeling as a decoy receptor for RANKL in the RANK/RANKL/OPG axis, inhibiting osteoclastogenesis and bone resorption. Melatonin reduces the expression of Rankl and enhances the expression levels of osteoclast inhibitory genes, Calcitonin and Opg, in microgravity conditions.*

Conclusion and Perspectives

Despite the preventive and therapeutic effects of various regimens designed to protect astronaut's musculoskeletal system (including intense physical exercise, a balanced diet, calcium and vitamin D dietary supplements combined with anti-resorptive, anti-catabolic and anabolic or bone-forming agents), they remain at risk of losing 1.0% to 1.5% of their bone mass for every month they spend in space [49,50]. Thus, identification of effective therapeutic agents with fewer side effects and minimal loss of bone mass is a high priority [51]. Microgravity induces osteoclastic activity and significantly increases the expression of genes involved in osteoclast differentiation and activation which leads to bone loss [5]. Administration of melatonin significantly enhances *Calcitonin* (an osteoclast-inhibiting hormone) mRNA expression and inhibits mRNA expression of *receptor activator of nuclear factor κB ligand* (*RANKL*, a promoter of osteoclastogenesis), supporting the inhibitory actions of melatonin on osteoclastic activation thereby suppressing bone loss induced in microgravity [5]. There are many favorable criteria concerning melatonin as an appropriate agent to prevent bone loss for the spacefaring population. Firstly, any useful medication should have a history of safe application in humans with minimal or negligible side effects. Secondly, possible side effects of the medication should be easily observable by an astronaut with minimal skill of health care. Thirdly, the medication itself should be easily administered and should have a long shelf life in the space environment [52]. Accordingly, melatonin which lacks any significant toxicity is worthy of consideration as an agent to prevent microgravity-induced osteoporosis-like bone loss during spaceflight [5].

In addition, osteoporosis is a systemic skeletal disorder characterized by low bone mass, reduced bone strength and loss of the bone microarchitecture/mineralization leading to bone fragility with a consequent increase in fracture risk [49]. Osteoporosis is classified as primary osteoporosis when associated with sex hormone deficiency and aging and as secondary osteoporosis when associated with underlying diseases or disuse, including microgravity-induced osteoporosis or bone loss occurring in the weightless environment of space [49,53].

With the technological advancements of stem cell-based therapy, mesenchymal stem cells (MSCs) with the capacity to differentiate into osteoblasts may serve as a novel approach for the treatment of osteoporosis [54]. The most commonly used MSCs are the bone marrow mesenchymal stem cells (BM-MSCs), which have been broadly investigated in bone regeneration and bone repair due to their easy accessibility and high osteogenic differentiation capacity [54]. Ichioka., *et al*. locally injected the allogeneic BM-MSCs into the bone marrow cavity of senescence accelerated mouse prone 6 (SAMP6) mice (a substrain of senescence-accelerated mice, an osteoporosis mouse model) and found that BM-MSCs increased trabecular bone, inhibited the loss of BMD, and prevented osteoporosis [55]. Kiernan., *et al*. demonstrated that systemic injection of allogeneic MSCs enhanced bone formation and strengthened microarchitectural competence in an age-related osteoporosis mouse model [56].

"Nanoparticles based countermeasures for Treatment of microgravity induced Osteoporosis" (NATO) project evaluated the effects exerted by the addition of calcium- and strontium-containing hydroxyapatite nanoparticles (nCa-HAP and nSr-HAP) suspensions on human bone marrow mesenchymal stem cells (hBM-MSCs) in three different conditions: on the earth's surface at 1g, in the Random Positioning Machine (RPM) and in space, onboard the International Space Station (ISS) [49]. Under 1g conditions, nSr-HAP enhanced the differentiation of hBM-MSCs into osteoblasts, as demonstrated by the increased alkaline phosphatase (ALP) activity and the up-regulation of the expression of bone marker genes (*ALP, Col1A1, Col3A1, IBSP, OCN, DCN, RUNX2*, etc.); this is consistent with an increased extracellular bone matrix deposition and mineralization. The nSr-HAP treatment provided a protective effect on the microgravity-induced reduction of ALP activity in RPM specimens, and a promotional action on the deposition of hydroxyapatite crystals in either ISS or 1g specimens. These findings suggest the addition of nSr-HAP to hBM-MSCs could be a potential strategy to promote bone regeneration for microgravity-induced osteoporosis [49].

Finally, Zhou., *et al*. investigated the effect of combined melatonin and bone marrow mesenchymal stem cell therapy on the treatment of osteoporosis [57]. The results revealed that melatonin up-regulated bone marrow mesenchymal stem cell osteogenic action by inacti-

vating MT2-mediated NF-κB signaling pathway and suppressed osteoclastogenesis by reducing the production of RANKL [57]. Combined melatonin and bone marrow mesenchymal stem cell therapy may be a highly useful and safe regimen for the treatment of osteoporosis [57]. The results of this study suggest a possible prospective strategy for both preclinical and clinical studies of combined melatonin and mesenchymal stem cells in the treatment of microgravity-induced osteoporosis or bone loss in order to preserve optimal health of astronauts on long-term space exploration missions.

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