

# Mimic Spaceflight: Microgravity Induces Bone Injury

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**Abstract.** Astronauts take the risk of health problems during spaceflight. One of the most severe problems is bone injury. Though astronauts experience exercises before spaceflight, bone injury is still induced by anti-gravity. To try best to mimic the condition free from the force of gravity, most studies have explored the mechanism of bone injury under microgravity condition. In microgravity environment, osteocytes, osteoclasts, osteoblasts, and mesenchymal stem cells are all identified to alter, contributing to bone loss and function defects of mineralization. Ulteriorly, three typical molecular regulatory pathways including NF- $\kappa$ B pathway, RhoA/integrin pathway, and calcium pathway have been discovered till now, which reveals the possible molecular mechanism. In this article, the specific alterations of cells associated with bone and the three pathways mentioned above will be presented. Different systems are found to related to bone alterations modulated by microgravity as well. So far, the mechanism has not been completely comprehended because of its complexity. However, the current discoveries about mechanism are still valuable. According to regulatory mechanism, new therapies are designed to improve bone injury aiming to help astronauts.

**Keywords:** Microgravity, Spaceflight, Bone.

## 1. Introduction

It has been commonly acknowledged that space flight has adverse effects on astronauts' body, particularly the bone. Since spacecrafts send humans into space, the influences caused by microgravity has never quitted exploring. With the development of aerial detection technology, profound space mysteries are increasingly revealed. At the same time, the data collected from astronauts are valuable to study how human body reacts to microgravity. Compared with human body on the ground, muscular system, vascular system and bone are all induced by microgravity. Bone alterations are mainly focused on this paper and worthy to be explored. However, the mechanism of bone injury is complex under microgravity condition. There are various bone injury including bone loss, mineralization defects and so on. Bone loss and mineralization defects are two key problems that are studied positively in microgravity environment. The two questions have been studying from phenomenon to molecular level, contributing to reveal the mechanism of experiencing weightlessness.

There are some features in bone injury induced in microgravity environment. Damage occurring in space remains when astronauts come back to ground. To be more specific, bone damage cannot vanish even if humans experience the normal gravity. More importantly, the study implicated that bone injury could not recover to the previous states and it would take long time to repair the bone. One experiment demonstrated that recovery from bone loss took longer than incur the loss. The time of complete recovery needs over four times than that of rebuilding 50% bone structure. It usually takes three to nine months to recovery after human returns to ground [1]. More severely, they found that the trabecular volumetric bone mineral density (BMD) of the femoral neck failed to return to preflight values even two years after return to Earth [1]. Impaired bone undergoing microgravity is considered to be difficult to re-establish. However, some study illustrated that the bone loss trended to be stable after experiencing dramatically loss. The result remains to be further confirmed. In addition, bone alterations occur at some specific sites under microgravity condition. Bone loss usually happens in weight-bearing sites, especially in hip and lumbar spine including tibia and radius. Gravity change has influences on the part of the body that forces itself originally on the ground. The metabolism regulated by gravity can be disturbed by the absence of gravity. The reaction of human body under microgravity is otherness of sites. So far, hip and lumbar have shown significant response

to gravity change and varieties of models such as hindlimb unloading model, which are applied to explore relevant aspects, using the cells of the two sites to test [2].

The study data was collected from not only astronaut detection during space flights but also various experimental models. Experimental environments are most microgravity which mimics space environment. According to bone injury features, weight-bearing sites unloading models are commonly used to carry out assessments. Except for human, the object of study also includes mice. Experiments use mice models to test in both vivo and vitro.

In recent years, the mechanism of bone loss has been studied further from the bone organ level through cells to molecules level, and kinds of possibility of regulation pathways have been reported. Osteocytes, osteoclasts, and osteoblasts modulate bone metabolism. Mesenchymal stem cells (MSCs) are related with differentiation process. Microgravity induces the balance of metabolism, thus ultimately affecting osteosis and bone resorption. The researches to discover bone injury in microgravity environment are essential, and the results can provide more proper exercise plan for astronauts and new therapy after they return. Nowadays, if astronauts have been in space for several months, they are considered to take larger risk of severe bone loss. A long period of space exploration makes astronauts undergo strong impact on bone quality. To meet the need of space exploration, the study of mechanism of bone induced by microgravity plays an important part.

## **2. Bone influenced by microgravity**

It has been known that microgravity leads to bone injury. There are various specific aspects of bone injury under microgravity condition. The most severe problem is bone loss. Microgravity decreases bone formation and increases bone resorption, making adverse effects on bone volume, thickness and density. Some evidences showed that there were bone mineralization defects under microgravity. The detail data from Russian Mir space craft first revealed that BMD had an average loss as 1%–1.5% per month and the loss even reduced faster than that of older individuals [3]. The quality of bone material is declined because of impaired tissue mineralization, maturation, and maintenance [4]. The material composition of bone was observed changing, which was identified by downregulating mineral, osteocalcin and collagen concentration. Some chemical elements can be regulated by microgravity, making phosphorous and magnesium decreased and sulfur increased on the contrary. Such alterations influenced maturation of newly formed bone. Calcium and phosphorus are two important mineral elements in bone formation. The concentrations decrease under microgravity condition, which lowers quality of bone material. To be more specific, bone modulus and hardness decrease while the mineral quality stays the same level compared with ground control.

Bone loss in microgravity is induced by cortical thinning. The study discovered that trabecular bone declined by 0.4–0.5% per month and cortical bone by 2.2–2.7% per month [5]. Notably, the data reported that volumetric bone mineral density in tibial trabecular reduced by 5.43% while cortical bone decreased by 1.78%, which illustrates that bone resorption is more severe in trabecular bone than in cortical bone [4]. Trabeculae responds significantly to microgravity. More importantly, once microgravity drives trabecular bone resorption, no mechanical signal produces by such stimuli and it means that cellular structure remodeling will not function. Damage to Trabecular bone caused by bone resorption is difficult to repair. This is consistent with the case that bone loss induced by microgravity is hard to recover even on the ground. There are reports that microgravity influences the number of blood cells [6]. Decreasing vascularization is also a barrier to recover from bone loss.

## **3. Changes induced by microgravity on cell level**

### **3.1. Osteocyte**

Bone loss has been assessed in microgravity environment and the large risk of osteoporosis taken by astronauts has been found. To discover the deeper alters, researchers have explored whether cells of bone respond to the change of gravity from ground to space. Osteocyte was observed at first and

scientists found that osteocyte apoptosis occurred three days after the cells treated in the microgravity and the speed of osteocyte apoptosis was faster than expected. Such phenomenon confirms bone loss because the number of osteocytes determines bone quality. Severe osteocyte apoptosis results in significant decrease in the number of osteocytes and breaks the balance of the metabolism of osteocyte. To further investigate the link between osteocyte apoptosis and bone loss, scientists tested pro-apoptotic markers. The result showed that osteocyte apoptosis contributed to bone loss, but there was not enough evidence to confirm that osteocyte apoptosis occupied the main status in bone loss in microgravity environment [7]. Besides, though they found some pro-apoptotic genes upregulated, it was unclear whether osteocytes automatically entered apoptosis process in the absence of weight.

### 3.2. Osteoclast

The metabolic balance of osteocytes on both osteoclasts and osteoblasts. Osteoclast is responsible to accelerate apoptosis of aging osteocyte with function deficit. Scientists predicted that abnormal osteocyte apoptosis was related to changes in osteoclasts. Osteoclast originates from mononuclear precursors and amounts of mononuclear precursors fuse to form osteoclasts. The mature osteoclast is the large multinucleated cell. Some evidence show that microgravity directly influences osteoclast structure and functions. It has been confirmed that an increase in bone resorption is caused by osteoclast treated in the microgravity in different methods.

Nabavi et al. were the first to prove that osteoclast experiencing weightlessness could promote bone resorptive activity by observing the improvement of osteoclast resorption pit formation [8]. They found differences in pit between the two conditions, on the ground and in microgravity. Cells cultured in microgravity had characteristics of larger pit sizes, greater depths and less uniform morphologies. Through quantitative analysis, the number of resorption pits of osteologic slides was significantly larger than that of resorption pits of ground controls. Enhancing osteoclast resorption pit formation demonstrates that osteoclast is related to bone loss in microgravity environment.

More experiments were designed to focus on markers of bone resorption, thus aiming to reveal the relationship between osteoclast and bone loss. The process of bone resorption is complex, and varieties of molecules can be markers tested in the experiments. Collagen cross-link products are commonly applied to determine bone resorption level. In an experiment analyzing astronaut data, an increase of urinary collagen cross-link products was observed, indicating the improvement of bone resorption [9]. Another experiment quantified bone resorption by mature osteoclast using the collagen I telopeptide concentration (CTX) as the marker [10]. The larger number of collagen I telopeptides was found in microgravity groups in this experiment, indicating that microgravity promoted bone resorption, which was contributed by osteoclasts.

Microgravity can also induce changes in immune regulation of osteoclasts. Osteoclasts respond to various pro-inflammatory cytokines providing that they are immunologically reactive cells [11]. Pro-inflammatory cytokines were assessed and they stimulated osteoclast genesis thus promoting bone resorption. The phenomenon of facilitating osteoclast genesis were tested by some genes and mRNA level. The expression of genes related to bone resorption dramatically increased in microgravity by PCR examination and the result is consistent with the above description that microgravity activated osteoclasts [10]. In addition to reacting to pro-inflammatory cytokines, osteoclast genesis is associated with autophagy in immune system in microgravity environment. To prove such result, modulation of autophagy markers was assessed in preosteoclast cells [12]. Cells under microgravity environment produced a large amount of autophagosome formation, providing evidence that autophagy was involved in promoting osteoclast genesis. It was proposed that microgravity induced osteoclast differentiation, but no more direct experiments confirmed that.

### 3.3. Osteoblast

Microgravity facilitates a reduction of osteoblast resulting in bone loss. Osteoblasts originate from local osteoprogenitor cells and they take over the duties of production of the inorganic bone matrix and modulation of its mineralization [13]. Therefore, the reduction of osteoblasts contributes to low

bone mineral density under microgravity caused by downregulating bone mineralization. It has been widely known that osteoblasts are more likely to shorten their survival spans and downmodulated their activities. To explain the reason, scientists were devoted to exploring the internal structural change of osteoblasts. Significant changes of osteoblasts in microgravity environment were observed, including alteration of cellular morphology, disruption of cytoskeleton and reduced focal adhesions [13]. The integrity of osteoblast cytoskeleton was broken down under microgravity and tissue was altered, contributing to a decrease of osteoblast surface area and its irregular shape.

The decrease in focal adhesions was confirmed to be the consequence of damage of focal adhesions maturation [8]. The experiment showed that un conspicuous focal adhesion morphological structures compared with cells remaining on ground through testing the substance recruited to focal adhesions at a later stage of their maturation. Such phenomenon can better prove the reduction of focal adhesions. Besides, the number of stress fibers in osteoblasts reduced when osteoblasts were treated in microgravity, and especially, actin stress fibers were influenced by weightlessness [14]. More importantly, focal adhesion sites interacted with actin stress fibers, thus determining osteoblast shape. Therefore, downregulation of the formation of stress fibers due to the absence of gravitational forces made osteoblasts appear in irregular shape. Other structures of cytoskeleton such as microtubule and actin filaments were assessed ineffective as well.

In addition to cytoskeleton disruption, changes in nuclear morphology were important concerns. Nuclei of flight osteoblasts showed much more variability, with many nuclei smaller and condensed, and some appearing fragmented [8]. Exposure in microgravity decreased the number of integrated nuclei of osteoblast and a few intact nuclei became more swollen. Nuclei shape were also shown to be elongation [15]. Nuclei alterations may be associated with cytoskeleton disruption since nuclei direct genes expression, which influences cellular structures. Microgravity affects cellular structures from focal adhesion sites through the cytoskeleton to the nuclei.

### **3.4. Mesenchymal stem cells (MSCs)**

Osteoblasts are terminally differentiated cells and they originate from MSCs through differentiation. The absence of weight causes osteoblast formation defects via inhibiting the function of MSCs differentiation. MSCs tend to develop into adipocytes and fibroblasts rather than osteoblasts in microgravity. Some experiments prove microgravity promotes adipocyte differentiation instead of osteoblast differentiation. One experiment using hindlimb unloading (HU) model displayed a decrease in osteogenic potential. They found that MSCs produced large amount of small lipid droplets in the group which was untreated to adipogenic inductors but under UH condition [2]. Microgravity induced independently MSCs adipocyte genesis. Another experiment assessed adipogenic markers and found that microgravity inhibited expression of osteoblastogenesis markers and stimulated expression of adipogenic markers along with an increase in adipogenic transcription factors [16]. Besides, MSCs differentiation were related to cytoskeleton disruption. Cytoskeleton was damaged in the process of MSCs differentiation and increased actin accompanied with a decrease stress fiber and cellular morphology alterations. All the results agree with the phenomenon that regulation of MSCs differentiation reduces osteoblasts. Some scientists also proposed that space radiation may modulate MSCs autophagy, thus influencing osteoblastogenesis.

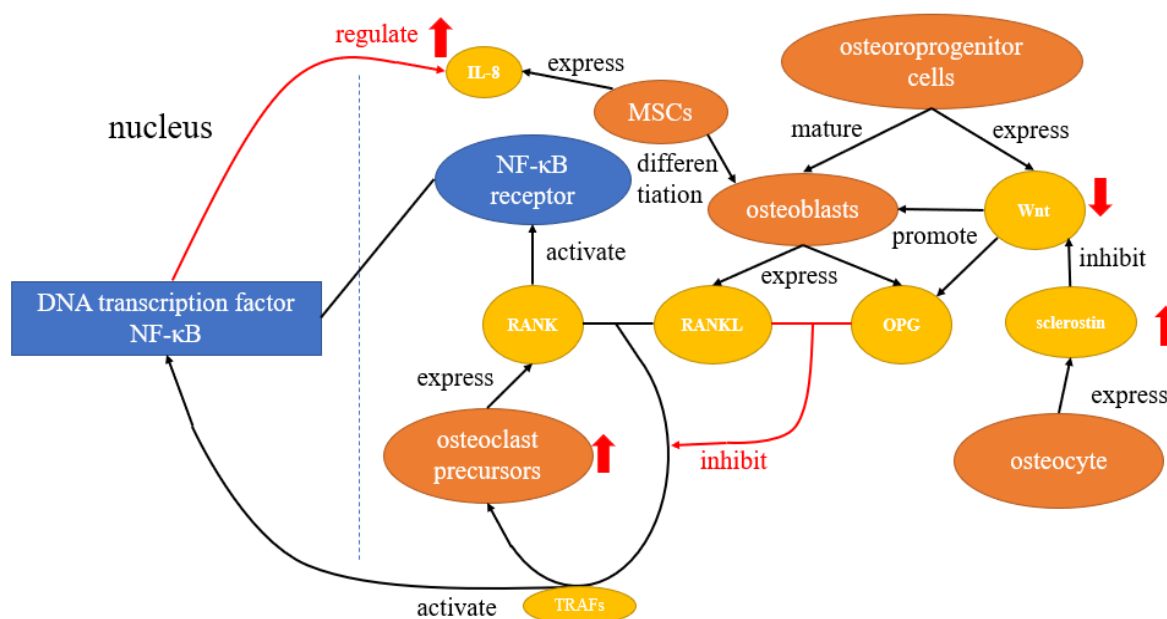
## **4. Microgravity regulating mechanism on molecular level**

### **4.1. NF- $\kappa$ B pathway**

The mechanism of signal pathway stimulated by microgravity is complicated. To explore the mechanism, various possible pathways and relevant molecules have been studied. So far, some essential factors have been identified to be involved in this regulating mechanism. DNA transcription factor NF- $\kappa$ B pathway is the key signal pathway to regulate bone formation and resorption [1]. NF- $\kappa$ B inducing proinflammatory gene expression produces in nuclear. Through nuclear pore, NF- $\kappa$ B binds with the receptor which is regulated by receptor activator for DNA transcription factor NF- $\kappa$ B

(RANK) expressed by osteoclast precursors. RANK ligand (RANKL) expressed by osteoblast is bound by RANK. RANK and RANKL are upstream signaling of NF- $\kappa$ B pathway [17]. The binding makes TNF receptor activating factors (TRAFs) increase and activates a kinase cascade which initiates transcription of NF- $\kappa$ B [11]. This pathway promotes osteoclast differentiation. Osteoprotegerin (OPG) is another significant element secreted by osteoblasts. OPG tends to bind to RANKL as well and it competes with RANK [18]. Wnt expressed by osteoprogenitor cells which also promote osteoblast maturation upregulates OPG and develop osteoblast differentiation. In turn, Wnt is inhibited by sclerostin secreted by osteocyte. The ratio of RANKL/OPG determines the balance of osteoclast and osteoblast, thus further controlling the balance of bone resorption and formation.

Microgravity influences the ratio of RANKL/OPG. The ratio of RANKL/OPG was observed to enhance in osteoporosis and some types of arthritis [19]. The elevated ratio of RANKL/OPG breaks down the balance of osteoblast and osteoclast. OPG concentration decreases and the joint probability of RANK and RANKL elevates, therefore inducing osteoclast formation and improving bone resorption through NF- $\kappa$ B pathway. The study reported that sclerostin increased under microgravity condition. Increasing sclerostin as the inhibitor of Wnt made osteoblast more difficult differentiation. The decreasing Wnt also reduced OPG concentration. The study also revealed that IL-8 secreted by osteoblast committed MSCs, a chemoattractant of neutrophils and macrophages promoting their migration to the inflammation foci, upregulated in microgravity environment [18]. The increasing IL-8 modulated by NF- $\kappa$ B pathway was shown to accelerate bone resorption [20]. The visualized regulatory process is shown in Figure 1.



**Figure 1.** NF- $\kappa$ B pathway regulation of microgravity. Black lines and arrows indicate the regulation under normal gravity, while red lines and arrows demonstrate the alterations induced by microgravity. (Photo credit: Original)

#### 4.2. RhoA/integrin pathway

Another key pathway is integrin signaling involved in RhoA kinase. Integrins are transmembrane heterodimers that reside on the cell surface and integrate extracellular signals into intracellular responses [21]. The study demonstrated that RhoA activity reduced in the absence of weight, leading to disruption of stress fibers and altered skeleton via decreasing phosphocofilin [16]. Skeleton disruption influenced integrin functions. Integrin signaling was activated and it regulated osteoblastic differentiation, which inhibited osteoblast formation. Another study first found that decreasing RhoA elevated nitric oxide synthase (NOS2) and further enhanced nitric oxide (NO) [13]. Increasing NO

not only threatened osteoblast survival and influenced functions, but also promoted dense-dependent adipocytic differentiation. Recognizably, NO was observed to provide negative feedbacks for RhoA.

### 4.3. Calcium pathway

Bone mineralization are modulated by calcineurin pathway. Calcium signaling is induced by a complex kinase cascade including Bruton's tyrosine kinase (Btk) and phospholipase C gamma (PLC $\gamma$ ) activated by microgravity and bone mineral component alterations are stimulated through calcineurin pathway activated by calcium signal [11]. Calcium signaling has also been displayed to directly regulate autophagy [12]. The experiment illustrated that PLC $\gamma$  dramatically increased in preosteoclast cells in microgravity environment. Calcium signaling stimulated by PLC $\gamma$  regulates osteoclast apoptosis. Increasing PLC $\gamma$  accelerates osteoclast apoptosis and inhibits bone formation.

## 5. Discussion

The researches of bone alterations on molecular level induced by microgravity are carried out from various aspects and show a large number of signal pathways. What mentioned above only involves some common acknowledged pathways and neglects some molecules with peculiarity. More possible pathways and possible relevant factors are being explored. The definite point is that the number of molecules participating in regulation mechanism under microgravity condition is enormous. The molecules which deserve attention are not only those who can directly modulate bone cells but also ones that are likely to influence other metabolism which indirectly control bone alterations. Moreover, many molecules are corelative and the exploration of interacting is not enough. So far, most experiments are focused on individual molecule affecting a specific reacting chain. Whether those molecules or cells having been confirmed to be induced by microgravity have interaction is still unclear. Various molecules remain to be integrated into a complete regulation pathway, which contributes to comprehensive mechanism of bone injury in microgravity environment.

Some evidences illustrate that there are links between cells and molecules related to bone regulation. In an experiment, researchers found that osteocytes apoptosis occurred ahead of the increasing expression of RANKL by tracking pro-apoptotic markers and analyzing organization of osteocytes in the mechanical unloading model [22]. For further study, they also discovered that the substances released by apoptotic osteocytes upregulated the expression of RANKL secreted by healthy osteocytes [7]. However, the result was only tested in vitro model of bone loss and has not assessed in the microgravity environment. In addition, another study showed that osteoclasts and osteoblasts were independently stimulated by microgravity. Microgravity directly induced osteoclastogenesis and increased bone resorption in the absence of osteoblasts. They proposed that the osteoclast was the direct target of mechanical forces [10]. Combined with regulation on the molecular level, microgravity is likely to not only induce relevant molecules to modulate osteoblasts and osteoclasts, but also influence the two kinds of cells directly.

Scientists have discovered that microgravity affects other systems which indirectly caused bone loss. Osteocytic mechanosensory system is influenced in the absence of weight, thus resulting in function defects of mechanotransduction. Osteocytes experience decreased liquid flow velocity and fluid shear stress, therefore receiving less nutrients and insufficient mechanical stimulation [7]. The alterations of the flow field of tissue fluid and the mechanical response to the flow field were studied, and the reduction of liquid transmission was considered as a cause of bone loss under microgravity condition [23]. There is also a correlation between bone and skeletal muscle. Microgravity induces skeletal muscle atrophy through protein loss [24,25]. Skeletal muscle, as the largest metabolic and secretory organ in the human body, participates in crosstalk with other tissues, one of which is bone. Microgravity has been confirmed to have effects on vascular system. There are some evidences displaying that bone, skeletal muscle, and vascular system are corelated with each other. Microgravity inhibits angiogenesis in skeletal muscle. Scientists also observed that NO participating bone response

to microgravity was responsible for angiogenesis [26]. All recent discoveries indicate that it remains certain co-relationship between bone and other systems, therefore being worthy to pay attention.

Notably, limited data are an essential problem on this field. Astronauts are the ideal experimental objects while low frequency of spaceflight and small number of astronauts make troubles in providing practical data of the research. It is difficult to acquire intuitive data due to the peculiar and strict condition of experiments. It is hard to take long period to monitor experimental phenomenon during spaceflight. Though most studies are carried out under microgravity condition mimicking spaceflight or apply unloading model of mice, the consequences cannot avoid inaccuracy. The results found in the microgravity cannot be guaranteed to be completely consistent in space, so it is necessary to improve similarity of experimental condition.

The influence of bone induced by anti-gravity is hard to vanish. More practical option is to limit the inflight bone loss enough to minimize any inflight risk rather than try to prevent the inflight bone loss [27]. It has been discussed which stage is the best moment to carry out intervention. However, there is not enough data showing whether an intervention is a need during spaceflight [3]. Some therapies of bone loss induced by inflight are proposed according to regulatory mechanism. Except for normal exercises, molecular treatments aiming to modulate relevant molecules seem to be practical therapies. Most therapies on molecular level are still under development.

## 6. Conclusion

Microgravity leads to bone injury which is hard to recover even under normal gravity condition. Bone injury most occurs at loading sites of bodies. Microgravity induces bone injury including bone loss and mineralization defects through cellular and molecular regulation. When bone is subjected to microgravity, BMD decreases and material components change. Microgravity also influences the rates of reconstructing bone and wound healing. On cellular level, osteocytes apoptosis accelerates, and the balance between osteoblast and osteoclast is broken, thus enhancing bone resorption. Microgravity leads to reduction of MSCs osteoblastic differentiation. On molecular level, there are three regulatory pathways that are generally recognized—NF- $\kappa$ B pathway, RhoA/integrin pathway, and Calcium pathway. In the microgravity environment, quantities of molecules participate in regulation and bone interplays with other systems as well.

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