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ORIGINAL ARTICLE

# Mechano-biological Coupling of Cellular Responses to Microgravity

**Mian Long[1,2] · Yuren Wang[1] · Huiqiong Zheng[3] · Peng Shang[4] · Enkui Duan[5] · Dongyuan Lü[1,2]**

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**Abstract** Cellular response to microgravity is a basic issue in space biological sciences as well as space physiology and medicine. It is crucial to elucidate the mechano-biological coupling mechanisms of various biological organisms, since, from the principle of adaptability, all species evolved on the earth must possess the structure and function that adapts their living environment. As a basic element of an organism, a cell usually undergoes mechanical and chemical remodeling to sense, transmit, transduce, and respond to the alteration of gravitational signals. In the past decades, new computational platforms and experimental methods/techniques/devices are developed to mimic the biological effects of microgravity environment from the viewpoint of biomechanical approaches. Mechanobiology of plant gravisensing in the responses of statolith movements along the gravity vector and the relevant signal transduction and molecular regulatory mechanisms are investigated at gene, transcription, and protein levels. Mechanotransduction of bone or immune cell responses and stem cell development and tissue histogenesis are elucidated under microgravity. In this review, several important issues are briefly discussed. Future issues on gravisensing and mechanotransducing mechanisms are also proposed for ground-based studies as well as space missions.

**Keywords** Microgravity · Mechanobiology · Mechanotransduction · Cells

✉ Mian Long
mlong@imech.ac.cn

1 Key Laboratory of Microgravity (National Microgravity Laboratory), Institute of Mechanics, Chinese Academy of Sciences (CAS), No. 15 North 4th Ring Road, Beijing 100190, China

2 Center for Biomechanics and Bioengineering and Beijing Key Laboratory of Engineered Construction and Mechanobiology, Institute of Mechanics, Chinese Academy of Sciences (CAS), No. 15 North 4th Ring Road, Beijing 100190, China

3 Laboratory of Photosynthesis and Environmental Biology, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, CAS, Shanghai, China

4 School of Life Sciences, Northwestern Polytechnical University, Xi'an, Xi'an, China

5 State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, CAS, Beijing, China

## Introduction

Microgravity-induced cellular responses are vital in space biological sciences as well as space physiology and medicine. It is crucial to elucidate the mechano-biological coupling mechanisms about how altered gravity regulates the biological responses at cellular, molecular or tissue levels. In 2010, a National Key Basic Research Project, "Mechano-biological coupling of cellular responses to (micro-)gravity", was approved by Ministry of Science and Technology of China. As one of the biggest program projects on basic sciences in China, this type of project attempts to make the innovative understandings in the related field. Fifteen scientists from various interdisciplinary fields in biomechanics and mechanobiology, cellular and molecular biology, plant and animal physiology, as well as space bioengineering and technology, are working collaboratively for five years, who are reformed into five subgroups to address the different issues (Fig. 1).

This aforementioned project attempts, from the viewpoint of space human physiology and life support system, to unravel the cell mechanotransduction mechanisms under (micro-)gravity. Scientific issues are focused on understanding the effect of gravity environment on evolution of terrestrial life and on the impact of space environment on physiological homeostasis of organisms. Three specific aims are: 1) How do the terrestrial lives sense (micro-)gravity signaling and what are the underlying mechanotransduction pathways? 2) How do the organisms adapt themselves to the (micro-)gravity environment? 3) How are the (micro-)gravity resources utilized to promote the perspective of space life science and the development of space biotechnology? (Fig. 1). In additional to those conventional biological approaches, a new strategy of mechanobiology is adopted for elucidating the mechanisms how external forces or changes in cell or tissue mechanical microenvironment contribute to development, physiology, and disease of an organism (Wang et al. 2008). The outcomes of this project would provide the fundamental understandings for space human physiology and life support system, propose new concepts, new ideas, and new methodology in space biological sciences, and establish the integrated platforms for the ground- or space-based studies. Expected results are to develop numerical or virtual simulation platforms and biologically-specific techniques and to further the understandings in gravisensation, mechanotransduction of gravity signaling, or gravimetric response of plant or animal cells and tissue histogenesis. This Special Issue would update collectively the progresses in cellular responses to microgravity and promote the studies for space life science and space biotechnology in China and even in the world.

## Biomechanical and Mechanobiological Approaches

Space microgravity is unique mechanical environment to induce significant biological and physiological alterations in organisms (Hu et al. 2014; Kang et al. 2014). This mechano-biological coupling nature raises various related issues, such as, are these physiological changes of astronauts adaptive or pathological and how can we clarify them, can we and how do we determine if these changes are reversible or irreversible, or is there a threshold and can the threshold be enhanced (van Loon 2009; Wang et al. 2014b). As a basic element of an organism, the cell is sensitive to its surrounding mechanical environment including types, patterns, and parameters of mechanical load (Wang et al. 2014b). Thus, caution should be taken when we attempt to address the issues of what gravitational change is, how space microgravity environment or effect is simulated, and how cellular responses to microgravity environment are mimicked. For example, a clinostat or a rotary bioreactor, which is frequently used by space life scientists to test the impacts of the so-called *stimulated microgravity* (SM), is unable to *stimulate* the microgravity from the view of mechanical/physical principles, since gravity could not be eliminated on ground as a body force. Instead, (partial) biological effects of space microgravity environment are able to be stimulated as long as the cell movement inside the clinostat or rotary bioreactor behaves like a rigid-body movement (Ayyaswamy and Mukundakrishnan 2007; Wang et al. 2014b). The capacities and limitations of the ground-based microgravity stimulators are thoroughly discussed (Herranz et al. 2013). Knowing that the simulators have considerable microgravity (μg)-like impacts on biological samples but they also induce a number of side effects, the term

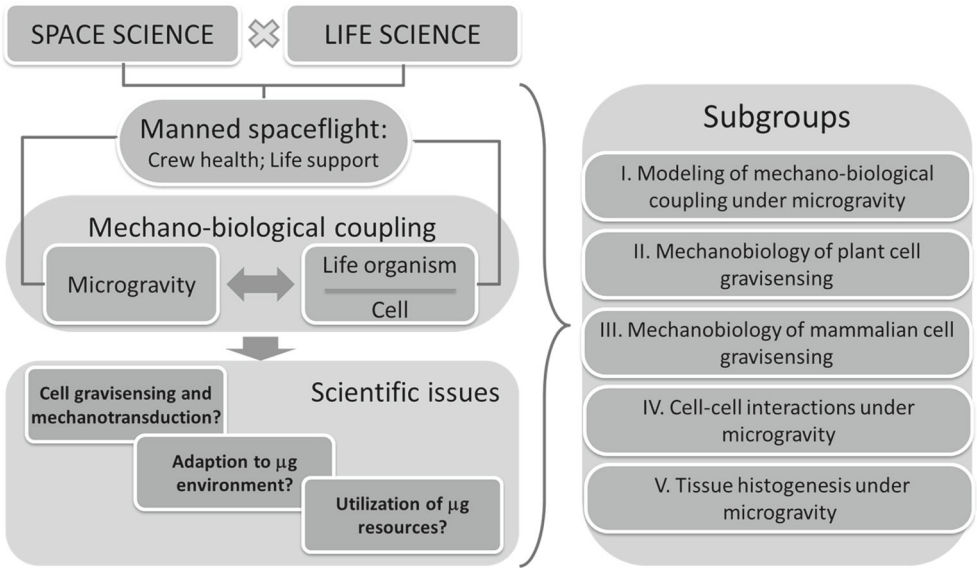


**Fig. 1** Overview for specific issues under microgravity ($\mu g$) and scientific framework of five subgroups

SM is used in this review for simplicity. Meanwhile, a virtual numerical platform upon computational fluid dynamics needs to be developed for evaluating the effect of SM on ground, optimizing the mechanical parameters for the clinostat or rotary bioreactor, and designing the novel bioreactors for mimicking the biological responses under microgravity. Moreover, a conceptual test on gravity-vector directed effects also indicate that downward- or edge-on-orientated substrate enhances the F-actin expression with shrinking morphology of rat osteosarcoma Ros 17/2.8 cells (Li et al. 2010). Such the conceptual clarification helps to elucidate the basic processes in space life science and biotechnology as well as in space physiology and medicine, and to present the scientific merits of related ground-based studies.

Different biomechanical and bioengineered approaches are also used to understand the biological responses under (micro-)gravity. These approaches coordinate multiple species (plant cell, bone cell, immune cell, stem cell, or substrate matrix), various systems (single cell, cell-cell or cell-matrix assembly), mechanical modeling (mass transportation, cell mechanics, or microgravity stimulation), mechanical stimuli (shear, tension, compression, rigid-body rotation, or magnetic levitation) with biological responses at cell, protein, gene, or tissue level (Fig. 2). One example is to validate the statolith hypothesis of plant gravisensing from the viewpoint of microrheological feature of amyloplast movement in the plant root gravity-sensing cells. While it is well known that the sedimentation movement of amyloplasts primarily triggers the asymmetric distribution of auxin and then leads to the differential growth of the plant root (Vanneste and Friml 2009; Leitz et al. 2009; Morita 2010; Sato et al. 2015), the inhomogeneous structures in statocytes are also found to significantly affect the movements of amyloplasts and the resulted gravimorphogenesis (Weise et al. 2000; Toyota et al. 2013a). Microrheological analysis, frequently used to detect micro-mechanical properties of cytoskeletal network, is employed for the first time and indicates that the intracellular environment of columella cells exhibits the spatial heterogeneity and the cage-confinement on amyloplasts. By comparing the distinct diffusive dynamics of amyloplasts in wild type, *dis1*-mutated, and Latrunclin B-treated plants with the behaviors of colloidal systems in different states (Zheng et al. 2011; Zheng et al. 2014), the influence of the actin reorganization-dominated intracellular environments is quantitatively characterized on amyloplast movement. The cage-confinement strength is measured by calculating the spatial fluctuation of local apparent viscosity within the columella cells through microrheological analysis. Finally, a linear correlation is observed among the initial mechanical stimulation strength in the columella cells estimated by the inverse local apparent viscosity, the subsequent intercellular signal transduction of the asymmetric accumulation of auxin ratio in root cap, and the final gravity response of the root curvature angle. A possible gravity sensing mechanism is suggested giving rise to a linear frustration of the actin cytoskeleton on the conversion of mechanical stimulation into gravitropic signals (Zheng et al. 2015).

Another example is to develop the new magnetic levitation technique for mimicking the biological effects of microgravity on the ground. Magnetic levitation of diamagnetic materials, also called diamagnetic levitation (DL), has been successfully applied in biological studies (Beysens et al. 2011; Shang et al. 2013). Various species (*i.e.*, macromolecules, cells, tissues, organisms) are such the diamagnetic materials that experience a magnetic force repelled from the source in a gradient field. When the samples are placed in a vertical gradient magnetic field, the apparent

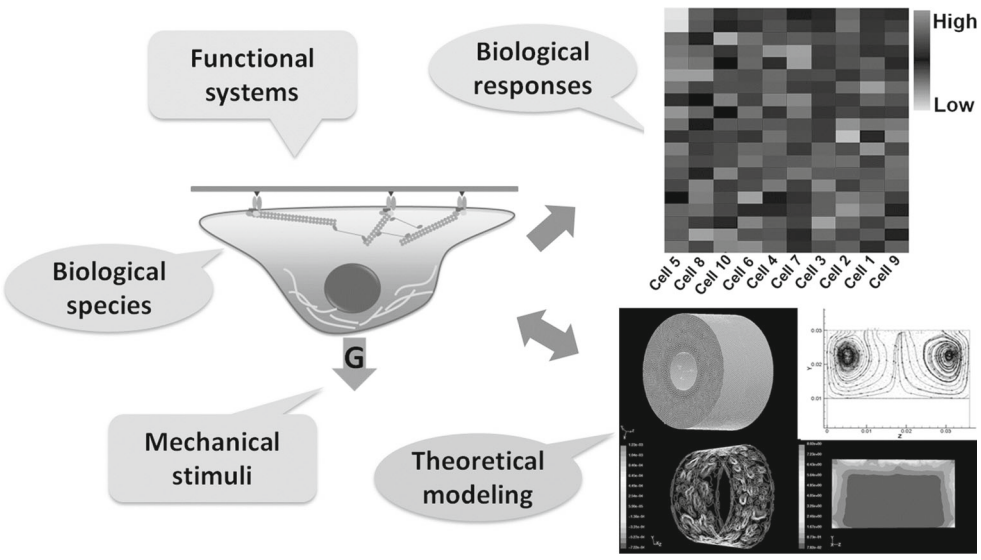


**Fig. 2** Schematic of working approaches demonstrating five aspects of biological species, functional systems, theoretical modeling, mechanical stimuli, and biological responses

gravity could be decreased or increased depending on the vector direction of magnetic force. Therefore, in a large gradient high magnetic field produced by a specifically designed superconducting magnet, a gravitational environment from microgravity ($\mu g$) to hypergravity ($2\times g$) could be achieved. This gravitational environment provides not only a long-term but also a stable weightlessness environment for space SM research on the ground (Qian et al. 2013a). Corresponding facilities have been developed for biological studies on cell culture, plant growth, protein crystallization, and others (Zhang et al. 2015a; Lu et al. 2015; Yin 2015). It should be noted that the biological effects in this high magneto gravitational environment (HMGE) are composed of gravity and high magnetic field simultaneously. Some organisms or biological processes may be more sensitive to high magnetic field. For example, osteoblast proliferation and differentiation are promoted in HMGE regardless of gravity level (Qian et al. 2013b; Zhang et al. 2014). But both space flight mission and random positioning machine (RPM) test have an inhibitory effect (Shang et al. 2013), indicating that positive effects exerted by magnetic field cover the inhibitory effects by microgravity for osteoblasts.

## Mechanobiology of Plant Gravisensing

Studies on plant gravisensing could be dated back to early nineteen century for the gravitropism of plant roots and leaves (Knight 1806). Recently, the underlying mechanisms of gravisensing are elucidated intensively in plant cells by focusing on the key issues about how plant cells sense, transmit and respond to (micro-)gravity (Boonsirichai et al. 2002; Blancaflor and Masson 2003; Morita 2010; Toyota and Gilrory 2013b; Sato et al. 2015). Proteomic, genomic and microRNA changes are analyzed in plants or cell cultures of *Arabidopsis* and rice in response to gravitational alteration (*i.e.*, microgravity, hypergravity, and clinorotation) (Barjaktarović et al. 2009; Paul et al. 2013; Mazars et al. 2014). Various approaches and systems are developed to quantify the mechanotransduction of plant cells (Silverberg et al. 2012). A recent study indicates that leaf dorsoventral polarity signals lead to mechanical heterogeneity that is sufficient to produce the asymmetry seen in planar leaves *via* altering cell wall mechanical properties (Qi et al. Under review). Altered gravity systems including horizontal and vertical clinorotations, hypergravity, and stationary control are used in studying proteomic responses of *Arabidopsis* root cells to different gravitational conditions (Tan et al. 2011). Comparisons of genomic and proteomic profiles of *Arabidopsis* and rice callus grown under microgravity on Chinese spacecraft ShenZhou (SZ)-8 with those grown under 1×g centrifugation (1×g control) in space and on ground are also performed (Zhang et al. 2015b, c). Scientifically, the following progresses have been made in the past years.

At protein level, the proteomic data of *Arabidopsis* wild-type (WT) and *pin2*-mutated root tips and cell cultures under SM or real microgravity conditions indicate that gravity can mediate the expression of particular classes of proteins involved in stress responses such as cytoskeleton organization, metabolic changes in plastids and mitochondria, gene activation/transcription, and cell wall biosynthesis (Tan et al. 2011; Qi and Zheng 2013; Zhang et al. 2015b, c). Among these gravity-responsible proteins, further attentions are focused on those potentially important proteins involved in the gravity signaling transduction and transmission pathways, such as ANN2, KNAT1, and myosin heavy chain-related protein (MHCRP). It is noticed that ANN2 could interact with MHCRP and regulate the early response of roots to the gravitational stimulation (Tan et al. 2011) *via* affecting the reorganization of actin-network in columella cells. This is further confirmed by the observation of differential increase of ANN2-GFP signals in the columella cells of WT or *pin2*-mutated plants subjected to SM. KNAT1, a homeobox family transcription factor, is found to inhibit the gravitropic growth direction of root tips and the pedicels, possibly by regulating auxin redistribution after gravity signal transduction and transmission (Qi and Zheng 2013).

At gene level, the genomic changes in rice seedlings and calli in response to gravitropic stimulation and microgravity on spacecraft SZ-8 are analyzed. The key transcripts and corresponding pathways in gravitropism in the shoot base of rice (*Oryza sativa sp. japonica*) are studied by using microarray, in which 167 transcripts at 0.5 h and 1202 transcripts at 6 h after 90° reorientation of the shoot are unraveled to be significantly different in abundance by 2-fold between upper and lower flanks of the shoot base. Among these, 48 transcripts are found to be changed at both 0.5-h and 6-h treatment, while only 119 transcripts are changed at 0.5 h and 1154 transcripts are altered at 6 h in association with gravitropism. Also demonstrated are the asymmetric regulations of transcripts related to phytohormones, signaling, RNA transcription, metabolism, and cell wall-related categories between upper and lower flanks. These data suggest that the induction of asymmetrical transcription, likely as a consequence of gravitropic reorientation, precedes gravitropic bending in the rice shoot base (Hu et al. 2013b; Jin et al. 2015). The functions of the gravity-regulated genes are ongoing analyzed.

At mRNA level, the gene expression changes in *Arabidopsis thaliana* seedlings are analyzed under either microgravity (spacecraft SZ-8) or SM (three-dimensional (3D)

clinostat). From deep sequencing and real-time PCR confirmation, it is found that the expression of several miRNA genes is significantly changed, and target genes of these miRNAs participate in the biological processes related to auxin response, environmental stress, DNA methylation, and other phenotypes. For example, miR397 and miR408 expressions are significantly up-regulated under both microgravity and SM, in which two target genes of miR 397, LAC4 and LAC17, are involved in the constitutive lignification of *Arabidopsis* floral stems. By contrast, the abundance of miR408 is responsive to copper supply, in which its target genes encode copper-containing proteins of three members of the laccase family (*LAC3*, *LAC12*, and *LAC13*) and plantacyanin. These results (Li et al. 2015) support a model that various space environmental stresses activate the respective signal transduction pathways that entail transcriptional induction of miR408 expression to modulate development and growth.

To further validate the statolith hypothesis, a time-lapse imaging system is set up to monitor the dynamic movement of amyloplasts in *Arabidopsis* root gravity sensing cells in response to the gravitational alteration. The statoliths, generally existing in animals, are vital for sensing balance and response to gravity, *e.g.*, statoliths in statocytes (sensing cells) for invertebrates and in inner ear for vertebrates. In plants, starchy-amyloplasts in gravity-sensing cells play the similar roles as statoliths. In the widely accepted starch-statolith hypothesis, the physical movement of amyloplasts in statocytes is believed to primarily trigger auxin redistribution and leads to differential growth across the organ during gravitropism. It has been gradually recognized that the inhomogeneous structures in statocytes arising from intracellular components such as cytoskeletons and endomembranes significantly affect the complex movement of amyloplasts and the final gravimorphogenesis. *In vivo* microrheological approach is, at the first time, applied in modeling plant *Arabidopsis* gravity-sensing cells by exploiting amyloplasts as native microprobes. The results reveal the spatial heterogeneity and anisotropy of apparent viscosity within columella cell. ARP2/3-dependent actin network dominates cage-effect on amyloplast movements and the cooperative cage-escaping motion of amyloplasts during sedimentation. This finding suggests a potential gravity-sensing mechanism dictating a linear frustration effect of actin cytoskeleton upon the conversion of mechanical stimulation of amyloplasts into gravitropic signals (Zheng et al. 2015).

## Mechanotransduction of Bone and Immune Cells Under Microgravity

Various physiological alterations often appear in long-term flight including bone loss, immunosuppression, and others. Specifically, mechanical unloading in space flight is proved to disrupt balance of bone remodeling and induce severe bone loss (Shang et al. 2013). Bone remodeling requires a highly coordinated collaboration of multiple bone cells, such as osteoblasts, osteocytes, osteoclasts and mesenchymal stem cells (MSCs). Evaluating the mechanotransduction of bone cells under microgravity is critical for understanding the possible mechanisms associated with balance of bone remodeling. Cytoskeletal proteins, fibronectin, microtubule actin cross-linking factor 1 (MACF1), and connexin 43 (Cx43) are found to be the promising gravity-

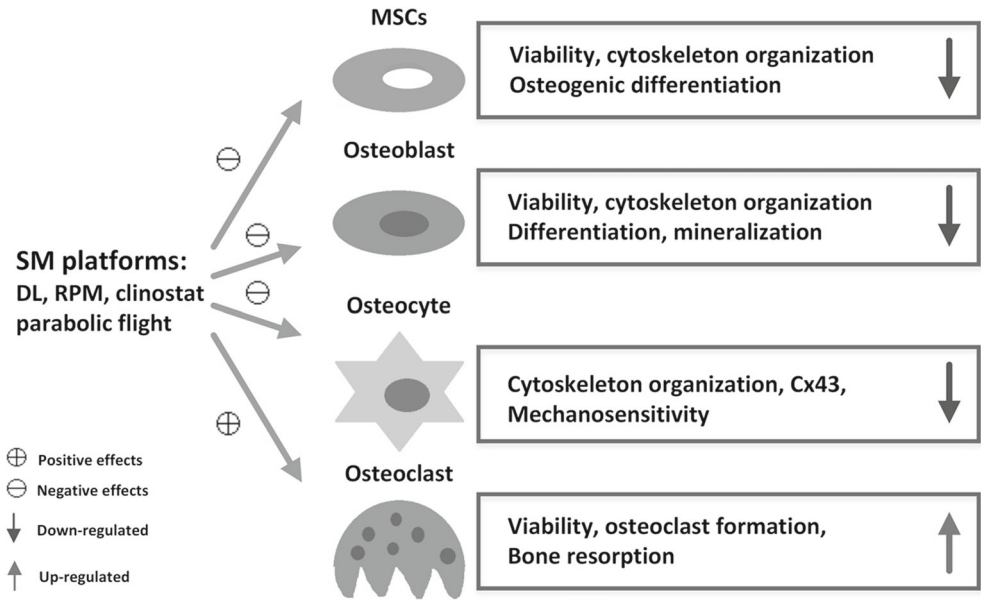


**Fig. 3** Responses of bone cells to SM effect. Microgravity has negative effects on osteogenic cells including MSCs and osteoblasts and on mechanosensitive osteocytes *via* cytoskeleton disruption. On the contrary, osteoclastogenesis is enhanced under microgravity. Reproduced from Fig. 1 of Zhang et al. 2015a. Copyright, Springer Science+Business Media Dordrecht 2015

sensitive pathways, which are crucial to understand the signaling pathway and cytoskeletal remodeling of bone cells under microgravity (Fig. 3).

Cells are assumed to sense and respond to mechanical loading through extracellular matrix (ECM)-integrin-cytoskeleton axis (Alenghat and Ingber 2002). As the load-bearing architecture of cells, cytoskeleton is believed to play the central roles in mechanosensation and then regulates integrin and ECM to adapt to altered mechanical environment. Under microgravity, cytoskeleton is no longer subjected to gravitational force and causes reorganization. Cytoskeleton, especially actin filaments, is critical for tensional integrity and determines cell shape. Multiple SM platforms, including DL, rotary cell culture system (RCCS), RPM, and two-dimensional (2D) or 3D clinostat, induce the reorganized, thin, and discontinuous actin filaments with decreased complexity and chaos in osteoblasts, osteocytes, and MSCs. Meanwhile, microtubules become more disperse and sparse, and correspondingly, cell area is decreased. Both the height and roughness of cytoskeleton are attenuated (Qian et al. 2010). Cytoskeleton-associated proteins are found to present altered expression in osteoblasts after being treated in HMGE, as seen in cDNA microarray analysis (Qian et al. 2009a). The distributions of actin-binding proteins (vinculin, paxillin, zyxin, and talin) are dispersed and the expressions are also reduced consistently (Nabavia et al. 2011; Qian et al. 2012). These results suggest that microgravity is first likely to cause cytoskeletal relaxation by disorganizing cytoskeleton, which then results in altered homeostasis. Cytoskeleton associated proteins are involved in gravisensing of bone cells.

Fibronectin and MACF1 are also important molecules in the mechanosensitive axis (Singh et al. 2010; Suozzi et al. 2012). Fibronectin is a kind of ECM protein and found to have significantly increased expression after RPM treatment in osteoblasts (Li et al. 2011). Microgravity effects impair the combination between fibronectin and integrin, which produces more fibronectins to complement the interactions with integrin. In this way, osteoblasts resist the disrupted cytoskeleton to a certain extent. MACF1 is widely distributed across the osteoblasts and co-localized with cytoskeleton (Becker and Souza 2013), but the colocalization is remarkably reduced under DL with accumulation at perinuclear region (Qian et al. 2009b), suggesting that MACF1 may function in graviperception. Connexin 43 (Cx43) forms gap junctions and hemichannels in bone cells directing cell-cell communication, which plays pivotal roles in bone development and remodeling (Plotkin and Bellido 2013). Cx43 expression is down-regulated when osteocytes are subjected to altered gravity produced by parabolic flight (Di et al. 2011), indicating that Cx43 may be involved in mechanotransduction of gravity and needs to be further studied.

In bone remodeling, bone formation and resorption are coupled tightly and depend on differentiation of osteoblasts and osteoclasts. After being normally cultured up to late phase of differentiation and then acutely subjected to RPM for 24 h, the mineralization of typical osteoblastic MC3T3-E1 and 2T3 cells is suppressed and the expression of osteogenic genes such as alkaline phosphatase (ALP), osteocalcin (OC), collagen I$\alpha$1 (Col I$\alpha$1), dentin matrix protein 1 (DMP1), and runt-related transcription factor 2 (Runx2) is reduced. Extracellular signal-regulated kinase (ERK) pathway may also be involved in this regulation (Hu et al. 2013a; Hu et al. 2015). For MSCs, studies on STS (space transportation system) project during spaceflight and other SMs on the ground reveal that apoptosis, cytoskeleton disruption and osteogenic differentiation inhibition are caused under microgravity (Blaber et al. 2014; Ulbrich et al. 2014). Besides, microgravity strongly inhibits osteoblastogenesis and increases adipocyte differentiation in MSCs (Rodriguez et al. 2008). Osteoclast differentiation is promoted by RPM and DL. RPM not only increases viability of human preosteoclast FLG29.1 cells, but also osteoclast formation (Di et al. 2012). DL has an inhibitory effect on osteoclast formation of preosteoclast Raw264.7 cells, but the inhibition at the initial stage is rescued when the cells are normally cultured for two more days (Sun et al. 2015). Moreover, differential regulations treated by cell roller and follow-up shear flow exertion are found for osteocyte-like MLO-Y4 cells in bone biomarker production and F-actin reorganization (Yang et al. 2013). These results indicate that bone remodeling is likely to be disrupted with reduced bone formation and enhanced bone resorption under microgravity. Further studies are needed to connect this altered differentiation with gravity sensitive pathways as mentioned above.

On the other hand, immunosuppression is a key issue in space biological and life sciences, but the underlying mechanisms how altered gravity regulate the immune responses remains poorly understood (Pecaut et al. 2000; Kaur et al. 2004; Kaufmann et al. 2011). To elucidate the mechanotransduction of innate and adaptive responses, an in-house MG-I rotary bioreactor system developed in the Institute of Mechanics, CAS is applied to test the time-dependent effects on activation of murine resting $CD4^+$ and $CD8^+$ T cells in the presence of concanavalin A stimulation. Flow cytometry analyses indicate that the pre-exposure to the SM simulation suppresses the expression of biomarkers of CD25, CD69 and CD71 and inflammatory cytokine secretion in a time-dependent manner and that the cell proliferation is more severely reduced in $CD4^+$ T cells than in $CD8^+$ T cells (Luo et al. 2014). Similar studies are done to elucidate the SM effects on inflammatory defects of murine RAW264.7 cells and primary macrophages using RCCS, resulting in the significantly reduced TNF-$\alpha$ expres-

sion together with marked activation of heat shock factor-1 after the cells are pre-exposed to the SM simulation in the presence of lipopolysaccharide stimulation (Wang et al. 2014a). Meanwhile, the rolling and adhesion of human promyelocytic leukemia HL-60 cells onto human umbilical vein endothelial cell monolayer under shear flow is also tested after being pre-exposed to the SM stimulation using RCCS, in which shear stress-dependent rolling and adhesion presents differential features between intact and dimethyl sulphoxide-stimulated HL-60 cells (Wang et al. Accepted).

## Mechanotransduction of Developmental Biology and Tissue Histogenesis Under Microgravity

In developmental biology and tissue histogenesis, attentions are focused on stem cell proliferation and differentiation in 3D microenvironment and embryo culture under microgravity. Stem cells and mouse embryos are ideal models to investigate the lineage-specific differentiation in cell and tissue development and also serve as a source of seed cells in tissue repairing (Daley and Scadden 2008). While scientists have long appreciated the roles of soluble factors (*e.g.*, growth factors and cytokines) in regulating stem cell differentiation (Vallier et al. 2005), recent evidences demonstrate that mechanical forces and related mechanotransduction play a critical role for in controlling cellular development and histogenesis, as seen that reduced gravitational force has effects on cell growth, gene expression, soluble factor production, cell signaling, and cytoskeletal organization (Discher et al. 2009).

In an attempt to investigate the effect of gravity/microgravity on cell growth and 3D histogenesis, a 3D dynamic SM culture system is used for culturing stem cells and the new experimental methods are developed for seeding and culturing 3D cell-biomaterial tissue constructs in bioreactors. One example is to perform with RCCS to investigate the effects on proliferation and differentiation of human epidermal stem cells (hEpSCs), indicating that RCCS may provide an ideal physical and chemical environment to guide hEpSCs proliferation and serve as an acceptable culture model to assemble 3D multilayer epidermis tissue (Lei et al. 2011). Another example on the impact of rotary suspension culture on development of embryonic stem cells (ESCs) demonstrates that the RCCS does play an important role in enhancing mesendoderm differentiation of mouse ESCs by modulation of Wnt/$\beta$-catenin signaling (Lei et al. 2014).

It is well known that many cells reside in a 3D ECM *in vivo*. A 3D scaffold culture system which could provide 3D geometry resemble *in vivo* is increasing interest in studying the mechanobiology of how stem cells sense and generate mechanical force in response to the surrounding environment (Koehler et al. 2013; Guilak et al. 2014). The self-renewal of mESCs is boosted by the introducing of dimensionality and the stemness maintenance of mESCs is promoted by 3D geometry of the scaffolds, supporting long-time culture in 3D scaffolds (Wei et al. 2014). Similarly, a hydrogel scaffold-based 3D culture system is proposed for human skin derived precursors (SKPs), where 3D hydrogel scaffolds are able to facilitate *in vitro* self-renewal of human SKPs and alleviate cell senescence (Wang et al. 2014c). Bone marrow MSCs (BMSCs), the most common type of MSCs, are good candidates for bone tissue construction and clinical application. Differences between traditional 2D culture and collagen scaffold-based 3D cultures present enhanced osteogenic and adipogenic differentiation efficiency (Han et al. 2012). Moreover, 3D collagen scaffolds are used as cell carriers combining with RCCS to create a stereoscopic dynamic environment for BMSC proliferation, which enhances the abilities of stem cell proliferation and colony formation and maintains the differentiation potential of the BMSCs compared with static traditional 2D and static 3D cell culture conditions. On the other hand, microarray analyses demonstrate the up-regulated genes involved in cell proliferation, survival, apoptosis, and differentiation in RCCS-3D condition (Tang et al., under review).

Although there are numerous biological experiments, which have been performed in space environment, to study the physiological effect of microgravity on living organisms (Pietsch et al. 2013), the potential effect of weightlessness or gravity on the reproductive system in most species, particularly in mammalian, is still unclear and controversial. Mouse early embryos can develop well in simulated microgravity with the use of a horizontal clinostat device (Kojima et al. 2000). In real microgravity experiment of embryo development, the effects of space microgravity on 4-cell mouse embryo development in Chinese ShiJian (SJ)-8 satellite are examined at the first time, but, unfortunately, there are no developed embryos found during space flight (Ma et al. 2008). Considering that the process of space experiment is quite different from most experiments done on earth in several aspects such as the vibration and short-term hypergravity in launching and landing phases, mimicking the short-term hypergravity during launching by using a centrifuge to investigate its influence on the development of early embryo (2-cell) in mice is performed accordingly. There are no significant effects on the normal development and actin filament structures of mouse embryos (Ning et al. 2015).

Taken together, the above studies provide insights in understanding the biological roles of stem cells in SM condition and 3D systems, the developmental capacities of early mouse embryos under microgravity environment, and the proliferation and differentiation potentials of BMSCs or

skin-derived stem cells under the combination of 3D culture and RCCS environment.

## Future Perspectives of Cell Mechanobiology Under Microgravity

Understanding cell mechanotransductive signaling and mechanobiological responses under microgravity has attracted more and more attentions. As a new emerging field, the outcomes from these studies would bridge the gap between the mechanical stimuli and the biological phenotypes of cell grown in space microgravity environment. From the viewpoint of biomechanical and biophysical principles, it is able to quantify the required sufficient mass transport and nutrient supply of cell growth and proliferation, determine the cell spreading and reorganization dynamics, and elucidate the molecular mechanisms of gravisensing, gravitransducing, and graviresponding pathways. Novel mathematical modeling and mechanical analysis help to design the well-controlled, parameter-optimized bioreactors that are more biologically relevant for understanding cellular responses to (micro-)gravity. Evidently, the studies on microgravity-induced mechanobiology at cellular level are still at the embryonic stage and need to be further accomplished.

For technical aspects, one also needs to know how a driven flow of culture medium in a cell bioreactor affects biological responses of mammalian cells to microgravity, since it is hard to isolate the unique impacts of microgravity from those habitat effects. Under microgravity, cell sedimentation and medium convection tend to disappear, and stable diffusive boundary layer of medium is formed at the vicinity of cell. This significantly reduces nutrient supply, gas exchange, and removal of metabolic waste. Driven flow is usually required to provide sufficient mass transport and nutrient supply for cell growth and cell-cell interactions under microgravity, which imposes additional effects on cellular structures and functions. These impacts of microgravity are possibly coupled with such experimental conditions as temperature, pH value, as well as strong mechanical vibrations during launching, which should also be isolated out as possible.

### Abbreviations

| | |
|---|---|
| 2D or 3D | two- or three-dimensional; |
| BMSC | bone marrow mesenchymal stem cell; |
| CAS | Chinese Academy of Sciences; |
| Cx43 | connexin 43; |
| DL | diamagnetic levitation; |
| ECM | extracellular matrix; |
| ESC | embryonic stem cell; |
| hEpSC | human epidermal stem cell; |
| HMGE | high magneto gravitational environment; |
| MACF1 | microtubule actin cross-linking factor 1; |
| MSC | mesenchymal stem cell; |
| RCCS | rotary cell culture system; |
| RPM | random positioning machine; |
| SJ-8/-10 | Chinese ShiJian-8/-10 satellite; |
| SM | stimulated microgravity; |
| SKP | skin derived precursor; |
| STS | space transportation system; |
| SZ-8 | Chinese ShenZhou-8 spacecraft; |
| WT | wild-type; |
| $\mu g$ | microgravity. |

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