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Impact of shear stress and simulated microgravity on osteocytes using a new rotation cell culture device

Xiao Yang [a], Lian-Wen Sun [a,*], Xin-Tong Wu [a], Meng Liang [a], Yu-Bo Fan [a,b,*]

[a] *Key Laboratory for Biomechanics and Mechanobiology of Ministry of Education, International Joint Research Center of Aerospace Biotechnology and Medical Engineering, Ministry of Science and Technology of China, School of Biological Science and Medical Engineering, Beihang University, No. 37 Xueyuan Road, Haidian District, 100191 Beijing, China*

[b] *National Research Center for Rehabilitation Technical Aids, 100176 Beijing, China*

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ABSTRACT

Osteocytes sense the mechanical loading and weightlessness, and orchestrate bone remodeling by directing both osteoblast and osteoclast functions. Previous studies mostly focused on the impact of mechanical loading or microgravity on the osteocyte changes in themselves. However as the mechanosensors, it is more meaningful to make clear whether and how the mechanosensitivity of osteocytes affected by weightlessness, since the alteration of mechanosensitivity may further affect the mechanotransduction process in osteocytes, and finally altered the bone remodeling process. Whereas until now, this aspect had long been overlooked and was still not clear yet.

To investigate how osteocytes respond to shear stress under weightlessness, a novel custom-made device was designed to simulate microgravity and load fluid flow on cells in vitro at the same time. The osteocyte-like cell line MLO-Y4 was loaded by this novel device at 15 dyn/cm^2 shear stress with 15 rpm rotating speed. There were two loading durations for different determinations: 30 min of loading for the detection of nitric oxide and Prostaglandin E2, and 6 h of loading for the detection of three bone formation biomarkers (alkaline phosphatase, osteocalcin and procollagen type I N propeptide). In order to preliminarily explore the mechanism of this altered response to mechanical loading with different gravity in osteocytes, an observation of cytoskeleton and the determination of the elements in the Wnt/β-catenin signaling pathway were also performed after 6 h of loading. The results showed that (1) the mechanical response of both NO and PGE2 were increased higher during 15–30 min of shear stress under simulated microgravity than that under normal gravity; (2) the mechanical response of ALP activity was decreased, while that of OC and PINP content were increased by simulated microgravity. Moreover, the ALP activity and the OC content were related to the activity of Wnt signaling pathway, which plays a key role in regulating the bone formation; (3) the exploration for the mechanism of altered mechanical bio-response in osteocytes showed that F-actin filaments enhanced by shear stress under simulated microgravity were not so robust as that showed under normal gravity, and some short dendritic processes at cell periphery were only observed within the simulated microgravity groups; (4) further, the simulated microgravity significantly inhibited the mechanosensitivity of the Wnt/β-catenin signaling pathway on protein level in the osteocytes.

These results suggested that the mechanosensitivity of osteocytes was altered by simulated microgravity, and this may be an adverse effect on the osteoblastic bone formation whereas be good for osteoclastogenesis. The findings in this study may provide

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* Correspondence to: No. 37 Xueyuan Road, School of Biological Science & Medical Engineering, Beihang University, Haidian District, 100191 Beijing, China. Tel.: +8610 82339349; fax: +86 10 82339349.

E-mail addresses: xiaoyang@buaa.edu.cn (X. Yang), sunlw@buaa.edu.cn (L.-W. Sun), yubofan@buaa.edu.cn (Y.-B. Fan).

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an important clue for searching an efficient approach to prevent osteocytes from changing the orchestration for bone remodeling under microgravity, and further this prevention may finally make the physical exercises capable against bone loss under microgravity.

1. Introduction

Physical activity (like exercise or physical training) would induce mechanical loading that is responsible for maintaining or improving bone mass and architecture [1] as well as for inhibiting bone loss with age [2]. However, weightlessness, or microgravity, is induced by spaceflight and leads to loss of bone mass invariably [3,4]. Even the resistance exercises in space usually cannot prevent this weightlessness-induced osteoporosis [5–8]. The main cause for this inability to recover bone mass is that the mechanism of microgravity-induced osteoporosis of bone is still unknown.

Bone is constantly renewed by collaborations of bone-forming osteoblasts and bone-resorbing osteoclasts, both of whose activities are determined mostly by locally produced signaling molecules [9]. The major producer of signaling molecules are osteocytes, which are the most abundant cells in mature bones [10]. Osteocytes sense the mechanical loading, especially shear stress caused by fluid flow [11]. Osteocytes also translate mechanical input into biochemical signals via the secretion of signaling molecules [12]. They then transmit these signaling molecules to effector cells [13–15] in order to orchestrate bone remodeling by directing both osteoblast and osteoclast function [16–19]. Conversely, the ablation of osteocytes rapidly leads to decreased bone strength, microfractures, and osteoporosis [20]. Therefore, osteocytes play a crucial role in bone remodeling as mechanosensors and modulators.

Previous studies have proven that not only mechanical loading but microgravity also affects the activity of osteocytes [21]. For example, mechanical unloading increases the prevalence of osteocyte apoptosis in vivo [22,23]; in addition, the osteolytic activity in mature osteocytes is intensified by microgravity in space flight [23,24] and further causes bone loss in both cortical and trabecular bone [25]. According to these researches, osteocytes' behaviors appear to cause the disorder of bone remodeling under weightlessness and are relevant to microgravity-induced bone loss. These studies mentioned above showed us that the viabilities of osteocytes are affected by microgravity. However, the study on osteocytes should not be limited to studying the microgravity impact on osteocytes in themselves, what is more important is to explore whether the function of osteocytes as the mechanosensors were affected by microgravity. One possible scenario is that osteocytes mechanosensitivity could be changed with the gravity alteration; this effect may further directly affect the mechanotransduction process in osteocytes and the instructions on osteogenesis or osteoclastogenesis in bone tissue. Whereas until now, the study on osteocytes capabilities of sensing the mechanical loading (namely their mechanosensitivity) under microgravity has long been overlooked. In our opinion, yet studying and understanding this may explain to us why the bone remodeling regularly adapted to the physical countermeasures (mechanical loading at macro-level) during spaceflight was unlike it showed on the earth.

Therefore, in this study we focused for the first time on whether the osteocytes respond to shear stress (a prevalent model of mechanical loading on osteocytes) altered under simulated microgravity. We hypothesized that osteocytes would have a different mechano-response to fluid shear stress under simulated microgravity than that under normal gravity. In this case, it was necessary to make osteocytes to be stimulated by fluid flow shear stress under simulated microgravity environment, and a device that can make the shear stress and the simulated microgravity load on cells simultaneously was needed in our study. As we all know, plenty of ground-based experimental devices may realize the function of microgravity simulation in cell culture, such as the Rotating Wall Vessel bioreactor (RWV, NASA, USA), the Rotary Cell Culture System (RCCS, NASA, USA), and 2D/3D clinostats in studying cell behaviors directly [26,27]. We have also used one of these rotators to simulate microgravity with loading the fluid shear on osteocytes subsequently [28], trying to study the mechano-response of osteocytes after simulated microgravity. However, neither those simulation apparatuses nor the studies we did before can truly reflect the actual circumstance of mechanical stimulations on cells during spaceflight.

In this case, a novel, custom-made ground-based experimental device was designed specifically for our study, which can achieve coupling shear stress with simulated microgravity. By using the device we made, two early mechanical secreted molecules, three bone formation biomarkers, the cytoskeletons, and seven primary components of the Wnt/β-catenin mechanical signaling pathway in osteocytes were investigated. By studying these, we could potentially improve the understanding on the mechanosensitivity of osteocytes under microgravity, which may further clarify the mechanism of osteoporosis induced by spaceflight, and can provide a clue for searching an efficient approach for the exercises more successfully countering the bone loss in space flight.

2. Materials and methods

2.1. Simulated microgravity with a novel custom-made device designed in our lab

We designed a device to make the horizontal axis rotating, and medium flowing through flow chamber fixed on the axis at the same time (Fig. 1A). The device comprised