Journal of Biomechanics xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Short communication

Continuous hypergravity alters the cytoplasmic elasticity of MC3T3-E1 osteoblasts via actin filaments

Shuai Zhou [a], Xueyi Yang [a], Jiliang Hu [a], Chi Mo [a,b], Yanping Cao [a], Chun Yang [a,*]

[a] Institute of Biomechanics and Medical Engineering, School of Aerospace, Tsinghua University, Beijing 100084, China
[b] Biodynamic Optical Imaging Center (BIOPIC), Peking University, Beijing 100871, China

ARTICLE INFO

Article history:
Accepted 15 February 2018
Available online xxxx

Keywords:
Cell mechanics
Hypergravity
Cell elasticity
Osteoblasts
Cytoskeleton

ABSTRACT

Osteoblasts are sensitive to altered gravity conditions, displaying changes in RNA and protein expression, proliferation, and differentiation; however, the effect of hypergravity on the mechanical properties of osteoblasts remains unclear. In this study, atomic force microscopy (AFM) was used to evaluate the effect of hypergravity on the elasticity of osteoblasts. We demonstrate that a continuous hypergravitational environment increased the elasticity of the cytoplasm, but not the nuclei zone, of MC3T3-E1 osteoblasts. Actin filaments, but not microtubules, dominated in the increased elasticity. These findings provide new insights on cellular gravity-sensing mechanisms.

1. Introduction

Space flight leads to osteoblast dysfunction, which can result in bone loss (Turner, 2000). Gravitational alterations affect osteoblast adhesion, protein expression, signal transduction, proliferation, and differentiation (Hughes-Fulford and Lewis, 1996; Kumei et al., 1996; Berezovskaia and Rodionova, 1998; Hughes-Fulford and Gilbertson, 1999; Harris et al., 2000). Hypergravity, accomplished by centrifugation, is a convenient way to study the effects of altered gravity on cells *in vitro*. Previous reports by us and other groups have indicated that hypergravity triggers biochemical changes and the activation of certain mechanotransduction pathways in osteoblasts (Furutsu et al., 2000; Fitzgerald and Hughes-Fulford, 1996, 1999; Zhou et al., 2015a,b; Searby et al., 2005; Gebken et al., 1999; Saito et al., 2003; Li et al., 2009). However, the effects of hypergravity on the mechanical properties of osteoblasts remain uninvestigated.

The mechanical properties of cells contribute to cell shape and structure, thereby regulating intracellular tension (Kuznetsova et al., 2007). Moreover, cell stiffness can indicate their sensitivity to force (Chowdhury et al., 2010). Therefore, studying the elasticity of osteoblasts in response to hypergravity can assist in understanding the mechanisms involved in gravity sensing.

Of the various mediators of cellular elasticity, the cytoskeleton plays a crucial role. Several previous studies have demonstrated that the disruption of actin filaments affects cell elasticity (Ketene et al., 2012; Moreno-Flores et al., 2010; Cai et al., 2010; Rotsch and Radmacher, 2000; Takai et al., 2005; Titushkin and Cho, 2007), while examinations of the contribution of microtubules have produced inconsistent results (Rotsch and Radmacher, 2000; Takai et al., 2005; Titushkin and Cho, 2007; Ingber et al., 1995; Wu et al., 1998, 2000). The aim of this study was to investigate the impact of continuous hypergravity on the elasticity of osteoblasts, and evaluate the contributions of actin filaments and microtubules.

Common methods to measure the elasticity of cells include micropipette aspiration, optical tweezers, and atomic force microscopy (AFM) (Kasza et al., 2007; Kollmannsberger and Fabry, 2011; Alsteens et al., 2017). AFM enables the measurement of both the topography and the mechanical properties of living cells (Alsteens et al., 2017). In this study, MC3T3-E1 osteoblasts were placed under hypergravitational conditions or 1*g*, after which the elasticity was investigated by AFM. In addition, we probed for cytoskeletal components involved in hypergravity-increased elasticity in the cytoplasm of MC3T3-E1 cells.

2. Materials and methods

2.1. Cell culture

MC3T3-E1 osteoblasts were cultured as described in Supplementary material. Once confluent, cells were trypsinized, seeded in confocal dishes (NEST Biotechnology) for 2 h, and then exposed to continuous hypergravity at 10*g* (279 rpm), 20*g* (396 rpm), or 30*g* (485 rpm) for 24 h at 37 °C. Control cells were subjected to the

* Corresponding author.
E-mail address: yangchun@mail.tsinghua.edu.cn (C. Yang).

https://doi.org/10.1016/j.jbiomech.2018.02.024
0021-9290/

same drug treatments, without exposure to hypergravity. After static culture or centrifugation, cells were assayed by AFM in culture medium or fixed for immunofluorescence.

2.2. Cell indentation experiments

The AFM setup described in Supplementary material was used to measure the elasticity of MC3T3-E1 cells. All measurements were conducted using pyramidal tips (DNP-10, Bruker) with a nominal stiffness of 0.06 N/m. One point in the nuclei zone and three to five points in the cytoplasm of each cell were randomly chosen, and force-indentation curves were acquired. In force mapping experiment, a force-indentation curve was acquired at each point in a 16 × 16 pixels scan area. Force-indentation curves were acquired at an indentation rate of 5 μm/s until a maximum load at 3 nN. In experiments to test strain rate effects, low level of strain rate (0.5 μm/s) was used.

2.3. Data analysis

All measured force curves were fitted with the Hertz/Sneddon model to determine the elastic modulus of the cells (Fig. S1). More experimental process was described in Supplementary material.

2.4. Statistical analysis

All data are shown as the mean ± standard error of the mean (SEM), and were analyzed by two-tailed analysis of variance with Student's *t*-test to compare cell elasticity in 1*g* and hypergravity. $P \leq 0.05$ was considered statistically significant.

Full methods are included in Supplementary material.

3. Results

3.1. Effects of hypergravity on the elasticity of MC3T3-E1 cells

We first examined the hypergravitational effects on the elasticity of MC3T3-E1 cells. Control cells were cultured at 1*g*, while experimental cells were subjected to continuous hypergravity by centrifugation at 10*g*, 20*g*, 30*g* for 24 h. Subsequently, one point in the nucleus zone and three to five points in the cytoplasmic area were randomly chosen and the elasticity of cells at each point was measured by AFM (Fig. 1A). Our results revealed that 24 h of 20*g* and 30*g* increased the average Young's modulus of MC3T3-E1 cells (Fig. 1B).

3.2. Effects of hypergravity on the spatial distribution of elasticity of MC3T3-E1 cells

To further analyze the hypergravitational effects on the spatial distribution of elasticity, the Young's moduli in the nucleus zone and cytoplasmic areas were quantified separately. Upon 20*g* and 30*g* hypergravity stimulation, the cytoplasmic elasticity of cells increased, while the elasticity of nucleus zone remained unchanged compared to control cells (Fig. 1C and D). In addition, the cytoplasmic elasticity of cells increased with increasing of the level of gravity (Fig. 1C). These results indicate that the level of hypergravity above 20*g* specifically increases the cytoplasmic elasticity of MC3T3-E1 cells. We therefore chose 20*g* to further investigate the effect of hypergravity on the elasticity of MC3T3-E1 cells.

The force mapping results confirmed that hypergravity specifically increases the cytoplasmic elasticity of MC3T3-E1 cells (Fig. 2A–C). The quantification of the average elasticity of the cytoplasm of each cell also confirmed that continuous hypergravity at

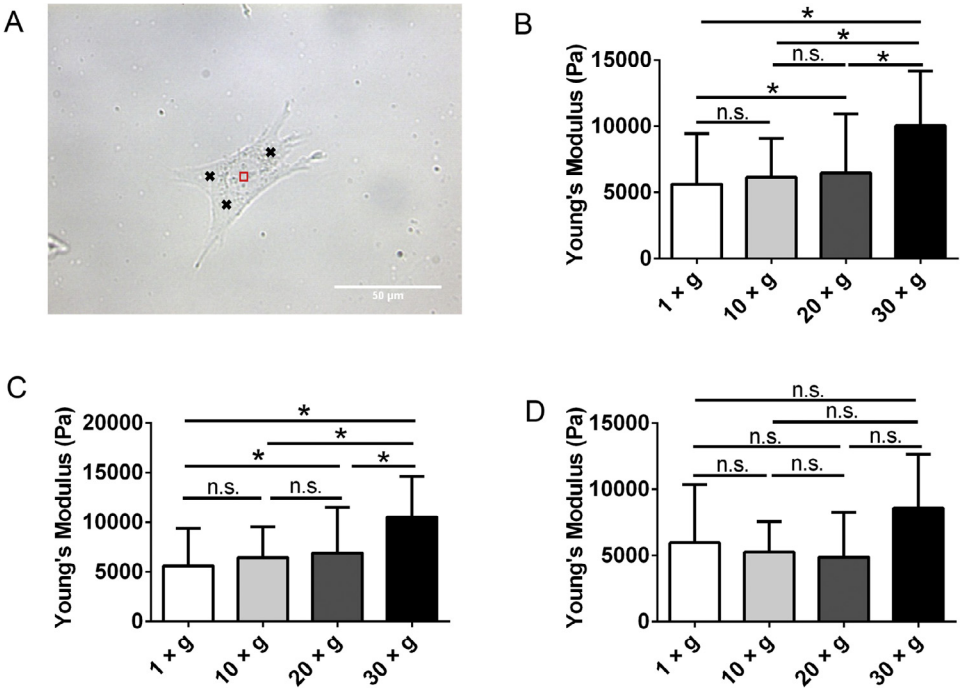

Fig. 1. Effect of hypergravity on the elasticity of MC3T3-E1 cells. (A) One point (square) in the nucleus zone and three to five points (crosses) in the cytoplasm of each MC3T3-E1 cell were chosen at random. (B) The average Young's modulus of the chosen points of each cell after 24 h (mean ± SEM; the total force curve numbers were 325, 96, 260, and 81 for the 1*g*, 10*g*, 20*g*, 30*g* conditions). (C) Average Young's moduli of cytoplasm of MC3T3-E1 cells after 24 h (mean ± SEM; the total force curve numbers were 304, 72, 205, and 60 for the 1*g*, 10*g*, 20*g*, 30*g* conditions). (D) Average Young's moduli of nucleus zone of MC3T3-E1 cells after 24 h (mean ± SEM; the total force curve numbers were 21, 24, 55, and 21 for the 1*g*, 10*g*, 20*g*, 30*g* conditions). n.s. indicates no statistical difference between 1*g* and hypergravity conditions. *$P < 0.05$.

Please cite this article in press as: Zhou, S., et al. Continuous hypergravity alters the cytoplasmic elasticity of MC3T3-E1 osteoblasts via actin filaments. J. Biomech. (2018), https://doi.org/10.1016/j.jbiomech.2018.02.024