

MECHANO-BIOLOGICAL RESPONSES OF ROS 17/2.8 CELLS TO DIFFERENT ORIENTATIONS OF CULTURE SUBSTRATE¹⁾

Lee Hong Long Mian²⁾

(National Microgravity Laboratory, Institute of Mechanics, Chinese Academy of Sciences, Beijing 100080, China)

Whether single cells or multicellular systems can sense and respond to gravitational changes becomes one of key issues in gravity sensing studies. Direct effects of microgravity at the single cell level were found in the cells without obvious gravity-sensing organelles^[1]. However, lack of reasonable experimental models still hampers the understandings to the mechanism of cell gravisensing. Those assays so far widely used to simulate gravitational environment are hard to filter out the unwanted side effects induced by much stronger forces or effects than cell weight^[2,3].

Here a novel and simple culture system was developed to test the effects of substrate orientations on mechano-biological responses of culturing cells, in which the culture substrate was oriented upwards (A), downwards (B), and vertically (C) (as seen in Fig. 1.). As a static gravisensing assay, three subgroups of cells were firstly seeded in A for 8 hrs and two of them were then inverted (B) and erected respectively (C) for further culture.

To test the reasonability of the culture system, morphology of Ros 17/2.8 cells, known to be sensitive to mechanical stress^[4] and to microgravity^[5], were measured and compared in three procedures of A, B and C. Measured cell area and cell perimeter increased with time in all procedures, and increasing gradients in B and C are the highest and lowest respectively. Shape factor, defined for cell sphericity, decreased with time in B and C but not in A. Cytoskeletal-immunostaining measurements demonstrated that in B and C actin stress fibers accumulated at cell periphery and vimentin network around nucleus loosed and rounded up, as compared to those in A. These data supported our hypothesis that cells can sense the orientating variations in morphology and cytoskeletal organization, suggested a novel gravisensing assay under a static condition.

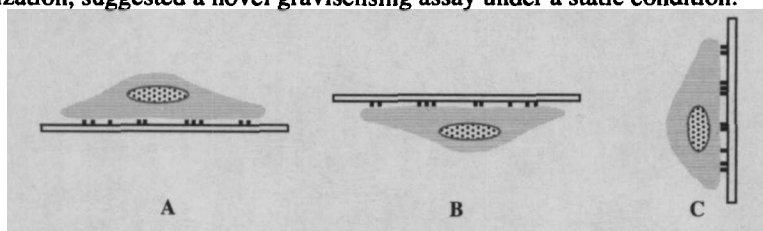


Fig. 1. Illustration of substrate orientations. (A) Upwards; (B) Downwards; (C) Vertically.

References

1. Ingber D. *FASEB J.* 1999,13:S3~15
2. Klaus DM. *Gravit. Space Biol. Bull.* 2001,14:55~64
3. Freed LE, and Vunjak-Novakovic G. *Adv. Space Biol. Med.* 2002,8:177~95
4. Kubota T, Yamauchi M, Onozaki J, et al. *Arch. Oral Biol.* 1993,38:23~31
5. Guignandon A, Vico L, Alexandre C, et al. *Cell Struct. Funct.* 1995,20:369~375

¹⁾ The project supported by grants from National Natural Science Foundation of China (10332060/30225027/10128205), and CAS projects from Chinese Academy of Sciences (KJCX2-SW-L06) and from Institute of Mechanics/CAS

²⁾ E-mail: mlong@imech.ac.cn