The Effect of Microgrooved Structures on Cell shape and Actomyosin Organization

***H. Miyoshi[1] M. Nishimura[2], Y. Yamagata[3], H. Liu[2], Y. Watanabe[1] and M. Sugawara[2]**

[1]RIKEN Center for Life Science Technologies, Japan
[2]Department of Mechanical Engineering, Chiba University, Japan
[3]RIKEN Center for Advanced Photonics, Japan
[*]hiromi-miyoshi@riken.jp

Abstract

The strategies to regulate cell migration, growth, and differentiation are fundamental for tissue engineering. It has been shown that cell shape and intracellular actomyosin organization regulate these cellular processes. The cell shape and actin cytoskeletal organization are affected by various cues in the tissue microenvironment [1]. Our aim here is to provide a basis for an external control of these cellular processes by clarifying the effect of extracellular microgrooved structures on cell shape and actin cytoskeletal tension.

We fabricated a polydimethylsiloxane (PDMS) cell culture substrate consisting of microstructured surfaces with of a major groove and branched grooves in order to control cell shape and actomyosin organization externally. The major groove was designed to guide cell body penetration, and the branched grooves were designed to guide cellular protrusion into them. Swiss3T3 fibroblasts were cultured on the substrate for 24 hours. Then, the cells were fixed and processed to visualize actin filaments and phosphorylated myosin.

On the microstructured substrate, about a half of the cells penetrated into the grooved structure as predicted. On the other hand, the other cells were grown on the grooves. In both of the two groups, the cells show various shapes. In the penetrated groups, disappearance of the stress fibers was observed. This observation indicated that the major groove had an effect to suppress actin stress fiber formation in the cell body by the downregulation of Rho. Furthermore, these cells had a tendency to form thin actin protrusions into the branched grooves. These actin protrusions contained no phosphorylated myosin, thus they were assumed to be filopodia, which indicated the upregulation of cdc42.

This result of actin filaments and phosphorylated myosin distribution in the cells in the microgrooved structure suggests that the microgrooves can be utilized for external control of local activities of Rho and cdc42. These small GTPases are known to be regulators of various cellular processes, involving cell migration, growth, and differentiation [2, 3]. Thus, our findings would be a basis for designing microstructures on cell and tissue culture substrate for tissue engineering.

Keywords: Microgroove, Fibroblast, Actin Cytoskeleton, Myosin Phosphorylation

References

[1] Miyoshi, H. and T. Adachi (2014) Topography Design Concept of a Tissue Engineering Scaffold for Controlling Cell Function and Fate Through Actin Cytoskeletal Modulation, *Tissue Engineering Part B-Reviews*. **20**, 609-627.

[2] McBeath, R., D.M. Pirone, C.M. Nelson, K. Bhadriraju, and C.S. Chen (2004) Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment, *Dev Cell*. **6**, 483-95.

[3] Raftopoulou, M. and A. Hall (2004) Cell migration: Rho GTPases lead the way, *Developmental Biology*. **265**, 23-32.