Atomic Force Microscopy Is A Valuable Tool In Studying Interactions Between Muscle Cells in Culture

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Atomic force microscope (AFM) is widely used for the molecular characterization of many synthetic materials, however, observing the biological materials is in it's infancy. Atomic force microscopy has been used for imaging a variety of cultured cells, and we have already reported the usefulness in the studying myogenesis in culture (Kim SJ, presented at the first ISMA meeting) [1]. The most promising aspect of AFM than scanning electron microscopy is the applicability on living wet samples, which would give us chances in observing the more natural cellular behaviors avoiding vacuum and in aqueous environment.

In this study we observed the lateral interaction between developing muscle cells with an AFM. Rat primary muscle cells were isolated from E20 embryo hind limbs and cultured on Thermanox® coverslips following the established protocol (Kim SJ, master thesis) [2]. As a preparatory step for wet sample observation, we used fixed and dried sample in this study. Cells were fixed and dried as in usual scanning electron microscopy. For AFM observation, a commercially available microscope (SPA400, Seiko Inc.) was employed. The measurements were performed in the non-contact mode or tapping-mode with Si cantilevers with the spring constant of 14 N/m, resonant frequency of 138 KHz and the tip radius of <10 nm. Myoblast preparations firmly attached to the coverslip were selected by observing with the light microscope, and the cantilever tip was positioned at an appropriate locus.

The prefusing myoblasts aligned in a chain were mostly spindle in shape and were characterized by the presence of many microprocesses along the facing edges of adjacent aligned myoblasts. The space between fusing myoblasts and between myotubes and myoblasts were often traversed by filopodia and cellular bridges formed by the connection of microvilli. These results suggest that microprocesses may be involved in the fusion of myoblasts.

Atomic force microscopy revealed the same structures as observed in scanning electron microscopy, which proved the value of AFM in the observation of cultured cells. The application on

the wet living cultures would be warranted.

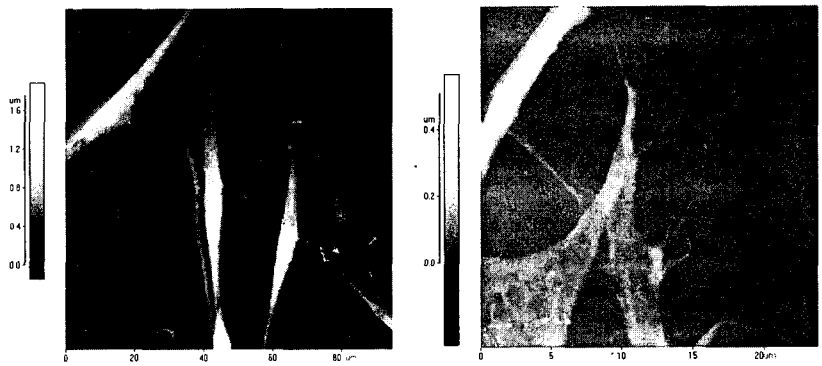

Figure 1. AFM image of myoblasts. Interface between myoblasts were showed by many microprocesses.

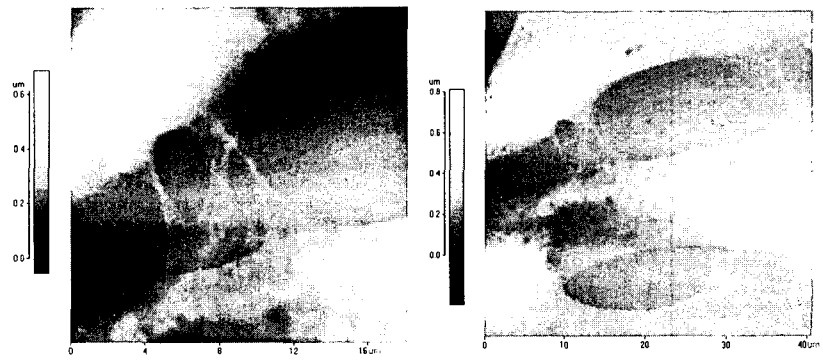

Figure 2. Interface of contacting myoblasts. Many microvilli, filopodia and ruffles travel through this space. Some microprocesses form bridge by fusion.

References

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2. Su-Jin Kim (Director Chang-Sub Uhm): Morphological and proteomic studies of rat myoblast fusion in culture. Thesis for Master of Sciences, Graduate School, Korea University, 2004.