Study of subcellular dynamics on cell-substrate interactions by live cell imaging

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Keywords: cell-substrate interaction, membrane expansion, live cell imaging, confocal microscopy

Cellular adhesiveness to biomaterial is one of the important properties to the success of tissue engineering. The cell-biomaterial interactions involve close cooperation of adhesion proteins, plasma membrane, and cytoskeletons in order to form focal adhesions during the process of anchoring. Initially, the suspension cells are round and floating. When suspended cells are seeded on a substrate, the cells touch the substratum by gravity and then extend the lamellipodia to explore the micro-environment for spreading or migrating. Successful attachment and spreading is important to determine the status of cellular physiology, such as morphogenesis, migration and proliferation that are related to the transition of cell shapes. Previous studies described that the cell-substratum interactions occurs mainly in cells attaching to the substrate at different stages of steady-state conditions [1][2][3]. Dynamic development of the plasma membrane in the process reflects the cellular biocompatibility and motility. The process of cell attachment beginning from seeding, contact, attachment, to spreading has not been investigated. In this study, we monitored the whole process of cells attaching to the substrate surface by time-lapse confocal microscopy.

We used M2-10B4, an attached fibroblast cell line, in this study. The cells were suspended and labeled by living cell reagents conjugated with fluorescence dyes. WGA-488 (W11261, Molecular Probes) was used to detect the plasma membrane and SYTO-61 DNA staining (S 11343, Molecular Probes) was used to label the nuclei. The cells were seeded on the planar culture dish (35 mm, μ-dish, Ibidi) and the substrate with 5 um (micro-meter) height pillars made with silicon [4]. Time-lapse images with Z-stack were acquired every 10 minutes starting at seeding for the total of 6 hours by confocal microscopy (TCS-SP5, Leica Microsystems).

The cell-attachment processes include contact (as 0 min), attachment, and spreading. We have analyzed the time-lapse images and then obtained the time points of this process (Figure 1). The cells grown on the plane showed attachment and spreading within 60 min. The cells seeding on pillars showed the attachment-spreading process within 105 min (Figure 1); the red dot line indicates the position of pillar substratum in Z axis and star signs label the focal adhesions. We observed that the surface configuration of the substratum effects the plasma membrane expansion. The pillars can be a resistance for cell attachment therefore cells spent more time to explore and anchor themselves and go on to the next step. The orthogonal sectioning images (X-Z) provided information for cells attaching on the plate or pillars substratum by discontinuous contacts as the focal adhesions (Figure 1). Contrast to cells grown on the plate, the cells attached on pillars are with rounded nuclei and with prominent lamellipodia spreading out (Figure 2). Membrane expansion is involved in dynamic development of the plasma membrane and lamellipodia formation for attachment, migration or proliferation and reflects the cellular physiology status of the cells. This study provides a platform to investigate cell behavior and dynamic development of subcellular structures about cell-biomaterial interactions.

References

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[5] The authors gratefully acknowledge for technical support by Core Facilities, ICOB, Academia Sinica.

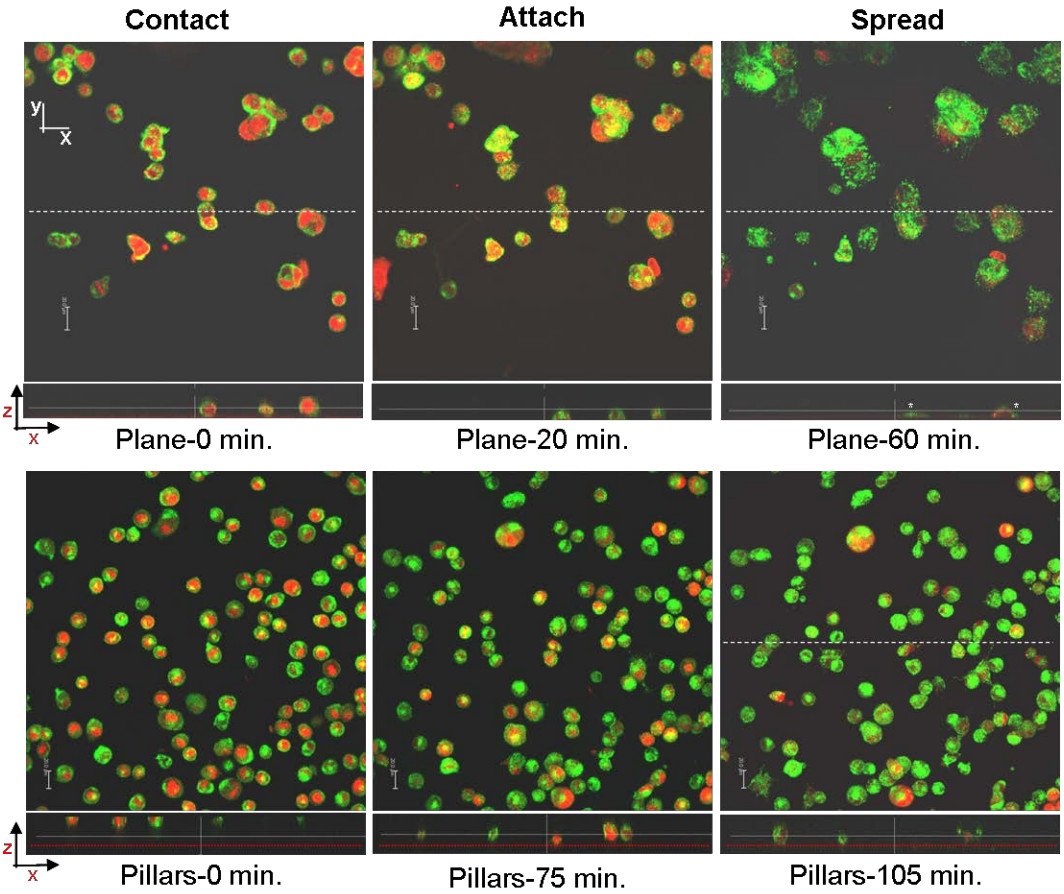

Figure 1. *Time-series images of cells attached to the plane and the pillar substrate. The X-Z images showed the cross-section of cells during contact, attachment and spread.*

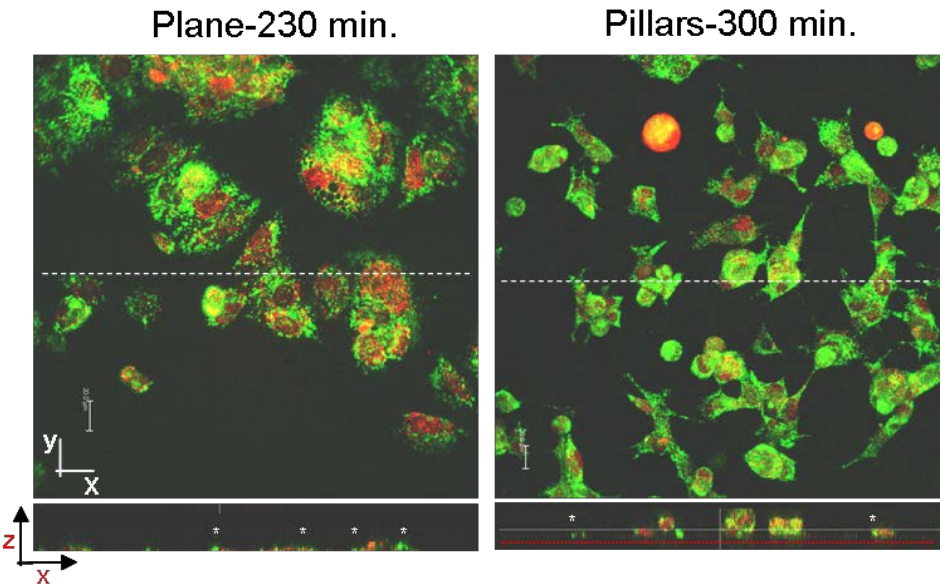

Figure 2*. The cells grown on the plane substratum showed well spreading of the cellular plasma membrane (left) but the cells grown on pillars were round and the plasma membrane extended and stretch out (right). Discontinuous contacts of the cellular plasma membrane showed the focal adhesion sites. The red dot line indicates the position of the pillar substratum and star signs label the focal adhesions.*