Observation of cell-material interface at cell detachment

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Abstract

A single cell adhering to glass surface was detached from material surface by applying shear for using a microcantilever. The interface between the cell and glass surface was observed by interference reflection microscopy during the cell detachment. As a result, it was found that focal adhesion proteins remained on glass surface after the detachment. Fibrous structures which may be stress fibers also remained attaching to the focal adhesion proteins. Immunofluoresccent staining confirmed that β_1-integrins remained on the glass surface after the detachment. There facts suggest that cell-material adhesion is fractured inside the cell when the cell is detached by shear force.

Introduction

Cell-material adhesion is one of the important events for the materials used in biomedical devices and diagnosis microchips because they are always used adjacent to living cells. Optimal strength of cell-material adhesion depends on the purposes of the devices. To develop new materials and chips with optimal affinity for cell, quantitative evaluation of the material affinity for cell and elucidation of the cell attachment/detachment processes is necessary.

Cell-material adhesion is generally constructed by a series of bindings; between material surface and adsorbing extracellular matrix proteins, between the extracellular matrix proteins and transmembrane integrins, between the integrins and focal adhesion associating proteins such as talin and vinculin, and between the focal adhesion associating proteins and cytoskeletons such as actin filaments, etc. In this study, we detached a single cell from material surface by applying shear force using a microcantilever[1], and observed cell detachment process by interference reflection microscopy (IRM) to identify the fracture points of cell-material adhesion.

Materials and Methods

About 2×10^4 cells of human normal diploid fibroblast, IMR-90, were seeded into the 10 mL of Eagle's minimum essential medium (MEM) supplemented with 10 vol. % of fetal bovine serum and 20 mmol/L of N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) on the glass-bottom dish (P50G-0-14-F, Mat Tek Corp., Ashland MA, USA) and cultured for 48h at 37 ℃ in a 5% CO_2-incubator prior to the detachment.

A cell adhering to the glass was detached by applying shear force with the equipment we developed[1]. The principle of the equipment is schematically shown in Figure 1. The glass-bottom dish was set onto a XY-stage of an optical microscope, which moves toward the cantilever

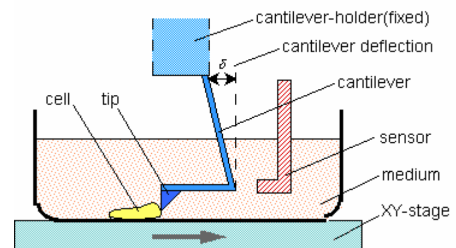

Figure 1. Principle of the system to detach a single cell from material surface.

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attaching a tip on its end. When the tip touches to the cell, shear force is applied to the cell and the cantilever is deflected corresponding to the load applied to the cell. The XY-stage keeps moving, and then, the cell is detached from the glass surface. The detachment process of the cell is observed by IRM and recorded on the videotape through a CCD camera equipped with the microscope.

Results and Discussions

An example of IRM images of the cell before and after detachment is shown in Figure 2. In the IRM image of IMR-90 before the detachment (Figure 2(a)), focal adhesion appears as a black region where the distance between the glass surface and the cell membrane is 10-15 nm. Close contact appears as a gray region where the distance between the glass surface and the cell membrane is about 30 nm. A white region is the place where the distance between the glass surface and the cell membrane is greater than 100 nm, that is, the cell membrane does not attach to the glass surface. In the IRM image after cell detachment (Figure 2(b)), focal adhesion remained on the glass surface. Most of close contact parts also remained on the glass surface. In the magnified image of the IRM image after cell detachment (Figure 2(c)), fibrous structures in which one end of them attaching to the focal adhesion remaining on the glass surface were observed. The other end of the fibrous structures was free, and most of them aligned into the direction of the detachment force applied to the cell.

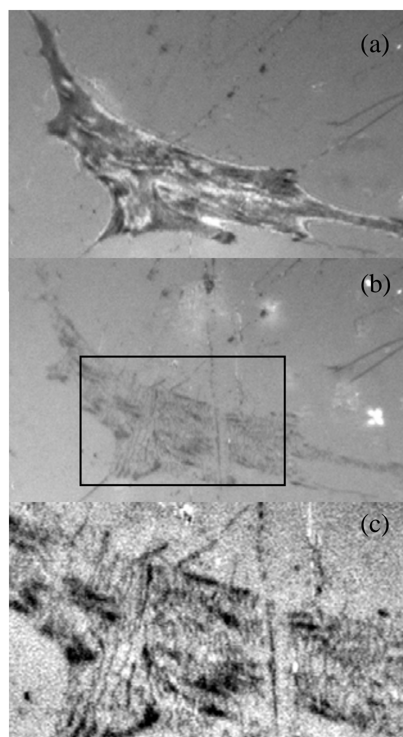

Figure 2. IRM images of IMR-90 before/ after detachment. (a) before detachment, (b) after detachment, and (c) magnified image of (b).

Proteins remaining on the glass surface after the cell detachment were fixed by 4% paraformaldehide and stained by FITC-labeled anti-β_1-integrin antibody. An example of the immunofluorescent staining is shown in Figure 3, indicating that some of the remaining proteins in focal adhesion are confirmed as transmembrane integrins. These facts suggest that the cell-material adhesion was not fractured at the binding between the material surface and extracellular matrix proteins or at the binding between the extracellular matrix proteins and transmembrane integrins, but fractured inside the cell. Regen et al. reported that some of integrins in focal adhesion were left on the substrate surface of cell rear after cell spontaneous movement.[2] These facts suggest that the binding between transmembrane integrin and extracellular matrix protein is relatively strong rather than that between integrin and focal adhesion associating protein.

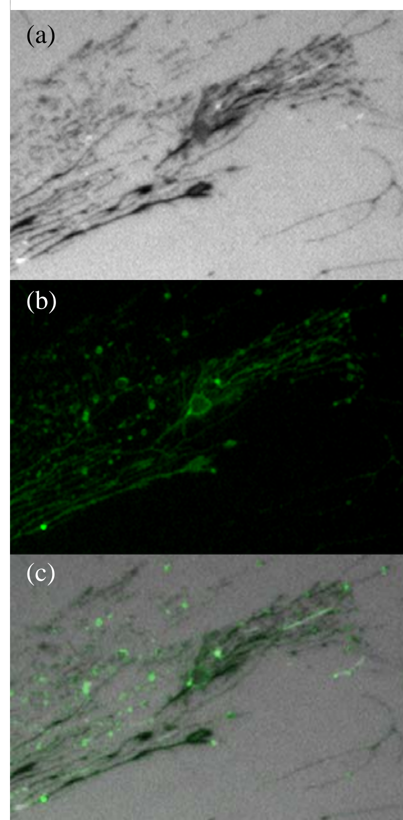

Figure 3. IRM and immuno-fluorescent images of proteins remaining on the glass surface after the detachment of IMR-90. (a) IRM, (b) β_1-integrin, and (c) merged (a) with (b).

References

1. A.Yamamoto et al.(1998) *Biomater*. **19**:871-879.

2. C.M.Regen and A.F.Horwitz(1992), *J.Cell Biol.*, **119**, 1347-1359.