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BIOMECHANICS OF CELL-MATERIAL ADHESION

A.Yamamoto

Biomaterials Research Team, Materials Engineering Laboratory, National Institute for Materials Science, Japan

INTRODUCTION: Cell adhesion to material surface has generally been evaluated by morphological observation of the cells adhering to the material surface using an optical and electron microscope, which is subjective, and not a quantitative method. Many researchers have tried to measure the adhesive strength between a cell and material surface to quantify the cell-material adhesion. A few methods have been developed to evaluate the strength of cell-material adhesion. One of them is determining the ratio of cells still adhering to the material surface after applying detaching force to a population of cells. A centrifuge is used to apply tensile detaching force to the cells, whereas a viscometer and a parallel flow chamber is used to cause a shear force to detach the cells by the flow of a physiological solution. In these methods, however, the force which a cell actually receives is non-uniform along the cell surface or between cells, and its magnitude is unknown. Therefore, this method evaluates the strength of cell-material adhesion qualitatively. Another method is micromanipulation, which is able to measure directly the force necessary to detach a cell from material surface. A micromanipulator with glass micropipette is used to pull out a single cell from material surface in normal direction. However, this method can only measure the detachment force of the cell which weakly attach to the material surface because it should have a spherical shape to be pulled by the micropipette. We developed a new system to measure the shear force necessary to detach a cell adhering and spreading onto the material surface in cell culture medium, observing the detachment process by an optical microscope[1]. In the present paper, the adhesive properties of murine fibroblasts to tissue culture polystyrene and extracellular matrix (ECM)-coated polystyrene dishes were examined[2]. The observation of the detachment process by interference reflexion microscopy (IRM) will also be discussed.

METHODS: A principle of the measurement of the adhesive shear force is shown in Figure 1. A cell adhering to the bottom of the dish on a XY-stage is moved to the tip attached to a cantilever. When the tip touches to the cell, a lateral load is applied to the cell. The cantilever is deflected corresponding to the load applied to the cell, and

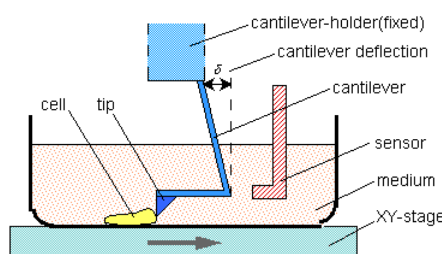

Figure 1. Principle of the measurement of cell adhesive shear force.

the deflection is recorded as a function of the displacement of the XY-stage. The cell adhesive shear force F is defined as the critical force to detach the cell from the material surface, and given by equation: $F = k\,\delta$, where k is the spring constant of the cantilever and δ is the maximum cantilever deflection. The cell adhesive shear strength S is defined as the cell adhesive shear force per a unit apparent cell adhesive area A, which was analysed on the optical-microscopic image of the cell.

RESULTS & DISCUSSIONS: Figure 1 shows the cell adhesive shear strength of L929 to ECM-coated and uncoated dishes after 24h-incubation. Among these surfaces, cells cultured on collagen (type I) have the highest cell adhesive shear strength, followed by those on fibronectin. This fact indicates that cells adhere strongly to ECM-coated surfaces than uncoated polystyrene surface. It was generally observed that coating the surfaces of glass and polystyrene by ECM increases the

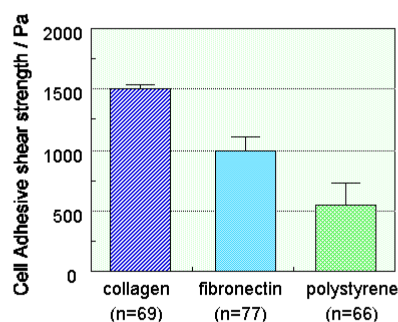

Figure 2. Cell adhesive properties of L929 on fibronectin-coated, collagen(type I)-coated, and uncoated polystyrene dish.

number and the morphology of the cells adhering to these surfaces. These fact suggest that morphological and qualitative evaluation of cell

adhesion onto ECM-coated and uncoated surfaces relates to the strength of adhesion between the cell and these surfaces.

A cell adheres to a substrate surface not the whole area facing to the substrate surface but with some parts of it, forming adhesion plaques. Adhesion plaque is the cluster of adhesive molecules such as transmembrane integrins binding to ECMs adsorbed onto the substrate surface. The integrins are connected to the cytoskeletal structures by attachment proteins such as talin, vinculin, and α-actinin. Cell-substrate adhesion is constructed by a series of bindings; the binding between a cytoskeletal protein and an integrin, the binding between the integrin and an ECM, and the binding between the ECM and the substrate surface. The differences in cell adhesive shear force are caused by the following factors; 1) the difference in the number of the complete set of serial bindings between the cell and the surface (an "entire" binding) per a cell or per a unit cell adhesive area, and 2) the difference in the strength of each "entire" binding.

Figure 3 shows the schematic explanation of the differences in cell adhesion to ECM-coated and uncoated dishes. In normal cell culture condition, cell adhesion onto glass or polystyrene is mediated not by fibronectin but by vitronectin(a).

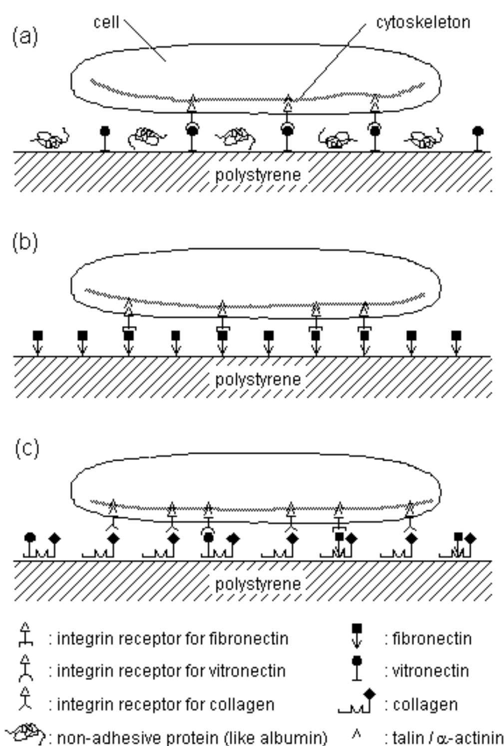

Figure 3. Schematic explanation of cell adhesion to polystyrene with cell adhesion molecules

When the polystyrene surface is coated with fibronectin, only a serial binding including fibronectin is formed (b). Comparing to the polystyrene uncoated surface, cells can easily form the "entire" bindings since the binding between the polystyrene surface and fibronectin is already done. In the case of collagen-coated dish, the binding between polystyrene and collagen is also done. Furthermore, collagen could bind directly to the cell and indirectly through fibronectin and vitronectin (c), which is an advantage for making the larger number of "entire" bindings comparing to fibronectin-coated and uncoated dishes.

The strength of the "entire" binding is decided by the strength of the weakest "fragmentary" binding in the "entire" binding. It is observed that an "entire" binding of the cell-cell or cell-substrate adhesion is frequently fractured at the binding between the transmembrane receptor and the cytoskeletal protein, resulting in the extraction of the receptors from the plasma membrane[3]. Transmembrane integrins are found on the substrate surface after the cell moved away spontaneously[4]. Furthermore, the force necessary to extract an transmembrane receptor from plasma membrane was measured as 10-20 pN[5], which is the same order of the magnitude as the force required to break another "fragmentary" binding such as the binding between a transmembrane receptor and its ligand[6]. These fact suggests that the "entire" binding of cell-substrate adhesion can be fractured at any "fragmentary" binding and that the strength of cell-substrate adhesion mainly depends on the number of the "entire" bindings formed. According to IRM observation of cell detachment process by this system, a part of cell surface proteins were remained on the substrate surface after cell detachment, suggesting that the extraction of transmembrane receptors is occurred. In future, the fracture point of the "entire" binding of cell-material adhesion should be confirmed by fluorescent microscopy to elucidate the mechanism of cell detachment from material surface.

REFERENCES: [1]A.Yamamoto, et al (1998) *Biomater*. **19**:871-879. [2]A.Yamamoto, et al (2000) *J. Biomed. Mater. Res.* **50**:114-124. [3]E.Evans, D.Berk, and A.Leung (1991) *Biophys J* **59**:838-848. [4]C.M.Rengen and A.F.Horwitz (1992) *J Cell Biol* **119**:1347-1359. 5L.Chu, L.A.Tempelman, C.Miller et al (1994) *AIChE J* **40**:692-703. [6]S.C.Kuo, D.A.Lauffenburger (1993) *Biophys J* **65**:2191-2200.