The Technique for Measurement of Cell Adhesion Force

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Abstract

After cells attach the substrata, cells begin to spread and form an organized actin cytoskeleton and complex transmembrane signaling regions, then cells start to express their functions, such as proliferation, differentiation and so on. Therefore, cell adhesion is very important. Recently, a number of studies have investigated cell adhesion from the viewpoint of biochemistry, materials, and mechanics. In this study, we observed the adhesion from mechanical point of view. The objective of this study was to develop a cytodetachment technique to quantitatively measure the adhesive force between cells and substrata. A holder was designed which can hold the probe of the AFM (atomic force microscope) probe, which was integrated to a laser tweezers workstation. A detach rig was setting, which is consisted of the probe of AFM, an AFM probe holder, and a cantilever arm mounted on the laser tweezers workstation. NIH/3T3 fibroblast seed on non-coated glass microscope slide, and test in different test mediums, such as Dulbeccos modification of eagles medium (DMEM)+10% fetal bovine serum (FBS) versus DMEM and Phospate-Buffered Saline (PBS). Detach NIH/3T3 fibroblast at room temperature, 25°C. Cell adhesion force of NIH/3T3 fibroblast in different test mediums. A novel cell-detachment apparatus to measure the adhesion force of a single cell was developed to incorporate into an optical tweezers workstation. The results demonstrate that a greater force is required to detach cells in DMEM+10% FBS versus DMEM and Phospate-Buffered Saline (PBS). In this study, we set a rig to quantify cell adhesion force. And the result of this study showed that adhesion force of NIH/3T3 fibroblast in DMEM+10% FBS is greater then cells in DMEM and PBS.

***Keywords*:** Cell adhesion force, Microcantilever, NIH/3T3 fibroblast

Introduction

With the development of the medical science, more and more artificial prostheses are implanted in human bodies to be replaced the original tissue. In these kinds of surgeries, the proliferation of human cells on the implants' surface will influence the success of the surgeries. Therefore, a complete understanding of the interaction behavior between cell and substrate is necessary. Studies found that cells must attach and adhesive to the substrate, then cells will start to spread on the substrata, and develop the functions of proliferation, differentiation, migration and so on. The investigation of the cell adhesion becomes a key issue to understand the cell-substrate's behaviors.

Cell-matrix adhesion is mediated by the membrane proteins such as integrin, and extracellular matrix (ECM) proteins such as fibronectin, collagen, etc. After cells seed on the substrate, the ECM proteins' amino acid, RGD (Arg-Gly-Asp), will bind with the membrane proteins by covalent bond, then the spread of cells will happen. After cells spread on the ECM, these proteins, which influence the adhesion of cell and ECM, will form an organized actin cytoskeleton and complex transmembrane signaling regions, and then cells start to express their functions, such as proliferation, differentiation and so on.

Because cell adhesion will affect cell differentiation and proliferation, biologists and chemists usually evaluate cell physiology by analysis of the cell adhesion phenomena. Until recent years, a few studies have investigated the cell adhesion from mechanical point of view. McClay et al.[1] and Thoumine et al.[2] utilized the centrifugation to produce tensile force to separate cells form the substrate. In addition, a micropipette is used to apply a tensile force directly to the single cell, then the cell can be pulled from the substrate[3-5]. Owens[6] and Truskey[7] designed a parallel flow chamber to create laminar flow over cells and to detach cells from substrate by shear force. Furthermore, a viscometer or its modification were used to produce shear force to detach cells from substrate[8-9]. Yamamoto[10] and Athanasiou[11] detached a single cell from its substrate by a microcantilever and the deflection of the microcantilever was detected using the optical technique. The detachment force to separate the binding of cell and substrate is assumed as the cell adhesion force.

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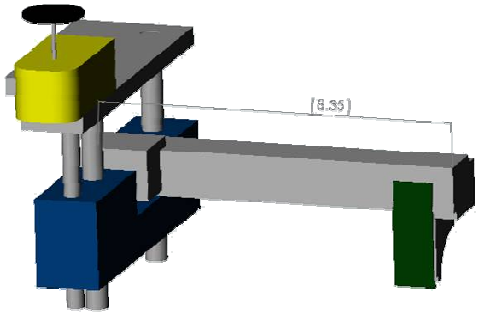

Figure 1: the detachment rig

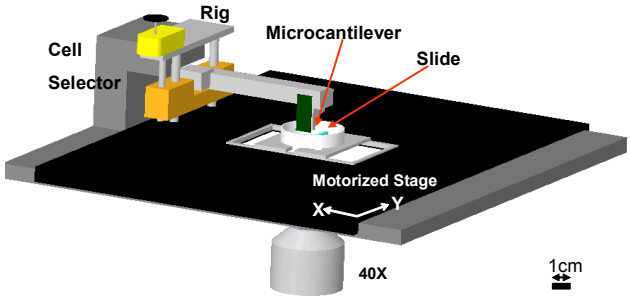

Figure 2: the complete cytodetachment apparatus

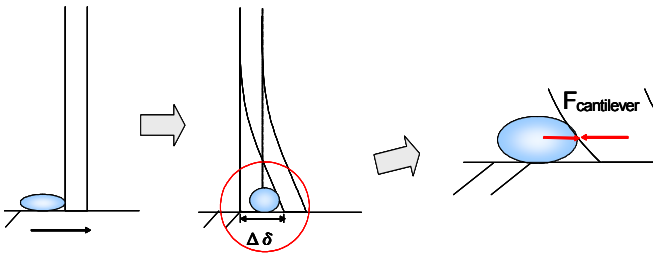

Figure 3: deflection of the cantilever

To date, the optical tweezers can be used to manipulate the cells and detach a cell from the substrata. The optical tweezers was used to trap fibroblast maintained on fibronectin-coated glass for measuring short-term (1-16s) binding force periods. Laser have opened a new era and provide a novel tool to micromanipulate cells and can be used to quantitative measure the short-term binding and adhesion force of single cell to different types of ECMs. However, the detachment of a cell from the substrate is only possible for within a minute short-term binding subject to the constraint of the laser power.

For long-term cell adhesion measurement, the other detachment technique is required. Therefore, the objective of this study was to develop a cytodetachment apparatus integrated with an optical tweezers workstation to quantitatively measure the adhesive force between cells and substrata. The developed detachment rig consisted of the probe of atomic force microscopy (AFM), an AFM probe holder, and a cantilever arm mounted on the laser tweezers workstation. The position of the probe can be adjusted at both axes in horizontal plane at the resolution of 0.2 μm and in vertical direction by adjusting the screw at resolution of 1μm to assure the precise detachment of the cell. A single cell was detached using the probe of AFM for quantitatively measurement of the adhesion force between the substrata and cell.

Methods

Literature available shows that the cell adhesion force is usually less than 10μN, therefore we design a rig with a microcantilever to measure the micro scale force (Figure 1). The complete detachment rig (Figure 2) consisted of the probe of atomic force microscopy with the spring constant from 0.016 to 0.020 N/m (Nanosensors™, NanoWorld AG, Switzerland), an AFM probe holder, and cellselector mounted on the laser tweezers workstation (Cell Robotics Inc, Albuquerque, NM, USA). The position of the probe can be adjusted by a motorized stage at both axes in horizontal plane at the resolution of 0.2 μm and at vertical direction by adjusting the screw at resolution of 1μm to assure the precise detachment of the cell. A single cell was detached using the probe of AFM for quantitatively measurement of the adhesion force between the substrata and cell.

Detachment force calculation

A probe of AFM was fixed on the holder, and then the cell approached the probe by constant velocity controlled by the motorized stage. During detachment, the cantilever of the probe provides a shear force ($F_{cantilever}$)to the cell, show in Figure 3. $F_{cantilever}$ can be calculated by Hock's Law

$$F_{Cantilever} = K \cdot \Delta\delta \qquad (1)$$

where K is the spring constant of cantilever and Δδ is the deflection of the end of the cantilever.

Experiment procedure

The cell in the experimental dish was moved using a motorized stage to contact the AFM probe, then the cell approached the AFM probe at the speed, 5μm/s, and the deflection of the AFM probe was recorded through a microscopy using a CCD camera (Figure 4). After a cell was detached from the microscope slide, image processing technique in Matlab 6.0 (The MathWorks, Inc., USA) was used to quantify the deflection of the probe, and then Hooks Law was used to calculate the $F_{cantileve}$ proportional to the deflection the probe. The typical force curve is shown in Figure 5 where the peak value is the cell adhesion force.

Cell culture

A microscope slide (Yung Song Co., Taiwan) was cut to the following dimension: 5×26×1 mm^3. After cleaning the surface of the microscope slide, NIH/3T3 fibroblasts were cultured on microscope slides with DMEM +10% FBS in 5 different dishes . After cells were cultured for 30min, 1h, 2h, 4h, and 12h, one of the microscope slides removed from one of

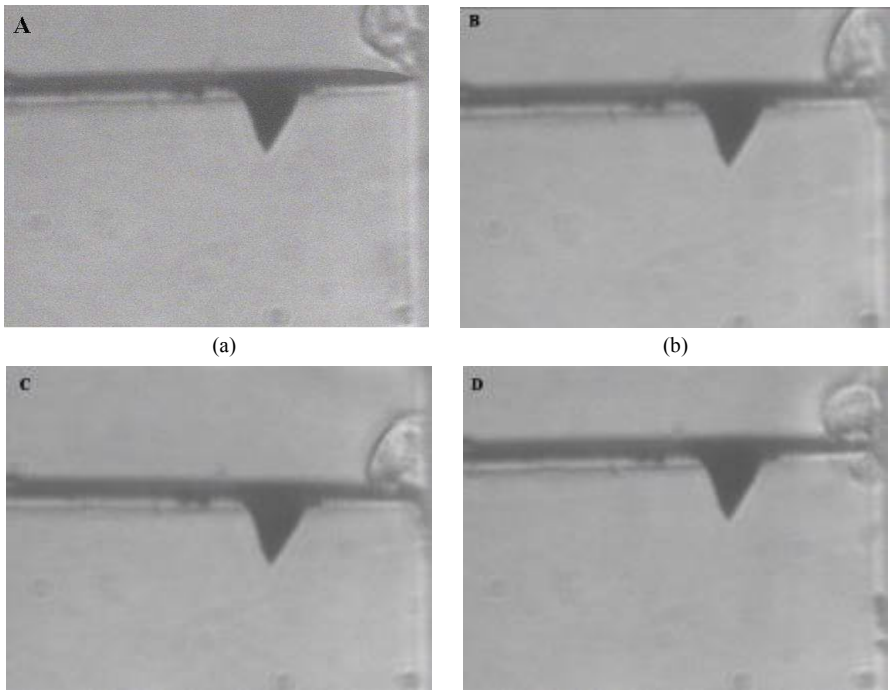

Figure 4: the image of the experiment. (a) the seeding cell approaches the cantilever. (b) The cell touches the cantilever and a shear force apply to the cell. (c) The cell is detached from the substrate, at the same time and the deflection of the cantilever is recorded by a CCD camera. (d) The cell was speared from the substrate.

Table 1. Mean value of cell adhesion force in differential mediums at 25℃, unit: μm=10^{-6}m

	Cell adhesion force		
	PBS	DMEM	DMEM＋10%FBS
30 min	n=9 0.0735（0.0145）	n=13 0.0675（0.0471）	n=14 0.1228（0.0536）
1 hour	n=5 0.0891（0.0160）	n=16 0.1558（0.0558）	n=10 0.1783（0.0894）
2 hour	n=6 0.1549（0.0497）	n=14 0.1784（0.0774）	n=11 0.1966（0.1001）
4 hour	n=10 0.1759（0.0485）	n=12 0.2084（0.0635）	n=13 0.2916（0.1017）
12 hour	n=12 0.1893（0.0899）	n=14 0.2391（0.0821）	n=6 0.3695（0.1059）

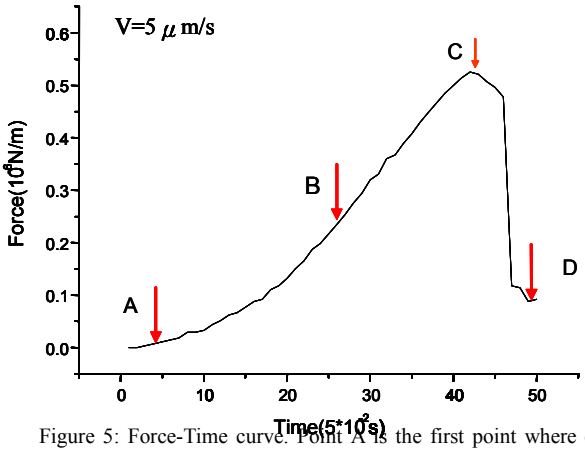

Figure 5: Force-Time curve. Point A is the first point where cell start attaches the cantilever. Point B shows that cell approaches the cantilever, and the cantilever starts to apply a shear force to the cell. Point C is considered as the point where cell start being detached from the substrate, and we name the value of this point as cell adhesion force. Point D means that cell is complete separated from the substrate.

the dishes and transferred to the experimental dish. We test the cell adhesion force in 3 different experimental medium, DMEM+10%(no phenol red), DMEM (no phenol red), PBS, at room temperature.

Results

After each experiment, a force-time curve can be obtained, as shown in Figure 5. In Figure 5, the peak point C is considered as the point, where cell was detached from the substrate, so we assume that the value of point C is cell adhesion force. Table 1 shows mean value of cell adhesion force in differential mediums at 25℃.

Figure 6 shows the relationship between adhesion force and seeding time by seeding cell in the same substrate. The results demonstrate that cell adhesion force increased with the time. Figure 7 shows the relationship between adhesion force and mediums and demonstrate that a greater force was required to detach cells in DMEM+10% FBS versus DMEM and PBS .

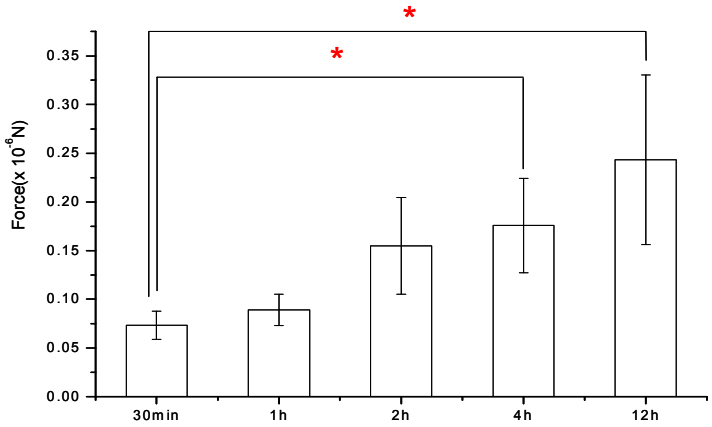

Figure 6: Cell adhesion force v.s. time (* : P＜0.05)

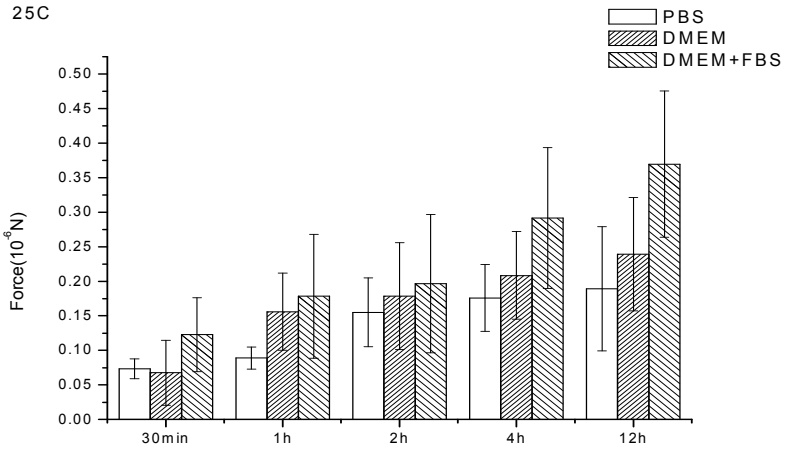

Figure 7: Cell adhesion force in three

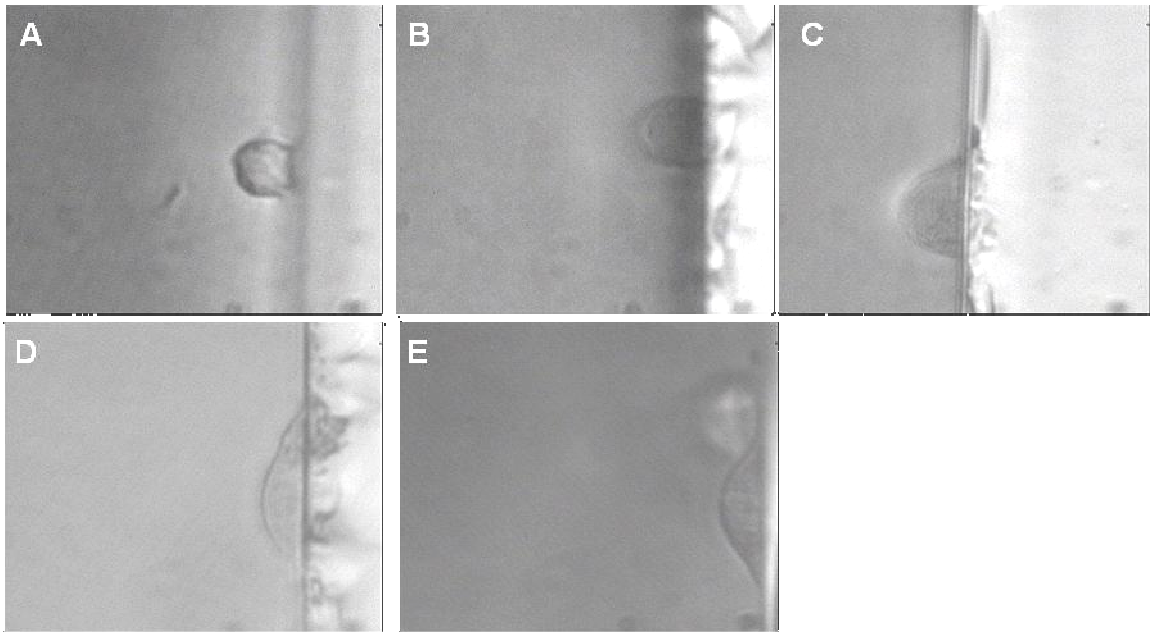

Figure 8: the lateral view of the cell with the change of time. From figure (A) to (E) are images of cells, which seeded after 30min, 1h, 2h, 4h, and 12h, respectively. These figures show that the seeded area of the cell increases with time.

Table 2: Comparison of cell adhesion force measurement

	Spring constant of the cantilever	Sensor to Measure Deflection	velocity	Adhesion force	Observation of the Crash of Slide
This study	0.016-0.02 N/m	Fiber-optic Probe	5 μm/s	NIH/3T3 369 nN （non-coated substrate）	Y
Yamamoto [10]	3.12 N/m	Light fiber	20 μm/s	Murine Fibroblasts L929 310－390 nN (Non-coated Dish)	Y
Athanasiou [11]	3×10^{-3} N/m	Dual Photodiode	1 μm/s	Rabbit Articular Chondrocytes 550±240 nN (Non-coated Coverslip)	N
Sagvolden [12]	0.34 N/m	Split Diode	2.5 μm/s	Human Cervical Carcinoma Cells 204 nN (Coated with Fibronetin)	N

Discussion

In this study, we developed a novel cell-detachment apparatus to measure the adhesion force of a single cell, which is incorporated into an optical tweezers workstation. The tendency of the force curve is same as other studies, which represented in Table 2. The image processing techniques was developed to measure the deflection with the reduction of noise and increase of the accuracy.

Results show that the cell adhesion force increases with the time, which can be proved by the lateral view of the cell shown in Figure 8. It agrees with the previous findings [3] [10] and [12]. This indicates that the linkage between cell and substrata increase with increased seeding time of cells.

Examining the effect of the experimental medium on the adhesion force, we found that the medium of DMEM+ 10% FBS has more growth factor and then the performance of cell in DMEM+ 10% FBS is best. The PBS doesn't have any nutriment such that the cell adhesion force in PBS has the smallest. Therefore, a greater force is required to detach cells in DMEM+10% FBS medium compared to in DMEM and PBS medium.

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細胞黏著力量測技術

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摘　要

細胞接觸基質或材料時，細胞首先發生貼附及黏著的現象，這個現象會決定細胞在基材上的生理表現，進而影響細胞所能發揮的特有功能，因此目前已有細胞生理學、生物化學等領域的學者藉由評估細胞的黏著能力，來評斷細胞的表現，近年來也有力學等領域的學者藉由定量細胞黏著力來評估細胞黏著能力，故本研究藉由設計一套可量測細胞黏著力的機構來定量細胞黏著能力。機構中採用原子力顯微鏡探針上的懸桿爲施力爲施力設備，藉由細胞以等速逼近懸桿，使懸桿對細胞施加剪力並將細胞從基材上刮下，再分析懸桿的偏移量求出探針對細胞的施力，藉此求得細胞的黏著力量。完成機構測試後，並利用此機構觀察 NIH/3T3 纖維母細胞在不同實驗溶液下，細胞黏著力量的改變，結果發現細胞在相同溫度下於 Dulbeccos Modification of Eagles Medium (DMEM，無酚紅) 培養基加 10％ 胎牛血清(Fetal Bovine Serum, FBS)中的黏著現最佳，其次爲在 DMEM(無酚紅)中，而在無機鹽溶液(Phospate-Buffered Saline, PBS) 中的表現則最差。

關鍵詞：細胞黏著力、微小懸桿、NIH/3T3 纖維母細胞

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