A STUDY ON CELL ADHESION BY USING MEMS TECHNOLOGY

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Abstract

Cells adhere or stick to each other or to an extra cellular matrix in an organized way to form tissues and organs by self assemble. A qualitative and quantitative understanding of cell adhesion is essential to understand not only the evolution of tissues and organs, but also to manipulate the adhesion by biochemicals to enhance or retard physiological formations. For example, heart attacks may be caused by a blood clot closing off a passage in the heart, which is a result of the adherence of cells. If the adherence of these cells could be controlled, then the risk of heart failure would decease.

Since cells and MEMS are in the same scale and also some cells can survive on artificial surfaces like silicon oxide, glass, ceramic or plastic, it is natural to think about applying MEMS system into cell adhesion study. Actually, some MEMS devices are already developed and proved powerful in measurement of the adhesion force between cells and some matrices.

In our project, we will introduce two MEMS systems in this area. One employs a MEMS sensor to study the adhesion properties of a single living bovine endothelial cell. The sensor consists of a cantilever beam made of single crystal silicon and a perpendicular plate at the end of the beam. The beam is deformed under the adhesion force applied by the cell. By measuring the deformation of the beam, the adhesion force is then quantified. The other micro-system device is composed of a regular array of vertical posts with cells cultured on top of it. Each post in the array can be independently bended in response to local forces exerted by the attached cell. Because the posts bend to the direction of the applied force, the magnitude of the adhesion force as well as the direction of it can be obtained through this system.

These two systems present some good trials in this area. But the disadvantage of these two models is that the cantilever and the posts are not small enough to capture the adhesion force from one contact point (focal contact) between the cell and the extra cellular matrix because the average spacing of focal contacts is around 1 µm while their spacing is much greater than this critical number. Thus they could not provide a precise measurement on the adhesion force. We propose a membrane-based device with high resolution to capture the adhesion force on each focal contact.

Keywords: cell adhesion, BioMEMS, PDMS, microcontact printing
Introduction

As we stated before, cell adhesion is of great importance in the development of disease and multi-cellular organisms [1]. For instance, cancer cells, unlike other typical cell, can form colonies on variety of tissues and organs. They have lost their addressing ability, in contrast to cardiac cells that adhere to only other cardiac cells to form the heart. That is the cardiac cells have their address (heart), and if detached from the address, they die. But cancer cells won’t. That’s why cancer can spread over a body. For the cancer cells, in order to form a colony, they need to adhere to some tissues or in general matrices. Therefore, their adhesion properties dictate whether cancer will spread or not [2,3]. So if the adhesive properties of the cancer could be turned off, the spread of cancer could be controlled. This is a case where adhesion is undesirable. While in other cases such as physiological integration of MEMS and other implantable devices, greater cell adhesion is favorable [4].

Interest in using MEMS and micro-fabrication technologies is growing because of the great properties of MEMS, such as small size scale, electrical nature, and ability to operate on short time scales. Furthermore, the digital capabilities of MEMS may allow potentially greater and more precise control in cell adhesion study.

Cell adhesion involves binding and clustering of integrins to extracellular matrix (ECM) ligands [5,6], active spreading of the cells across the substrate [7,8], and contraction of the actomyosin cytoskeleton. The total effect of this process is that traction forces are generated at the sites of adhesion. Although these mechanical interactions between cells and their substrates dictate how cells organize and function in their environment [9-11], our understanding of the biology of traction forces, cell adhesion, and substrate mechanics remains incomplete.

To study these minute forces, investigators have relied on soft materials such as lightly crosslinked hydrogels or silicone elastomers, where the crosslinking chemistry is used to control mechanical compliance [12, 13]. The deformations generated by cells are tracked by the displacement of beads or microfabricated markers embedded in the substrate. Then the forces are calculated from the displacement. However several fundamental limitations remain. The forces are generated on a continuous plane, which
means many adhesion sites are in contact with the substrate and they all contribute to the
deformation of the substrate. It is hard to differentiate each contribution from any
adhesion site. Moreover, using discretely distributed beads or microfabricated markers to
capture the deformation of a continuous plane is an approximation which will further
introduce some error into the final result, not to speak of error in the measurement of
displacements of individual markers and uncertainty of deformations in the space
between markers. To circumvent these disadvantages associated with that kind of
structure, the two models mentioned in abstract use cantilever beam and vertical micro
posts, respectively. In later sections, we will introduce these two models and make a
comparison between them. Because of the low
resolution associated with these two models, we
propose a model which has higher resolution and
can capture the force by each adhesion site.

Model 1:

**In-situ adhesion studies of a single living bovine endothelial cell
using MEMS sensor [14]**

**I. Micro Mechanical Experimental Procedure**

The MEMS sensor consists of a cantilever beam made of Single Crystal Silicon
(SCS), 1200 $\mu$m long $\times$ 11.5 $\mu$m deep $\times$ 4 $\mu$m wide, coated by a thin native silicon
dioxide layer. At the end of the MEMS cantilever is a 100 $\mu$m long perpendicular plate
that has similar width and depth (see Figure 1 micrograph). The lateral stiffness of the
beam at the end is 18 nN/$\mu$m and was determined from its resonant frequency and mass.
The beam is supported by an anchor which is attached to the substrate of a silicon chip.
The chip is held by an x-y-z stage controlled by a piezo driven motor. The stage has a
motion resolution of 20nm, Figure 2.
The cell tested by the MEMS sensor was a Bovine Endothelial Cell. The cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM). The DMEM contained 1,000 mg/L of D-glucose, 110 mg/L of sodium pyruvate, and pyridoxine hydrochloride. Later added to the DMEM was 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin to feed the cells and protect them from bacteria, respectively. The cells were cultured in a large, 10 cm diameter, culture dish. A sparse population of cells was utilized so that neighboring cells would not interfere with an ongoing experiment. Once the cells were cultured, they were stored in an incubator at 5% CO₂, 37°C, and 98% relative humidity for 14 hours.

The Bovine Endothelial Cell was attached to a substrate by integrin activation. The MEMS cantilever was coated with fibronectin, an ECM protein, and then allowed to incubate for 14 hours. The small area, 11.5 µm × 4 µm, located at Site A of Figure 1 was used as the bonding location.

The MEMS beam was brought in contact with the cell by the x-y-z stage (see Figure 2). Note that the beam was submerged in the liquid media to interface with the endothelial cell at Site A of Figure 1. Figure 3 shows the MEMS actuator interfacing with
the cell. The observations were made by an inverted optical microscope and the images recorded digitally. A fifteen-minute period was allotted for adhesion between the MEMS cantilever and cell. After this period the piezo-actuator, located on the Y-axis of the X-Y-Z stage, was displaced incrementally. Images were taken at each displacement increment and stored for later analysis. As the cantilever was moved away, the cell began to deform applying a restoring force on the cantilever. The cantilever began to bend in response. The amount of deformation of the cantilever beam was obtained from the difference between the motion of the stage and the motion of the cantilever tip. From the known spring constant of the cantilever, the force is obtained.

II. Experimental Results

Using this MEMS sensor, the adhesion properties of a single living bovine endothelial cell has been studied quantitatively and qualitatively. Figure 4 is the plot of the force versus deformation of the endothelial cell at the point of contact with the cantilever. Figure 5 shows the micrograph when the cantilever was brought in contact

Figure 3: MEMS Cantilever and Bovine Endothelial Cells

Figure 4 Endothelial Cell Force Versus Piezo-Actuator Displacement
with the cell with no load being applied. Figure 6–8 are a series of pictures display the process of the cell deformation. They respectively correspond to points A, B, D in Figure 4.

In-situ observation of the experiment has shown that focal adhesion sites are spaced on the order of 1 µm intervals. Quantitatively it has been found that a focal adhesion site, prior to failure, applies 37 nN of force onto the MEMS sensor. It has also been observed during in-situ experiments that focal adhesion sites do not detach from the substrate, as the cell is being pulled, rather the cell necks followed by the failure of the cell membrane.
III. Discussion

This work is among the earliest studies on cell adhesion and it provides some vivid pictures on the behavior when a cell is in touch with a cantilever beam. They determined the magnitude of the adhesion force between a cell and a substrate, which is around 37 nN. But this device doesn’t have much control on the contact of the cell and the beam. They have to wait until one cell, which is on the bounding location, to make a contact with the beam and then deform it. Only after that could they measure the adhesion force between the cell and the beam. Although the device is quite simple and they only measure the force from one contact region (we call it region because they did not depart the contact region into an array of contact points as the researches did later on), it is a nice start in finding the adhesion force between a cell and a substrate.

Model 2:

Cells lying on a bed of microneedles: An approach to isolate mechanical force[15]

1. Materials and Methods

   Fabrication and Preparation of mPADs. mPADs were fabricated by replica-molding (Fig. 9B). To make a template containing an array of holes, prepolymer of poly was poured over an array of SU-8 posts made on silicon wafers by standard photolithography, cured at 65°C overnight, peeled off, oxidized in an air plasma for 1 min, and silanized with (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane vapor overnight under vacuum to aid subsequent release of PDMS from the template. To make mPADs, prepolymer of PDMS was poured over the template, degassed under vacuum, cured at 110°C for 20 h, and peeled off the template. The substrates were then either immersed for 1 h in 50 µg/ml human fibronectin or printed with ECM protein.

   As described in the paragraph above, some special treatments are needed to form a micropost-shape substrate, which are a little different from a general MEMS device since here the device is designed to work with an organic system---cell.
Here microfabrication techniques are applied to pattern a silicon wafer to a desired shape which is used as a template to produce the final microposts substrate because the fabrication techniques to pattern silicon wafers are very mature and we can produce a silicon template of any shape. Why not use silicon wafer directly instead of PDMS? Because silicon is too stiff compared with the magnitude of the force exerted by the cell, it is practically impossible to use silicon to measure the adhesion force. So the silicon wafer is used here as a template or mold.

Calibration of mPADs. Spring constants can be obtained by following the procedures. Briefly, deflection of the tip of a micropipette was measured under the weight of small crystals of \( p \)-nitrophenol (Sigma). The mass of the crystal was determined by dissolving it in bicarbonate buffer and diluting until the absorbance of the
solution at 400 nm was within the range of a linear absorbance curve generated from solutions containing known concentrations of \( p \)-nitrophenol. Calibrated glass micropipettes were then mounted onto a piezoelectric manipulator fitted on a microscope stage. Viewed under a \( \times 100 \) objective, the tip of the pipette was maneuvered into contact with the top of an individual post. The entire glass pipette was then repeatedly moved various set distances by the piezomanipulator and the deflection of the post in response (1–4 \( \mu \text{m} \)) was recorded. The ratio of the deflection of the pipette tip to the deflection of the post equals the ratio of the spring constant of the post to the calibrated spring constant of the pipette.

**Measurement of Traction Forces.** The tips of posts in a field of view were manually assigned coordinates. A regularly spaced grid of coordinates representing the ideal undeflected positions of the posts was formed by minimizing the difference between the assigned coordinates of posts and the ideal grid. Once minimized, the standard deviation of these differences was 0.2 \( \mu \text{m} \) for the posts used in this study. To calculate the force on each post, the distance between real and ideal position of the post was multiplied by the spring constant of the post (measured to be 32 nN/\( \mu \text{m} \) for the 3-\( \mu \text{m} \)-diameter, 11-\( \mu \text{m} \)-tall posts used in Figs. 9 and 10). The resolution of force was limited by the deviation of the undeformed posts from the ideal grid (0.2 \( \mu \text{m} \)); thus, they were able
to resolve forces greater than 12 nN. The spatial resolution, defined by the periodicity of the post array, was 9 µm.

**Cell Culture and Reagents.** Bovine pulmonary artery smooth muscle cells and NIH/3T3 mouse fibroblasts were cultured in Dulbecco’s modified Eagle’s medium containing 2 mM glutamine, 100 units/ml penicillin, and 100 mg/ml streptomycin with 10% calf serum. Bovine pulmonary artery endothelial cells were cultured in DMEM with 5% calf serum. BPASMCs were used in all studies; 3T3s and BPAECs were used to confirm results in Figs. 10 and 11 A–D. Lysophosphatidic acid, 2,3-butanedione monoxime, and cytochalasin D were used as described. The expression construct for pEGFP-RhoA-G14V was mutagenized from pEGFP-WT-RhoA. Transfection was carried out by using Lipofectamine for 24 h, and transfected cells were enriched by flow cytometry; sorted cells were seeded onto mPADs and fixed after 10 h.

To restrict cell adhesion to the tips of the posts, and thereby precisely define the surface mechanics, microcontact printing is used to deliver fibronectin from a stamp onto the tips of the posts (Fig. 10B), and adsorbed Pluronics F127 onto the remaining unstamped regions of the array to block nonspecific protein adsorption and cell adhesion. Using this method, they can print fibronectin onto specific posts within the array to spatially pattern the adhesiveness of the surface toward cells (Fig. 10C). On substrates where the tips of all of the posts were printed with fibronectin by using a flat stamp, cells attached, spread, and migrated selectively across the tips of the posts, bending the posts centripetally toward the interior of the cell (Fig. 10D). The general morphology of the cells on posts was similar to that of cells cultured on planar substrates (Fig. 10E).

**II. Result**

By using this system, they demonstrated that a positive correlation exists between the size of focal adhesions and the force generated at those adhesions. They also observed in the same cells a subset of smaller adhesions (<1 µm² in area) that exerted significant traction forces in which the magnitude of the exerted forces did not correlate with adhesion size. Their findings suggest the existence of two classes of force-supporting
adhesions that exhibit distinct force–size relationships. Importantly, they show that both classes of adhesions can coexist within the same cell (Fig. 11).

**III. Discussion**

The device they designed is a very good one, which can capture the magnitude also the direction of the force at the same time. Compared with the first one, it has a much better spatial resolution, which in their paper is $9 \mu m$. Furthermore by changing the geometry of the posts they can easily change the mechanical property of the substrate without change the material of the substrate. This is a very good improvement because changing material may inadvertantly affect surface hydration, chemistry, and adhesiveness, making it difficult to isolate the effects of substrate mechanics on cell adhesion and behavior. They explored engineering substrates with mechanical properties that are difficult to generate with planar substrates. By varying the heights and therefore the stiffness of specific posts within an array, they defined spatial changes in local substrate stiffness (Fig. 9D). Because the stiffness of posts varies as the inverse cube of
their height, decreasing the height by half caused a local change in stiffness by 8-fold (Fig. 9E). By using geometric anisotropy they generated mechanical anisotropy in the substrate, creating oval posts that required 8 times more force to deflect along the long axis than along the short axis (Fig. 9 F and G). Thus, geometric parameters, which are easily defined by our fabrication processes, can be used to engineer well-defined mechanical terrains for cellular studies.

They mentioned in their paper that the resolution of mPADs could be further improved by using available technologies to increase the density of posts in the array and the precision in measuring deflections. But actually the spacing of the posts is not only restricted by the techniques available but also by the space needed to prevent the contact of posts, which are in a close neighborhood. As they said when they calibrated the spring constant of a post, they deformed the post to a displacement of 1-4 μm. So the resolution of this method can’t be in submicron region. But as we know, the size of a focal contact is in a micron magnitude. Using their device can’t accurate determine the adhesion force from one focal contact. Maybe this is why they find two kinds of adhesion forces: one is increasing with the size of the contact region, the other is the magnitude of the exerted forces does not correlate with adhesion size. Based on this, one of the goals of studying the adhesion force is try to improve the spatial resolution of the devices. That’s what we will do in the next subsection.

Model 3. Micropatterned Substrata[16]

I. Materials and Methods

- Microfabrication

Prepare two silicon wafers. One is p type and the other is n type. Use photolithograph and dry etching to fabricate an array of rectangular holes on wafer 2, the thinner wafer (Fig 12). Then bond two wafers together. Prepolymer of poly (dimethylsiloxane) was poured over the array of holes. Deposit a layer of silicon dioxide on the backside of wafer 1 as a mask in wet etching of silicon and use p-n junction etching stop. Remove the substrate and the PDMS above the wafer 2. Then a PDMS stamp is prepared with a hydrophobic alkanethiol. The thiol pattern is stamped onto the membrane, forming self-
assembled monolayers. The remaining regions are blocked with a protein-resistant hydrophilic alkanethiol. This process is called microcontact printing. This is a special technique when it comes to the bio-MEMS area. In the following section, we will give a brief introduction of this technique.

![Microfabrication process diagram]

**Microcontact printing**

Because we want to restrict the cells to the specific small membranes, we differentiate the membrane region and other regions by microcontact printing different materials. The membrane region is “inked” with a hydrophobic alkanethiol which cells like to stay on. The other regions are “inked” with a protein-resistant hydrophilic alkanethiol. Silicone stamps were produced by the thin stamp technique. The procedure for microcontact printing is summarized in Fig. 12. The stamp was ‘inked’ with a 1.5 mM solution of a hydrophobic alkanethiol [octadecylmercaptan (ODM) Aldrich] in ethanol and pressed onto a gold-coated coverslip, forming self-assembled monolayers at the protruding parts of the stamp. Uncoated regions of the coverslip were blocked with a solution of a hydrophilic alkanethiol [tri-(ethyleneglycol)-terminated alkanethiol. Protein solutions of ECM molecules in phosphate-buffered saline were applied to the coverslips for 1 hour at 4°C and bound specifically to the ODM via hydrophobic interaction. Coverslips were
then blocked in 1% bovine serum albumin (BSA; Sigma) in Dulbecco’s modified Eagle’s medium (DMEM; Gibco).

![Image](image.png)

**Mesurement**

We culture cells on top of the membrane structure. Cells will contact with the surface only at the small membrane spots because the protein-resistant hydrophilic alkanethiol “inked” outside of the membranes will prevent the contact of cells with these regions. By measuring the displacement of each membrane, we can know the adhesion force between the cells and the substrate at each contact point. Since the membrane can be made to a scale of 0.1µm² and of 1 µm spacing, which is smaller than the size of a focal contact site (of several micron), then the measurement of the displacement of each small membrane dictates the adhesion force at each site.

We use optical readout to detect the displacement of each membrane. The system of one membrane and the corresponding measuring device looks like Figure 13

![Figure 13 Optical Readout](image.png)

We know that there is a relation between the force on the membrane and the displacement of the membrane, which is

\[
\frac{Fr^4}{Eh^4} = \frac{16}{3(1-v^2)} \left( \frac{\Delta d}{h} \right) + \frac{7-v}{3(1-v)} \left( \frac{\Delta d}{h} \right)^3
\]
By measuring the displacement, using this device, we can derive the force applied on the membrane through the above equation. As there is an array of membranes, corresponding measuring devices array will need to gather all the membranes deformations. By using the optical readout devices, the immediate response of cells can also be captured by our membrane system.

**II. Discussion**

By using this device, we can improve the spatial resolution and determine the adhesion force from each focal contact site. This can also provide a force distribution when the cell is in contact with a certain surface. Based on the knowledge, the relation between the cell morphology and the adhesion force distribution can be studied. But when improving the resolution, we kind of sacrifice other information such as the direction of the force. Also, the readout device is another problem in this model, because for each small membrane we need an independent optical device. We think by using the micromirror technique we can get an array of laser beams. With the same device mentioned above, the corresponding array of displacements can be measured. The rest part is data analysis. After that, we will have the information of force field.

**Conclusion**

In this paper, we introduced two MEMS systems which were used to investigate the cell adhesion behavior. After studying these two models, we addressed some advantages and disadvantages associated with them. As we mentioned before, the first one is too simple to give much information, while the second one may encounter problem to improve the resolution. In order to further improve the resolution of the adhesion force measuring device, we try to design a new one. Following the idea of the reference [16], we proposed a new device using membrane instead of cantilever or posts to measure the adhesion forces. Although we don’t have access to experiments, thus we can not practically determine the resolution of this system, based on the experiments carried out in [16], we expect it to be in submicron region.
In order to achieve higher resolution, the size and spacing of the membranes have to be further reduced. It is natural to think of using NEMS to investigate the cell adhesion. This could be a future direction in this area.
Reference


