Micromachined High Aspect Ratio PDMS Elastic Micropost Arrays for Studying Traction Forces of Vascular Smooth Muscle Cells

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ABSTRACT

PDMS micropost arrays were fabricated, characterized, and used to analyze the traction force generated by vascular smooth muscle (VSM) cells. The micropost array was fabricated with diameters between 3-7µm, with edge to edge spacing of 5 and 7µm, and height to diameter aspect ratio up to 8. The spring constant of micropost used in cell culturing experiment is 20.94 nN/µm and the smallest traction force exerted from VSM cell is 8.05nN. The force generated by VSM cells cultured on top of micropost was in the nano Newton (N) range and it can be obtained by measuring the micropost deflection using optical microscopy images. The high aspect ratio micropost can be used to measure the traction forces of cells with weaker contractile forces in the nN-pN range.

Keywords: vascular smooth muscle cell (VSMC), micropost array, PDMS, AFM, phase contrast image.

1 INTRODUCTION

There is a great interest in studying the traction forces of vascular smooth muscle (VSM) cells that possess specific mechanisms to sense mechanical forces and respond accordingly, a phenomenon known as mechano-transduction. Although the mechanical stresses can be transmitted to cells, the force transmitted inside the cell to the extracellular environment plays a more important role in this mechanism [1-4]. The recent advances in micropost technologies and atomic force microscopy (AFM) have made it feasible to start addressing the mechano-transmission processes experimentally.

There have been many attempts to measure the intracellular force transmission on continuum substrate such as polydimethylsiloxane (PDMS) where cell deforms it by applying intracellular forces and produce wrinkled pattern on it, or uses fluorescent beads-embedded polyacrylamide gels where the cultured cells generate traction forces that deform it. The deformation can be detected by analyzing wrinkled pattern or measuring the movement of the embedded beads. These techniques have led to a significant improvement in our understanding of spatial and temporal traction response in cells. However, they can not determine the location and direction of point forces, and the magnitude of the force is difficult to calculate [5-11]. Recently, some experiments have applied high density elastomeric micropost array to measure the intracellular force in nano Newton range and successfully determined the location and direction of the force [12-16]. Micropost array technologies have been used to analyze cells such as cardiac fibroblasts or myocytes, smooth muscle cells, and cell monolayer with significantly large contractile forces at the edge of the cell [9, 12, 15, 17] and cells such as tendon fibroblast [18] with weaker contractile force.

The objective of this work is to fabricate high density array of high aspect ratio microposts with very high measurement sensitivity and is able to detect and measure traction force of VSMC in pN-nN range. In this paper, we successfully fabricated high aspect ratio (up to 8) PDMS micropost arrays and used them to map the VSM cell traction forces at single cell level. The cell’s traction force on each micropost was determined by measuring the displacement of post top surface position away from its bottom surface position in the corresponding phase contrast images. This measurement was accomplished by using matlab image processing software. The smallest measured traction force is 8.05nN and the spring constant of the micropost can be as low as 0.75nN/µm.

2 DESIGN, FABRICATION AND CALIBRATION

2.1 Design of micropost array

Micropost can be treated as a cylindrical cantilever beam that one end is fixed to the substrate and the other end is free to move. It should be noted that the deflection-force relation of micropost stays linear when force is below several tens of nano Newton. Therefore, the bending property of micropost is similar to spring and the deflection $x$ at the free end can be obtained from the following Euler-Bernoulli equation:

$$x = \frac{64L^3}{3\pi ED^4}F = \frac{1}{k}F$$

(1)
where $E$ is the Young’s modulus of PDMS, $D$, $L$ and $x$ are the diameter, the height, and deflection of the micropost, respectively; and $k$ is the spring constant of the micropost. According to the above equation, spring constant $k$ can be modified by varying the size of the post or changing the Young’s modulus of PDMS. In order to adapt to post fabrication techniques and cell culturing experiment, the post diameter is varied from 3 to 7 μm and the post height is varied from 5 to 25 μm. The spacing between posts was also varied to achieve different array densities.

2.2 Fabrication

To fabricate high aspect ratio micropost (See figure 1), the following microlithography and replica-molding techniques were used in the following sequence: a photoresist layer (Shipley 1813) was patterned on a 3” silicon wafer which was initially cleaned with Pirhana solution, to form openings at locations corresponding to the microposts; silicon micromold was formed by etching micro-holes through the wafer with high aspect ratio using Deep Reactive Ion Etching System; the wafer was treated with a vapor of hexamethyldisilazane reagent in vacuum desiccators for 15 minutes to facilitate peeling off the posts. Figure 1: 3D view of the micropost array

from the mold after being cured. Finally, a certain amount of PDMS, prepared by mixing the resin (Sylgard 184 Silicon Elastomer Kit, Dow Corning) with curing agent (10:1), was poured over them. The PDMS- wafer mold were placed inside a vacuum oven at 65°C for 24 hours. After the PDMS was cured, it was peeled off manually from the mold creating the micropost arrays. Figure 2 shows the SEM micrographs of microposts with 3μm diameter and 25μm height.

2.3 Calibration of micropost

The Young’s Modulus of the microposts were determined experimentally by applying predetermined forces at the free end of the micropost using an atomic force microscope (AFM) cantilever (Veeco Company). The displacement of the micropost free end was then recorded. The spring constant of the micropost can be obtained dividing the applied force by the measured displacement. The calibrated Young’s Modulus (994KPa) is used in Euler-Bernoulli beam theory to determine the spring constant of each micropost. The micropost arrays can be used not only for analysis of cells generating small traction force but also for cells with large contractile forces.

![Figure 2: SEM micrographs of micropost with 3μm diameter and 25μm height](image)

3 EXPERIMENT AND RESULTS

3.1 Cell experiment using micropost array

VSM cells isolated from male Sprague-Dawley rats (250-350g) were cultured on top of a micropost array. Cells placed on micropost array were incubated for 3-5 days before image acquisition experiment. Cells growing on the post are able to push or pull the top end of the posts as they grew, spread or reacted to the change of their physical surroundings and applied traction forces to their external environment. A Fluoview1000 confocal microscope was used to collect images from top to bottom of the post with 1μm step size. Since the bottom of the post was fixed, only

![Figure 3: A) Cell traction force distribution after image processing, B) SEM micrograph of cell cultured micropost array with 7μm diameter and 25μm height. Scale bars correspond to 10μm](image)
its top was shifted by the cell traction force. Pattern recognition program using Matlab was used to compare the position of micropost bottom with the micropost top to determine the bending of each individual micropost underneath the cell. Figure 3A shows the traction forces map of a VSM cell growing on the micropost array with diameter, height and spacing 7µm, 25µm and 5µm, respectively. Figure 3B shows a micrograph of a cell cultured micropost array with diameter, height and spacing of 7µm, 25µm and 3µm respectively. These figures clearly show the force distribution of VSM cells grown on the top of the micropost array.

3.2 Results

The calculated spring constant of the micropost in figure 3 is 20.94nN/µm and the resolution of the phase contrast images is 400nm/pixel. The measured largest and smallest traction forces were 50.55 nN and 8.05nN generated by the cell on those posts in figure 3. The arrows in figure 3A accurately indicate the relative amount and the direction of the cell traction force. Apparently, the traction forces around the center region of the cell are small, but they are large near the cell edge. It was also observed that the cell tends to contract toward its physical center to balance itself in order to grow well on top of the micropost array. In future experiment, the functionality of integrin-linked kinase (ILK), a serine/threonine protein kinase involved in VSM cell proliferation and migration will be studied by using high aspect ratio micropost array. Two types of cells (typical VSM cell and VSM cell absent of ILK molecules) will be grown on the micropost array with sufficient sensitivity. The effect of ILK molecule on cell’s mechano-transduction can be studied by comparing change of traction forces of those different VSM cells.

4 CONCLUSION

In conclusion, we have successfully fabricated high aspect ratio PDMS micropost array and used it to measure the traction force generated by VSM cells. Micropost deflections were detected by optical microscopy images and equivalent forces were calculated by applying experimentally calibrated Young’s modulus of PDMS and post geometry to classic beam bending theory. It was found that the smallest detectable force depends on the geometry of the micropost and the accuracy of the phase contrast image processing.

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REFERENCES


