

MEASUREMENT OF CELL FORCES USING A POLYMER MEMS SENSOR

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Abstract: Cellular mechanics are responsible for execution and regulation of a number of cell functions. Mechanical forces generated within the cytoskeleton are transmitted via transmembrane linkages to the underlying substrate. Measurement of these forces could lead to a wealth of additional information about the role of cell mechanics in regulating cell function and signal transduction. Here we describe the design, fabrication, and testing of a polystyrene cantilever beam array for measuring forces generated by WS1 human skin fibroblasts. Finite element analysis was used to guide the design of a compound cantilever beam. Sensors were fabricated from polystyrene to provide a well-studied and biocompatible surface for cell attachment. Soft lithography based techniques were used for microfabrication of the sensors. Cells were placed on four and eight probe cantilever sensors and deflection of the probes was measured optically during attachment and spreading of the cells. The device was successfully used to measure time varying mechanical forces generated by fibroblast cells.

1 INTRODUCTION

Mechanical forces generated by adherent cells play an important role in execution and/or regulation of a host of cellular processes. When anchorage dependent cells attach to a surface, forces generated in the cytoskeleton are transmitted to the underlying substrate via transmembrane protein linkages. These mechanical forces are involved in controlling cell functions including adhesion, morphology, and motility (Galbraith and Sheetz, 1998; Chicurel et al., 1998) as well as apoptosis (Chen et al., 1997) and wound healing (Wrobel et al., 2002) among others. Measurement of mechanical forces generated by adherent cells could provide additional insight into the basic role of cell mechanics in regulating cell function. In addition, monitoring time dependent cell mechanics could lead to new routes of cell-based sensing focused on mechanical changes in the cell brought about by externally applied chemical or mechanical stimuli.

Several devices have been utilized for observing and measuring cellular forces. Some of the first approaches involved growing cells on deformable elastic substrates, which wrinkled in response to mechanical forces (Harris et al., 1980; Beningno and

Wang, 2002). More recently, microfabrication techniques have been used to fabricate force measurement devices. This is an attractive approach due to the ability to make precise structures on the same size scale as biological cells. Galbraith and Sheetz (1997) used micromachined silicon cantilevers to measure localized forces generated by fibroblasts. Single cantilevers with one direction of motion were used, thus limiting the ability to measuring forces directed along the axis of the cantilever or determine the direction of the force. Soft lithography based microfabrication techniques (Xia and Whitesides, 1998) have also been used to fabricate devices for measuring cell forces (Tan, et al., 2003). In this case, elastic poly(dimethylsiloxane) (PDMS) pillars acted as vertical cantilevers. Cells were grown on top of the pillars and deflections were measured and used to calculate the force on each pillar.

Our approach to measurement of cell forces involves the use of a polystyrene cantilever array with a compound beam design. The compound beam allows the forces to be measured in all directions, thus allowing calculation of both the force magnitude and direction. The choice of polystyrene as the structural material also has a significant impact on the function of the device. Polystyrene is

a well-characterized biocompatible material that is used ubiquitously in cell culture applications. In addition, it is well known that the stiffness of the substrate can significantly affect the mechanical behaviour of cells (Lo et al., 2000, Choquet et al., 1997). Most devices to date have been fabricated from relatively flexible (silicone rubber) or relatively stiff (silicon) materials. In this case we use a materials with intermediate stiffness.

The device consists of a four or eight probe cantilever array fixed to a glass substrate at the base of the beams. The ends of the beams were designed to provide adequate surface area for cell spreading. The fixed post at the center of the device was included to provide a location for initial cell attachment as well as provide a fixed reference point for probe deflection analysis. As the cell attaches to the beams and exerts forces, the deflection of each cantilever is measured optically over time to give spatially and temporally resolved measurement capabilities.

2 MATERIALS AND METHODS

2.1 Device Fabrication and Characterization

Devices were fabricated using sacrificial layer micromolding as described in (Ferrell et al., 2007). A water-soluble sacrificial layer was first patterned by photolithography and reactive ion etching. A layer of poly(vinyl alcohol) (PVA) was dissolved in water to a final concentration of 10% (wt/wt). The PVA solution was spin coated on 18 mm glass coverslips at 1000 rpm. A protective layer of poly(methyl methacrylate) (PMMA) was then spin coated on top of the PVA. The PMMA layer protected the PVA from the developer in the upcoming photolithography process. Photolithography was then used to pattern an etch mask on the PVA/PMMA films. Reactive ion etching in an O₂ plasma was used to removed both the PVA and PMMA in the unmasked regions. The remaining photoresist and PMMA layers were then removed by sonication in acetone, leaving only the patterned PVA.

A PDMS mold of the device was fabricated by replica molding (Xia and Whitesides, 1998) of a photolithographically patterned master. The PDMS mold was spin coated with a solution of polystyrene in anisole (7.5% wt/wt). The polystyrene was removed from the raised portions of the mold by

contact with a glass slide heated to 200 °C. The remaining polystyrene was left in the recessed portion of the mold. The mold was aligned with the sacrificial layer and heat (120 °C) and pressure (75 psi) were used to transfer the device onto the sacrificial layer. The device was then annealed at 115 °C for 15 minutes to improve adhesion of the anchor regions and remove any residual stress in the beams.

The thickness of each device was characterized using a stylus profilometer. The thickness range for the above processing parameters was 1.31-1.75 µm.

2.2 Design and Simulations

Finite element simulations (ANSYS) were used to guide the design of the cantilever beam. The beam was designed to give reasonable x,y deflection response while still conforming to the geometrical constraints of the devices circular configuration. The deflection plot for a 5 nN force applied to an area at the end of the cantilever beam at 10° increments from 0° to 360° is shown in Figure 1.

An ideal beam response would be a circular deflection profile with no offset between the direction of the beam deflection and the force direction. The plot shows a slight offset. The plot also shows that the beam is stiffer in the 90° and 270° directions compared to the 0° and 180° directions. This leads to slightly less sensitivity to forces in those general directions, but the overall response of the beam is adequate for the application described here.

2.3 Cell Culture and Image Acquisition

The cells used in this study were WS1 human skin fibroblasts (ATCC). Cells were cultured in Minimum Essential Medium, Eagle (ATCC) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were cultured at 37°C in a 5% CO₂ atmosphere. To obtain cells for measurements, cells were detached from T75 tissue culture flasks using .25% trypsin-EDTA.

Prior to performing measurements, the devices were modified by a brief exposure to O₂ plasma in a reactive ion etcher to make the surface more hydrophilic and improve cell attachment. Devices were fixed to a PDMS coated petri dish. The PDMS coated dish allowed fixation of the device without the use of a chemical adhesive. The devices were placed in cell culture medium to dissolve the sacrificial layer. After complete dissolution of the

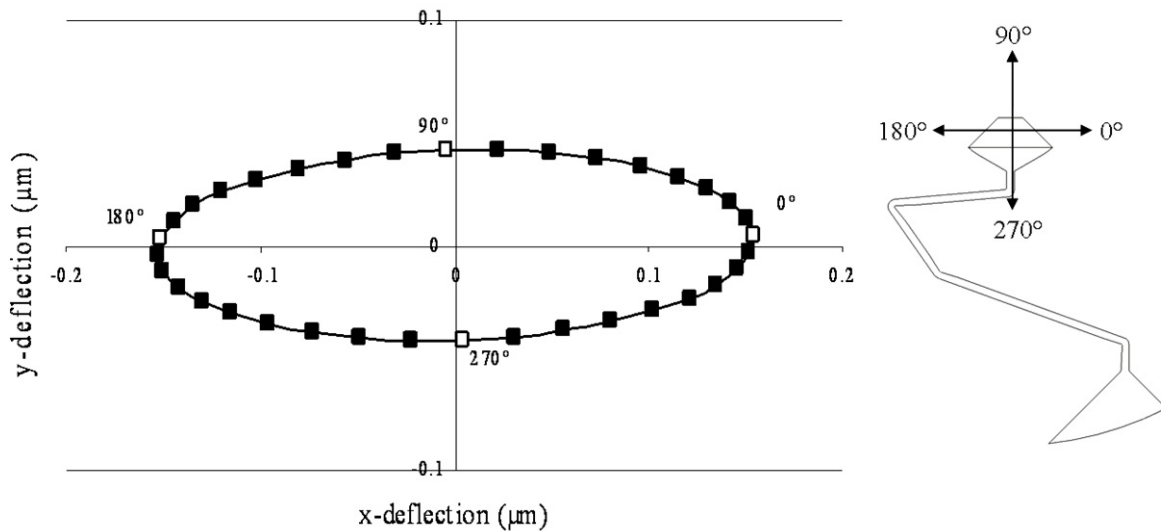


Figure 1: Deflection plot for the compound cantilever beam with a 5 nN applied load.

PVA layer, the medium was aspirated and fresh medium was added and aspirated three times to remove the majority of the dissolved PVA. 20 ml of fresh medium without cells was added to the petri dish. A few drops of cell suspension were then added to the dish. This provided a low cell density and minimized the likelihood of having multiple cells on a single device.

Measurements were performed on an inverted microscope (Nikon TS100) with a custom stage incubator. The incubator consisted of an acrylic enclosure with the temperature regulated at 37°C and supplied with 5% CO₂. A manual micromanipulator (World Precision Instruments) with a 2 μm inner diameter glass micropipette was used to position a single cell on the center region of sensor. Cells were moved onto the device with the microscopy in phase contrast mode to allow better visualization. A 6.6 megapixel CCD camera (Pixelink) was set to capture images at 30 second intervals for the duration of the experiment. For analysis, the phase contrast filter was removed and brightfield images were captured to facilitate easier edge detection.

2.4 Image Analysis and Force Calculation

Images were analysed using NIH Image J software (download available at <http://rsb.info.nih.gov/ij/>). The x,y position of the end of the probe as well as a fixed point on the device were determined prior to cell attachment. The x,y displacement of the end of each the cantilever was then monitored over time.

The x,y position of the fixed point was also monitored to determine and correct for image shift. After determining the magnitude and direction of the cantilever deformation, the force magnitude and direction were calculated based on the finite element simulations.

3 RESULTS AND DISCUSSION

Scanning electron micrographs of the four probe sensor prior to removal of the sacrificial layer are shown in Figure 2. Figure 2(a) shows the entire device with the anchor region at the outer perimeter of the device. The close-up of the center of the device shows the four adhesion pads as well as the fixed post.

The force versus time plots for two different experiments are shown in Figure 3 (a,c). The plots show the force magnitude for each of the four probes. Figure 3(a) shows that force is exerted on each of the four probes. The graph also indicates that the cell adhered to the sensor relatively quickly after being placed on the device. Figure 3(c) shows that force is only exerted on three of the four probes and the magnitude of the force is significantly higher for probes 1 and 3 compared to probe 4. This is likely due at least in part to a smaller adhesion area on probe 4 as compared to probes 1 and 3. This could be a result of off center cell attachment and spreading. In addition, Figure 3(c) shows that there is a period of time prior to cell attachment with no force generation. The plot clearly shows the onset of

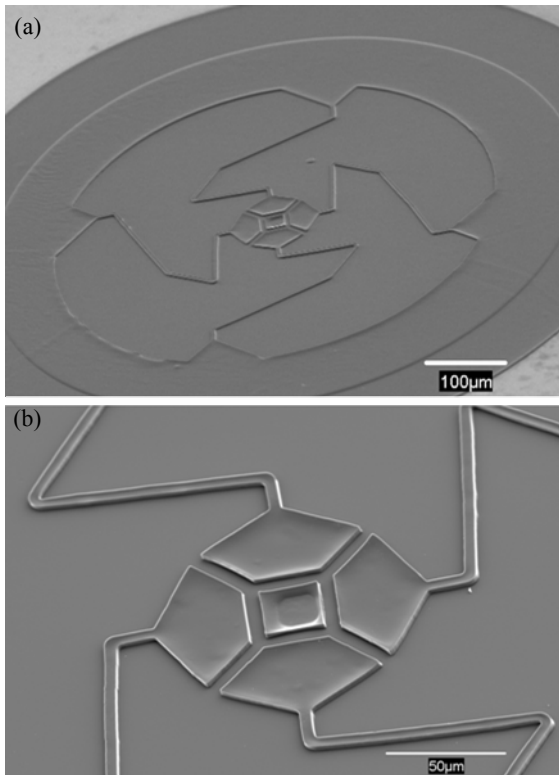


Figure 2: SEM micrographs of the cell force sensor.

cell attachment and force generation for each of the probes. Probe 2 showed no force/deflection response attributed to cell mechanics. The data is included to

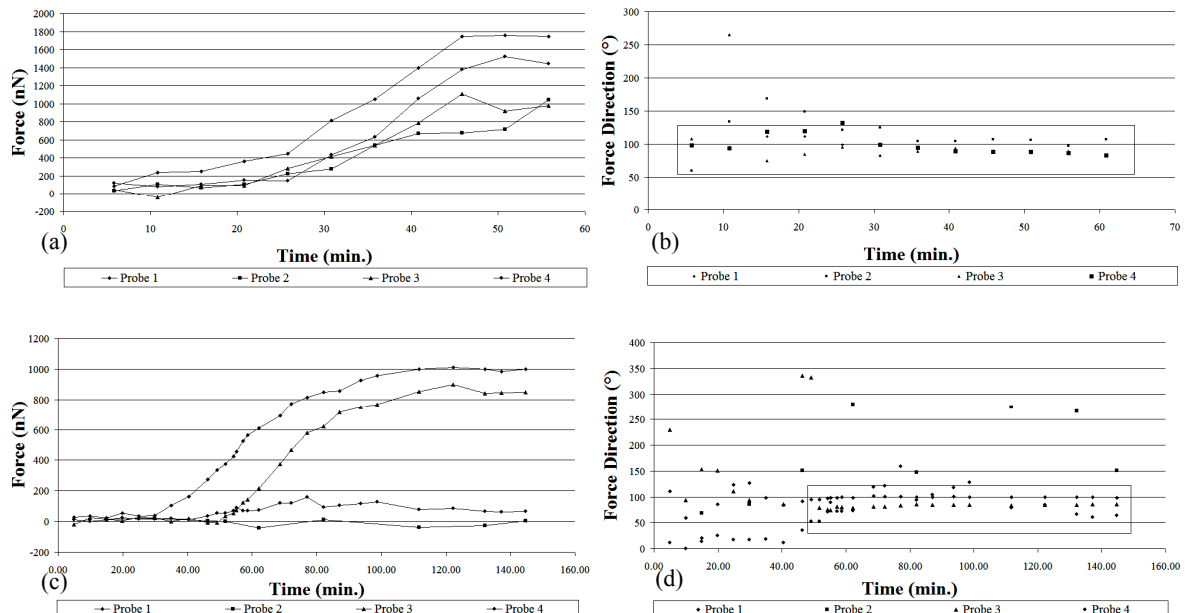


Figure 3: (a,c) Force magnitude versus time for two separate experiments. (b,d) Force direction corresponding to the forces in (a,c). The boxes highlight that the majority of the forces are oriented in the direction toward the center of the structure.

show the noise in the measurement and analysis system.

The angle of the force vectors are shown in Figure 3(b,d). The boxes highlight that most of the forces are oriented around 90° or toward the center of the devices. This is expected given the nature of the forces. In figure 3(d) the random orientation of the angle prior cell attachment and for probe 2 are due to noise.

Figure 4 shows optical phase contrast micrographs of the cantilevers corresponding to the force and direction plots in Figure 3 (a,c). The force vectors for each probe are overlaid on the images. The images show the changes in the both the magnitude and direction of the deformation at 0, 30, 37.5, and 50 minutes.

4 CONCLUSIONS

A novel force sensor was designed and fabricated for measurement of mechanical forces generated by fibroblast cells. The sensor was designed with the aid of finite element simulations of the sensor behavior. The device was fabricated from polystyrene using a soft lithography based fabrication procedure. Force magnitudes and directions were measured using WS1 skin fibroblast and show the ability to measure variation in the cell mechanics over time.

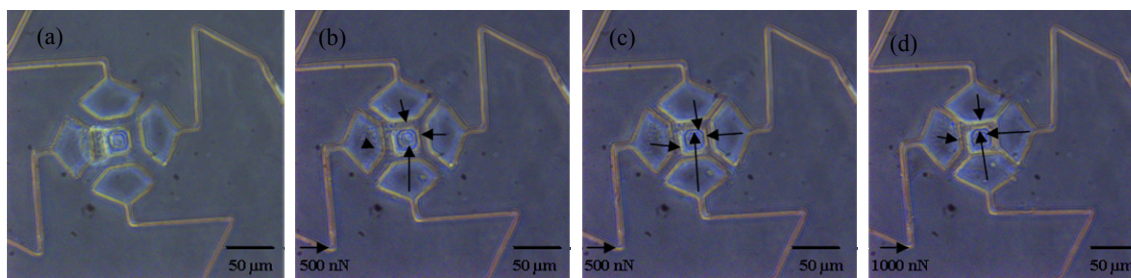


Figure 4: Phase contract micrographs with vector force overlays at (a) 0 min. (b) 30 min. (c) 37.5 min. and (d) 50 min. Note the difference in the force vector scale in (d).

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