A new approach for measuring protrusive forces in cells

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Mechanical factors, such as force, rigidity and geometry, play a crucial role in determining the function and behavior of cells. A change in the force exerted by a cell on a surface could be an indication of disease. For example, it has been reported that cancer cells exhibit larger protrusive forces than normal cells by ~ 20% – 50 %.[1] Such forces are the result of actin polymerization at the leading edge of the cell. We present here a simple and novel way to measure cellular protrusive forces.

Protrusive forces in keratocytes have previously been measured using different approaches. Bohnet et al. used fluid flow from a micropipette to estimate the protrusive force of a moving fish keratocyte.[2] Prass et al. used a soft SiN AFM (atomic force microscopy) cantilever in the path of a migrating fish keratocyte.[3] We have developed a polydimethylsiloxane (PDMS)-based device for measurement of the protrusive forces in NIH 3T3 fibroblasts. The advantage of this approach is that multiple sensors can be integrated into a single device. Figure 1 shows a schematic of the device, which consists of alternating pads and hexagonal arrays of pillars, with the pillar height slightly (~150 – 300 nm) above the plane of the pads. The reason for this is the size of the lamellapodia where actin polymerization occurs is in the range of 200 nm. When cells are plated on the pads and begin spreading. A cell spreading on the edge of a pad encounters the arrays of pillars and bends them. The protrusive can thus be calculated from the deflection of the pillar using simple mechanical considerations.

The device fabrications process is shown in Fig. 2. Molds for casting PDMS were prepared using photolithography and reactive ion etching. Fabrication was a two-step process in which the holes were first patterned in oxide. Using the oxide as a mask, the Si was given a shallow etch. The next step consisted of patterning the pads in oxide, for which an aligned photolithography step was performed, and the pads were etched using an oxide mask. Once this was done the whole area (including the pads and array of holes) was etched together (Fig. 3). The key challenge in the fabrication was in simultaneously controlling the etch depth of the large area pads (250 μm) and the small holes (diameter = 1 μm).

Cells were seeded on PDMS coated with fibronectin. Figure 4 shows the bending of the pillars in pixels and frames captured from a time-lapse movie. Protrusive forces, calculated using simple cantilever theory, were found to be in the range of 1.5 – 2 nN. Correct quantification of protrusive forces can be potentially used for developing novel cancer therapies and diagnostic tools.

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1. Zhou Li, et.al., *Nano Letters, 9* (10), 3575-3580 (2009**).**
2. Bohnet, S, et.al., Biophys. J. 90:1810–1820 (2006).
3. Prass M, et.al., J Cell Biol. Sep 11;174(6):767-72 (2006).

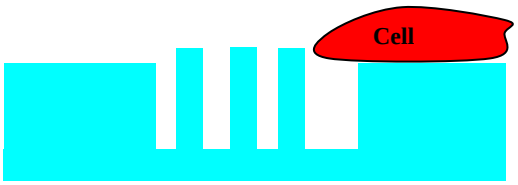

Fig. 1. Schematic of the cell on PDMS device.

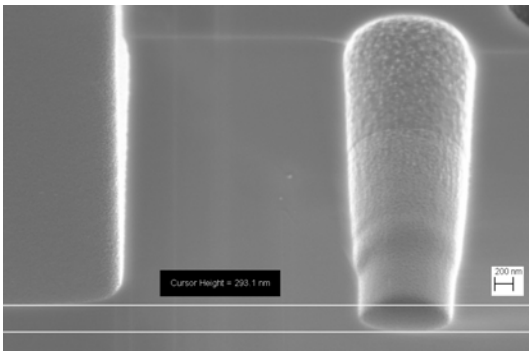

Fig. 3. Cross-sectional view of etched hole and pad in silicon. This is a mold for PDMS. Scale bar 200 nm.

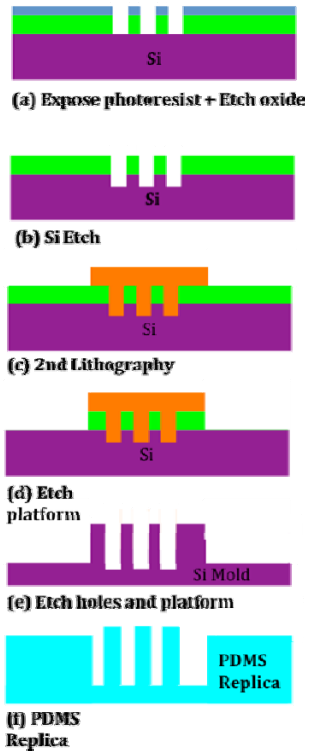

Fig. 2. Schematic of fabrication process.

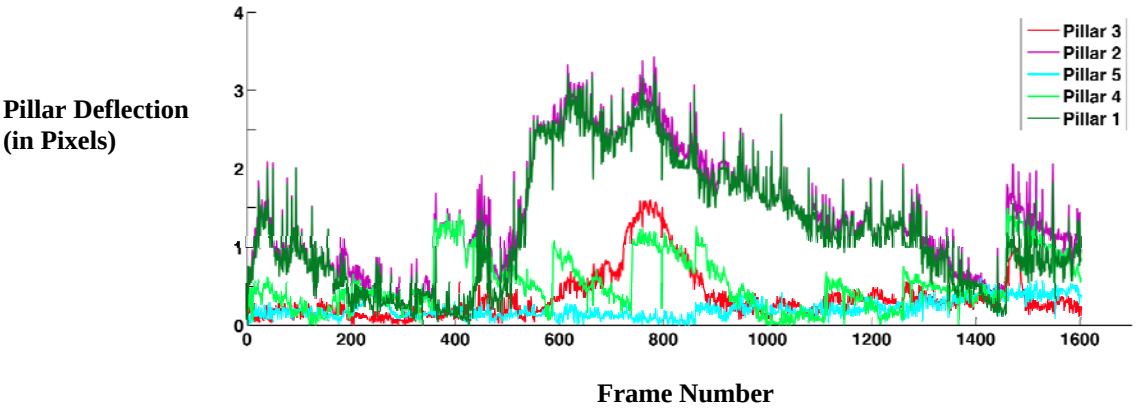

Fig. 4. Plot showing deflection of pillars with frame rate. Each frame was captured after an interval of 3 second. Different colors represent different pillars. Cyan pillar is control – no bending was observed on this pillar.