Fabrication of Elastometer Pillar Arrays with Modulated Stiffness for Cellular Force Measurements

S. Ghassemi[1], N. Biais[2], K. Maniura[4], S. J. Wind[3], M. P. Sheetz[2] and J. Hone[1]

[1]Department of Mechanical Engineering, [2]Department of Biological Science,
[3]Department of Applied Physics and Applied Mathematics
Columbia University, New York, NY 10027
[4]EMPA, Switzerland

Cells generate traction forces in the nN range during adhesion and migration [1]. In order to quantify the mechanical interaction of cells with their environment, the traction forces between a cell and its underlying substrate need to be measured. To this end, we use a high density microfabricated array of elastometric pillars which provides direct measurement of the traction forces. The forces are deduced from the measurement of the mechanical deflection of these pillars by cells as they spread on the surface. Previous studies have demonstrated the importance of substrate rigidity in numerous cellular processes (ranging from migration to adhesion as well as mesenchymal stem cell differentiation) [2,3,4].

In this work, we focus on the effect on cell behavior of a boundary between two different rigidity substrates. In order to properly study the influence of a sudden change in rigidity, pillars with different stiffness but the same contact surface area need to be designed on a single substrate. To achieve this goal, we sought to fabricate PDMS pillar arrays in which the pillar diameter and top surface remains constant, but the pillar height, and therefore the stiffness, changes abruptly (Fig. 1d). Molds for these arrays consist of etched silicon substrates, as shown in Fig. 2. Figure 1 shows the process used to fabricate these molds.

The fabrication process begins with the growth of a 950 nm-thick thermal oxide. The pillar pattern is formed in a positive photoresist by photolithography. The resist is treated with a long post development bake in order to smooth the sidewalls, followed by an O_2 plasma descum step. The oxide layer is etched, using the resist as a mask, in a fluorine-based RIE system. The silicon is then etched to the desired depth in a Cl_2-based ICP-RIE system using the oxide as a hard mask. After removal of the oxide mask, SiO_2 is deposited conformally over the entire wafer. The surface of wafer is then planarized by chem-mech polishing. A second layer of photoresist is applied, and half of the originally patterned area is exposed. After development, the Si is etched to the desired step height using a Bosch process. The remaining oxide is removed in a buffered oxide etchant (BOE), leaving a multi-height mold (Fig. 2). This mold is used to form arrays of pillars in poly-dimethyl siloxane (PDMS) having two different heights (Fig. 3), and therefore different mechanical response. Figure 3 shows the behavior of a cell as is crosses over a boundary separating regions of different rigidity. This work helps us understand the role of rigidity in the response of cells to external physical factors.

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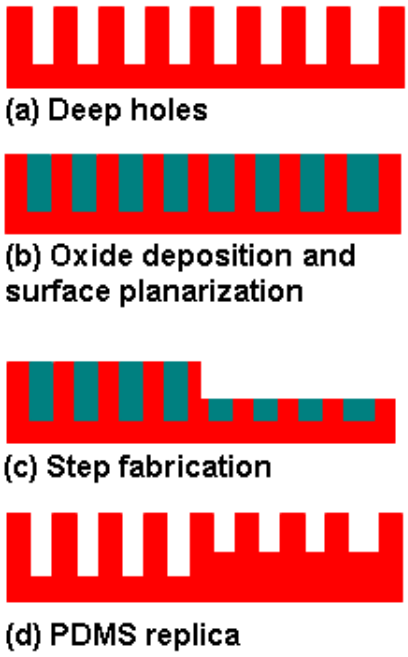

Fig. 1. Schematic drawing of the fabrication of PDMS pillars

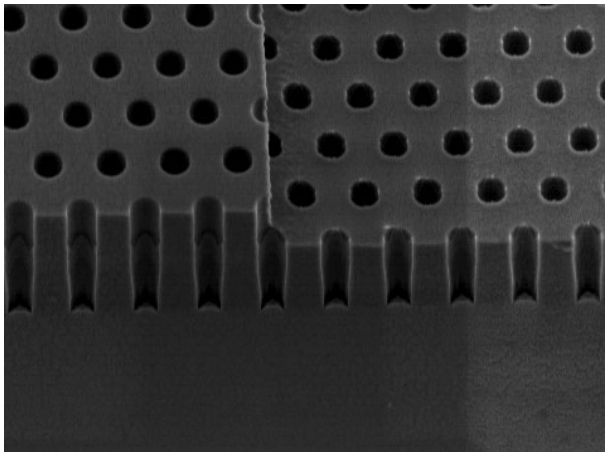

Fig. 2. SEM image of double height holes in silicon substrate.

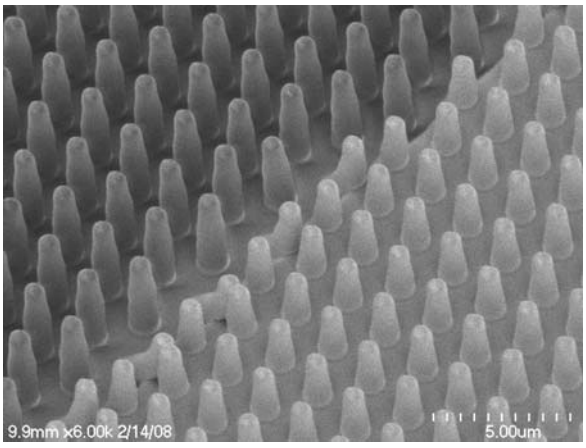

Fig. 3. SEM image of double height PDMS pillars.

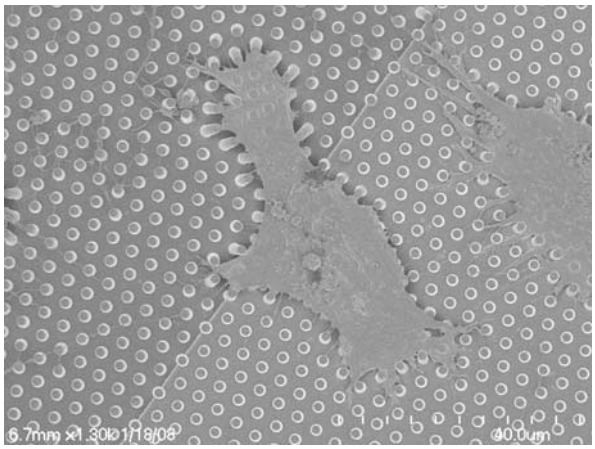

Fig. 4. SEM image of a cell attached to an array of pillars with different height. The cell spreads differently in the regions with different rigidity.