Gold-Tipped Elastomeric Pillars for Celllar Mechanotransduction

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The physical properties of the cellular environment are key factors in determining cell function and behavior.[1] Previous studies have shown that the topography and physical properties of the surrounding matrix have a significant influence on cell response.[2-4] In order to study the mechanical interaction of cells with their environment, we culture cells on substrates with different topographical surfaces using a variety of geometries and topographies consisting of microfabricated arrays of elastometric pillars.

One concern regarding the use of such structures is that a cell's mechanical response may differ depending on whether it is in contact with the tops of the pillars or the sides (or bottoms) because of different mechanical signals (e.g., rigidity, geometry, force response) from these features. We have therefore devised a way to restrict cell spreading exclusively to the tops of the pillars by selective chemical functionalization of the top surface. Cells will therefore adhere to and spread only on the tops of the pillars and will not be influenced by the sides or bottoms. To this end, we coat the tops of the pillars with a thin Au film which can then be functionalized by a thiolated biomolecule, such as extracellular matrix proteins or protein fragments, while the rest of the sample features are passivated with a non-adherent molecule.

The fabrication process is shown schematically in Fig. 1. A rigid template is created for elastomer molding. The template is formed from a Si substrate with a 950 nm-thick thermal oxide. Optical lithography is used to form an array of micron-scale holes in resist. After developing, the resist is treated with a long post exposure bake in order to smooth the sidewalls, followed by descumming in an O_2 plasma. The oxide layer is reactive ion etched using a fluorine-based etch chemistry. The oxide layer is used as a hard mask for Cl_2 etching of the underlying Si to the desired depth (which ultimately determines the height of the elastomer pillars). The oxide is then stripped in BOE, leaving the Si template shown in Fig. 2. The template is cleaned in piranha solution and an O_2 plasma, followed silanization with vapor phase tridecafluoro-trichlorosilane. Cr is deposited at a 30 ˚angle by thermal evaporation. This shadow evaporation results in Cr deposition on the top surface and sidewalls of the etched holes but not on the bottoms, as in Fig. 1b. A 20 nm layer of Au plus 5 nm of Ti is then deposited normal to the template surface. Removal of the Cr sacrificial layer results in a Si mold with Au+Ti at the bottoms of the etched holes (Fig. 1c). Poly-dimethyl-siloxane (PDMS) is then poured over the mold, cured at 70 °C for 12 h and peeled off in ethanol. The Au-tipped PDMS pillars (Fig. 1d) are then treated with a solution of HS-C18 thiol and incubated with fluorescently labeled fibronectin. Pluronics, a protein-repellent substance, is adsorbed on the remaining regions of the array to passivate those areas against non-specific binding.

Figure 3 shows arrays of functionalized pillars as imaged by confocal microscopy. The fluorescently-labeled fibronectin is seen only at the tops of pillars and not on the sides or bottoms. This new technique enables improved cell spreading assays by (a) restricting cell adhesion to the tops of the pillars and (b) allowing accurate visualization of the pillar tops for more precise measurement of deflection induced by cellular traction forces.

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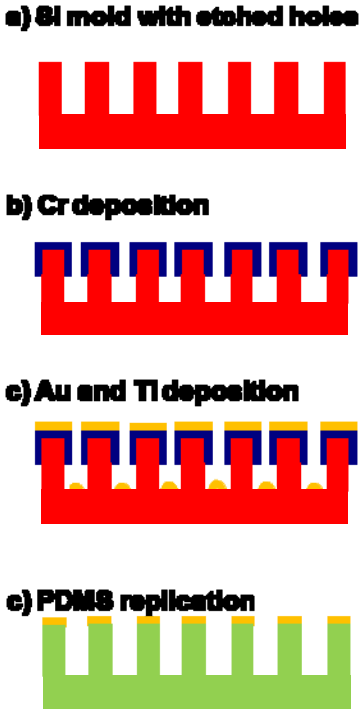

Figure 1. Schematic drawing of the fabrication of PDMS pillars with gold layer on the top.

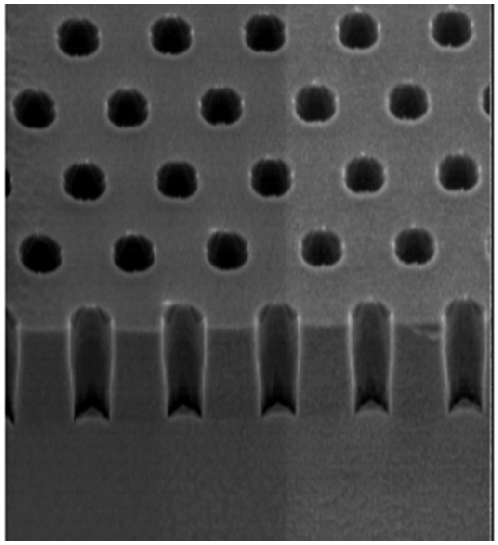

Figure 2. SEM image of holes in silicon substrate.

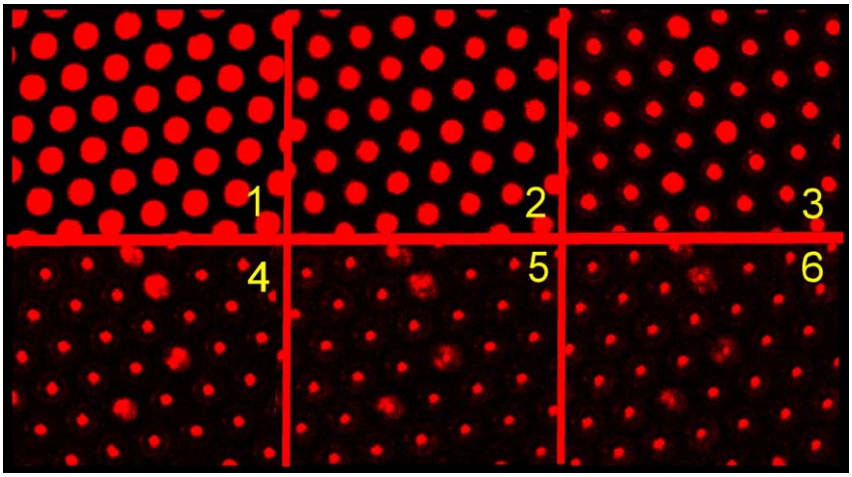

Figure 3. Confocal microscopy images of fluorescently labeled fibronectin on arrays of PDMS pillars (d = 5 μm; pitch = 10 μm). The reduced fluorescence seen for the different heights indicates that the fibronectin is only on the top of the pillars.