Published in final edited form as:

J Vac Sci Technol B Microelectron Nanometer Struct Process Meas Phenom. 2009 November 1; 27(6): 3088–3091. doi:10.1116/1.3259953.

Gold-Tipped Elastomeric Pillars for Cellular Mechanotransduction

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Abstract

We describe a technique for the fabrication of arrays of elastomeric pillars whose top surfaces are treated with selective chemical functionalization to promote cellular adhesion in cellular force transduction experiments. The technique involves the creation of a rigid mold consisting of arrays of circular holes into which a thin layer of Au is deposited while the top surface of the mold and the sidewalls of the holes are protected by a sacrificial layer of Cr. When an elastomer is formed in the mold, the Au adheres to the tops of the molded pillars. This can then be selectively functionalized with a protein that induces cell adhesion, while the rest of the surface is treated with a repellent substance. An additional benefit is that the tops of the pillars can be fluorescently labeled for improved accuracy in force transduction measurements.

Introduction

The physical properties and topography of the cellular environment are key factors in determining cellular function and behavior.^{1–4} In order to study the mechanical interaction of cells with their environment, we culture cells on microfabricated arrays of elastomeric pillars. The pillar geometry and material can be easily modified to control the bending stiffness and hence the mechanical properties of the cellular environment. At the same time, the pillars can be used as independent force sensors: in the linear bending regime, the force exerted by the cell on each pillar can be determined by measuring its lateral deflection.^{4–8} Figure 1a shows a scanning electron micrograph of a uniform array of elastomeric pillars, and Fig. 1b shows an optical micrograph of a fibroblast cell on this array.

For many such experiments, it is advantageous to selectively functionalize the tops of the pillars for two purposes. First, application of proteins that promote cellular adhesion (such as fibronectin) encourages cell spreading and help to restrict the adhesion points to the tops of the pillars, making the force measurements much more straightforward to interpret. Second, fluorescent labeling of the tops of the pillars can facilitate detection of the pillar displacements. The most common technique for selective functionalization of the pillar tops is microcontact printing, i.e., stamping.^{5–7} However, this technique can only be used for relatively rigid pillars; flexible pillars tend to adhere to one another or collapse as a result of the stamping process.

We have developed an alternative method for selective chemical functionalization of the top surface of elastomer pillars. We coat the tops of the pillars with a thin gold film that can then be functionalized using thiol chemistry, facilitating both cellular adhesion and fluorescence. This functionalization can be performed while the sample is kept in liquid to prevent adhesion

of the pillars to one another. In addition, the rest of the surface (i.e., the sidewalls of the pillars and the interstitial spaces) can then be functionalized with an anti-adhesion coating to further discourage spreading on these surfaces. In this work, we describe fabrication and initial testing of substrates with arrays of gold-tipped pillars.

Fabrication Process

A. Fabrication of Arrays of Deep Holes

The central part of the work described below involves fabrication of a Si structure, which is used as a mold for the fabrication of the gold-tipped elastomer pillars. The mold is made by first etching a uniform array of holes in a Si wafer. A silicone elastomer is poured into the mold to yield the reverse structure, namely an array of micron-scale pillars whose mechanical properties are a function of their dimensions and the Young's modulus of the elastomer material. The latter can be tuned by adjusting the degree of crosslinking of the elastomer. The new feature introduced in this work is the deposition of a thin layer of Au at the bottom of the etched holes in the Si mold prior to the curing of the elastomer. When the elastomer is thermally crosslinked, the Au adheres to the tops of the pillars, yielding into an array of gold-tipped elastomeric pillars (Fig. 2i).

The fabrication process is illustrated in Fig. 2. Uniform array of holes in silicon wafer was made by photolithography as described previously.⁹ Briefly, RCA-cleaned Si wafers were oxidized at 1100 °C for 2 h to form a 950 nm-thick SiO₂ film. The wafers were coated with 1.2 μm-thick photoresist (Shipley SPR 7000) using HMDS as an adhesion promoter. The resist was then soft-baked on a hot plate at 90 °C for 60 sec., followed by another bake at 115 °C for 60 sec. to remove residual solvent and mechanical stress in the film. The hexagonal array of holes was replicated in positive photoresist by UV photolithography (Fig. 2b). After developing, it was treated with a post-development bake at 90 °C for one hour in order to smooth the sidewalls then descummed in O₂ plasma for one minute. The oxide layer was etched, using the resist as a mask, in fluorine based system (Fig. 2c). The Si holes were etched to the desired depth in a Cl-based ICPRIE system using the SiO₂ as a hard mask (Fig. 2d–e). The resulting wafer was then immersed in BOE, leaving the Si mold shown in Fig. 2e.

The mold was then cleaned in piranha solution for 6h at room temperature, followed by a one minute O₂ plasma clean and overnight silanization in vapor phase tridecafluoro-trichlorosilane in vacuum (100 μL in a glass vial inside a vacuum jar). This facilitates the subsequent release of elastomer and gold from the wafer after curing.

Following formation of the Si mold structure, a layer of Cr was deposited onto the mold at a 30° angle, using a rotary sample holder in an electron beam evaporator. Rotating of the substrate is necessary for achieving a homogeneous deposition of Cr around the holes. This shadow evaporation results in Cr deposition on the top surface and upper portion of the sidewalls of the etched holes but not on the bottoms, as in Fig. 2f. A 20 nm layer of Au followed by 5 nm of Ti was then deposited normal to the mold surface, using electron beam evaporation. Because the sidewalls are not perfectly vertical, the thin layer of Cr on the top lip of each hole is used to prevent deposition of the Au/Ti onto the sidewalls; the thickness of the layer was adjusted depending on the depth of the hole. Removal of the Cr sacrificial layer results in a Si mold with Au+Ti at the bottoms of the etched holes (Fig. 2g). Figure 3b shows the final Si mold with a thin layer of Au and Ti on the bottom of the holes.

The Si structures were used as molds for fabrication of gold tipped pillar arrays using a liquid silicone prepolymer, Poly(dimethylsiloxane) (Dow Corning Sylgard). The PDMS was mixed with its curing agent, poured over the mold and cured at 70 °C for 12 hours, in order to achieve

a Young's modulus of 2 ± 0.1 MPa. The PDMS was then peeled off in ethanol. The silane treatment previously applied to the Si allows both the PDMS and the gold to be removed easily. In addition, the titanium on top of the gold acts promotes adhesion of the metal to the PDMS. Therefore, the gold at the bottom of each hole is removed by the PDMS, resulting in gold-tipped pillars (Fig. 2i). Figure 4 shows a scanning electron micrograph of the PDMS pillars with the gold on their tips.

Using the process described above, samples were fabricated with a range of pillar diameters and pitches as well as different heights. The diameter of the holes varied from 1 to 5 μm to achieve different pillar stiffness range of the substrate.

B. Surface Chemistry

Having gold on the tips of the pillars allows the use of thiol-based chemistry, which permits a quasi-covalent bond between a variety of molecules (e.g. fibronectin) and the gold-coated pillars.¹⁰ Long chain alkanethiol, $\text{HS}(\text{CH}_2)_n\text{X}$, adsorb from solution onto gold surfaces and form a densely packed monolayer. The specific interaction of the sulfur atom in the thiol binds strongly to the gold substrate, while a wide range of chemical functionalities can be designed into the other end of alkanethiol molecule by attaching different functional end-groups. In this work, we control the chemical functionality of the surface using a methyl group (CH_3) tail. This group confers hydrophobicity to the substrate, which is used to adsorb fibronectin on the substrate. To this end, the Au-tipped PDMS pillar substrates were immersed in 2 mM ethanolic solution of HS-C18 thiol overnight at room temperature. The substrates were washed extensively with ethanol followed by PBS (Phosphate Buffered Saline) washes. Substrates were incubated in a solution of 0.1% Pluronic F127, a block copolymer of ethylene oxide and propylene oxide, in PBS (2h at room temperature) to passivate the rest of the areas against fibronectin and non-specific protein binding. Under these conditions, we obtained an array of gold-tipped PDMS pillars offering a surface prone to adsorb fibronectin on the top of the pillars, while the remaining areas were rendered non-adhesive by the Pluronic treatment. The substrates were immersed in 50 $\mu\text{g}/\text{ml}$ fluorescently labeled fibronectin in PBS for one hour. As a control experiment, we incubated non-functionalized pillars with fluorescently labeled fibronectin under the same conditions.

Results

In order to examine adsorption of fibronectin to the tops of the pillars, immunofluorescence microscopy was used to image the pillars. Figure 5 shows an image of the tops of pillars. The pillar diameters were 5 μm with a center-to-center distance of 10 μm . To determine whether the fluorescence signal was indeed restricted to the top of the pillars, we used confocal laser scanning microscopy (CLSM). Figure 6 shows confocal microscopy images of the bottom of the pillars in a functionalized substrate as well as a control sample which were not treated with the gold at the tops of the pillars. The fluorescence signal in Fig. 6a is seen to decrease as the focus is moved downward along the length of the pillar, indicating that the signal originates at the top of pillars. Rings of fluorescence signal in control experiments were observed along the sidewalls of pillars, indicating the complete coating of the sidewall. These images confirm that the fibronectin coating was restricted to the tops of the gold-coated pillars.

Conclusions

We have described a fabrication process of elastomeric pillar substrates with a thin layer of gold on the top of the pillars. The Au-tipped PDMS pillar substrates have the advantage that the chemistry of the top of the pillars can be specifically controlled by selective chemical functionalization. This approach facilitates cell spreading exclusively on the top of the force

sensing pillars. Fluorescent labeling improves detection of the tops of the pillars, enabling and precise monitoring of cellular force generation during motile processes.

Acknowledgments

The authors would like to thank Dr. Katharina Maniura, of EMPA, Switzerland and Dr. Otte Homan, of ETH Zurich, Switzerland for their valuable help in performing the experimental procedures. This publication and the project described were supported by the National Institutes of Health through the NIH Roadmap for Medical Research (PN2 EY016586). Partial support was provided by the Nanoscale Science and Engineering Initiative of the National Science Foundation under NSF Award No. CHE-0641523 and by the New York State Office of Science, Technology, and Academic Research (NYSTAR). This work was performed in part at the Cornell NanoScale Facility, a member of the National Nanotechnology Infrastructure Network, which is supported by the National Science Foundation (Grant ECS-0335765).

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Figure 1.

(a) Scanning electron micrograph of PDMS posts. The diameter of the posts is 1 μm and the pitch is 2 μm . The height of the posts is 6.6 μm . The stiffness of the post is about AAA. (b) Bright-field micrograph of spreading of RPTP cells on pillar substrate.

Figure 2. Schematic of the process flow for fabricating arrays of PDMS posts with functionalized Au on the top surface.

Figure 3.

(a) SEM image of holes in silicon substrate. The diameter of the holes is 1 μm and the pitch size is 2 μm . The height of the hole is 6.6 μm . (b) SEM image of final Si mold with a thin layer of Au and Ti on the bottom of the holes.

Figure 4. Scanning electron micrograph of Au-tipped PDMS pillars. The tops of the pillars can be selectively chemical functionalized.

Figure 5.
Epifluorescence image of the tip of the functionalized pillars with diameter of 5 μm and center-to-center distance of 10 μm . The tops of the pillars are coated homogeneously with fluorescently labeled fibronectin and there is no signal from the bottom of the pillars.

Figure 6. Image of the bottom of the pillars using confocal laser scanning microscopy. (a) The fluorescence signal of functionalized pillars decreases as along the length of the pillar, indicating that signal is coming from the top of pillars. (b) Rings of fluorescence signal around the sidewall of pillars in a non functionalized substrate, indicating the complete coating of the sidewall