Sub-micron scale gold-tipped elastomeric pillar arrays for human T cell activation and culture

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Cell growth and differentiation are critically dependent upon matrix rigidity, yet many aspects of the cellular rigidity sensing mechanism are not well understood. By using arrays of elastomer pillars with dimensions extending to the sub-micron scale, where molecular sensing mechanisms are likely to reside, we observed that the cellular response is fundamentally different on micron-scale and sub-micron pillars. We are currently integrating this approach to determine the impact of substrate stiffness on the ex-vivo activation and expansion of human T cells.

In this work, we fabricated sub-micron pillars with varying heights in order to achieve different stiffness. To selectively functionalize the top surface of these pillars, pillar tips were coated with gold (Fig. 1g). A self assembled monolayer of thiol presenting biotin end groups can adsorb to the gold-tipped pillars. Stimulatory ligands can then be immobilized on the tip of the pillars through biotin-streptavidin interaction, providing an interface for T cell adhesion and activation.

The fabrication process is shown schematically in Fig. 1. This process involves the creation of a rigid mold consisting of arrays of sub-micron scale circular holes. The fabrication process starts with patterning the photoresist by DUV photolithography. After developing, the resist was descummed in O_2 plasma. Silicon was then etched to the desired depth in Chlorine-based ICP-RIE system using resist as a mask. The remaining resist was then removed and the wafer was piranha cleaned, as well as plasma cleaned, and coated with FOTS in a molecular vapor deposition system. A layer of Chromium mask was deposited at a 30-degree angle in e-beam evaporator. A 10 nm layer of gold was deposited directly, followed by 3 nm Titanium. Cr sacrificial layer was removed in Cr etchant, resulting in a mold containing a thin layer of gold at the bottom of the holes (Fig. 2). PDMS was poured over silicon substrate, cured at 70 °C for 12 h. As PDMS forms in the mold, Au adheres to the tops of pillars. The gold-topped PDMS pillars were then treated with an ethanolic solution of HS-$(CH_2)_{11}$-$(C_2H_6O_2)_3$-OH and HS-$(CH_2)_{11}$-$(C_2H_6O_2)_3$-Biotin. This results in the formation of self assembeled monolayer of thiolated alkanes presenting biotin end-groups, which can be used for the subsequent immobilization of streptavidin. Pillars can then be selectively functionalized with ligands that activate T cells, while the remainder of the surface is treated with a repellent substance to prevent T cell migration into the spaces between pillars (Fig. 3).

Studying primary human T cell behavior at the nano-scale regime will provide mechanistic insight into T cell activation and enhance our understanding of signaling events in relation to substrate rigidity. The development of sub-micron pillar substrates may also provide a unique platform for creating patterned rigidity that could be useful in T cell culture for adoptive immunotherapy for cancer and Infectious disease.

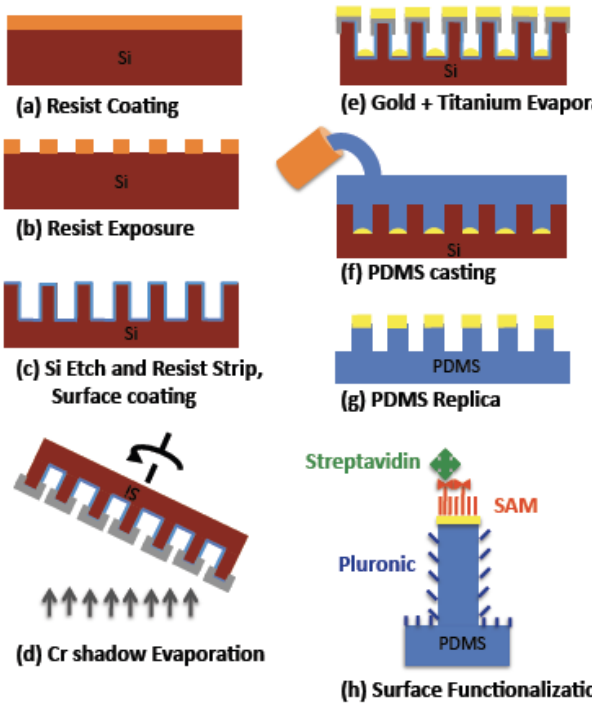

Fig. 1. Schematic drawing of the fabrication of PDMS pillars with gold layer on the top

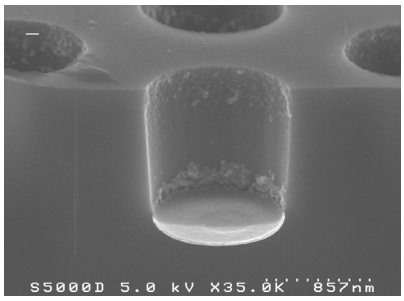

Fig. 2. SEM image of holes containing a thin layer of gold at the bottom of the holes in silicon substrate.

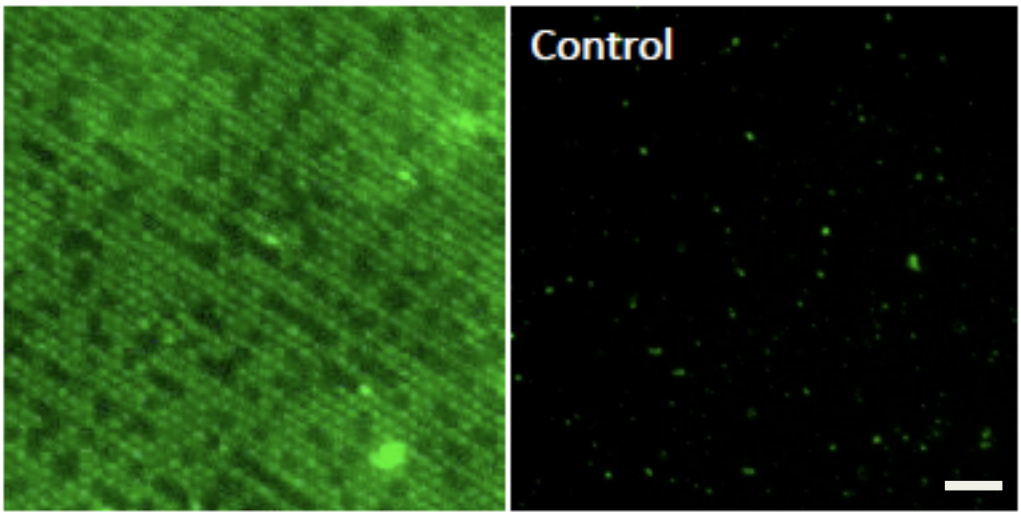

Fig. 3. (a) Image of the tip of pillars with fluorescently labeled Streptavidin with the control experiments missing the biotin link. The diameter of the pillars are 0.5 µm.