Micro and Nano Pillar Assay for T cell Activation

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Mechanical interaction of cells and their immediate environment is one of the basic cellular signaling pathways. Physical forces exerted on a cell can be translated into biochemical signals to influence cell behavior. The study of mechanotransduction in immune cells has been scarce, usually with polymer beads. We demonstrate a multi-well plate with micron and submicron pillars on well bottoms to assay effects of pillar geometry on T cell activation.

Naïve T cells communication with antigen presenting cells is an important event in the immune system response to infections. The cell-cell communication takes place in a temporary binding of various ligands between the cells in the immune synapse (IS)[1]. The spatial organization and mechanical properties of bound receptor/ligand complexes is believed to be important to proper signaling between the cells. Artificial T cell activation is required in certain therapeutic approaches against cancer[2]. Micro beads have been found to be more efficient presenters of signaling molecules than flat substrates. The bead diameter has been found to make a significant difference in efficiency of the activation signal[3]. We have developed pillar multi well plates, which allow us to study efficiency of mechanical signal transduction to T cells by varying pillar size, spacing and length. The extra dimensions could let us mimic *in vivo* synapse *in vintro* and possible allow better control of T cell activation and differentiation.

We tested activation of naïve CD4 T cells after stimulation with αCD3 and ICAM1 coated on polydimethylsiloxane (PDMS) pillar plate. The pillars were molded from etched Si master. To make pillars sufficiently stiff, they were molded from a thin layer of stiff (9 MPa) hPDMS supported by softer (2 MPa) PDMS. The pillars diameter ranged from 1um to 0.25 um. T cells were stimulated on pillar substrates for 6 hours in a growth medium, Figure 1. After stimulation, the cells were stained with fluorescently tagged IL2 antibodies. The stained cells were analyzed with flow cytometry, Figure 2.

We found that pillar diameter and effective rigidity influences IL-2 secretion. Pillars, in general enhanced IL-2 secretion in comparison to flat PDMS. Optimal diameter appears to be 0.75 um. Softer effective rigidity appears to stimulate more than stiffer effective rigidity.

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[1] S.K. Bromley, et al., Annu Rev Immunol 19:375–396, (2001)
[2] J. Sadelain, Practice of Oncology: Recent Advances, 15, 6, (2009).
[3] M. Mescher, J. of Immun., 149, 7, (1992)

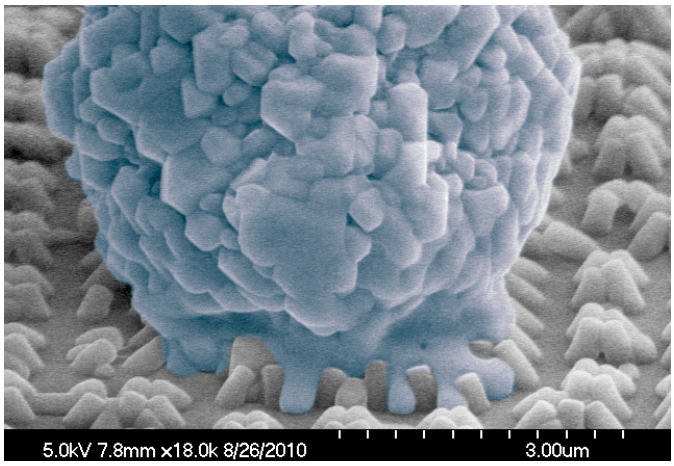

Figure 1: T cell resting on PDMS pillar array. The pillars are 250 nm in diameter, and 700 nm high.

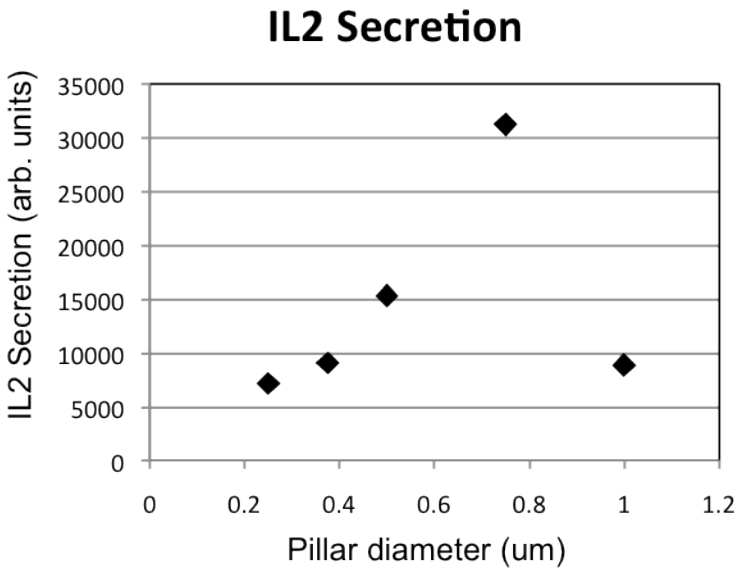

Figure 2: Average fluorescence of phycoerythrin labeled IL2 antibody. At least 2000 cells were scanned for each pillar size. The pillars are 500nm high.