

Screening T-Cell Activation with Nanostructured Substrates

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Mechanical interaction of cells with the extracellular matrix is one of the basic cellular signaling pathways. Physical forces exerted on a cell translate into biochemical signals and alter cell behavior. The study of mechanotransduction in immune cells has been scarce, usually with polymer beads¹. We demonstrate a multi-well plate with micron and submicron pillars on well bottoms to assay effects of pillar geometry on T cell activation.

During a physiological immune response to a pathogen, naïve T-cells are activated via a physical contact with antigen presenting cells (APCs)². Mechanical and chemical signals are continuously exchanged between the cells throughout the immune response. After exposure to the APCs, the T-cells divide and differentiate into various phenotypes to fight the offending pathogen³. Identifying these differentiation pathways is critical to the fundamental understanding of immunology and clinical treatment of cancer and immunodeficiency disorders.

We screened effects of T-cells mechanical environment during the first 3 days of in vitro activation and division. We were able to identify a direct relation between pillar geometry and inhibition of T-cell activation, Figure 1a. Our screening system allowed us to study the time response of mechanotransduction and identify different signaling regimes during early T-cell activation.

The screening assay consists of a standard 96 well plate with nano pillars in the well bottoms. The pillar geometries are varied across different plate columns. The pillars are molded from polydimethylsiloxane (PDMS). The pillar diameter and spacing are varied from 275 to 1000 nm. The plate is used to culture cells in a parameter matrix, Figure 1bc. On one axis we have different nano scale geometry. On the other we vary composition and concentration of signaling molecules. We also perform an 'exposure matrix' where we transferred cells between wells with different signaling molecules and geometry combination to identify the time frame of chemical and mechanical signal transduction.

¹ M. Mescher, *J. of Immun.*, 149, 7, (1992)

² S.K. Bromley, et al., *Annu Rev Immunol* 19:375–396, (2001)

³ J. Sadelain, *Practice of Oncology: Recent Advances*, 15, 6, (2009)

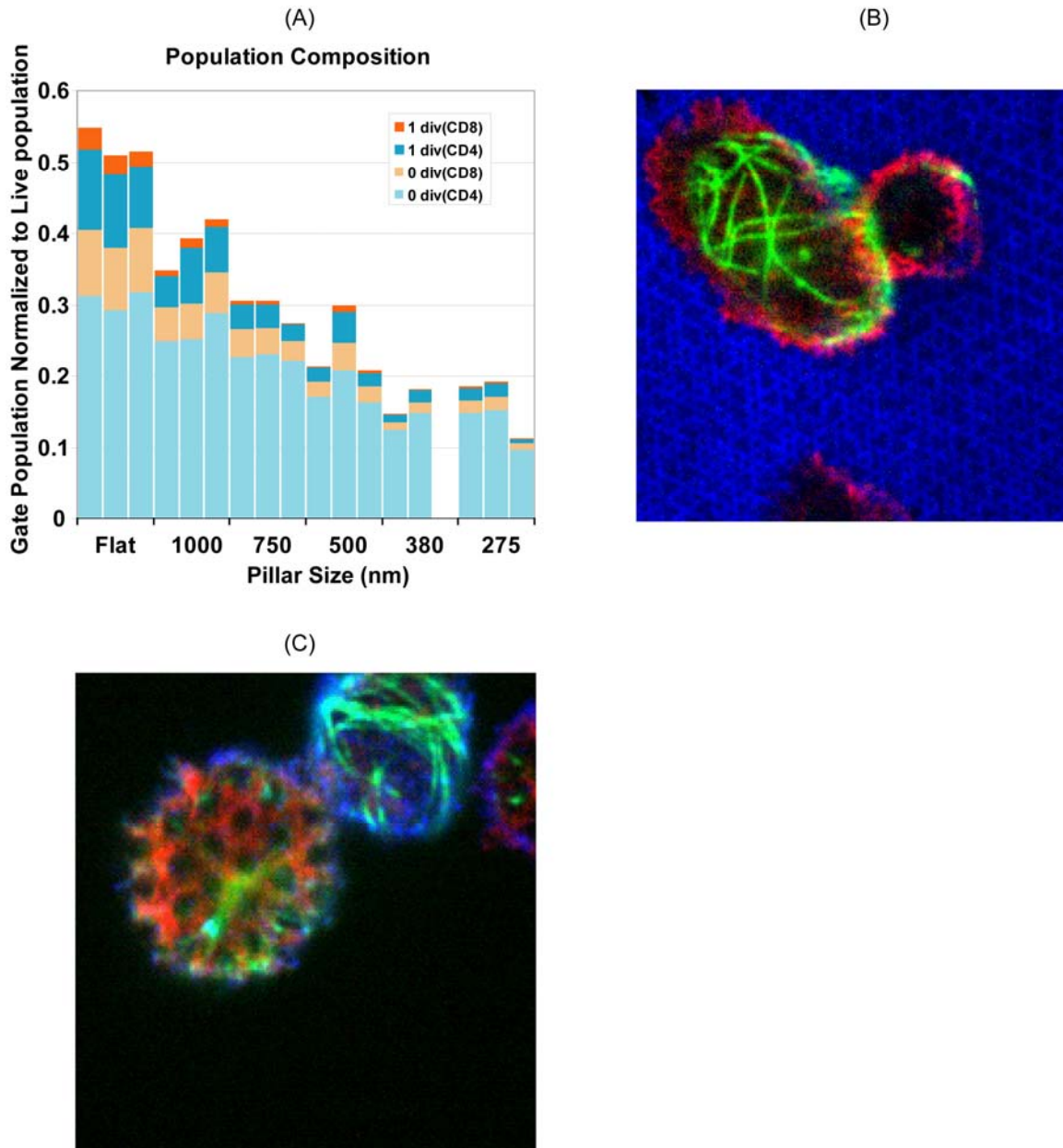


Figure 1 (A) T-Cell subpopulations after 3 days of activation on pillars with different diameter, (pitch=2*diameter). 1 div(CD4)/1 div(CD8), T-cell which have undergone a single division. 0 div(CD4)/ 0 div(CD8), T-cells which have undergone expansion but did not divide. Populations are normalized to all of the living cells detected in flow cytometry. (B),(C) Confocal images of T-cells on 275nm and 1000nm pillars respectively. Staining: Red-Phalloidin, Green-Tubulin, Blue-Mouse antibodies (Signal molecules OKT3, and 9.3 clone).