Probing Immune Cell Response to Heterogeneous Rigidity at the Nanoscale

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Physical factors in the environment of a cell regulate cell function and behavior and are involved in the formation and maintenance of tissue. There is evidence that substrate rigidity plays a key role in determining cell response in culture. Previous studies have demonstrated the importance of rigidity in numerous cellular processes including migration and adhesion. Atypical response to rigidity is also a characteristic of transformed (cancerous) cells. Immune cells have recently been shown to respond differently to surfaces having different rigidity, depending upon phenotype, with memory T-cells preferring softer surfaces, and effector T-cells displaying an affinity for hard surfaces.[1] Understanding the mechanisms that support cellular rigidity sensing can lead to new tissue engineering strategies and potential new therapies.

We have developed a new technique for the creation of biomimetic surfaces comprising regions of heterogeneous rigidity on the micro- and nanoscale. The surfaces are formed by exposing an elastomeric film of polydimethylsiloxane (PDMS) to a focused electron beam to form patterned regions of micro- and nanoscale spots. Finite element analysis of nanoindentation measurements performed on irradiated PDMS films show that in a thin layer near the film surface, where approximately 90% of the electron energy is absorbed, the Young's modulus of the elastomer undergoes an increase as a function of electron beam dose of up to two orders of magnitude (Fig. 1a). Surface chemical analysis indicates a depletion of oxygen in the exposed regions, rendering them more "glass-like," although there was little difference in contact angle between exposed and unexposed regions, and under the proper conditions, protein adhesion is uniform over the entire surface (Fig. 1b). Topographic measurements find shallow depressions (up to ~ 140 nm) with a large radius of curvature (~ 200 μm) in the exposed regions, resulting in a gently undulating surface (Fig. 1c).

$CD4^+$ T-cells plated upon PDMS electron beam-exposed in a pattern of spots with diameters ranging from 2 μm to 0.25 μm displayed different co-localization of the adhesion protein pCAS-L to the exposed features, depending on both rigidity and feature size. On spots with diameters of 2 μm, the pCAS-L appeared to accumulate on the edge; For spots ~ 1.5 μm and below this behavior was lost, and the cells appeared unable to identify the rigid regions (Fig. 2a). Similarly, T-cell polarization was observed on 2 μm-wide lines but not on 1 μm-wide lines (Fig. 2b). Further, Ca^{2+} release, and indicator of immunoresponse, was significantly enhanced by mixed-rigidity patterned PDMS (Fig. 3). These results are suggestive of possible new approaches to adoptive immunotherapy based on rigidity modulation.

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[1] *R. S. O'Connor, X. Hao, K. Shen, K. Bashour, T. Akimova, W. W. Hancock, L. C. Kam, and M. C. Milone, J. Immunol. 189, 1330 (2012.*

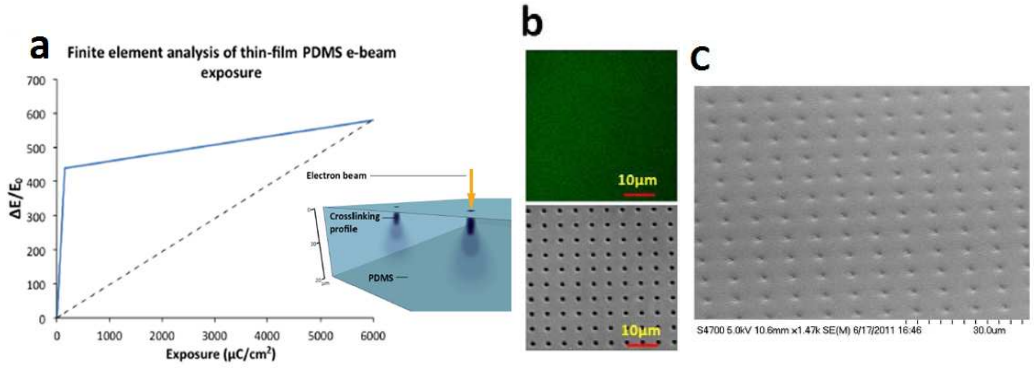

Figure 1. Physical and surface chemical characteristics of e-beam exposed PDMS. (a) Relative increase of Young's modulus of a 3 μm-thick layer near the surface (inset: schematic of PDMS exposure). (b) Top: Fluorescent image of OKT3 on pattered surface showing a uniform signal; Bottom: DIC image of same. (c) SEM image of exposed PDMS showing gentle depressions up to 140 nm at maximum exposure dose. The radius of curvature of these depressions is ~ 200 μm.

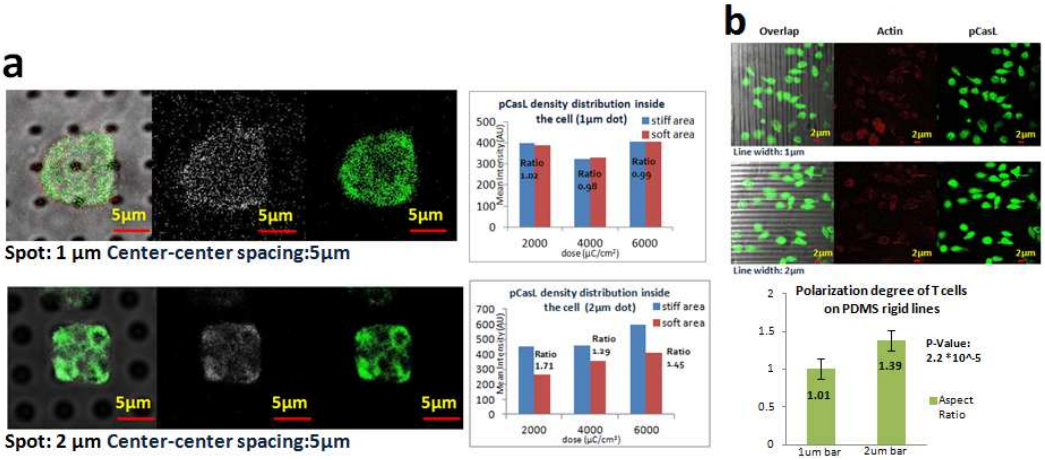

Figure 2. T-cells spreading on heterogeneous rigidity surfaces. (a) pCAS-L on 1 μm (top) and 2 μm (bottom) spots. At right are the relative distributions of pCAS-L on hard and soft regions for each. (b) Top: T-cells spread on 1 μm (top) and 2 μm (bottom) rigid stripes. Bottom: Degree of T-cell polarization.

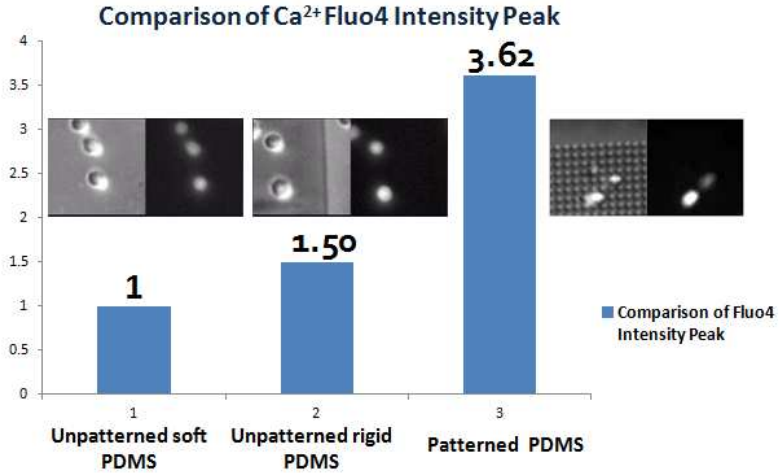

Figure 3. T- cell functional response to heterogeneous rigidity - Comparison of Ca^{2+} release peak on soft, rigid and spot patterned PDMS.