Patterned Surfaces to Investigate Spatially Regulated Mechanisms in Immune Cell Signaling

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Abstract
Micro- and nano-fabricated surfaces have been widely used for applications in cell and tissue engineering. However, the full potential of these technologies has not been explored, particularly in the area of molecular cell biology. Employing these technologies, we are investigating fundamental mechanisms in immune cell signaling, specifically IgE receptor (FceRI) signaling involved in allergic responses on mast cells. We are interested in learning the spatial regulation mechanisms for intracellular signaling events and the role of the actin cytoskeleton in these processes.

Summary
Recently, we established the use of patterned surfaces as a tool for visualizing spatial distribution of signaling molecules, as well as providing new insights in the structural and functional relevance of membrane compartmentalization [1, 2]. We have used standard photolithography techniques and the polymer lift-off method to fabricate surfaces containing patterned lipid bilayers with haptons that serve as antigens [3]. Antigens on the lipid bilayers bind and cross-link FceRI-bound IgE on the surface of mast cells, thus activating signaling events in these cells (Figure 1). By spatially clustering receptors on the surface of mast cells, we are able to control and observe the local environment in which signaling molecules undergo a series of biochemical events. Previously, we showed that early signaling components such as Lyn kinase selectively co-redistribute with patterned receptors [2]. Currently we are studying the dynamics of the actin cytoskeleton following FceRI mediated activation and we have identified possible new adapter proteins that may be involved.

We found that F-actin and other actin binding proteins such as vinculin (Figure 2) and paxillin are recruited to the clustered receptor sited and that this local recruitment may be mediated by interactions with Lyn kinase. This approach combined with more standard biochemical analysis is yielding new insight into the role of the actin cytoskeleton in FceRI receptor signaling.

References
Figure 1: Cartoon representation of the interaction between receptors on the cell surface and the patterned lipid bilayers.

Figure 2: Visualization of the redistribution adapter proteins upon stimulation with fluorescence microscopy.