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. By using these technologies we are investigating fundamental mechanisms in immune cell signaling, specifically IgE receptor (FcεRI) 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 and early signaling components in these processes.

Summary:
We have been using patterned surfaces as a tool for visualizing spatial distribution of signaling molecule upon IgE receptor activation on the cell surface [1,2]. We have used standard photolithography techniques and the polymer lift-off method to fabricate surfaces containing patterned lipid bilayers or immobilized protein carriers with haptens that serve as antigens [3]. Immobilized antigens/haptens bind and cross-link FcεRI-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. We are studying the dynamics of the actin cytoskeleton and other signaling components including Syk and Protein Kinase C (PKC) following FcεRI mediated activation. We have found that F-actin and other actin binding proteins commonly associated with focal adhesions such as vinculin, talin and paxillin are recruited to the clustered receptor sited (Figure 2) and that this local recruitment may be mediated by interactions with Lyn kinase. Biochemical data confirms that paxillin plays a role in IgE receptor signaling and that activation of vinculin and paxillin is specific to IgE clustering and not due to mechanical tension. In addition, we have found that Syk but not PKCβ is locally recruited towards the activated IgE receptor and PKCβ shows an oscillatory translocation to the plasma membrane.

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 of signaling components upon stimulation with fluorescence microscopy.