Utilizing Micro-Patterned Protein Surfaces to Investigate the Properties of the Lysosomal Synapse

CNF Project # 1726-08
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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 techniques has not been explored, particularly in the area of molecular cell biology. Employing these technologies, we are now investigating the mechanisms of uptake of aggregated low-density lipoproteins (agLDL) by the macrophages by creating an extracellular hydrolytic acidic compartment [1]. An understanding of the mechanism of agLDL uptake that doesn’t require receptor mediated endocytosis may play an important role in foam cell formation and lead to new therapeutic approaches to prevent atherosclerosis. We use the micro-patterned protein arrays to investigate the properties of a novel cell-associated structure – the lysosomal synapse. These patterns are also used to investigate the formation of the lysosomal synapse with the fibrillar beta-amyloid plaques that are formed in brains of patients suffering from Alzheimer’s disease.

Summary of Research:

In the past, we established the use of micro-patterned protein surfaces as a tool for visualizing spatial distribution of signaling molecules, such as FcεRI bound immunoglobulin E (IgE) in Rat Basophilic Leukemia (RBL) mast cells [2]. We are now extending this technique to study the interactions of macrophages, which are a part of the immune system, with aggregated low density lipoproteins (agLDL). Among the various functions of macrophages in the human body, one of the medically important functions is their interaction with agLDL in the walls of blood vessels. This results in massive uptake of cholesterol and conversion of macrophages to foam cells, which is an early step in development of atherosclerosis [3]. The use of micro-patterned protein surfaces provides us a unique opportunity to investigate the properties of a novel cell-associated structure – the lysosomal synapse. We also look at the formation of lysosomal synapse with the beta-amyloid plaques that are often found in patients suffering from Alzheimer’s disease.

Previously, we prepared micro-patterned proteins on silicon surfaces. However, some fluorescence microscopy techniques, such as total internal reflection fluorescence microscopy (TIRFM) for live cell imaging, require micro-patterned proteins on glass surfaces (Figure 1). We use standard photolithography techniques and the polymer lift-off method to fabricate surfaces containing spatially defined fluorescently labeled streptavidin-LDL on glass cover slips.

Figure 1: Surfaces micro-patterned with A568-streptavidin. Each patterned feature is 1 µm x 1 µm² and separated by 5 µm.
In initial experiments J774 macrophages were incubated with micro-patterned protein arrays of agLDL for 30 minutes. Upon interaction of the macrophages with agLDL, regions of low pH were seen at contact sites (Figure 2). Since the agLDL is tightly bound to the surface this demonstrates that the acidic domains are extra-cellular.

In addition to the agLDL studies, we have used proteins micro-patterned on glass surfaces to image lysosomal synapses formed by the interaction of J774 macrophages with fibrillar beta-amyloid. The plaques that accumulate in the brain of Alzheimer’s disease patients are composed of fibrils of this beta-amyloid protein. Fibrillar Cy3 (red)-labeled beta-amyloid was conjugated to the micro-patterned surfaces. The lysosomes of the macrophages incubated on these surfaces were labeled with FITC-dextran. The green signal of FITC is strongest at neutral pH and is less intense at the more acidic pH that exists in the lysosome (Figure 3A). TIRFM movies of the macrophages interacting with the Cy3-fibrillar beta-amyloid patterns show that the green FITC signal increases in intensity as the lysosomal synapses form (Figure 3B), then eventually fades as the labeled dextran diffuses away from the lysosomal synapse into the media (Figure 3C).

In future work, we will investigate secretion of lysosome contents into the lysosomal synapse formed between macrophages and the patterned agLDL surface. We will also examine the signal transduction mechanisms involved and the SNARE proteins that participate in the plasma membrane fusion events.

References: