Design and Characterization of Micro-cavity Suspended Lipid Bilayers: their Applicability as Platforms for Incorporation and Study of Membrane Proteins.

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Abstract

Fabrication of supramolecular assemblies that are capable of mimicking biological functions has been on the surge since the past few years. In particular, systems resembling the cytoplasmic cell membrane have attracted the attention of scientists, owing to the fact that cell membrane performs myriads of important cellular processes such as signal transduction, mediating the transport of ions and metabolites across the cell, cell-cell interactions, etc. via their membrane proteins. However, the greatest challenge in mimicking this "intelligent" supramolecular assembly lies in retaining its functional integrity.[1, 2] In this context, artificial lipid bilayers that possess intrinsic 2-D fluidity are known to be the easiest route to the goal. In that, the construction of a Supported Lipid Bilayer, in which the lipid film is directly attached to its support, has been frequently employed. However, in these models the bilayer being in very close proximity to the surface restricts the incorporation, mobility and henceforth the stability of the membrane proteins inserted in the bilayer.

Herein, we present the fabrication of a system, where lipid bilayers are suspended over micro-cavities that are structured on polydimethylsiloxane (PDMS) surfaces (Fig. 1).[3] These micro-cavities were pre-filled with an aqueous buffer and the lipid bilayer was spanned over them by employing a combination of Langmuir-Blodgett technique and vesicle fusion. It was observed by Fluorescence Light Correlation Spectroscopy (FLCS), that the bilayers suspended over a cavity were highly diffusive in nature as compared to bilayers supported on a flat surface, and the diffusion co-efficient values of the bilayer above a cavity was very close to that of free vesicles in solution. This result indicated that the presence of the underlying buffer filled cavity greatly reduced the interactions between the attached lipid film and the substrate (PDMS), thereby, reducing the diffusional constraint on the lipids in the bilayer. These results illustrate the validity of these systems as potential cell membrane models in which the lipid bilayer is distinctly decoupled from the underlying surface, thereby, providing enough room to accommodate the intracellular domain of inserted membrane proteins. Encouraged by these results we also tested the insertion of a platelet membrane protein, $\alpha_{IIb}\beta_3$ integrin into these cavity-suspended bilayer systems. The diffusion behavior of the integrin and its activity was found to drastically improve upon its insertion in the cavity spanning bilayers.

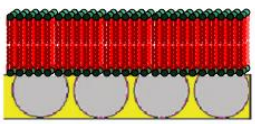

Fig. 1 Cartoon representation of a bilayer spanning prefilled cavities.

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