Specific cholesterol depletion through membrane-substrate interactions

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Introduction: Substrate induced cholesterol depletion

Supporting lipid membranes and cells on biomimetic substrates is a common tool for biological research and technological advances. However, such substrates often portray various biophysical parameters of the membrane, including phase behaviour [1], lateral fluidity [2], and nano-scale cracking [3].

Here we report on a previously observed membrane-substrate interaction, induced by lipid bilayer contact with nanoscale hydrophilic and hydrophobic domains at a polymer substrates interface.

Using plasma oxidation, we alter the surface hydrophobicity of poly(dimethylsiloxane) (PDMS) substrates to create an interface capable of selectively removing cholesterol. We verify that the presence of nanoscale hydrophilic and hydrophobic domains on the substrate surface induce a depletion of membrane cholesterol. Finally, we demonstrate how to control the initiation of cholesterol depletion through mechanical extension of the plasma treated PDMS substrates.

Specific depletion of cholesterol via substrate interactions

Cholesterol-containing phospholipid bilayers undergo distinct morphological changes when fused to partially plasma oxidised PDMS. These changes appear in the form of poncho patch shrinkage and increase in fluorescence intensity.

We extracted values for cholesterol mole fraction of each patch by fitting our data to a simple model for specific cholesterol depletion (derived from Liu et al 2016 [4]). The fitting matched our observations, validating our hypothesis that PDMS substrate can induce specific cholesterol depletion from the fused lipid membranes, while leaving other components stably supported.

Cholesterol depletion inducing a change in phase

Using a bilayer composition of DPPC-Cholesterol (60:40 mol%) the ability of partially oxidised PDMS to modify the bilayer fluidity through a depletion of cholesterol is exemplified. Substrate induced cholesterol depletion significantly alter membrane composition, transforming a fluid membrane to a gel-like state.

Characterisation of PDMS-membrane interaction

Chemical force mapping

We use chemical force mapping to characterise the surface hydrophobicity of the fully and partially plasma oxidised PDMS. Using hydrophobic AFM tips, we confirmed the presence of nanoscale hydrophobic/hydrophilic domains on the partially oxidised PDMS.

Next, we characterised the PDMS-cholesterol interaction using functionalised AFM tips coated with a cholesterol analogue and probed relevant interaction forces between the substrate and model supported membranes. Using this technique, we identified characteristic adhesion forces between cholesterol and silica-like substrates, and show these forces can out-compete cholesterol affinity to the surrounding lipid membranes. This causes cholesterol to partition out of the lipid membranes and then be lost in the porous PDMS matrix through the nanoscale hydrophilic domains.

Summary: Influence of substrate surface

- PDMS substrates can extract cholesterol from supported lipid membranes. This effect is induced by membrane contact with nanoscale hydrophobic and hydrophilic domains present at the PDMS interface.
- The Force magnitudes required to extract cholesterol via substrate interactions are accessible to cellular support structures such as the cytoskeleton or extra cellular matrix.
- Cells should be taken in mechanical studies of biomembrane/cells using flexible PDMS substrates. Unwanted cholesterol depletion from membranes could be perturbing measurements.
- These interfacial effects could be used for controlled drug release, and protective coatings.

References


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