EPRSC Centre for Doctoral Training in Soft Matter and Functional Interfaces

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Specific cholesterol depletion through membrane-substrate interactions

e.j.miller@dur.ac.uk

Ethan J. Miller , Kislon Voitchovsky , Margarita Staykova

Soft Matter Physics Group, Department of Physics, University of Durham

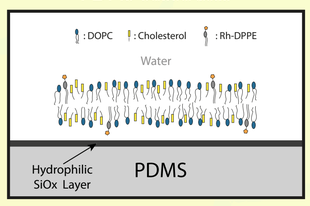
Introduction: Substrate induced cholesterol depletion

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

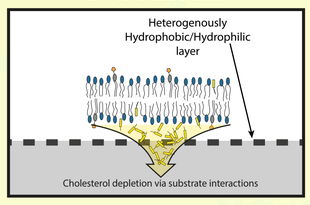
Here we report a previously unobserved membrane – substrate interaction, induced by lipid bilayer contact with nanoscale hydrophobic and hydrophilic domains at a polymer substrate interface.

Using plasma oxidation, we alter the surface hydrophilicity of polydimethylsiloxane (PDMS) substrates to create an interface capable of selectively removing cholesterol. We verify that the presence of nanoscale hydrophobic and hydrophilic 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 substrate.

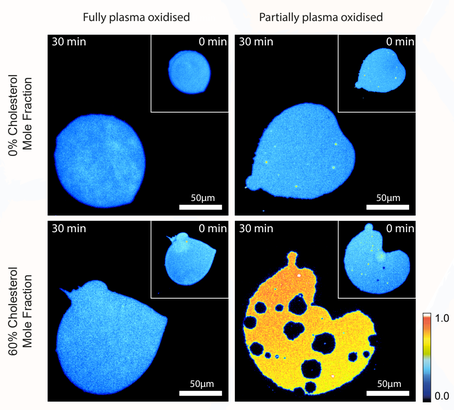
Fully Plasma Oxidised PDMS Substrates



Partially Plasma Oxidised PDMS Substrates



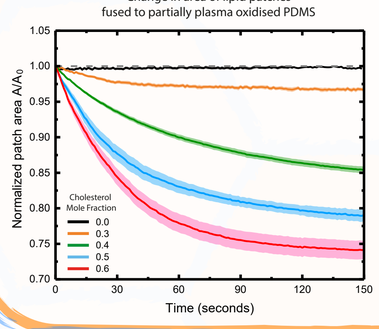
Specific depletion of cholesterol via substrate interactions



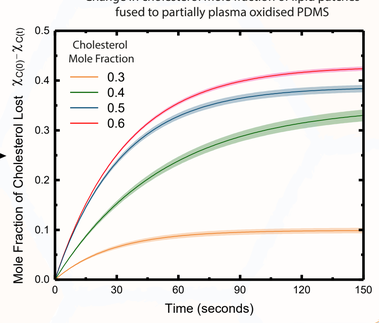
Cholesterol-containing phospholipid bilayers undergo distinct morphological changes when fused to partially plasma oxidised PDMS. These changes appear in the form of pores, patch shrinkage and increase in fluorescence intensity.

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

Change in area of lipid patches fused to partially plasma oxidised PDMS

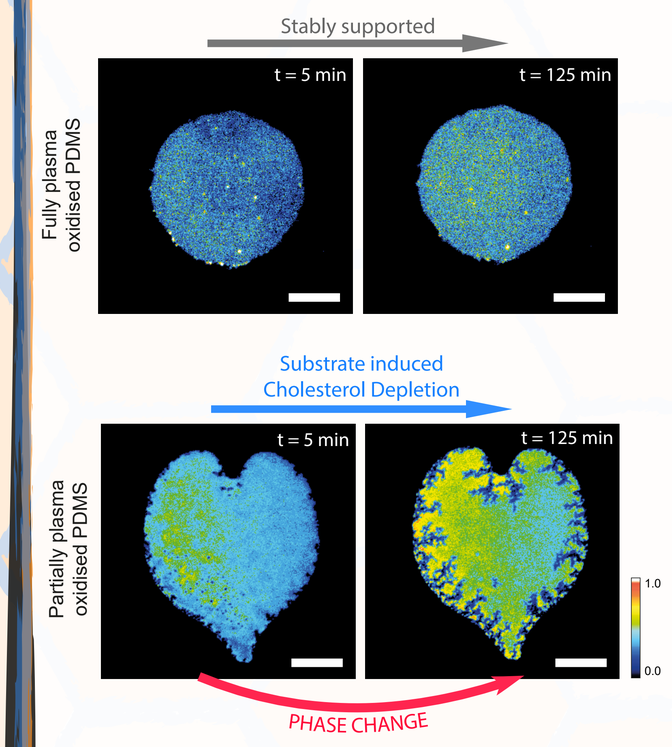


Change in cholesterol mole fraction of lipid patches fused to partially plasma oxidised PDMS

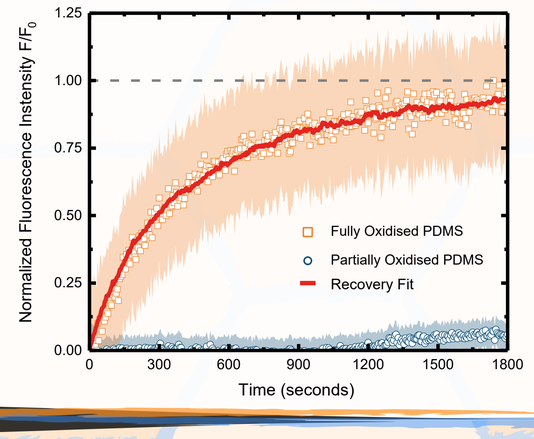


Cholelesterol depletion inducing a change in phase

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 alters membrane compsition, transforming a fluid membrane to a gel-like state.

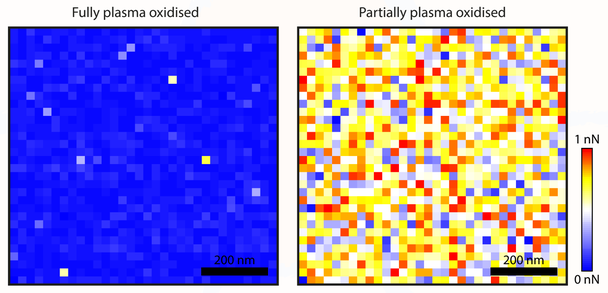


Fluorescence recovery after photo bleaching (FRAP) measurements verify a loss of membrane fluidity for patches fused on partially oxidised PDMS indicating a solidifaction of the membrane due to choleserol depletion

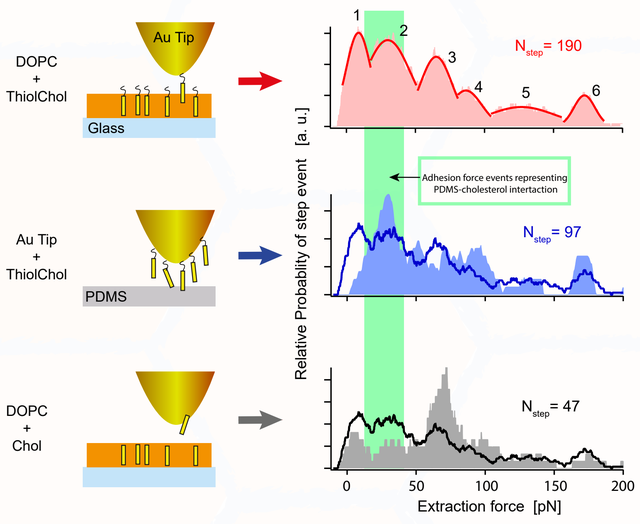


Chracterisation of PDMS-membrane interaction

Chemical force mapping



Adhesion force event distributions



We use chemical force mapping to characterised 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.

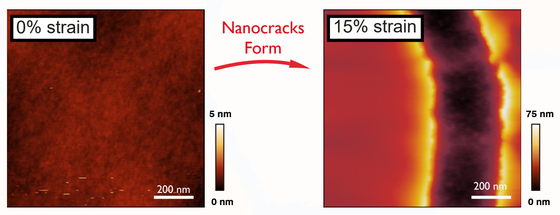
Next, we characterised the PDMS-cholesterol interaction using functionalised AFM tips coated with a cholesterol analogue and probed relevant interaction forces between the substrate and model supported membranes. Using this technique, we identified characteristic adhesion forces between cholesterol and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

Summary: Influence of substrate surface

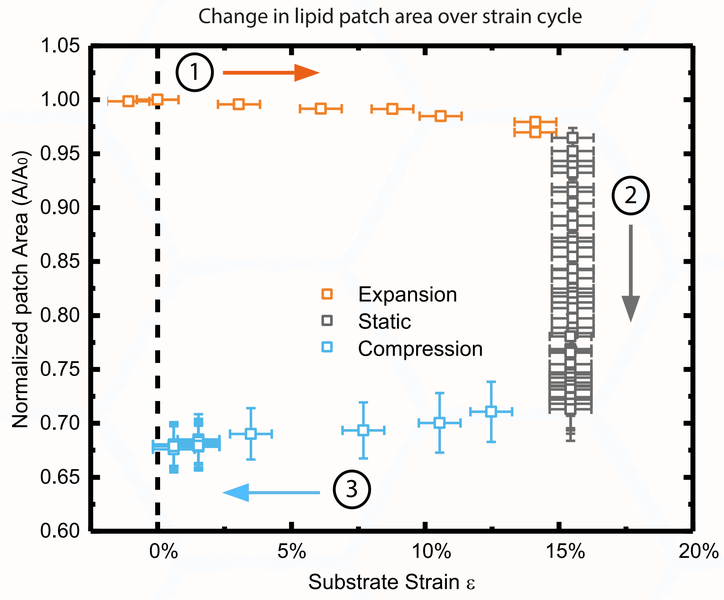
- PDMS substrates can extract cholesterol from supported lipid membranes. This effect is induced by membrane contact with nanoscale hydrophobic and hydrophilic domains present at the PDMS interface.
- The Force magnitudes required to extract cholesterol via substrate interactions are accessible to cellular support structures such as the cytoskeleton or extra cellular matrix.
- Care should be taken in mechanical studies of biomembranes/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.

Triggered cholesterol depletion in supported membranes

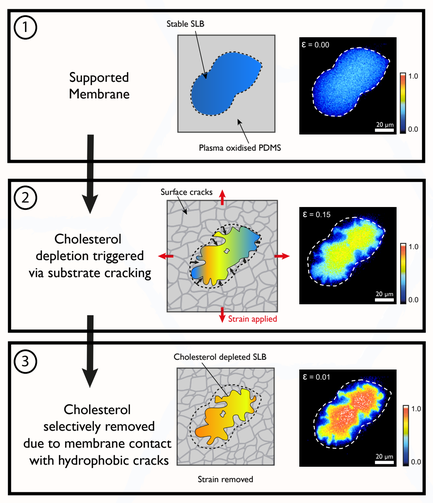
We demonstrated that, by utilising mechanical strains, cholesterol depletion can be intiatied in plasma treated PDMS substrates. Using controlled substrate strains, nano-cracks in the substrate surface can be created. This is because plasma treatment of PDMS substrates creates a brittle hydrophilic silica surface layer. These cracks expose the underlying hydrophobic native PDMS. This creates a hydrophilic surface interspered wih nanoscale hydrophobic cracks; an interface capable of selectively depleting cholesterol from supported membranes.



Change in lipid patch area over strain cycle



Cartoon of crack induced cholesterol depletion



References

[1] H. M. Seeger, G. Marino, A. Alessandrini, and P. Facci, Effect of physical parameters on the main phase transition of supported lipid bilayers.," Biophys. J., vol. 97, no. 4, pp. 1067–76, Aug. 2009
[2] F. Pincet et al., "FRAP to characterize molecular diffusion and interaction in various membrane environments," PLoS One, vol. 11, no. 7, pp. 1–19, 2016.
[3] H. M. Seeger, A. D. Di Cerbo, A. Alessandrini, and P. Facci, Supported lipid bilayers on mica and silicon oxide: Comparison of the main phase transition behavior," J. Phys. Chem. B, vol. 114, no. 27, pp. 8926–8933, Jun. 2010.
[4] J. P. Litz, N. Thakkar, T. Portet, and S. L. Keller, Depletion with Cyclodextrin Reveals Two Populations of Cholesterol in Model Lipid Membranes," Biophys. J., vol. 110, no. 3, pp. 635–645, 2016.

Acknowledgements

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