

EVALUATION OF SUPPORTED PHOSPHOLIPID BILAYER PERSISTENCE AND MOBILITY IN CELL CULTURE

[N.Tymchenko](#), [D.Thid](#), [J.Gold](#)

Department of Applied Physics, Chalmers University of Technology, Göteborg, Sweden

INTRODUCTION: The use of supported synthetic lipid membranes as cell culture substrates is an increasingly popular approach to investigate cell-molecular interactions, as well as to model cell-cell interactions in a controlled manner. Whilst unmodified phosphocholine (POPC) membranes are inert to protein adsorption and cell adhesion, they may be functionalised with ligands in order to present cells with specific cues for attachment, or for signalling of proliferation, differentiation, or apoptosis.

The present study is an assessment of supported POPC bilayers over 10 days in culture with adult rat-derived hippocampal progenitor (AHP) cells. The aims were to (1) investigate stability, morphology and fluidity of the POPC membranes with or without the attachment-promoting IKVAV peptide, and (2) identify any interactions between the cells and the fluorescently labelled bilayer.

METHODS: Cell experiments were performed on borosilicate glass cover-slips incubated with sterile-filtered solutions of 30 nm POPC vesicles doped with 1-5% (n/n) NBD-DHPE, with or without 3% maleimido-EG₂-POPE lipid. Cysteine-terminated IKVAV peptide was covalently bound to the maleimide terminated lipids^{1,2}.

Quartz crystal microbalance with dissipation monitoring (QCM-D) analysis showed characteristic bilayer formation from vesicle adsorption onto silicon oxide. The bound peptide was stable upon rinsing and binding behaviour was as expected^{1,2}.

Fluorescence recovery after photobleaching (FRAP) was used to assess the mobility of bilayers every day for 10 consecutive days. FRAP measurements and cell imaging was performed on live cultures. Images were acquired at various intervals after a 15s bleach.

RESULTS: POPC bilayers were largely cell resistant, supporting in general few, often very large, sedimented clusters (lack of cell processes). The IKVAV-functionalisation promoted AHP attachment followed by growth in clusters. At later time points, cells grew out forming an interconnecting network between clusters. AHP growth morphology on these surfaces was as previously reported².

With time, we observed losses in fluorescence intensity and lateral mobility of both IKVAV-functionalised and non-functionalised bilayers, indicating that the integrity of the bilayers was compromised. This occurred more rapidly (within 48 hours) for the functionalised bilayers than for POPC (day 7). Bilayers on the back side of the cover-slips remained as mobile as on day 1, out to day 6-8. Bilayer recovery was observed at early time points under sedimented/non-spread cells (Fig 1.), whereas in other cells there was loss of fluorescence and/or reduced mobility in areas under, as well as immediately surrounding, cells and their processes. We have also observed fluorescent cells on both bilayers already from day 1. Fluorescence intensity was both diffuse as well as specifically located to certain regions of the cell, eg. processes.

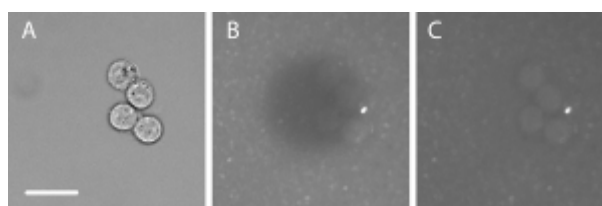


Fig. 1: POPC (1%NBD) Bilayer mobility under cells after 24 hours in culture. Bright field (A) Bleach spot (B) Recovery after 5 minutes (C). Scale bar indicates 20 micrometers.

DISCUSSION & CONCLUSIONS: We report a more rapid loss in lateral mobility for a bilayer supporting cell spreading/extension of processes compared to those without specific attachment. Furthermore, uptake of fluorescent lipid from the underlying bilayer into cells was observed. Loss of bilayer fluorescence under and around cells might be a direct result of this uptake, and/or an indication of bilayer destruction in these regions. Chemical analysis of bilayers in cell culture is needed to clarify the loss of fluorescence and changes in composition with time.

REFERENCES: ¹S Svedhem et al, Langmuir, 2003; ²D Thid et al, JBMRA, 2007.

ACKNOWLEDGEMENTS: Financial support was received from the Swedish Foundation for Strategic Research (SSF), the Swedish Council for Research (VR), and the Chalmers Bioscience Initiative.