PROGRESS REPORT FOR ALNGRA10080

**PROJECT TITLE**
Using X-ray reflectometry to understand the structure of the surface of the tear film

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**SCIENTIFIC OBJECTIVES**

Meibomian lipids are a complex mixture of wax esters, cholestereryl esters and other lipids. They are produced by the eyelids facilitate the spreading of tears across the ocular surface. The objective of this work was to gain insight into the arrangement of these different lipids as they form a film at an air liquid interface in a Langmuir trough. A particular interest was in determining if wax esters in the outer layer of the tear film align in a trans or a gauche conformation and how temperature and $\beta$-carotene affect this arrangement, and whether the strong hydrophobic property of $\beta$-carotene causes the wax esters to be strongly attracted to the $\beta$-carotene and the trans conformation is more stable and as a result these lipids orientate vertically.

**PROGRESS REPORT and RESEARCH OUTCOMES**

Baseline data were collected using known systems:

(i) Dipalmitoylphosphatidylcholine (DPPC)
(ii) 75% palmitoleate (a wax ester), 15% cholesterol stearate (sterol ester) and 10% cholesterol in hexane
(iii) 75% palmitoleate (a wax ester), 24% cholesterol stearate (sterol ester) and 1% $\beta$-carotene in hexane.

These were compared with results from meibomian lipids and meibomian lipids mixed with

(iv) 90% human meibomian lipids and 10% cholesterol in hexane and
(v) 99% human Meibomian lipids and 1% $\beta$-carotene in hexane.

The data obtained for the control lipids (DPPC) were consistent with a hydrophobic tail aligned at the air interface and the phosphate head group being associated with the subphase (Fig 1). A surprise was that meibomian lipids self assemble into multiple layers even at the lowest pressure (Fig 2), because a monolayer was expected at the lowest pressure. Individually, films of cholestereryl or wax esters did not emulate meibomian lipids in their arrangement. These showed Bragg style peaks (Fig 3). Mixtures of wax and cholestereryl esters also did not emulate meibomian lipids, but instead seemed to form micelles that were displaced from the surface. While $\beta$-carotene showed some effects on the meibomian lipids, the data were not strong enough to be conclusive. A reason was the limitation of the current set-up to a limited compression ratio. The effects of $\beta$-carotene are only observed under high pressures (small surface areas) which were not available. Although attempts were made to obtain data on heated films (34°C), evaporation from the subphase meant that the surface level of the trough was changing. This made recordings difficult and more time and major changes to the equipment would be required to resolve this issue. Therefore the trans/gauche question remains to be resolved.

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Figure 1: X-ray reflectivity profile of a DPPC monolayer (0.5mg/mL; 20µL) at the air-buffer interface. Inset shows SLD profile.

Changes to scattering length density indicate different layers/ phases. Individual points represent raw data. Line represents model using parameters.

Figure 2: X-ray reflectivity profiles of human meibomian lipid films (1mg/ml; 50µL) at the air-buffer interface (a) in the uncompressed state (0.6 mN/m, blue data); (b) at 3.7 mN/m surface pressure (red data); and (c) at 9.0 mN/m surface pressure (green data). Inset shows SLD profiles.

Meibomian lipids self assemble into multiple layers even at the lowest pressure. Water is squeezed out of lipid film when surface pressure increases from 0.6 mN/m to 3.7 mN/m.
Figure 3: X-ray reflectivity profiles of compressed films of: (a) the wax ester (stearyl oleate; 0.5mg/ml, 50µL; red profile) and (b) the sterol ester (cholesterol oleate, 0.5mg/ml, 220 µL; blue profile), showing Bragg peaks indicative of multilayer formation normal to the surface of the subphase; (c) a 1:1 mixture of the wax ester and sterol ester (0.5 mg/ml, 40 µL; green profile). The data for (b) and (c) are offset by factors of 0.1 and 0.01 for clarity.

Bragg style peaks of wax and cholesteryl esters illustrate that the major constituents of the TFLL are poor models for its structure. Absence of peaks suggest that mixtures of wax and cholesterol esters form lipid micelles.

Signature of Investigator preparing the report for
After signing this report please fax this page with your signature for our files

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Date:

PUBLICATIONS / REPORTS arising as a result of your work.

At this stage there are no publications, but we have prepared a draft manuscript which we are hoping to submit to an international journal before the end of the year.

PhD STUDENTS

Shiwani Raju Anticipated date of submission February 2011 Title: Viscoelasticity of meibomian lipids at an air interface

Chendur Palaniappan Anticipated date of submission January 2011 Effects of various lipids on the structure and surface properties of meibomian lipid films.