Biophysical characterization of monofilm model systems composed of selected tear film phospholipids

While our eyes are open they are constantly exposed to bacterial infections, injury, and dehydration. To provide the necessary protection against these hazards the outer surface of our eyes is covered by a thin layer known as tear film. The tear film must not only protect the eye but also provide a high quality optical surface that is capable of rapidly respreading and reforming with every blink.

The structure of tear film contains three relatively distinct layers with a total thickness ranging from 1.5 to 4.7 microns at the centre over the cornea. Closest to the eye's surface is the mucus which is predominately made up of proteins with attached sugar groups. In the middle, a water layer contains different salts and bacteria fighting proteins, like lysozyme, lactoferrin, and lipocalin. The outer most layer is the fat or lipid layer that can be further divided into two more layers (Fig. 1). Directly on the surface is a monolayer of polar lipids which can better interact with the water layer, in contrast to the thicker and really water repellent non-polar lipid layer. During every blink, the polar lipids help to spread the non-polar lipids which would otherwise form droplets much like oil on water.

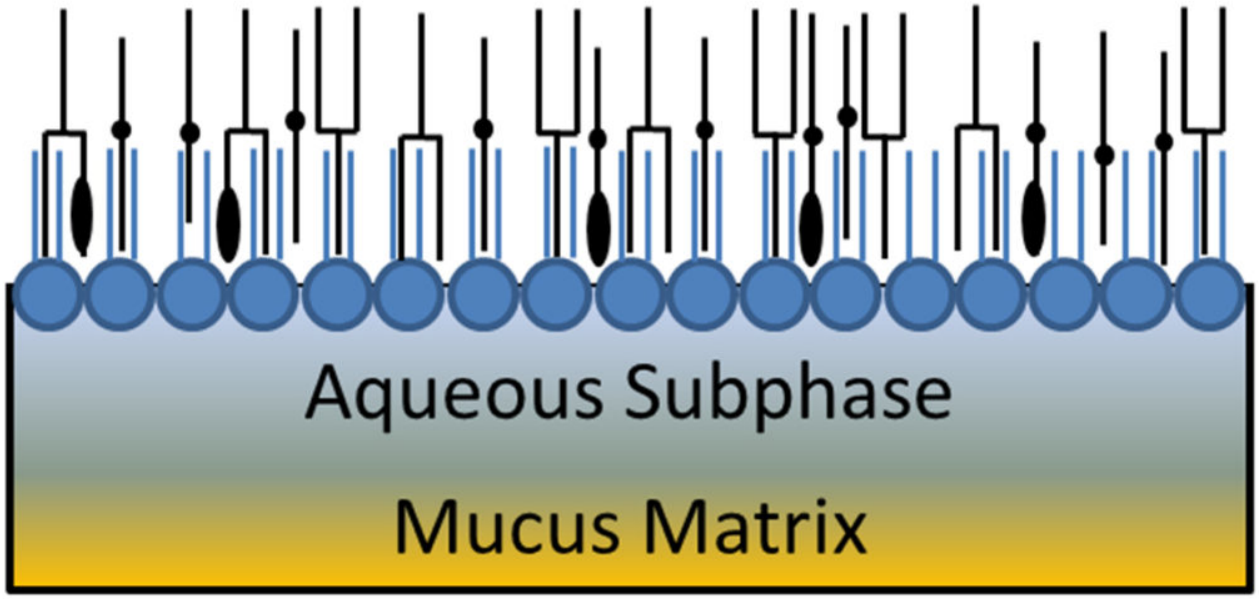

Fig. 1. Cartoon of the tear film. The blue circle and stick structures represent the polar lipid layer while the black structures represent non-polar lipid layer.

Our recent paper published in *BBA Biomembranes* focused on the polar lipid layer of tear film with

an emphasis on understanding the role the different polar lipids play during blinking. To study this our lab created a polar tear film model using synthetic polar lipids. This mixture was spread on the water surface of a Teflon container, commonly known as a Langmuir trough. Upon spreading, the lipids form a film consisting of a single layer. This so called monolayer was compressed by moveable barriers to mimic the closing of the eyelid. The pressure created in the film can be measured by a sensor.

We have selected relevant polar lipids that contain 1 or 2 defined side chains. In all cases these chains had 16 carbons and no double bond which are termed palmitoyl. Two important classes, cholines (PC) and sphingomyelins (SM) are the main components of many human cell membranes. A choline with 2 palmitoyl side chains is named in short DPPC while the sphingomyelin has only one variable side chain and is referred to as PSM. As in other membranes, both primarily provide stability to the tear film to withstand high pressure during blinking. In addition, two other lipids were tested, ethanolamine (PE) which is more similar to PC and a sugar-containing ceramide which is related to SM. With the same palmitoyl side chains DPPE and PGC also stabilized the tear film but over a wider pressure range.

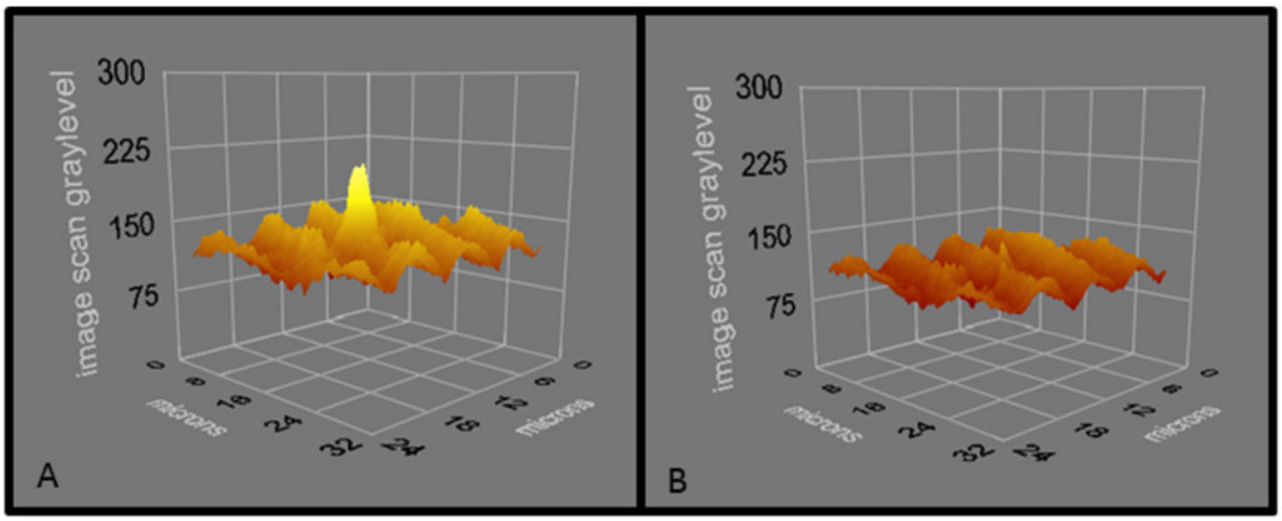

Fig. 2. Relative film thickness of DPPC, DPPE, and PGC film mixtures. The x and y axes represent the length in microns and the z-axis relates to the intensity of reflected light. Panel A represents a part of the film undergoing multilayer formation while B represents an area that isn't.

Finally we also investigated mixtures of DPPC, DPPE and PGC films and found that they were able to form multilayers. Figure 2 shows 3D images of the relative film thickness recorded by a laser microscopy, called Brewster Angle Microscopy. Images are created when a laser is reflected off of a lipid film. Any structures that stick out reflect more light and appear brighter as shown in Figure 2A. This image was recorded during the compression of a mixture of DPPC, DPPE, and PGC film at a surface pressures well below expected film collapse. These areas of the film were thicker

when compared to the surrounding film that is shown in figure 2B.When the film area is reduced during a compression, the formation of outward pointing structures would allow storage of film material that could be easily reused during eye opening. This process could be very important for tear film stability during blinking.

Elmar Josef Prenner
Biochemistry Research Group, Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada

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