Published in final edited form as:

Ocul Surf. 2013 October ; 11(4): 236–245. doi:10.1016/j.jtos.2013.05.003.

Four Characteristics and a Model of an Effective Tear Film Lipid Layer (TFLL)

P. Ewen King-Smith, PhD¹, Melissa D. Bailey, OD, PhD¹, and Richard J. Braun, PhD²

¹College of Optometry, Ohio State University, Columbus, Ohio

²Department of Mathematical Sciences, University of Delaware, Newark, Delaware, USA

Abstract

It is proposed that a normal, effective tear film lipid layer (TFLL) should have the following four characteristics: 1) high evaporation resistance to prevent water loss and consequent hyperosmolarity; 2) respreadability, so it will return to its original state after the compression-expansion cycle of the blink; 3) fluidity sufficient to avoid blocking secretion from meibomian glands; 4) gel-like and incompressible structure that can resist forces that may tend to disrupt it. These characteristics tend to be incompatible; for example, lipids that form good evaporation barriers tend to be disrupted by compression-expansion cycles. It is noted that clues about the function and organization of the TFLL can be obtained by comparison with other biological lipid layers, such as lung surfactant and the lipid evaporation barrier of the skin. In an attempt to satisfy the conflicting characteristics, a “multilamellar sandwich model” of the TFLL is proposed, having features in common with the skin evaporation barrier.

Keywords

blinks; evaporation resistance; evaporative dry eye; lipid layer structure; lipid monolayers; meibomian gland dysfunction; skin lipid barrier; X-ray analysis

I. COMPARISON OF BIOLOGICAL LIPID LAYERS

Deficiency of the tear film lipid layer (TFLL) is the basis of evaporative dry eye,¹ and therefore an understanding of this layer is fundamental to the diagnosis, management, and

© 2013 Elsevier Inc. All rights reserved

Corresponding author: P. Ewen King-Smith, PhD, College of Optometry, Ohio State University, 338 West 10th Ave., Columbus, Ohio 43210, USA. Tel: 614-292-3939. king-smith.1@osu.edu..

Single-copy reprint requests to P. Ewen King-Smith, PhD (address below).

Editor's note to compositors: The outline and headings throughout are unconventional, as the main heads are in “topic” format and the sub-heads are complete sentences. The authors and editors agree that this is a useful format. Instead of using our usual format with capitalization of all words in the heads, please set the outline and heads in the text as shown, with capitalization of first word only and periods at the ends of the sentences.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors have no proprietary or commercial interest in any product or concept described in this article.

treatment of dry eye disorders. Here, it is proposed that a normal, effective TFLL should have the following four characteristics:

1. It should have a high evaporation resistance to prevent water loss and consequent hyperosmolarity.
2. It should have good respreadability, so that the lipid layer returns to its original state after the compression-expansion cycle of the blink.
3. It should be sufficiently fluid that it does not block secretion from meibomian glands.
4. It should be gel-like and incompressible so that it can resist forces that may tend to disrupt it.

Some of these characteristics tend to be incompatible. The gel-like, incompressible property (characteristic 4) would evidently tend to be at odds with fluidity (characteristic 3).

Additionally, monolayers having high evaporation resistance (characteristic 1), such as saturated fatty acids and their esters,² tend to show poor respreadability (characteristic 2).^{3,4} Thus, lipid layer composition and structure must involve compromise and trade-off between apparently conflicting characteristics.

Rantamaki et al discussed how a comparison of two biological lipid layers, namely, the TFLL and lung surfactant, can lead to insights into the relation of their function and composition.⁵ Both layers are “respreadable” in that they withstand compression-expansion cycles, either from breathing or from blinking. However, evaporation resistance is important for the TFLL, but not for the lung lipid layer; given the small size of the alveoli, the enclosed air would rapidly become saturated, even if the evaporation resistance were comparable to that of the tear film lipid layer. Here, we extend this approach to the comparison of biological lipid layers involved in the prevention of evaporation, as summarized in Table 1.

A. Function

Table 1 compares function and composition for six different biological lipid layers. Although the TFLL and lung surfactant are respreadable and withstand compression-expansion cycles, the other four layers are not subjected to such cycles. However, they, like the TFLL, are all thought to be barriers to evaporation,⁶⁻¹⁰ as indicated in Column 3 of Table 1.

B. Composition

The TFLL and lung surfactant layer have some similarity of composition in that they both contain polar lipids (such as phospholipids)¹¹⁻¹⁵ and surfactant proteins, particularly the hydrophobic proteins SP-B and SP-C.^{13,15-16} These surfactant proteins are thought to have a role in the resreading of lung surfactant during the breathing cycle,^{13,15} and while their function in the tear film is unknown, they may play a corresponding role in the blink cycle.

Whereas the main components of lung surfactant are polar phospholipids,^{13,15} the other five layers, which are all thought to be evaporation barriers, are composed mainly of nonpolar

lipids.⁶⁻¹⁰ There is considerable variation in the type of nonpolar lipids in different types of layer (Table 1). Whereas wax and cholesteryl esters are the main nonpolar lipids in the TFLL, other types of nonpolar lipids are common in the other four evaporation barrier layers. However, all five evaporation barriers have one striking feature in common; they all contain many, very long (> 19 carbons) saturated hydrocarbon chains.⁶⁻¹⁰ The saturated chains in lung surfactant, which does not function as an evaporation barrier, have generally fewer than 19 carbons. The corresponding entries in Table 1, Column 5 match those in Column 3, to emphasize the correlation between saturated chain length and evaporation barrier function.

II. ROLE OF LONG, SATURATED HYDROCARBON CHAINS IN EVAPORATION RESISTANCE OF MEIBUM

A. In a normal eye, the lipid layer has a high evaporation resistance compared to lipid monolayers

“Evaporation resistance” is a measure of the ability of a surface layer to retard evaporation of water. It is described in the Appendix (Section VI), together with estimates of the evaporation resistance of rabbit and human tear film lipid layers. In Table 2, these estimates are compared with the evaporation resistance of monolayers of hexadecanol and octadecanol, which are the lipids most commonly used to retard evaporation from reservoirs.¹⁷ Evaporation resistance of monolayers is not significantly improved by increasing average thickness to more than that of a monolayer; either the regular array of molecules in a monolayer is disturbed in the thicker layer,¹⁸ or the additional lipid forms thick “lenses” within the monolayer.¹⁹

B. Evaporation resistance increases exponentially with saturated chain length.

Lipid monolayers that form good evaporation barriers have two characteristics. First, the molecule must contain saturated rather than unsaturated hydrocarbon chains. For example, Jarvis et al found that saturated fatty acids containing 16 or more carbon atoms, and saturated alcohols containing 14 or more carbon atoms, caused considerable resistance to evaporation at temperatures of 16 to 20°C.²⁰ For comparison, all the unsaturated fatty acids that they tested – elaidic, oleic, linoleic, and stearolic acids – showed no detectable resistance to evaporation. The second important characteristic is that evaporation increases greatly with increasing length of the saturated hydrocarbon chain. Figure 1 shows measurements of evaporation resistance of saturated fatty acids as a function of carbon chain length (from Archer and La Mer²¹). Evaporation resistance is an *exponential* function of chain length. Adding one carbon atom increases the evaporation resistance by a factor of 1.65, so increasing the chain length by 3 carbon atoms from 17 to 20 atoms increases evaporation resistance by a factor of $1.65^3=4.5$. Thus, the increase in evaporation resistance as a function of chain length is much greater than expected by simple proportionality between resistance and chain length, which would predict an increase in resistance of a factor of only 1.18 for an increase from 17 to 20 carbon atoms.

C. Meibum contains long, saturated hydrocarbon chains.

Analysis of the lipid molecules in meibum shows that they have the two characteristics for forming a good barrier to evaporation, namely saturated hydrocarbon chains which are abnormally long. Thus, McCulley and Shine found that most of the fatty acids in cholesteryl esters are saturated, as are most of the fatty alcohols in wax esters.²² Additionally, they found that most of the fatty acids in cholesteryl esters and most of the fatty alcohols in wax esters have chain lengths greater than or equal to 19 carbons. (The fatty acids of wax esters differ in that they are often shorter and unsaturated.) More quantitative data has been provided by Butovich, who found that the most common fatty acids in cholesteryl esters are saturated and have 24, 25, and 26 carbon atoms, and the most common fatty alcohols in wax esters are also saturated and have 24, 25, and 26 carbon atoms.¹¹ Given the exponential increase in evaporation resistance as a function of chain length shown in Figure 1, it seems probable that these abnormally long chains help to provide the high evaporation resistance of the lipid layer (Table 2). A clinical implication is that any substitute for the natural lipid layer might need to contain long, saturated hydrocarbon chains in order to simulate natural meibum and provide a good barrier to evaporation.

It is important to note that lipids containing saturated hydrocarbon chains, particularly DPPC, play an important role also in lung surfactants.^{13,15,23} These lipids are capable of reducing lung surface tension to low values, hence preventing collapse of alveoli, which is a major cause of respiratory disorders.²³ Although these saturated chains are not as unusually long as in the TFL (palmitic acid has 16 carbons), the presence of saturated chains in both lung surfactants and the TFL could raise problems for the respreading of the lipid layers in the breathing and blinking cycles, respectively, as discussed in the next section.

III. SPREADABILITY OF LIPID LAYER AFTER BLINK

During a blink, the TFL does not pass under the lids but is trapped between the lids,²⁴ so it thickens considerably during the compression phase and thins during the expansion phase of the blink. Bron et al demonstrated that the TFL often has a remarkable ability to return to its original pattern after a blink.²⁵ After other blinks, the lipid pattern does change considerably, but it is still expected that characteristics of the TFL, such as its resistance to evaporation, are not consistently degraded by the blink cycle (otherwise extensive degradation would occur after the thousands of blinks that occur during a day).

The ability of meibum to withstand compression/expansion cycles can be studied using a Langmuir trough. A thin layer of meibum is spread on an aqueous sub-phase, and then the "surface pressure" (two-dimensional equivalent of pressure) of the meibum is measured as a function of its surface area as it is compressed and expanded through one or more cycles.³ Meibum is little altered by compression-expansion cycles; thus, for any surface area, surface pressure during expansion is not much different from the surface pressure during compression.³ More importantly, the surface pressure functions for repeated compression-expansion cycles are similar to that for the first cycle,^{3-4,26} indicating that meibum is "respreadable."

Although the TFL is typically thicker than a monomolecular layer,²⁷ insights into its compression-expansion cycles can, nonetheless, be obtained from monolayer studies. The mechanisms involved in compression-expansion cycles of the TFL can be elucidated by studying the “collapse” of monomolecular layers of lipids when they have been compressed so much that there is insufficient space for all the lipid molecules in the monolayer. Some of the lipid molecules must then be expelled from the plane of the monolayer. Monolayer collapse may be reversible (ie, expelled lipids are re-incorporated when the film is expanded again) or it may be irreversible (ie, expelled lipids are not re-incorporated on expansion); examples will be discussed below. As implied above, the collapse of a monolayer of meibum is largely reversible.⁴

Collapse of a lipid monolayer can occur by formation of a bilayer fold perpendicular to the surface, either extending upward into the air (Figure 2A)²⁸ or downward (Figure 2B).²⁹ Collapse may also cause folds that are parallel to the surface, either above (Figure 2C)³⁰ or below (Figure 2D)³¹ the main monolayer. Folds can occur along straight lines,²⁹ or they can have a circular outline.³⁰ Respreading of lung surfactants after collapse is aided by the surfactant proteins, SP-B and SP-C.¹³

A. Some lipids collapse irreversibly on compression and do not return to their original state after expansion

Substances containing saturated hydrocarbon chains tend not to be respreadable in a Langmuir trough study. Thus, in the first compression-expansion cycle, the surface pressure during expansion is often much lower than for the original compression of the same surface area. Moreover, surface pressures in the second and later cycles are often much different from that of the first cycle. Example lipids include stearic acid, cholesteryl palmitate, and dipalmitoyl phosphatidylcholine (DPPC).³ In addition to the changes in surface pressure, the appearance of the lipid layer at the end of the first compression-expansion cycle may be quite different from the original appearance.⁴ If an artificial meibum substitute were composed of lipids that collapsed irreversibly, then the substitute would fail to provide a barrier to evaporation.

B. Other lipids return close to their original states after a compression/expansion cycle

Substances containing *unsaturated* hydrocarbon chains tend to show more respreadability than those containing saturated chains. Thus, in the first compression-expansion cycle, the surface pressure during expansion is usually similar to that during compression for the same surface area. Additionally, surface pressures in the second and later cycles are usually similar to the first cycle. As an example, cholesteryl nervonate, derived from the mono-unsaturated nervonic acid, has much more repeatable compression-expansion cycles than cholesteryl palmitate, derived from the saturated palmitic acid.⁴

In simulations of lung surfactants, lipids containing unsaturated chains (eg, palmitoyl-oleoyl phosphatidylcholine) improve the respreadability of lipids containing only saturated chains, such as DPPC.^{13,15,23,32} It thus is possible that the unsaturated chains in meibum, particularly the fatty acid components of wax esters, which are commonly oleic acid,¹¹ aid in the respreading of the TFL after blinking. Since surfactant proteins SP-B and SP-C aid

in the respreading of lung surfactant during breathing,^{13,15} they may have a similar role for the TFL in blinking.

IV. VISCOSITY OF MEIBUM

Obstructive meibomian gland dysfunction (MGD) may involve an obstruction of the gland orifice and duct by hyperkeratinization,³³ but it may also involve a pathological, more viscous or rigid meibum.³⁴ In young normal subjects, expressed meibum is a clear fluid, but in eyes with advanced MGD, it can appear opaque and thick, like toothpaste.³⁵ Borchman et al have quantified the difference between meibum from MGD and normals by comparing the “phase transition temperatures” above which hydrocarbon chains change from a rigid, ordered “trans” configuration to a more fluid, disordered “gauche” configuration.³⁶ The phase transition temperature was significantly increased from 28.9°C in normals to 32.2°C in MGD. Correspondingly, the degree of chain order was significantly increased in MGD (at 33.4°C, close to the surface temperature of the eye).³⁷ These results indicate that meibum in MGD is typically more rigid or less fluid than in normals. It should be noted, however, that Raman and NMR spectroscopies do not support the suggestion that the increased viscosity observed in meibomian gland dysfunction is due to an increase in the ratio of saturated to unsaturated chains.³⁸⁻³⁹

A. Unsaturation and branching of hydrocarbon chains affect melting temperature of lipids

Lipids containing unsaturated chains melt at lower temperatures than those with saturated chains and therefore tend to be more fluid. Thus, as Table 3 shows, for 18 carbon fatty acids, the melting point of mono-unsaturated oleic acid is about 55°C lower than for the saturated stearic acid. Similarly, branched-chain lipids tend to have lower melting points than the corresponding straight-chain lipids. In the simplest example, the melting point of branched iso-butane is 21°C lower than for the normal, straight-chain butane (Table 3).

B. Meibum contains unsaturated and branched hydrocarbon chains

In human meibum, the majority of fatty acid components in cholesteryl esters and the majority of fatty *alcohol* components in wax esters have saturated hydrocarbon chains.^{11,22} However, 82% of fatty *acid* components of wax esters are unsaturated⁴⁰ of which 90% are oleic acid. Additionally, the majority of the saturated fatty *acid* components in wax esters are branched.⁴⁰ In conclusion, meibum contains both unsaturated and branched hydrocarbon chains, which can be expected to lower the melting point and make it more fluid at the temperature of the lipid layer.³⁶ These considerations have relevance to the development of artificial tears with a lipid supplement – addition of unsaturated and/or branched lipids may help to maintain lipid fluidity.

V. “DEWETTING” OF THE LIPID LAYER WITH EXCESSIVE FLUIDITY OF MEIBUM

A. The normal lipid layer resists “leveling” (smoothing) by surface tension, indicating a gel-like structure

The floating lipid layer, comprised of meibum and other ingredients from elsewhere on the ocular surface,^{11-12,41} is much more viscous than the aqueous layer. Using a capillary tube to estimate the viscosity of meibum, Tiffany and Dart found that it was 10^3 to 10^4 times more viscous than water.⁴²

Observations at relatively low resolution with color interferometry show that the lipid layer may not spread evenly in the presence of MGD or other dry eye conditions.^{1,43,44} High resolution images from King-Smith et al show some of the details of this poor spreading and may suggest at least some causes.⁴⁵ Broadband interferometry from a strobe is used to catch images of lipid layer dynamics during the blink and in subsequent interblinks. In some images, such as those in Figures 5 and 11 from that paper,⁴⁵ it can be clearly seen that there are so-called “droplets” with corners and edges that appear within the lipid layer; these droplets are often quite thick compared to the lipid layer. They may also be quite long-lived, over 10 seconds with no apparent change in aspect. Thus, one may reasonably conclude that these objects are definitely not a Newtonian liquid (like water), and that they may be more like a gel (or “jello”), in that they can support mechanical stresses and not deform like a liquid. We propose that the source of these droplets may be the organized lipid droplets observed in the acinar cells of the meibomian gland as seen in electron micrographs and discussed in Section VI.⁴⁶⁻⁴⁷

B. Dewetting of the lipid layer can occur when the lipid layer is unusually fluid

A healthy lipid layer is generally spread to a fairly uniform thickness rapidly after a blink. In some cases, the rapid spreading does not occur or is very rapidly undone. As depicted in Figures 8 and 9 of King-Smith et al,⁴⁵ for less than 10% of the subjects they observed, the lipid layer was liquid enough that it could dewet and separate into liquid “islands” on the surface of the aqueous layer. The dynamics of this process can be quite rapid. One liquid island shaped like an upper case “C” (shown in their Figure 8⁴⁵) relaxes to a circular outline in a subsequent image that is 0.27s later. The process may begin from a complete or majority coverage of the lipid layer; Figure 13 in that paper shows an image that would correspond to an earlier stage in this dewetting process because much of the lipid is still connected.⁴⁵

Why does this dewetting occur in only some cases? We propose that the cause is an abnormal chemistry of the lipid layer; there appear to be two contributing factors, and either or both may be critical to these cases. The first factor is that such subjects may have lower concentrations of polar lipids or other surface active components of the tear film than are present in healthy subjects. The lack of an adequate amount of these surface active components would hinder spreading of the lipid layer on the aqueous tear film in the first place and fail to assist in maintaining the layer spread. The second factor could be a lack of higher melting point lipids that increase the viscosity of the lipid layer; a lower viscosity of

the lipid layer would enable it to retract more quickly after being spread. Both factors could be present, especially in severe cases, such as those shown in Figures 8, 9, and 13 of King-Smith et al.⁴⁵

VI. CONCLUSIONS – IMPLICATIONS FOR LIPID LAYER STRUCTURE: A MULTILAMELLAR SANDWICH MODEL

Good biological barriers to evaporation involve dense, rigid, two-dimensional arrays of long, saturated hydrocarbon chains (Table 1 and Figure 1). However, monolayers of lipids with saturated chains, such as cholesteryl palmitate, tend to be rigid and poorly respreadable.^{3,4} These observations imply that the TFL should include dense arrays of long, saturated chains, organized in such a way that the overall TFL structure is fluid and not disrupted by blinking. We propose a “multilamellar sandwich model” as a way of satisfying these requirements.

Two possible structures for the lamellae are proposed in Figure 3. In both cases, stacks of lamellae are represented by blue lines running parallel to the TFL plane. Therefore, evaporating water must pass through several lamellae, thus increasing the evaporation resistance compared to a single lamella. The skin lipid barrier has been shown to have a rather similar multilamellar structure by electron microscopy.⁹ Given the saturated hydrocarbon chains in the lamellae, they may be relatively rigid, but the binding between faces of neighboring lamellae may be relatively weak, allowing them to slip over each other during blinking. In Figure 3.A, the lamellae are organized as flat plates. During the compression phase of the blink, the plates may slip between each other, like a deck of cards being shuffled. In Figure 3.B, the lamellae are derived from foldings of one or more larger sheets. In this case, compression during a blink may produce new lamellae by generating additional folds, as may occur during compression of lung surfactants.¹³ Leiske et al have used X-ray reflectivity on meibum spread on saline to demonstrate a multilamellar structure with lamellae parallel to the film surface⁴⁸; this supports the models of Figure 3.

Our model includes the proposal that polar lipids form an interface between nonpolar lipids and the aqueous layer.²² The red lines in Figure 3 represent polar lipids such as (O-acyl) omega hydroxy fatty acids^{11,12} and phospholipids.¹⁴ The polar lipids have been represented as a folded sheet, and additional folds could be generated during blinking. Folds are shown both above and below the main monolayer surface, as in Figures 2.C and D, respectively, but it is possible that only one of these types could exist.

Possible molecular arrangements of cholesteryl and wax esters in the nonpolar lamellae are shown in Figure 4. It is proposed that the lamellae are in the form of “sandwiches” made from “interdigitated bilayers,”⁴⁹ where the center of the lamellar thickness consists of a dense array of the long, saturated hydrocarbon chains of cholesteryl and wax esters. The proposed structure is similar to the “sandwich model” of the skin lipid barrier, in that the core of the sandwich consists of densely packed, saturated chains providing the evaporation resistance.⁵⁰ In Figure 4.A, the interdigitation of saturated fatty acid chains of cholesteryl esters is similar to that in cholesteryl ester films deduced by Alonso et al,⁴⁹ and is also consistent with the “bilayer” type of structure observed in crystals of long-chain, saturated

cholesteryl esters.⁵¹ Rectangles in Figure 4.A represent the cholesterol rings, while the side chain of cholesterol is represented by short lines at top and bottom of the lamellae. The long, saturated chains have a cross-sectional area of 0.18 to 0.2 nm², whereas the cross section of the cholesterol ring structure is 0.38 nm² – about twice as large⁴⁹; thus, the interdigitated structure of Figure 4.A, where a section through the saturated chains contains twice as many molecular cross-sections as a section through the cholesterol rings, is a compact, stable form. The long saturated chains slope at an angle of 24° corresponding to the molecular tilt observed in meibum films by grazing incidence X-ray diffraction.⁴⁸

For a cholesteryl ester based on typical saturated fatty acid chain lengths of 24-27 carbon atoms,⁵² the total thickness of this lamella would be about 5.5-6 nm. However, this value might be reduced if there was bending of the cholesterol side chains. It may be noted that small angle X-ray scattering of bulk human meibum⁵³ indicates multilamellar structures with a lamellar thickness of 4.9 nm, and multilamellar structures of a similar thickness have been demonstrated in meibum spread on saline.⁴⁸ It may also be noted that a small quantity of human meibum spread on saline at 20°C (20 µg over 80 cm²) forms a uniform layer (with included “lakes”) of about 5.2 nm thick.⁴ For a TFL lipid layer thickness of 40-50 nm,^{27,54} there could thus be about 10 lamellae, as in the models of Figure 3. Given the considerable variation in TFL thickness,²⁷ there could be a corresponding variation in the number of lamellae.

A possible corresponding interdigitated bilayer of wax esters is proposed in Figure 4.B, where, in this case, the long, saturated chains come from the alcohol components; connecting oxygen atoms are given as circles, and unsaturated fatty acids (eg, oleic) are represented at top and bottom. The overall structure of a lamella might consist of “domains” of cholesteryl and wax esters (Figure 3.C), or a dense intermixing of the two ester molecules (Figure 3.D). It is also conceivable that cholesteryl and wax ester might be arrayed on opposite sides of the interdigitated chains (Figure 3.E). (It should be noted that, at a molecular level, the lamellae are three-dimensional structures and so are difficult to represent on a two-dimensional figure; thus, for example, the bent tails of the wax ester acids, which appear to collide with cholesterol side chains in Figures 3.C and D, could actually extend into the third dimension, in or out of the figure.) An important aspect of these lamellae might be that, whereas the long, saturated chains are bound strongly together to form a good barrier to water penetration, the outer surfaces of neighboring layers will not have such strong, regular binding and so may readily slip over each other during the blink. The unsaturated fatty acids of wax esters, and the iso-branching of the cholesterol side chain, which are both at the lamellar surfaces, may help to increase fluidity in the interface region between two lamellae (cf. Table 3).

It is possible that the lamellae in Figure 4 could form spontaneously by self-assembly. For example, McIntosh et al showed that the *in vivo* structure of the skin lipid layer, with a laminar repeat period of 13.0 nm, may be replicated *in vitro* by a mixture of ceramides, cholesterol and fatty acid in proportions similar to those found *in vivo* (other mixtures deviated from the *in vivo* structure).⁵⁵

An alternative origin of the nonpolar lamellae in Figure 4 may be proposed. Sirigu et al used freeze-fracture electron microscopy to study “fat droplets” in human meibomian glands, which are the main source of meibum lipid.⁴⁷ These fat droplets were seen to have an onion-like structure of very thin lamellae (perhaps similar in thickness to the proposed lamellae). Jester et al used transmission electron microscopy to show how the fat droplets (or “meibomian vesicles”) are generated by concentric layers of smooth endoplasmic reticulum which surround the droplets.⁴⁶ It thus seems possible that the proposed lamellae are derived from the lamellae of the fat droplets in the meibomian glands and that their structure, as in Figure 4, is generated and controlled by the smooth endoplasmic reticulum.

A remarkable finding about the evaporation resistance of the TFL (Appendix and Table 2) is that it has not been possible to reproduce it in vitro by spreading meibum on saline.^{56,57} Similarly, artificial TFL compositions have little or no effect on evaporation.^{58,59} Herok et al therefore proposed that prevention of evaporation by the TFL requires a complex interaction between components of aqueous tears and meibum.⁵⁷ The in vitro evaporation resistance of meibum may also be readily disturbed by experimental procedures. In this respect, it may be noted that high evaporation resistance is difficult to achieve even in laboratory conditions. For example, in determining the evaporation resistance of a monolayer of a saturated fatty acid, the measured value increased by up to a factor of 20 with improved experimental procedure.⁶⁰ Particularly relevant to the TFL is the finding that low concentrations (eg, 0.0125 molar fraction) of cholesterol can greatly reduce the evaporation resistance of a monolayer of octadecanol⁶¹; it seems possible that breakdown of cholesteryl esters in the TFL by bacterial esterases could cause a similar reduction of the evaporation resistance of the TFL.

It should be emphasized that the current model is proposed as a basis for discussion, and the model will need further development and analysis. The role of less common lipids, such as triglycerides,^{11,12} needs to be considered. Additionally, studies of wax ester structures^{62,63} have not shown the interdigitation of chains in Figure 4.B, so the origin of such an organization of wax esters needs to be considered; eg, can the known interdigitated structure of cholesteryl esters in Figure 4.A^{49,51} form a “framework” for a similar interdigitation of wax esters in Figure 4.B?

In summary, the proposed lamellar structure of the TFL avoids the potential incompatibility between the rigid arrays of long saturated chains needed for evaporation resistance and the fluidity required for respreading of the TFL after a blink. The rigid evaporation barrier forms the central thickness of the nonpolar lamellae, while the surfaces of these lamellae allow easy slippage and provide the fluidity needed for respreading the TFL. It would have an important clinical implication if the lamellae were disrupted by factors such as biosynthetic anomalies in meibomian glands,⁶⁴ inclusion of keratin proteins,⁶⁵ structural disorder associated with TFL deposition on contact lenses,⁶⁶ or the ocular surface, bacterial lipases⁶⁷ and oxidative stress.⁶⁸ Such lamellar disruption could lead to increased evaporation, hyperosmolarity and dry eye.

Acknowledgments

Financial support: R01 EY017951 (King-Smith), NSF 0616483 and 1022706 (Braun).

VII. APPENDIX: EVAPORATION RESISTANCE OF THE TEAR FILM LIPID LAYER

In this appendix, rough estimates of the evaporation resistance of the lipid layer of the tear film are given, so that the values obtained can be compared with published values for lipid monolayers used in evaporation control.

A useful analogy to evaporation from the tear film is the flow of electric current through a chain of resistors. For a given potential difference between the ends of the chain, the current is given by Ohm's Law, namely:

Current = (potential difference) / (total resistance).

Correspondingly, evaporation rate is given by a similar equation^{21,69}:

$$Flux = (water \ vapor \ concentration \ difference) / (total \ evaporation \ resistance), \quad (A1)$$

where flux is the rate of flow of water per unit area, in g/(s.cm²), "water vapor concentration difference" is the difference between saturated water vapor concentration at the anterior eye temperature and the concentration for room air, in g/cm³, and "total evaporation resistance" is the sum of resistances for the lipid film and the overlying air; units of evaporation resistance are s/cm (reciprocal velocity).

In algebraic terms, Equation A1 may be written

$$F_{LA} = (C_E - \rho C_A) / (r_L + r_A) \quad (A2)$$

where F_{LA} is flux through lipid and air layers, C_E is saturated water vapor concentration at the anterior eye temperature, r_L is the evaporation resistance of the lipid layer, r_A is the evaporation resistance of the overlying air, ρ is fractional relative humidity of room air and C_A is saturated water vapor concentration at room air temperature (thus ρC_A is the water vapor concentration of room air).

The evaporation resistance of the lipid layer can be derived as follows. Equation A2 can be re-written

$$r_L = (C_E - \rho C_A) / F_{LA} - r_A \quad (A3)$$

The evaporation resistance of the overlying air, r_A , can be derived from Equation A2 by setting $r_L=0$,

$$r_A = (C_E - \rho C_A) / F_A \quad (A4)$$

where F_A is the corresponding evaporative flux (i.e., no lipid resistance).

Substituting this expression into Equation A3 gives

$$r_L = (C_E - \rho C_A) (1/F_{LA} - 1/F_A) \quad (A5)$$

Equation A5 shows that estimation of lipid resistance requires knowledge of five quantities: 1/. evaporative flux when lipid is present, F_{LA} ; 2 and 3/. eye and room air temperatures (hence determining saturated water vapor concentration at these temperatures, C_E and C_A); 4/. relative humidity of room air, ρ ; 5/. flux from a lipid free surface, F_A . In practice, not all these quantities are usually well known.

Mishima and Maurice measured evaporative flux from the rabbit tear film before and after removal of the lipid layer allowing a relatively complete estimate of lipid evaporation resistance using Equation A5.⁷⁰ Measured values of flux were:

$$F_{LA} = 7.78 \times 10^{-7} \text{ g/(s.cm}^2\text{)}$$

$$F_A = 1.18 \times 10^{-5} \text{ g/(s.cm}^2\text{)}$$

Other parameters in Equation A5 were not recorded, so the following values were assumed, based on an equation for saturated water vapor density⁷¹:

$$C_E = 3.97 \times 10^{-5} \text{ g/cm}^3 \text{ based on anterior eye temperature of } 35^\circ\text{C}^{37}$$

$$C_A = 1.73 \times 10^{-5} \text{ g/cm}^3 \text{ for assumed room temperature of } 20^\circ\text{C}$$

$$\rho = 0.5 \text{ (i.e., 50\% assumed relative humidity)}$$

Substituting all the above 5 values into Equation A5 gives a lipid resistance of 37.3 s/cm. This value, of course, depends on the assumed values of temperature and relative humidity. For example, an increase in anterior eye temperature of 1°C would increase the calculated lipid resistance by 6.8%, and increase in room temperature of 1°C would reduce the resistance by 1.7%, and an increase in humidity of 10% would reduce the resistance by 5.6%.

Iwata et al estimated the evaporation resistance of the lipid layer of the rabbit tear film to be 12.9 s/cm, only 35% of the above value.⁶⁹ Their procedure, which involved gluing a plastic chamber to the cornea, seems more invasive than the above method. It may also be noted that disturbance of the lipid layer of the tear film is likely to reduce evaporation resistance. Both these observations indicate that the higher resistance calculated above is closer to the true value. However, it is certainly possible that the higher value is an overestimate, eg, if the eye temperature was less than 35°C . It may be noted again that only approximate values of lipid evaporation resistance are needed here, for order-of-magnitude comparison with corresponding values for monolayers used for evaporation control.

From a review of published values, Tomlinson et al found an average evaporation rate for normal human eyes of $F_{LA} = 13.57 \times 10^{-7} \text{ g/(s.cm}^2\text{)}$.⁷² If the other four parameters in Equation A5 have the values given above, then evaporation resistance, r_L , would be 20.2 s/cm. King-Smith et al have argued that published evaporation rates are underestimates because of the restriction of air flow over the cornea by the pre-ocular chambers used in the measurements⁷³; it has been proposed that the thinning rate of the tear film between blinks may be a more accurate measure of evaporation rate which does not suffer from this restriction of air flow.⁷⁴ While a histogram of thinning rates⁷⁵ is a skewed distribution with a few cases of very rapid thinning, thus increasing the mean rate, the most common thinning rates give an evaporation rate similar to Tomlinson's value above, which will therefore be retained for comparison with monolayer studies.

REFERENCES

1. Tomlinson A, Bron AJ, Korb DR, et al. The international workshop on meibomian gland dysfunction: report of the diagnosis subcommittee. *Invest Ophthalmol Vis Sci.* 2011; 52:2006–49. [PubMed: 21450918]
2. Rosano HL, Lamer VK. The rate of evaporation of water through monolayers of esters, acids and alcohols. *J Phys Chem.* 1956; 60:348–53.
3. Mudgil P, Millar TJ. Surfactant properties of human meibomian lipids. *Invest Ophthalmol Vis Sci.* 2011; 52:1661–70. [PubMed: 21051693]
4. Millar TJ, King-Smith PE. Analysis of comparison of human meibomian lipid films and mixtures with cholesteryl esters in vitro films using high resolution color microscopy. *Invest Ophthalmol Vis Sci.* 2012; 53:4710–9. [PubMed: 22695957]
5. Rantamaki AH, Telenius J, Koivuniemi A, et al. Lessons from the biophysics of interfaces: lung surfactant and tear fluid. *Prog Retin Eye Res.* 2011; 30:204–15. [PubMed: 21352946]
6. Gibbs AG. Water-proofing properties of cuticular lipids. *Am Zool.* 1998; 38:47182.
7. Kunst L, Samuels AL. Biosynthesis and secretion of plant cuticular wax. *Prog Lipid Res.* 2003; 42:51–80. [PubMed: 12467640]
8. Madison KC. Barrier function of the skin: "la raison d'etre" of the epidermis. *J Invest Dermatol.* 2003; 121:231–41. [PubMed: 12880413]
9. Madison KC, Swartzendruber DC, Wertz PW, Downing DT. Presence of intact intercellular lipid lamellae in the upper layers of the stratum corneum. *J Invest Dermatol.* 1987; 88:714–8. [PubMed: 3585055]
10. McClanahan LL, Stinner JN, Shoemaker VH. Skin lipids, water loss, and energy metabolism in a South American tree frog (*Phyllomedusa sauvagei*). *Physiol Zool.* 1978; 51:179–87.
11. Butovich IA. Lipidomics of human Meibomian gland secretions: Chemistry, biophysics, and physiological role of Meibomian lipids. *Prog Lipid Res.* 2011; 50:278–301. [PubMed: 21458488]
12. Chen J, Green-Church KB, Nichols KK. Shotgun lipidomic analysis of human meibomian gland secretions with electrospray ionization tandem mass spectrometry. *Invest Ophthalmol Vis Sci.* 2010; 51:6220–31. [PubMed: 20671273]
13. Perez-Gil J. Structure of pulmonary surfactant membranes and films: the role of proteins and lipid-protein interactions. *Biochim Biophys Acta.* 2008; 1778:1676–95. [PubMed: 18515069]
14. Rantamaki AH, Seppanen-Laakso T, Oresic M, et al. Human tear fluid lipidome: from composition to function. *PLoS One.* 2011; 6:e19553. [PubMed: 21573170]
15. Zuo YY, Veldhuizen RA, Neumann AW, et al. Current perspectives in pulmonary surfactant--inhibition, enhancement and evaluation. *Biochim Biophys Acta.* 2008; 1778:1947–77. [PubMed: 18433715]
16. Brauer L, Johl M, Borgermann J, et al. Detection and localization of the hydrophobic surfactant proteins B and C in human tear fluid and the human lacrimal system. *Curr Eye Res.* 2007; 32:931–8. [PubMed: 18027169]

17. Barnes, GT.; LaMer, VK.; LaMer, VK. Retardation of Evaporation by Monolayers; Transport Processes. Academic Press; New York, NY: 1962. The evaporation resistance of monolayers of long-chain acids and alcohols and their mixtures; p. 9-33.
18. Langmuir I, Schaefer VJ. Rates of evaporation of water through compressed monolayers on water. J Frankl Inst. 1943; 235:119–62.
19. Harkins WD. A general thermodynamic theory of the spreading of liquids to form duplex films and of liquids or solids to form monolayers. J Chem Phys. 1941; 9:552–68.
20. Jarvis, NL.; Timmons, CO.; Zisman, WA.; LaMer, VK. Retardation of Evaporation by Monolayers: Transport Processes. Academic Press; New York, NY: 1962. The effect of monomolecular films on the surface temperature of water; p. 41-58.
21. Archer RJ, LaMer VK. The rate of evaporation of water through fatty acid monolayers. J Phys Chem. 1955; 59:200–8.
22. McCulley JP, Shine W. A compositional based model for the tear film lipid layer. Trans Am Ophthalmol Soc. 1997; 95:79–88. discussion 88-93. [PubMed: 9440164]
23. Notter, RH. Basic Science and Clinical Applications. Marcel Decker; New York, NY: 2000. Lung Surfactants.
24. Holly FJ. Physical chemistry of the normal and disordered tear film. Trans Ophthalmol Soc U K. 1985; 104:374–80. Pt 4. [PubMed: 3862270]
25. Bron AJ, Tiffany JM, Gouveia SM, et al. Functional aspects of the tear film lipid layer. Exp Eye Res. 2004; 78:367–70. [PubMed: 15106914]
26. Leiske DL, Raju SR, Ketelson HA, et al. The interfacial viscoelastic properties and structures of human and animal Meibomian lipids. Exp Eye Res. 2010; 90:598–604. [PubMed: 20156438]
27. King-Smith PE, Hinel EA, Nichols JJ. Application of a novel interferometric method to investigate the relation between lipid layer thickness and tear film thinning. Invest Ophthalmol Vis Sci. 2010; 51:2418–23. [PubMed: 20019370]
28. Ries HE, Swift H. Twisted double-layer ribbons and the mechanism for monolayer collapse. Langmuir. 1987; 3:853–5.
29. Ybert C, Lu WX, Moller G, Knobler CM. Collapse of a monolayer by three mechanisms. J Phys Chem B. 2002; 106:2004–8.
30. Schief WR, Antia M, Discher BM, et al. Liquid-crystalline collapse of pulmonary surfactant monolayers. Biophys J. 2003; 84:3792–3806. [PubMed: 12770885]
31. Schurch S, Green FHY, Bachofen H. Formation and structure of surface films: captive bubble surfactometry. Biochim Biophys Acta. 1998; 1408:180–202. [PubMed: 9813315]
32. Wang ZD, Hall SB, Notter RH. Dynamic surface-activity of films of lung surfactant phospholipids, hydrophobic proteins, and neutral lipids. J Lipid Res. 1995; 36:1283–93. [PubMed: 7666006]
33. Jester JV, Nicolaidis N, Kisspalvolgyi I, Smith RE. Meibomian gland dysfunction . 2. The role of keratinization in a rabbit model of MGD. Invest Ophthalmol Vis Sci. 1989; 30:936–45. [PubMed: 2470694]
34. Knop E, Knop N, Millar T, et al. The International Workshop on Meibomian Gland Dysfunction: Report of the Subcommittee on Anatomy, Physiology, and Pathophysiology of the Meibomian Gland. Invest Ophthalmol Vis Sci. 2011; 52:1938–78. [PubMed: 21450915]
35. Foulks GN, Bron AJ. Meibomian gland dysfunction: a clinical scheme for description, diagnosis, classification, and grading. Ocul Surf. 2003; 1:107–26. [PubMed: 17075643]
36. Borchman D, Foulks GN, Yappert MC, et al. Human meibum lipid conformation and thermodynamic changes with meibomian gland dysfunction. Invest Ophthalmol Vis Sci. 2011; 52:3805–17. [PubMed: 21398284]
37. Purslow C, Wolffsohn JS. Ocular surface temperature: a review. Eye Contact Lens. 2005; 31:117–23. [PubMed: 15894878]
38. Foulks GN, Borchman D, Yappert M, Kakar S. Topical azithromycin and oral doxycycline therapy of meibomian gland dysfunction: A comparative clinical and spectroscopic pilot study. Cornea. 2013; 32:44–53. [PubMed: 22668581]
39. Oshima Y, Sato H, Zaghoul A, et al. Characterization of human meibum lipid using Raman spectroscopy. Curr Eye Res. 2009; 34:824–35. [PubMed: 19895310]

40. Butovich IA, Arciniega JC, Lu H, Molai M. Evaluation and quantitation of intact wax esters of human meibum by gas-liquid chromatography-ion trap mass spectrometry. *Invest Ophthalmol Vis Sci.* 2012; 53:3766–81. [PubMed: 22531701]
41. Green-Church KB, Butovich I, Willcox M, et al. The International Workshop on Meibomian Gland Dysfunction: Report of the Subcommittee on Tear Film Lipids and Lipid-Protein Interactions in Health and Disease. *Invest Ophthalmol Vis Sci.* 2011; 52:1979–93. [PubMed: 21450916]
42. Tiffany JM, Dart JKG. Normal and abnormal functions of meibomian secretion. *R Soc Med Int Congr Symp.* 1981; 40:1061–4.
43. Danjo Y, Hamano T. Observation of precorneal tear film in patients with Sjogren's syndrome. *Acta Ophthalmol Scand.* 1995; 73:501–5. [PubMed: 9019372]
44. Di Pascuale MA, Goto E, Tseng SCG. Sequential changes of lipid tear film after the instillation of a single drop of a new emulsion eye drop in dry eye patients. *Ophthalmology.* 2004; 111:783–91. [PubMed: 15051213]
45. King-Smith PE, Nichols JJ, Braun RJ, Nichols KK. High resolution microscopy of the lipid layer of the tear film. *Ocul Surf.* 2011; 9:197–211. [PubMed: 22023815]
46. Jester JV, Nicolaidis N, Smith RE. Meibomian gland studies: histologic and ultrastructural investigations. *Invest Ophthalmol Vis Sci.* 1981; 20:537–47. [PubMed: 7194327]
47. Sirigu P, Shen RL, Pinto da Silva P. Human meibomian glands: the ultrastructure of acinar cells as viewed by thin section and freeze-fracture transmission electron microscopies. *Invest Ophthalmol Vis Sci.* 1992; 33:2284–92. [PubMed: 1607240]
48. Leiske DL, Miller CE, Rosenfeld L, et al. Molecular structure of interfacial human meibum films. *Langmuir.* 2012; 28:11858–65. [PubMed: 22783994]
49. Alonso C, Kuzmenko I, Jensen TR, et al. Self-assembly of crystalline films of interdigitated long-chain cholesteryl esters at the air-water interface. *J Phys Chem B.* 2001; 105:8563–68.
50. Bouwstra JA, Honeywell-Nguyen PL, Gooris GS, Poncet M. Structure of the skin barrier and its modulation by vesicular formulations. *Prog Lipid Res.* 2003; 42:1–36. [PubMed: 12467638]
51. Ginsburg GS, Atkinson D, Small DM. Physical properties of cholesteryl esters. *Prog Lipid Res.* 1984; 23:135–67. [PubMed: 6399750]
52. Butovich IA. Fatty acid composition of cholesteryl esters of human meibomian gland secretions. *Steroids.* 2010; 75:726–33. [PubMed: 20470811]
53. Leiske D, Leiske C, Toney M, et al. Temperature-induced transitions in the structure and interfacial rheology of human meibum. *Biophys J.* 2012; 102:369–76. [PubMed: 22339874]
54. Olsen T. Reflectometry of the precorneal film. *Acta Ophthalmol (Copenh).* 1985; 63:432–8. [PubMed: 4050364]
55. McIntosh TJ, Stewart ME, Downing DT. X-ray diffraction analysis of isolated skin lipids: reconstitution of intercellular lipid domains. *Biochemistry.* 1996; 35:3649–53. [PubMed: 8619983]
56. Brown SI, Dervichian DG. The oils of the meibomian glands. Physical and surface characteristics. *Arch Ophthalmol.* 1969; 82:537–40. [PubMed: 5344949]
57. Herok GH, Mudgil P, Millar TJ. The effect of Meibomian lipids and tear proteins on evaporation rate under controlled in vitro conditions. *Curr Eye Res.* 2009; 34:589–97. [PubMed: 19899972]
58. Borchman D, Foulks GN, Yappert MC, et al. Factors affecting evaporation rates of tear film components measured in vitro. *Eye Contact Lens.* 2009; 35:32–7. [PubMed: 19125046]
59. Rantamaki AH, Javanainen M, Vattulainen I, Holopainen JM. Do lipids retard the evaporation of the tear fluid? *Invest Ophthalmol Vis Sci.* 2012; 53:6442–7. [PubMed: 22930719]
60. Archer RJ, LaMer VK. The effect of monolayers on the rate of evaporation of water. *Ann NY Acad Sci.* 1954; 58:807–29.
61. McNamee CE, Barnes GT, Gentle IR, et al. The evaporation resistance of mixed monolayers of octadecanol and cholesterol. *J Colloid Interf Sci.* 1998; 207:258–63.
62. Kreger DR, Schamhart C. On the long crystal-spacings in wax esters and their value in micro-analysis of plant cuticle waxes. *Biochim Biophys Acta.* 1956; 19:22–44. [PubMed: 13304067]
63. Simpson TD, Miwa TK. X-ray Sstudy of hydrogenated Jojoba wax. *J Am Oil Chem Soc.* 1977; 54:54–8.

64. Shine WE, McCulley JP. Association of meibum oleic acid with meibomian seborrhea. *Cornea*. 2000; 19:72–4. [PubMed: 10632012]
65. Jester JV, Nicolaides N, Smith RE. Meibomian gland dysfunction .1. Keratin protein expression in normal human and rabbit meibomian glands. *Invest Ophthalmol Vis Sci*. 1989; 30:927–35. [PubMed: 2470693]
66. Lorentz H, Jones L. Lipid deposition on hydrogel contact lenses: how history can help us today. *Optom Vis Sci*. 2007; 84:286–95. [PubMed: 17435512]
67. Dougherty JM, McCulley JP. Bacterial lipases and chronic blepharitis. *Invest Ophthalmol Vis Sci*. 1986; 27:486–91. [PubMed: 3957566]
68. Dogru M, Wakamatsu T, Kojima T, et al. The role of oxidative stress and inflammation in dry eye disease. *Cornea*. 2009; 28:S70–S74.
69. Iwata S, Lemp MA, Holly FJ, Dohlman CH. Evaporation rate of water from the precorneal tear film and cornea in the rabbit. *Invest Ophthalmol*. 1969; 8:613–9. [PubMed: 5359578]
70. Mishima S, Maurice DM. The oily layer of the tear film and evaporation from the corneal surface. *Exp Eye Res*. 1961; 1:39–45. [PubMed: 14474548]
71. Wagner W, Pruss A. International equations for the saturation properties of ordinary water substance - revised according to the International Temperature Scale of 1990 (Vol 16, Pg 893, 1987). *J Phys Chem Ref Data*. 1993; 22:783–7.
72. Tomlinson A, Doane MG, McFadyen A. Inputs and outputs of the lacrimal system: review of production and evaporative loss. *Ocul Surf*. 2009; 7:17–29.
73. King-Smith PE, Nichols JJ, Nichols KK, et al. Contributions of evaporation and other mechanisms to tear film thinning and breakup: a review. *Optom Vis Sci*. 2008; 85:623–30. [PubMed: 18677230]
74. Kimball SH, King-Smith PE, Nichols JJ. Evidence for the major contribution of evaporation to tear film thinning between blinks. *Invest Ophthalmol Vis Sci*. 2010; 51:6294–7. [PubMed: 20688724]
75. Nichols JJ, Mitchell GL, King-Smith PE. Thinning rate of the precorneal and prelens tear films. *Invest Ophthalmol Vis Sci*. 2005; 46:2353–61. [PubMed: 15980222]

Figure 1.

Evaporation resistance as a function of carbon chain length for saturated fatty acids at 25°C. An exponential curve has been fitted to the experimental results. (Redrawn from Archer and LaMer.²¹)

Figure 2.

Collapse of lipid monolayers. Circles represent polar headgroups of lipid molecules whereas attached lines represent hydrophobic hydrocarbon chains. A. Upward folding towards the air. B. Downward folding towards the aqueous sub-phase. C. A parallel fold above the main monolayer surface. D. A parallel fold below the main monolayer surface.

Figure 3.

Possible structures of the tear film lipid layer. Red lines represent polar lamellae and blue lines represent nonpolar lamellae. A. “Plate” model of nonpolar lamellae. B. Folded model of nonpolar lamellae. See text for details.

Figure 4.

Possible molecular arrangement of cholesteryl and wax esters in the proposed nonpolar lamellae. For both cholesteryl and wax esters, the center of the lamellar thickness consists of interdigitated long, saturated chains. A. Cholesteryl esters. Long, saturated chains come from the fatty acid component. Rectangles correspond to the cholesterol rings, and the cholesterol side chain is represented by short lines at top and bottom. B. Wax esters. Long, saturated chains come from the alcohol component. The combining oxygen atoms are represented by circles, while unsaturated fatty acids (eg, oleic) are represented by kinked chains at top and bottom. C. Overall structure consisting of “domains” of cholesteryl and wax esters. D. An alternative overall structure where cholesteryl and wax esters are densely mixed on a molecular scale. E. A structure in which cholesteryl and wax esters are arrayed on opposite sides of the interdigitated chains.

Table 1

Comparison of different biological lipid layers by function and major components

Biological lipid layer	Respreadable?	Evaporative barrier?	Major components	Saturated hydrocarbon chain length 19?	References
Tear film lipid layer (TFLL)	Yes	Yes	Wax esters Cholesteryl esters Polar lipids Surfactant proteins	Yes	11,12,22
Lung surfactant	Yes	No	Phospholipids Surfactant proteins	No	13,15
Human skin lipid	No	Yes	Ceramides Cholesterol Fatty acids	Yes	98
Skin lipid secretions (tree frog)	No	Yes	Wax esters Triglycerides	Yes	10
Plant lipid layer (cuticle)	No	Yes	Hydrocarbons Ketones Alcohols	Yes	7
Arthropod lipid layer	No	Yes	Hydrocarbons	Yes	6

Table 2

Evaporation resistance of tear film lipid layers and monolayers

Layer	Evaporation resistance, s/cm	Reference
Rabbit lipid layer [#]	37.3	59
Rabbit lipid layer	12.9	58
Human lipid layer [#]	20.2	61
Hexadecanol, C ₁₆ H ₃₃ OH [#]	0.70	17
Octadecanol, C ₁₈ H ₃₇ OH [#]	1.73	17

[#] See Appendix^{*} For 35°C and a surface pressure of 30mN/m on distilled water, based on the equations of Barnes and La Mer¹⁷

Table 3

Melting temperatures of unsaturated and branched-chain substances

Molecule	formula	type	Melting point
Stearic acid	C ₁₇ H ₃₅ COOH	Saturated	71.5°C
Oleic acid	C ₁₇ H ₃₃ COOH	Mono-unsaturated	16°C
N-butane	C ₄ H ₁₀	Straight-chain	−138°C
Iso-butane	C ₄ H ₁₀	Iso-branched	−159°C