Do Lipids Retard the Evaporation of the Tear Fluid?

Antti H. Rantamäki,[1] *Matti Javanainen,*[2] *Ilpo Vattulainen,*[2–4] *and Juha M. Holopainen*[1]

Purpose. We examined in vitro the potential evaporation-retarding effect of the tear film lipid layer (TFLL). The artificial TFLL compositions used here were based on the present knowledge of TFLL composition.

Methods. A custom-built system was developed to measure evaporation rates at 35°C. Lipids were applied to an air-water interface, and the evaporation rate through the lipid layer was defined as water loss from the interface. A thick layer of olive oil and a monolayer of long-chain alcohol were used as controls. The artificial TFLLs were composed of 1 to 4 lipid species: polar phosphatidylcholine (PC), nonpolar cholesteryl ester, triglycerides, and wax ester (WE). Brewster angle microscopy (BAM) and interfacial shear rheometry (ISR) were used to assess the lateral structure and shear stress response of the lipid layers, respectively.

Results. Olive oil and long-chain alcohol decreased evaporation by 54% and 45%, respectively. The PC monolayer and the four-component mixtures did not retard evaporation. WE was the most important evaporation-retardant TFLL lipid (~20% decrease). In PC/WE mixtures, an ~90% proportion of WE was required for evaporation retardation. Based on BAM and ISR, WE resulted in more condensed layers than the non-retardant layers.

Conclusions. Highly condensed, solid-like lipid layers, such as those containing high proportions of WEs, are evaporation-retardant. In multi-component lipid layers, the evaporation-retardant interactions between carbon chains decrease and, therefore, these lipid layers do not retard evaporation. (*Invest Ophthalmol Vis Sci.* 2012;53:6442–6447) DOI:10.1167/iovs.12-10487

Tear fluid forms a thin film over the exposed ocular surface. The main functions of this film are to protect the cornea and to lubricate the eye. The tear film can be divided roughly into two distinct layers: an aqueous layer, with dissolved proteins, metabolites, and electrolytes; and a lipid layer, consisting of several differing lipid species.[1–4] Because lipids dissolve poorly in water, the high-energy state of lipid molecules in aqueous solutions drives individual lipid molecules into low-energy lipid aggregates, such as micelles and liposomes. The form of spontaneously formed lipid aggregates is determined mainly by hydrophobic, electrostatic, and steric forces between neighboring molecules.[5] One of the suggested functions of the tear film lipid layer (TFLL) is retarding evaporation, but the experimental evidence for this function is scarce.[6] The defective secretion of tear film lipids potentially can lead to faster evaporation of water from the aqueous layer.

The properties influencing the evaporation-retarding effects of simple lipid monolayers are well known.[7] The compressibility of the lipid monolayer affects the ability of the layer to retard evaporation.[8] The more incompressible a monolayer, the higher its retardation of evaporation. Typically, monolayers composed of polar lipids, such as fatty alcohols and fatty acids, which have a small head group and long saturated carbon chain, are rather incompressible and, therefore, are good water retardants. A maximum of a ~60% decrease in evaporation rate has been achieved outside laboratory-controlled conditions (i.e., in lakes and ponds) using long-chain alcohols.[9] The ability to retain water is due to the Van der Waals interactions among hydrophobic carbon chains. Furthermore, these incompressible lipid layers have very low free volumes, preventing diffusion of small molecules through the layers.[10] An increase in surface pressure increases the retardation of evaporation by decreasing the chain tilt and, therefore, increasing the thickness of the layer.[11] To a certain extent, evaporation-retardant lipids can be mixed with non-retardant lipids and still hold their ability to retard evaporation.[8] No comprehensive studies, however, exist to our knowledge regarding the evaporation-retarding effects of multicomponent lipid mixtures.

The composition of meibum, an oily secretion produced by the meibomian glands lining the lid margins, has been studied extensively.[12] Meibum mainly contains nonpolar wax esters, cholesteryl esters, and triglycerides, and only minute amounts of polar lipids, such as phospholipids. The composition of meibum, however, does not resemble the one of the TFLL. Based on our research and on other recent studies, the lipid layer also contains significant amounts of phospholipids, such as phosphatidylcholine and phosphatidylethanolamine, accompanied by meibomian lipids.[1–4] A mixture of polar and nonpolar lipids forms a layered structure, in which the polar lipids at the air-tear interface provide a suitable interface for the spreading of nonpolar lipids.[2,13,14] This layered structure is believed to retard the evaporation of water from the ocular surface. Evaporation of water from the ocular surface has been studied in vivo[15–33] and to a lesser extent in vitro.[34,35] Although lipids seem to have a role in evaporation in vivo, the same effect should apply to in vitro models. It should be emphasized that simpler systems (in vitro) are less prone to systematic and technical problems. Therefore, if tear fluid evaporation is dependent on lipid layer structure, this relationship also should be observed in model systems.

The purpose of our study was to provide novel information on the chemical and physical properties of multicomponent lipid layers that should be considered when assessing the

From the [1]Helsinki Eye Lab, Department of Ophthalmology, University of Helsinki, Helsinki, Finland; the [2]Department of Physics, Tampere University of Technology, Tampere, Finland; the [3]Department of Applied Physics, Aalto University School of Science and Technology, Helsinki, Finland; and the [4]MEMPHYS Centre for Biomembrane Physics, University of Southern Denmark, Odense, Denmark.

Supported by The Finnish Eye Foundation, the Academy of Finland, and the Sigrid Juselius Foundation.

Submitted for publication June 28, 2012; revised August 11 and August 23, 2012; accepted August 23, 2012.

Disclosure: **A.H. Rantamäki**, None; **M. Javanainen**, None; **I. Vattulainen**, None; **J.M. Holopainen**, None

Corresponding author: Juha M. Holopainen, Helsinki Eye Lab, Department of Ophthalmology, University of Helsinki, PO Box 220, 00290 HUS, Finland; juha.holopainen@hus.fi.

Investigative Ophthalmology & Visual Science, September 2012, Vol. 53, No. 10

potential evaporation-retarding effect of TFLL. We explored the potential evaporation-retarding effects of lipid layers that resemble closely TFLL, using a custom-built system. In addition, we used Brewster angle microscopy and interfacial shear rheometry to assess the structure and shear stress response of the lipid layers.

Methods

L-α-phosphatidylcholine from egg yolk (PC), cholesteryl oleate (CE), behenyl oleate (WE), behenyl alcohol (BA), and bovine submaxillary gland mucin (BSGM) were purchased from Sigma-Aldrich (St. Louis, MO); triglyceride mixture (TG), containing 20 mass-% of each of the 8:0, 10:0, 12:0, 14:0, and 16:0 compositions, from Supelco (Bellemonte, PA); and bovine serum albumin (BSA), from MP Biomedicals (Aurora, OH). Commercial extra virgin olive oil contained, according to the product label, 10% (wt/wt) saturated fatty acids, 80% monounsaturated fatty acids, and 10% of polyunsaturated fatty acids. PBS was purchased from Medicago AB (Upsala, Sweden).

For evaporation measurements, a custom-built system was developed. The system was built around a Langmuir trough (KSV Instruments Minitrough, Helsinki, Finland). The trough area, that is the area of evaporation, without barriers was ~273 cm^2. The temperature of the trough was controlled using a heating thermostat (Lauda Eco E4, Königshofen, Germany). Additionally, the temperature of the subphase was measured with a thermometer connected to the Langmuir device. The subphase temperature varied between 34°C and 37°C, depending on the evaporation-retarding effect of the lipid layer under study. The trough was enclosed inside a poly(methyl methacrylate) cabinet. The airflow of dried and filtered air was used to remove humid air from the cabinet and, therefore, diminish the effect of humidity variations on the evaporation rate. An approximate air mass flow was determined by measuring the speed of the airflow through an airflow meter (Testo 410-1, Lenzkirch, Germany) orifice. The airflow was set to 1.5 to 1.8 L/s. The relative humidity of the air inside the cabinet was measured at a close distance from the air-water interface using a digital hygrometer (Testo 608-H1, Lenzkirch, Germany). The humidity values were measured during the last 5 minutes of evaporation measurement, and they varied between 25% and 40% with lipid layers that did not retard evaporation. With evaporation-retardant lipid layers, the humidity varied between 15% and 30%.

The evaporation rate determination was based on the change in subphase (PBS) mass. The mass of the subphase was measured before the evaporation measurement. The subphase was placed into the trough, the timer was started, the lipid was applied in 10 mM chloroform solution to the air-water interface, and the surface pressure was measured with the Langmuir balance. The scale paper plate was removed from the air-water interface, and the front door of the cabinet was closed 10 minutes after the start. After 90 minutes, the trough was emptied, and the subphase was weighed again. For mass-to-volume conversions, an approximate density of 1 g/mL was used for PBS.

Four lipid species were used for preparing lipid mixtures for application to the air-water interface: PC, CE, TG, and WE. For lipid mixtures, 2 to 4 lipid components were mixed in differing molar ratios. The amount of lipid applied to the air-water interface was 500 nmol if not stated otherwise. Olive oil (5 mL) and BA were used as reference materials producing effective retardation of evaporation. The effects of 100 μg/mL BSGM and 10 mg/mL BSA dissolved in the subphase also were studied. BSGM was used as a model substance for the tear fluid mucins, and BSA was used as a model substance for the other dissolved proteins in tear fluid.

The evaporation-retarding effect of commercial Systane Balance lubricant eye drops (Alcon Laboratories Inc., Fort Worth, TX) also was studied because, according to company information, the solution decreases excessive evaporation of the tear fluid. The volume of one drop extracted from the eye drop bottle was ~30 μL. Because the approximate surface area of a human eye is 2.2 cm^2, the volume of the Systane Balance solution applied to the interface of 273 cm^2 was 3.7 mL. Moreover, double the volume (i.e., 7.4 mL) of the eye drop was tested. The contents provided by the manufacturer in the instructions sheet included boric acid, dimyristoyl phosphatidylglycerol, edetate disodium, hydroxypropyl guar, mineral oil, polyoxyl 40 stearate, POLYQUAD (polyquaternium-1) 0.001% preservative, sorbitan tristearate, sorbitol, and purified water.

Evaporation measurements were performed at least three times for each lipid layer. A one-way ANOVA was used to compare the evaporation rates generated by the pure and the lipid-covered air-water interfaces. A P value < 0.05 was considered significant.

A KSV NIMA microBAM and KSV Mini trough (Helsinki, Finland) were used to assess the lateral packing of selected lipid layers at 35°C under differing surface pressures. The lipids were applied to the air-water interface in 10 mM chloroform solution, and the layers were compressed at a rate of 10 mm/min. The surface pressures resembled those of the evaporation experiments, except for the 4:2:2:2 layer, because the 47 mN/m surface pressure could not be achieved by compression.

A KSV NIMA Interfacial Shear Rheometer (Helsinki, Finland) was used to measure the dynamic surface viscosities by using a frequency sweep at 22°C. The position amplitude of the magnetic probe was set to 100 μm, and the frequency sweep was performed from 2.0 to 0.5 Hz (2.0, 1.59, 1.26, 1.0, 0.8, 0.63, and 0.5 Hz). The subphase was PBS. The lipids were applied to the air-water interface and were compressed as described above.

Results

Evaporation

The evaporation rate of PBS was 10.90 ± 0.12 μm/min (mean value ± SD). The evaporation rates through the lipid layers were compared to this value. The measured rates are presented in Figure 1. The respective evaporation rates through the reference lipid layers, olive oil, and BA, were 4.99 ± 1.09 μm/min (~54% decrease in evaporation rate) and 6.02 ± 0.28 μm/min (~45% decrease). PC monolayers did not retard evaporation, but the evaporation rate through the WE layer was 8.40 ± 0.32 μm/min (~23% decrease). This retardation of evaporation was maintained when WE was mixed with 10% PC (1:9 PC/WE, 8.50 ± 0.10 μm/min). Lipid layers containing larger amounts of PC mixed with WE (4:6 and 9:1 PC/WE) did not retard evaporation. Mixtures containing four lipid species in differing ratios demonstrated no significant retardation of evaporation. Neither dissolved BSGM nor BSA decreased the evaporation rate. Systane Balance solution applied to the air-water interface did not retard evaporation. In summary, the evaporation rates through olive oil, BA, pure WE, and 1:9 PC/WE layers decreased evaporation significantly compared to the clean PBS interface ($P < 0.001$, one-way ANOVA). However, those layers that resembled the TFLL did not retard evaporation.

The respective surface pressures generated by 500 nmol of lipid are presented in Figure 1. The surface pressures for PC-containing lipid layers varied between 21 and 47 mN/m, depending on the amount of PC in the mixture. After reaching the 47 mN/m surface pressure, further addition of lipid had no effect on surface pressure. The surface pressure for 4:2:2:2 PC/CE/TG/WE (BSA and BGSM) is not shown, because BSA is surface-active protein and, therefore, interfered with the measurement. No surface pressure is shown for olive oil or Systane Balance solution because the large volumes of these samples interfered with the measurement.

Brewster Angle Microscopy (BAM)

The BAM images for 4:2:2:2, 1:3:3:3, 1:1:1:7 PC/CE/TG/WE, and 1:9 PC/WE are shown in Figure 2. The 4:2:2:2 layer

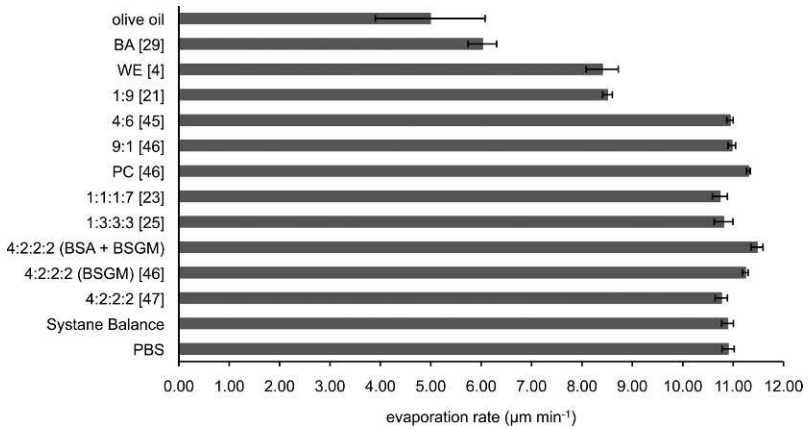

Figure 1. Evaporation rates of water after applying differing lipid compositions to the air-water interface. The amount of the lipid at the interface was 500 nmol. The four-component mixture compositions were PC/CE/TG/WE, and the two-component mixtures were PC/WE. The surface pressures produced by the lipid layers are presented in brackets (mN/m). The respective volumes of olive oil and Systane Balance were 5.0 and 3.7 mL. The respective concentrations of BSA and BGSM in the subphase were 10 and 0.1 mg/mL.

showed very little condensed phase. The area of the condensed phase increased with the increasing nonpolar lipid ratio. 1:3:3:3 and 1:1:1:7 PC/CE/TG/WE showed similar areas of condensed phase. The evaporation-retardant 1:9 PC/WE, however, was highly condensed.

Interfacial Shear Rheometry (ISR)

The dynamic surface viscosities for pure PC, 4:2:2:2, and 1:1:1:7 PC/CE/TG/WE are shown in Figure 3 as functions of surface pressure. The viscosities of the pure PC and 4:2:2:2 layers were of similar magnitude, and they increased with increasing frequency. The viscosity of the 1:1:1:7 lipid layer increased with increasing frequency, but it also increased as a function of surface pressure, particularly at 0.5 Hz. At 25 mN/m, the 1:1:1:7 lipid layer was in a highly condensed, solid-like phase. This phase was observed first as a loss of harmonic oscillation and finally as a complete stopping of the probe (thus dynamic surface viscosity became infinite, as shown in Fig. 3). The 1:9 PC/WE layer could not be measured either, due to the highly condensed phase of the lipid layer at a surface pressure of >1 mN/m (data not shown).

Discussion

We studied the evaporation-retarding effects of differing TFLL-like lipid layers, and we aimed to show the chemical and physical properties that generate an evaporation-retardant lipid layer. The custom-built system used for our evaporation measurements produced repeatable results, considering that the system was not isolated from the changing humidity conditions of the lab. In addition, the wide variation in the measured humidity values can be explained by the close proximity of the hygrometer to the air-water interface. Based on the measurements, however, the air flowing through the cabinet effectively decreased the effect of the variation in humidity. The studies showed that retardation of evaporation is

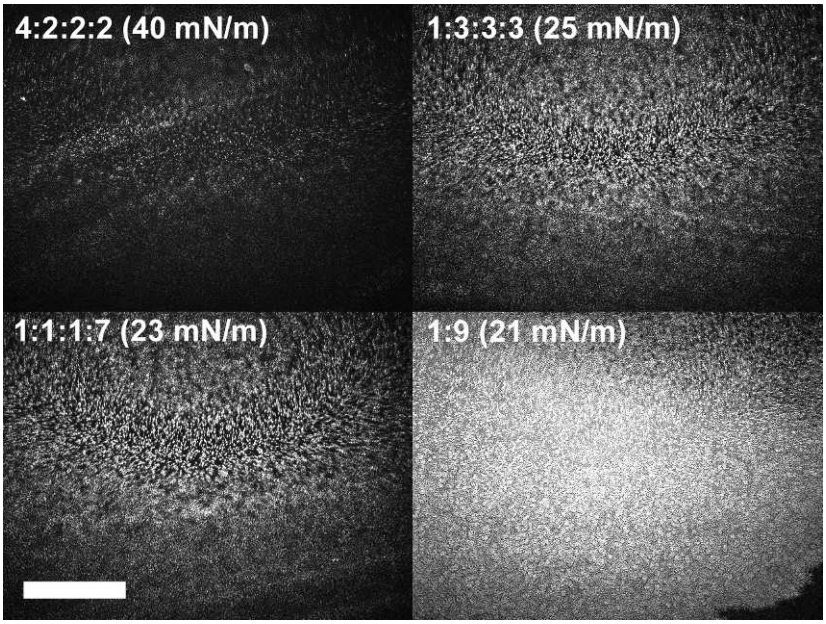

Figure 2. BAM images of selected lipid layers at 35°C. The condensed phase shows as bright domains. The four-component mixture compositions were PC/CE/TG/WE, and the two-component mixture was PC/WE. Surface pressures are presented in *brackets*. *Scale bar*: 1000 μm.

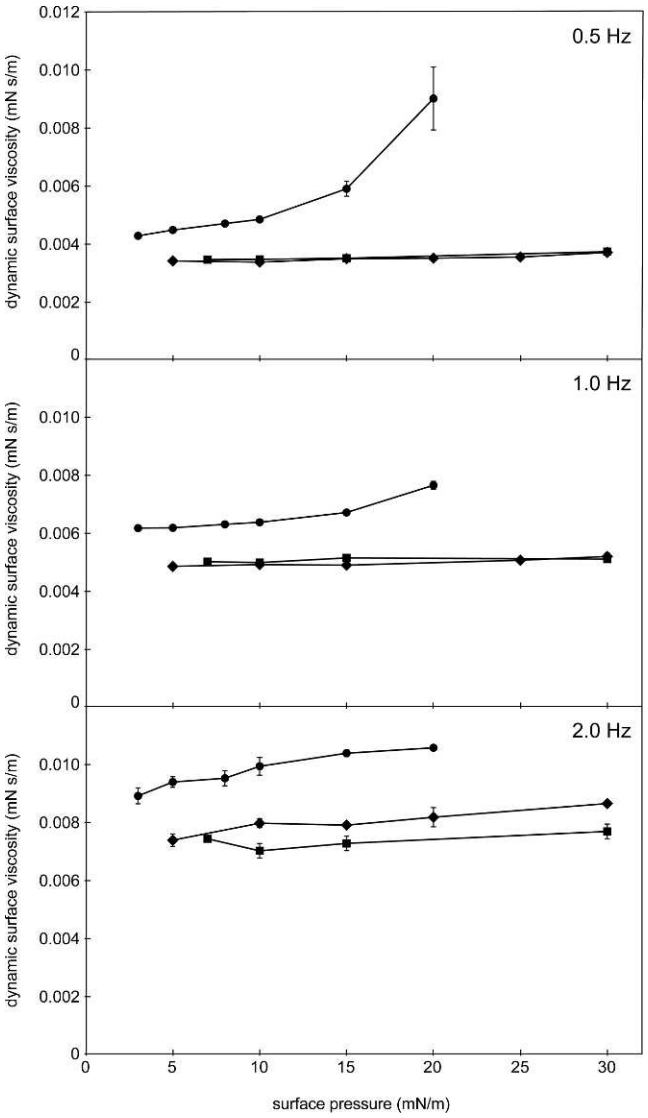

Figure 3. Dynamic surface viscosity as a function of surface pressure at three shear stress frequencies. *Circle*: 1:1:1:7 PC/CE/TG/WE; *Square*: 4:2:2:2 PC/CE/TG/WE; *Diamond*: PC.

highly dependent on the composition and, therefore, the properties of the lipid layer. WEs proved to be the most prominent evaporation-retardant lipids in TFLL, but mixing WE with other lipids resulted in loss of the retardation of evaporation. Based on the present knowledge of the TFLL lipid composition, an evaporation-retarding effect of the TFLL does not seem very likely.

The evaporation-retarding effect of olive oil was studied to show that the retardation of evaporation could be achieved by using a large volume of any oil at the air-water interface. Here, the volume of 5 mL produced an oily layer with a thickness of ~180 μm, if uniform spreading was assumed. However, the spreading was not uniform. In contrast, the thickness of the human lipid layer is tens of nanometers.[36]

A simple monolayer of egg yolk PC, a mixture of saturated and unsaturated PCs, did not retard evaporation. This lack of evaporation retardation most likely is due to the larger cross-sectional area of phospholipids, as well as the larger intra-lipid-layer free volume, compared to long-chain alcohols or long-chain fatty acids. Thus, bulkier phospholipids do not form lipid layers as dense as their smaller relatives do. In this sense, a multilayered model, suggesting a second nonpolar, evaporation-retardant lipid layer on top of the polar lipid layer, is compelling.[13,14]

We studied behenyl oleate layers, because WEs are known to retard evaporation. WEs have important roles in plant-leaf cuticle, a waxy substance, that among other functions, prevents non-stomatal water loss from the leaf surface.[37] WEs also retard evaporation at the air-water interface.[8,34] Whereas wax esters are packed on solid supports in plant leaves, the orientation of the wax ester molecules at the air-water interface is unclear. Based on the relatively hydrophobic nature of behenyl oleate, it is not expected to form a stable monolayer at the air-water interface. If the spreading at the air-water interface is observed by visual inspection, one can see that the spreading is uneven, and the lipids form raft-like aggregates at the interface. The rafts spread slowly with time in near-physiologic temperature, but do not disappear completely. Behenyl oleate at the air-water interface generated a surface pressure of ~4 mN, indicating poor surface activity. When mixed with egg yolk PC (1:9 PC/WE) the ability to retard evaporation was maintained, but it was lost when mixed with higher amounts of PC. The 1:9 PC/WE mixture showed increased surface activity, but spreading occurred slowly when the mixture was applied to the interface. In tear film, the rapid spreading of the compressed TFLL is required for the formation of a uniform lipid layer. Therefore, WEs alone in high concentrations are not the optimal lipids for the TFLL with regard to spreading.

The evaporation-retardation experiments with multicomponent lipid mixtures were begun with a 4:2:2:2 PC/CO/TG/WE composition. This four-component mixture was an approximation based on our current knowledge of tear film lipid composition,[2–4] meibum composition,[12] and the physical behavior of similar monolayers.[13,14] Because this composition did not demonstrate any evaporation retardation, we added more nonpolar lipids. However, 1:3:3:3 and 1:1:1:7 PC/CE/TG/WE compositions did not retard evaporation. Although this finding was somewhat of a surprise to us, it can be explained easily by the loose packing of these lipids at the interface, as we showed previously.[13,14]

Because tear fluid contains high levels (25 μg/mL) of mucins, which are high-molecular-weight water-binding glycoproteins, we studied whether they potentially have an effect on the evaporation rate. The concentration of BSGM used was 100 μg/mL, four times greater than the physiologic concentration of tear mucins.[38] High mucin concentration, however, did not slow down the rate of evaporation. Moreover, the effect of high protein concentration was studied by dissolving 10 mg/mL of BSA into the subphase, because the total tear fluid protein concentration is ~7 mg/mL. BSA as a surface-active agent lowered the surface tension of the air-water interface, but it did not retard evaporation. Based on this finding, the presence of proteins in the subphase does not change the properties of the aqueous phase in a more water-retardant direction. If the tear film contains proteins that decrease the evaporation rate from the tear film, the effect is more likely based on certain specific interactions with the lipid layer.[39,40] However, this theory seems quite unlikely, as the presence of bulky proteins would easily break the order of the evaporation-retardant monolayer.

The ability of Systane Balance eye drops to retard evaporation was examined because this solution contains lipids and carbohydrates that potentially could retard evaporation by forming a lipid layer at the air-water interface. In addition, Systane Balance solution is, at the moment to our knowledge, the only commercial drop containing phospholipids and, therefore, it could be compared to our model lipid layers. Based on the molecular structures of the ingredients, its

potential lipid-layer-forming components include dimyristoyl phosphatidylglycerol, mineral oil, and polyoxyl 40 stearate. However, based on this study, the dry-eye-relieving effect of these drops is not based on the formation or renewal of an evaporation-retardant lipid layer. Such a lipid-layer-independent effect, for instance, has been reported for gel-forming hydroxypropyl guar in vitro.[41]

Brewster angle microscopy illustrated well the differences in the lateral structures among the four studied lipid layers. The evaporation-retardant 1:9 PC/WE was clearly more condensed than the four-component lipid layers. Regardless, the 70% WE proportion of the 1:1:1:7 PC/CE/TG/WE was insufficient to retard evaporation. This finding suggests that the network of Van der Waals interactions required for evaporation retardation does not form between the lipid carbon chains in complex lipid mixtures, that is the complex lipid layers are heterogeneous mixtures of disordered and ordered phases. This finding is not surprising considering our previous findings with three-component lipid layers.[13,14]

All of the tested lipid layers in ISR showed non-Newtonian shear thickening, that is the viscosities of the layers increased with increasing shear rate. This finding means that the layer adapted to the rate of shear stress by changing viscosity. Applied to the TFLL, this kind of behavior suggests that the layer is able to absorb deformations caused by physical stress, such as blinking and vibrations induced by the environment, and thus retain uniform structure. The high proportion of WE in 1:1:1:7 PC/CE/TG/WE also increased the viscosity (i.e., condensation) of the lipid layer as a function of surface pressure, particularly at a frequency of 0.5 Hz. At 22°C, a phase transition from a fluid to a solid-like phase occurred, in both the 1:1:1:7 and 1:9 layers, and this transition caused the harmonic oscillation of the probe (and therefore the measurement) to stop. Because of the measurements taking several hours to perform, the broad area of the trough, and the rapid evaporation rate of the subphase at 35°C, the measurements were performed at 22°C. At 35°C, the layers most likely would have maintained the fluid phase for a longer duration under compression. However, the trend of increasing viscosity of the 1:1:1:7 layer also was apparent at lower temperatures. As expected, based on the ISR and BAM results, the wax esters seemed to have a condensing effect on the layers. The condensed phase most likely promoted retardation of evaporation, but it also might have an effect on the stability of the tear film. The effect of WE on viscosity seemed to be more evident at a low shear rate.

The tear film lipid layer has functions other than the potential retardation of evaporation. Because of the dynamic nature of the tear film lipid layer, it must respond to the shear stress induced by the changing area of the air-tear interface during blinking. The lipid layer must be rather compressible during compression (down-sweep of the lid)[13,14] and must spread rapidly after expansion (up-sweep of the lid).[42] The functions of the polar lipids are to decrease the surface tension of the air-tear interface and to increase the spreading rate of the lipid layer. In contrast, the functions of the nonpolar lipids are, in addition to potential retardation of evaporation, to increase the compressibility and stability of the lipid layer.[14] Imbalances in composition show as impaired viscoelastic properties. Thereby, the retardation of evaporation and the required viscoelastic properties do not necessarily support each other. In both of these cases, the properties are very sensitive to the quantitative (molar fractions of the lipid species) and qualitative (such as the saturation, polarity and cross-sectional area of the lipids) composition of the lipid mixture.

In summary, whether the tear film lipid layer has an ability to retard evaporation is not a trivial task to prove in vitro. This study suggests that building up an evaporation-retardant multicomponent (or even bi-component) mixture, containing individual lipid species that do not retard evaporation, is extremely difficult, if not impossible. If the tear film lipid layer prevents evaporation, its composition must be controlled precisely, because the composition definitely is not irrelevant relative to the evaporation-retarding effect of the layer. In addition to these requirements, viscoelastic properties should not be overlooked. These properties might have a greater role than expected in the stability of the entire tear film.

In our study, we showed that the retardation of evaporation could be achieved in vitro using lipid layers, with either a large volume of oil or a monolayer composed of a specific lipid, such as a long-chain alcohol or a wax ester. However, lipid layers consisting of several differing lipids resembling the TFLL do not retard evaporation, because they cannot attain the highly condensed phase required for evaporation retardation. As the TFLL in vivo also possesses a very complex composition of polar and nonpolar lipids, it most likely does not retard evaporation from the ocular surface.

References

1. Rantamäki AH, Telenius J, Koivuniemi A, Vattulainen I, Holopainen JM. Lessons from the biophysics of interfaces: lung surfactant and tear fluid. *Prog Retin Eye Res*. 2011;30: 204–215.
2. Rantamäki AH, Seppänen-Laakso T, Oresic M, Jauhiainen M, Holopainen JM. Human tear fluid lipidome: from composition to function. *PLoS One*. 2011;6:e19553.
3. Dean AW, Glasgow BJ. Mass spectrometric identification of phospholipids in human tears and tear lipocalin. *Invest Ophthalmol Vis Sci*. 2012;53:1773–1782.
4. Saville JT, Zhao Z, Willcox MD, Ariyavidana MA, Blanksby SJ, Mitchell TW. Identification of phospholipids in human meibum by nano-electrospray ionisation tandem mass spectrometry. *Exp Eye Res*. 2011;92:238–240.
5. Luisi PL, de Souza TP, Stano P. Vesicle behavior: in search of explanations. *J Phys Chem B*. 2008;112:14655–14664.
6. Mathers W. Evaporation from the ocular surface. *Exp Eye Res*. 2004;78:389–394.
7. Barnes GT. The potential for monolayers to reduce the evaporation of water from large water storages. *Agric Water Manage*. 2008;95:339–353.
8. Rosano HL, Mer VKL. The rate of evaporation of water through monolayers of esters, acids and alcohols. *J Phys Chem*. 1956; 60:348–353.
9. La Mer VK, Healy TW. Evaporation of water: its retardation by monolayers: spreading a monomolecular film on the surface is a tested and economical means of reducing water loss. *Science*. 1965;148:36–42.
10. Patra M, Salonen E, Terama E, et al. Under the influence of alcohol: the effect of ethanol and methanol on lipid bilayers. *Biophys J*. 2006;90:1121–1135.
11. Henry DJ, Dewan VI, Prime EL, Qiao GG, Solomon DH, Yarovsky I. Monolayer structure and evaporation resistance: a molecular dynamics study of octadecanol on water. *J Phys Chem B*. 2010;114:3869–3878.
12. Butovich IA. Lipidomics of human meibomian gland secretions: chemistry, biophysics, and physiological role of meibomian lipids. *Prog Lipid Res*. 2011;50:278–301.
13. Kulovesi P, Telenius J, Koivuniemi A, et al. Molecular organization of the tear fluid lipid layer. *Biophys J*. 2010;99: 2559–2567.
14. Kulovesi P, Telenius J, Koivuniemi A, Brezesinski G, Vattulainen I, Holopainen JM. The impact of lipid composition on the stability of the tear fluid lipid layer. *Soft Matter*. 2012;8:5826–5834.

15. 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;8:613–619.
16. Nichols JJ, Mitchell GL, King-Smith PE. Thinning rate of the precorneal and prelens tear films. *Invest Ophthalmol Vis Sci*. 2005;46:2353–2361.
17. Tomlinson A, Trees GR, Occhipinti JR. Tear production and evaporation in the normal eye. *Ophthalmic Physiol Opt*. 1991; 11:44–47.
18. Mathers WD, Lane JA, Sutphin JE, Zimmerman MB. Model for ocular tear film function. *Cornea*. 1996;15:110–119.
19. Mathers WD. Ocular evaporation in meibomian gland dysfunction and dry eye. *Ophthalmology*. 1993;100:347–351.
20. Mathers WD, Binarao G, Petroll M. Ocular water evaporation and the dry eye. A new measuring device. *Cornea*. 1993;12: 335–340.
21. Mathers WD, Daley TE. Tear flow and evaporation in patients with and without dry eye. *Ophthalmology*. 1996;103:664–669.
22. Uchiyama E, Aronowicz JD, Butovich IA, McCulley JP. Increased evaporative rates in laboratory testing conditions simulating airplane cabin relative humidity: an important factor for dry eye syndrome. *Eye Contact Lens*. 2007;33:174–176.
23. Tsubota K, Yamada M. Tear evaporation from the ocular surface. *Invest Ophthalmol Vis Sci*. 1992;33:2942–2950.
24. McCulley JP, Aronowicz JD, Uchiyama E, Shine WE, Butovich IA. Correlations in a change in aqueous tear evaporation with a change in relative humidity and the impact. *Am J Ophthalmol*. 2006;141:758–760.
25. Shimazaki J, Sakata M, Tsubota K. Ocular surface changes and discomfort in patients with meibomian gland dysfunction. *Arch Ophthalmol*. 1995;113:1266–1270.
26. Liu DT, Di Pascuale MA, Sawai J, Gao YY, Tseng SC. Tear film dynamics in floppy eyelid syndrome. *Invest Ophthalmol Vis Sci*. 2005;46:1188–1194.
27. Goto E, Endo K, Suzuki A, Fujikura Y, Matsumoto Y, Tsubota K. Tear evaporation dynamics in normal subjects and subjects with obstructive meibomian gland dysfunction. *Invest Ophthalmol Vis Sci*. 2003;44:533–539.
28. Matsumoto Y, Dogru M, Goto E, Endo K, Tsubota K. Increased tear evaporation in a patient with Ectrodactyly–Ectodermal Dysplasia–Clefting syndrome. *Jpn J Ophthalmol*. 2004;48: 372–375.
29. Rolando M, Refojo MF. Tear evaporimeter for measuring water evaporation rate from the tear film under controlled conditions in humans. *Exp Eye Res*. 1983;36:25–33.
30. Craig JP, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. *Optom Vis Sci*. 1997;74:8–13.
31. Craig JP, Singh I, Tomlinson A, Morgan PB, Efron N. The role of tear physiology in ocular surface temperature. *Eye*. 2000;14: 635–641.
32. Arciniega JC, Wojtowicz JC, Mohamed EM, McCulley JP. Changes in the evaporation rate of tear film after digital expression of meibomian glands in patients with and without dry eye. *Cornea*. 2011;30:843–847.
33. McCulley JP, Uchiyama E, Aronowicz JD, Butovich IA. Impact of evaporation on aqueous tear loss. *Trans Am Ophthalmol Soc*. 2006;104:121–128.
34. Borchman D, Foulks GN, Yappert MC, Mathews J, Leake K, Bell J. Factors affecting evaporation rates of tear film components measured in vitro. *Eye Contact Lens*. 2009;35: 32–37.
35. 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;34:589–597.
36. 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.
37. Samuels L, Kunst L, Jetter R. Sealing plant surfaces: cuticular wax formation by epidermal cells. *Annu Rev Plant Biol*. 2008; 59:683–707.
38. Millar TJ, Tragoulias ST, Anderton PJ, et al. The surface activity of purified ocular mucin at the air-liquid interface and interactions with meibomian lipids. *Cornea*. 2006;25:91–100.
39. Saaren-Seppälä H, Jauhiainen M, Tervo TM, Redl B, Kinnunen PK, Holopainen JM. Interaction of purified tear lipocalin with lipid membranes. *Invest Ophthalmol Vis Sci*. 2005;46:3649–3656.
40. Glasgow BJ, Abduragimov AR, Farahbakhsh ZT, Faull KF, Hubbell WL. Tear lipocalins bind a broad array of lipid ligands. *Curr Eye Res*. 1995;14:363–372.
41. Uchiyama E, Di Pascuale MA, Butovich IA, McCulley JP. Impact on ocular surface evaporation of an artificial tear solution containing hydroxypropyl guar. *Eye Contact Lens*. 2008;34: 331–334.
42. Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. *Exp Eye Res*. 2004;78:347–360.