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Lens-care-solution-induced alterations in dynamic interfacial properties of human tear-lipid films

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ABSTRACT

Purpose: To evaluate the influence of lens care solutions (LCS) on interfacial dynamics and rheological properties of human tear-lipid films.
Methods: Tear lipids were extracted from Schirmer strips collected from 6 healthy subjects. Sessile bubble tensiometry was used to study interfacial properties at 22 °C. Lipids were deposited on an air bubble immersed into electrolytes solution to form 90 ± 20 nm films. Lipid films were subjected to expansion-compression cycles for dynamic interfacial properties and to step-strain relaxations for assessments of rheological properties. LCS (BioTrue [BT], PureMoist [PM], Revitalens [RL], ClearCare [CC]) were injected into optical chamber and equilibrated for 2 h without or with lipid films. Dynamic interfacial properties of films were measured. Then electrolyte solution was pumped through chamber and properties of films were re-evaluated.
Results: Equilibrium surface tension (EST), elasticity modulus (E), and relaxation times (τ) of tear lipids were 22 ± 2.1 mN/m, 10.7–14.8 mN/m, and 80–150 s, respectively. EST for LCS was 45.3 ± 0.8 for CC, 40.3 ± 0.8 for BT, 33.4 ± 1.0 for PM, and 30.1 ± 0.8 mN/m for RL. E for LCS varied within 0.5–6.7 mN/m, and τ varied from 49 to 68 ± 5 s. For mixed lipids + LCS films, EST remained unchanged whereas E and τ were reduced for all LCS types. Exposure to PM and RL noticeably altered the shape of lipid-film iso-cycles. These changes persisted after LCS washout.
Conclusions: Some components of LCS bind irreversibly to lipid films and make them less viscous and less elastic. These findings suggest the possibility of tear-film destabilization upon LCS exposure.
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1. Introduction

An intricate mixture of oily substances known as tear lipids forms the outermost layer of human and other mammalian tear films. These lipid films play a vital role in maintaining ocular surface homeostasis by facilitating spreading of the tear aqueous over a cornea [1–7]. The interactions between tear-lipid films and tear-aqueous constituents (e.g., natural tear proteins, or ingredients added in topical ophthalmic medications or lens care solutions) are important and may influence overall tear-film stability [8–10]. It has been established that the tear-lipid layer plays a crucial role in tear-film stabilization, and surface physical properties such as interfacial elasticity and viscosity of tear-lipid films are the key factors determining thin-film stability [11–13]. Tear-film instability and ultimately film ruptures can cause ocular surface irritation or inflammation and dry eye symptoms. Therefore, understanding mechanisms responsible for tear-film instability is of public health significance as dry eye is one of the leading ocular complaints that eye doctors encounter in the United States [8].

All contact lenses significantly destabilize tear film as indicated by reduced pre-lens tear film break-up time (in contrast to pre-corneal). Contact-lens wear is often discontinued because of gradual development of dry-eye symptoms. The concern regarding adverse effects of multipurpose LCS on tear film properties and ocular health has been raised in the literature [14,15]. Most market-available lens care solutions (LCS) contain significant amounts of surface-active substances (surfactants or detergents) added as either preservatives (e.g., benzalkonium chloride, polyaminopropyl biquanide, polyquaternium) or cleansing and wettability-improving agents (e.g., polyoxyethylene-polyoxypropylene block co-polymers such as Tetronics™ or Pluronics™). These substances adsorb and accumulate at the interface of tear-film lipids and aqueous layers [16,17].

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In our previous study [17] we reported a novel technique to examine multi-layered tear-lipid films and found that a minimum of 20 nm-thick multilayer of tear lipids was required to exert an equilibrium surface pressure of 50 ± 1 mN/m. The physics of thin-liquid films demonstrates that the higher the surface pressure of a liquid film, the more spreadable and stable this film is. These thick multilayered lipid films studied in our previous project exhibited visco-elastic behaviors when subjected to small and uniform radial dilatational deformations [17], qualitatively similar to Meibum monolayer (one-molecule thick films) studied by other authors [12]. We also showed that interfacial rheological characteristics of these films were altered by interactions between the tear lipids and LCS (e.g., OptiFree Express and OptiFree Replenish) [17]. These findings were acquired using thick films composed of reconstituted tear lipids extracted from worn silicone-hydrogel contact lenses as a model for human tear-lipid films.

To further expansion of our knowledge about human tear-lipid properties and their interaction with LCS, this study focused on evaluation of dynamic interfacial and rheological behaviors of human tear lipids extracted from whole tears collected using Schirmer strips rather than tear lipids deposited on worn contact lenses as previously published [17]. We hypothesize that the dynamic and visco-elastic interfacial properties of human tear-lipid films may be influenced by the presence of surface-active ingredients of these new LCS, as these ingredients are introduced into the eye when lenses are inserted onto the eye after overnight soaking/cleaning. We designed and executed an in vitro study aimed to address the following questions:

(1) Are there differences between these new LCS in respect to their surface activity at water/air and water/lipids interfaces?
(2) Will these newly introduced LCSs alter interfacial properties of healthy human tear lipids in vitro?

By examining the physical properties of tear lipids associated with stable and healthy tears as well as the potential impact of LCS on these properties, our findings may in part identify causes leading to tear film instabilities that eventually lead to ocular discomfort/dryness during contact lens wear. Results obtained from this pilot study can be helpful for planning future clinical studies that examine effects of these LCS on tear film stability in vivo.

2. Materials and methods

Distilled and deionized water from a MilliQ® filter system (Millipore Co., Bedford, WA) was used for solution preparations. The aqueous phase was a buffered model tear electrolytes (MTE) solution composed of 5 g/l NaCl (Sigma, USA) with 4 g/l of sea salts (Sigma, USA) added to provide other ions found in human tears (i.e., K^+, Ca^{2+} and Mg^{2+}, phosphate and bicarbonate) [17]. The pH value of MTE solution was adjusted to 7.3 by additions of small amounts (0.5–0.8 ml) of 250 mM KH_2PO_4 so that the aqueous phase in our experiments simulated the salt content and pH of human tears.

2.1. Lipid collection and extraction procedures

Human tear lipids were extracted from Schirmer strips saturated with human tears (two strips from each eye, four strips total) collected from each of 6 subjects during one visit. All subjects were free from ocular disease or any ocular abnormality that contraindicated contact lens wear. A complete explanation of the study goals, procedures, risks, and benefits was given to each prospective subject, and informed consent was obtained. This study adhered to the tenets of the Declaration of Helsinki and was approved by the University of California, Berkeley Committee for Protection of Human Subjects. The Schirmer strips were inserted onto subjects' outer canthi and then collected by clinicians wearing powder-free examination gloves. A brief ocular surface examination was conducted before and after tear collection to ensure that none of the subjects had shown any adverse consequences from tear collection. Post-collection Schirmer strips were stored in a freezer at −80 °C.

The lipids were extracted using 4 ml of chloroform + methanol (2:1, v/v) mixture in glass vials submerged into ultrasound bath for 15 min at medium power intensity. Extracts were dried under vacuum and stored in a freezer at −20 °C. The samples were re-dissolved in 100 μl of toluene + iso-propanol (5:1, v/v) solvent prior to their deposition on the air–water interface for tensiometric and interfacial rheological measurements. Each tear-lipid sample was exposed to 4 different LCS. Each time a new lipid film was formed and aged, its initial properties were measured before LCS exposures. Data from physical properties of tear-lipid film after LCS-exposure were averaged for each LCS. The choice of LCS concentration was based on the following data and assumptions:

(1) total tear volume in healthy eye is typically around 10 μl and
(2) the amount of LCS retained on a soft contact lens after it is taken out of LCS and handled in a standard manner varies from 5 to 20 mg (equivalent to 5–20 μl) according to our gravimetric measurements. Consequently, once a contact lens with retained LCS is inserted onto an eye, the initial ratio of (LCS):(tear fluid) volumes might be in the range between 1:2 and 2:1. As LCS concentration in tear fluid decreases with time due to natural tear turnover rate, the rate of LCS washout depends on tear production rate and on contact lens material. It is known that HEMA lenses tend to accumulate and then leach out some polymeric surfactants present in LCS, whereas silicone hydrogel lenses retain much less of surface active substances. The concentration chosen for our experiments—a dilution of 1:10 (LCS to buffer solution)—is significantly lower than the initial dilution right after lens insertion. With basal tear secretion rate of 0.5–2.2 μl/min [18], this concentration might be reached within a couple of hours after lens insertion. The effect of LCS concentration in aqueous phase has been checked with two other concentrations, one twice as high (1:5), and the other half of the used dilution (1:20). No significant differences in interfacial properties of LCS alone as well as for in mixed lipid/LCS films have been observed.

2.2. Tensiometry

A sessile captive-bubble configuration (a small air bubble pinned to the underside of a straight hydrophobic capillary vertically immersed into an aqueous phase) was used to create an air–water interface and to examine the interfacial properties of reconstituted human tear lipids. This method has been employed in studies of surface activity and protein–lipid interactions in pulmonary-surfactant systems [19]. A detailed description of the sessile bubble apparatus and technique has been presented elsewhere [17,20–22]. The major advantage of this method over the widely used Langmuir-trough technique is that a very small amount of lipid sample (0.5–1 μg) is required for small interfacial areas (5–10 mm^2) on the bubble surface to create 100 nm-thick multilayered film.

A Ramé-Hart tensiometer (Ramé-Hart Instrument Co., Netcong, NJ, USA) with DropImage Advanced software, v.2.2, and an automated dispensing system was used for real-time surface tension data acquisition. Fig. 1 displays the experimental setup. Calculated amounts (based on ellipsometric measurements [17], enough to provide the initial film thickness of ~80–100 nm) of reconstituted lipids solution were deposited onto the surface of the air bubble from underneath using a 5 μl-high precision syringe (Hamilton

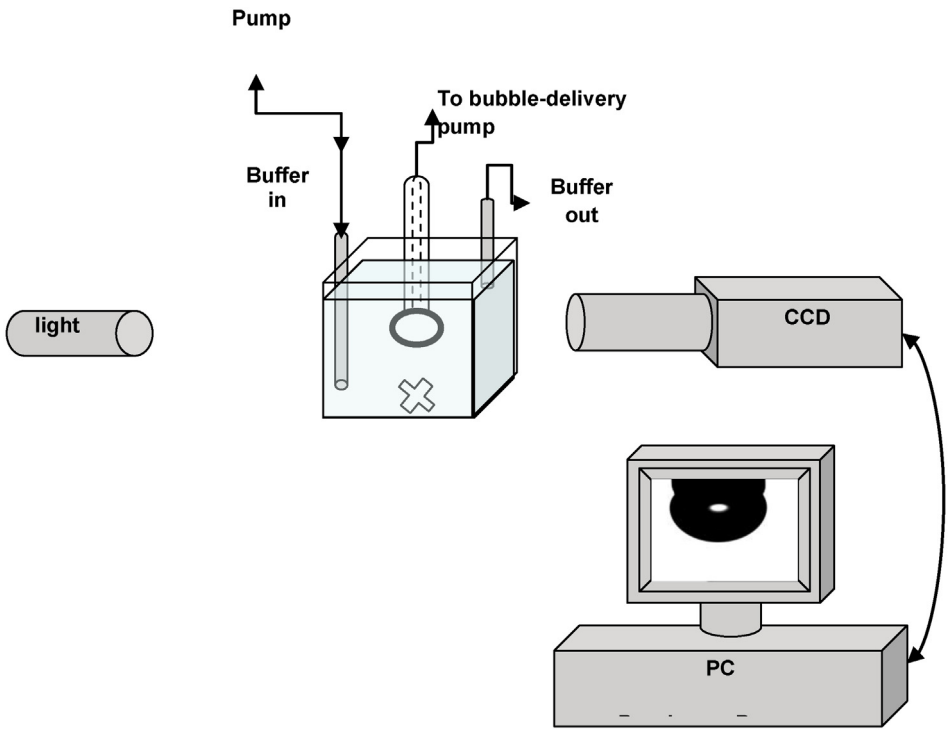

Fig. 1. Schematics of sessile bubble experimental set up.

Co., NV, USA). The aqueous phase in the optical chamber was stirred to provide a uniform distribution of lipids at the air–bubble aqueous interface and to accelerate dissolution of solvents into the aqueous phase. The aqueous phase was then displaced with 200 ml of MTE solution at flow rate 2–4 ml/min, to remove any traces of organic solvents. After the solvents were washed out, the bubble, coated with the lipid film, was left to equilibrate for 17–24 h (overnight) without stirring. Interfacial tension was monitored during each of these steps and surface area of the bubbles was kept constant during equilibration processes. Interfacial dynamic and rheological properties were evaluated after 17–24 h of equilibration. The interfacial properties of reconstituted lipids were found to be reproducible within 10 days after sample dissolution and no significant changes in viscoelastic properties were detected.

Surface-pressure ***vs*** film-thickness (for lipids and lipids + LCS 190 mixed films) and surface pressure ***vs*** surface-area (for dilute LCS alone) iso-cycles were recorded while the area of the bubble was slowly increased from its initial ~5 mm^2 to ~50–60 mm^2, which corresponded to lipid film thicknesses changing from ~100 nm to 5–10 nm and pressures ranging from 50 ± 2 down to 10 mN/m. In compression, the bubble size was gradually reduced to its initial value; rate of area change was 0.07 mm^2/s in both directions. Those iso-cycles were used for evaluation of lipid-film compressibility in wide range of lipid-film thicknesses. Analogously to the bulk compressibility, the compressibility of monolayer is defined as

$$C = -1/A(dA/d\pi) \tag{1}$$

where A is the area per molecule. Thus the compressibility properties of Langmuir monolayer can be determined in a simple way from the slope of the π–A isotherms [23]. In our case of thick multilayered films, we characterized compressibility C as the slope of π–h, surface pressure vs film thickness isotherms.

2.3. Interfacial rheology

A dilation step-strain technique was used to study the interfacial dilatational visco-elastic properties of lipid layers. The air bubble, previously coated with lipids and equilibrated for 17–24 h, was expanded or contracted very fast, within 0.2 s, so that its change in surface area (ΔA) was 5–7% of the initial surface area (A_0). The process of interfacial tension relaxation after surface perturbation was monitored and recorded by the DropImage software for 30 min. This time was typically sufficient for the interfacial tension to reach a nearly constant value. The decay over time of the transient elasticity ($E(t)$) was then determined as:

$$E(t) = \frac{A_0 \Delta\gamma(t)}{\Delta A}, \tag{2}$$

where A_0 is the initial bubble surface area (mm^2) and $\Delta\gamma(t)$ (mN/m) is the change in surface tension induced by the change in surface area [17,19–21]. Lipid layers examined in this project were approximately 50 times thicker than a monolayer and 5–8 times thicker than the reconstituted lipid layers we studied in our previous publication [17]. To quantify the lipid-layer response to dilatational perturbations, we adopted a combined Maxwell visco-elastic and diffusion-relaxation model used earlier [20,22] to describe dilatational behavior of mixed polymers and surfactants thick layers:

$$E(t) = E_\infty + A_M \exp\left(-\frac{t}{\tau_M}\right) + A_D \exp\left(\frac{2t}{\tau_D}\right) erfc\sqrt{\frac{2t}{\tau_D}} \tag{3}$$

where the first two terms on the right account for visco-elastic contribution in relaxation of interfacial layer and the last term reflects diffusion of polar lipid surface active species from interface into the bulk layer when compressed and in the other direction when the film is expanded. τ_M and τ_D represent the characteristic times for visco-elastic relaxation and diffusive exchange, respectively. E_∞ is the elastic modulus of the interface at time $t \to \infty$, and A_M and A_D are the constants characterizing the relative contributions of

Table 1
Multi-purpose lens care solutions studied.

Brand name	Surface-active components	Equilibrium surface tension and *surface pressure*, mN/m (1:10 diluted)
ClearCare (CC)	Pluronic 17R4 (polyoxyethylene-polyoxypropylene block copolymer)	43.6 ± 0.15
CIBA Vision		*28.9 ± 0.15*
Biotrue (BT), Bausch&Lomb	Poloxamine (polyoxyethylene-polyoxypropylene block copolymer), sulfobetaine (zwitterionic surfactant), hyaluronane, edetate disodium. Preservatives: polyaminopropyl biquanide −0.00013%, polyquaternium-1 −0.001%	38.3 ± 0.17
		34.2 ± 0.17
Revitalens (RL), Abbott	Tetronic 904 (polyoxyethylene-polyoxypropylene block copolymer with amino-groups), edetate disodium. Preservatives: alexidine dihydrochloride −0.00016%; polyquaternium-1 −0.003%	36.5 ± 0.18
		36.0 ± 0.18
PureMoist (PM), Alcon	Tetronic 1304 (polyoxyethylene-polyoxypropylene block copolymer with amino-groups), HydraGlyde (EOBO-4™ polyoxiethylene-polyoxybutylene block copolymer); Preservatives: POLYQUAD −0.001%; ALDOX (myristamidopropyl dimethylamine) 0.0006%	32.8 ± 0.23
		39.7 ± 0.23

visco-elastic and diffusion mechanisms into transient elastic modulus $E(t)$, respectively. The rheological experiments were conducted for each lipids/LCS combination, and the measurements were repeated three to five times and averaged for each lipids/LCS combination and for each LCS.

Temperature was kept constant (22 ± 0.5 °C) during all the experiments. The aqueous phase in the optical cell was stirred using a magnetic stirrer (EchoTherm™ HS50, Torrey Pines Scientific, Inc., Carlsbad, CA, USA) at a rate of 400 rpm during equilibration and washout procedures. The temperature 22 °C was chosen, based on our observations indicating that no significant changes in tear-lipid interfacial properties were observed when the temperature was increased to 35 °C.

3. Results

Human tear lipids from 6 subjects (3 female and 3 male; Mean ± SD age = 22 ± 4 years) were collected using Schirmer strips. As determined by ellipsometry measurements [17], an average amount of tear lipids extracted from 4 Schirmer strips collected from each subject was 95 ± 15 μg. The interfacial visco-elastic and dynamic properties of the tear-lipid films were first examined in MTE solution and then aged for 20–24 h without any LCS, followed by exposure to one of LCS for 2 h with 300 rpm stirring prior to interfacial properties re-evaluation. For the next LCS test, a new lipid film was formed, aged, and its initial properties measured before LCS exposure (Table 1).

Fig. 2a depicts two curves for lipids from two different subjects. These curves represent transient elasticity (calculated according to Eq. (2)) as a function of time after step-wise increase of tear-lipid film surface area with an initial thickness of 80–90 nm. These experimental data points were fit with Eq. (3) to obtain numerical values for interfacial rheological parameters such as elastic modulus E_∞ and relaxation time τ_M. Fig. 2b depicts transient elasticity for diluted LCS alone, without tear-lipid films deposited prior to LCS injection. The results of rheological experiments summarized in Table 2 along with data from Fig. 2a and b show significantly lower E_∞ and shorter τ_M for both LCS alone and for mixed human tear-lipid + LCS films, as compared to initial human tear-lipid films ($p < 0.05$, Student t-test).

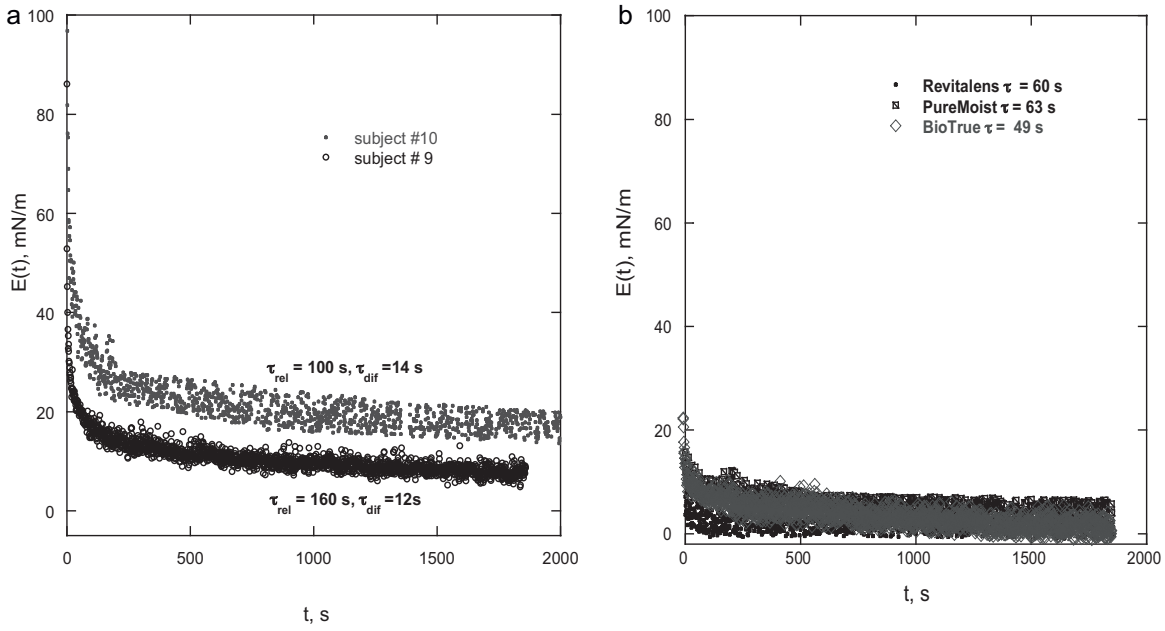

Fig. 2. (a) Transient elasticity as a function of time for 2 human tear lipids samples. (b) Transient elasticity as a function of time for 4 studied LCS (1:10 diluted).

Table 2
Rheological parameters of human tear lipids, LCS and mixed TL + LCS films.

Films constituents	Elasticity modulus, E_∞, mN/m (average ± StD)	Relaxation time, s (average ± StD)
Human tear lipids (TL)	12.5 ± 2.5	126 ± 25
ClearCare (CC)	0.8 ± 0.43	68 ± 3.5
Revitalens (RL)	0.5 ± 0.25	60 ± 2.9
PureMoist (PM)	6.7 ± 0.38	63 ± 3.6
BioTrue (BT)	2.8 ± 0.23	49 ± 4.5
TL + CC	0.9 ± 0.14	74 ± 2.5
TL + RL	0.5 ± 0.15	62 ± 3.5
TL + PM	0.9 ± 0.31	68 ± 4.1
TL + BT	1.1 ± 0.45	56 ± 3.8

As established earlier for the tear lipids extracted from worn lenses [17], the equilibrium surface tension of the tear-lipid films decreased with increasing thickness and reached a constant value of 21.5 ± 1.2 mN/m (surface pressure ~50 mN/m) for the films with thickness above 20–25 nm. In Fig. 3, we report examples of dynamic surface pressure as a function of film-thickness curves called iso-cycles [9–11]. As seen from this figure, a maximum surface pressure of 50 ± 2 mN/m was subject-independent; however, the shape of iso-cycles varied between human tear-lipid samples collected from two individuals. The difference in iso-cycle shapes correlated with the variations in rheological properties between the two samples reported in Fig. 2a.

Fig. 4a–d exhibits the surface pressure vs surface area dependencies obtained for diluted and post-wash CC, BT, PL and RL solutions alone. One can see that all interfacial films formed by LCS solutions have noticeable hysteresis between compression and expansion branches, and that the overall shape of these iso-cycles is very different from the iso-cycles for human tear-lipid films reported in Fig. 3.

Figs. 5–8 display examples of the iso-cycles corresponding to initial human tear-lipid films in the presence of four different LCS, and 2 h after the LCS was washed away (post-wash) by pumping MTE solution through the cell. These figures demonstrate that exposure to all LCS altered the dynamic interfacial properties of human tear lipids, but to a different extent. For PM and RL, these changes remained evident even after complete removal of LCS from aqueous media in the cell.

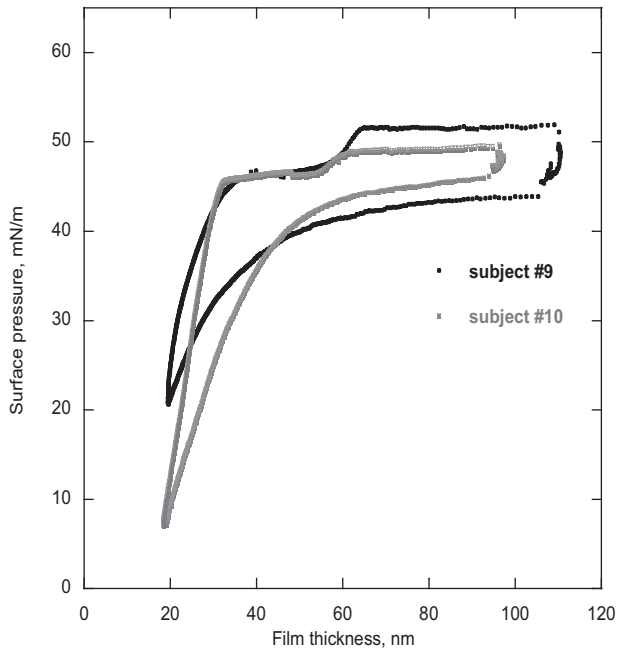

Fig. 3. Surface pressure vs film thickness iso-cycles for 2 different human tear lipid samples.

4. Discussion

It is generally recognized that human tear-lipid films are composed of at least 20 molecular layers and healthy stable lipid films are from 40 to 100 nm thick [23–25]. The most current data regarding the structure and chemical composition of the Meibomian and tear lipids were recently summarized in several reviews [23–25]. The polar double-tailed phospholipids detected in tear lipids [26–29] are one of the most surface-active compounds found in nature; they reduce surface tension of water down to 21–22 mN/m, corresponding to a surface pressure of 50 mN/m, when spread as a densely packed layer at the air–aqueous interface [30] or when these lipids are mixed with Meibomian gland secretions [31].

Despite the generally accepted assumption that human tear-lipid films are multilayered and are from 40 nm to 100 nm thick, most of the published studies on surface properties of Meibomian-lipid films have examined only monolayers (i.e., single-molecule layers ~2 nm thick). The expanded (i.e., less than 2 nm thick) Meibomian-lipid monolayer with relatively low surface pressures (up to 30–35 mN/m) was explored quite extensively [9–13,31]. In contrast, our work focused on interfacial properties of *condensed* tear-lipid multilayers with initial thickness close to 90 ± 20 nm. We found that these thick layers exerted significantly higher surface pressures of 50 ± 2 mN/m. Besides, the multilayered structure of tear-lipid films may be responsible for characteristic surface properties of these films, for instance, their high lateral elasticity [12].

Moreover, it has been shown in literature that compositions of human tear lipids are rather different from that of forcibly expressed Meibomian lipids [30,34]. Human tear lipids extracted from whole tears are substantially enriched with polar substances and contain up to 12% of phospholipids, which are known to possess high surface activity [28,31,32].

We stipulate that multilayered lipid films formed by using lipids extracted from whole tears with initial thickness close to the ocular lipid-film thickness (as in the present study) are better models for examining human tear-lipid behavior than single-layered Meibomian-lipid films [11–13,32].

In our previous study [17] we reported that equilibrium surface tension of human tear lipids extracted from worn soft contact lenses is a function of film thickness; the minimum surface tension of these thick tear-lipid films was 22 ± 1 mN/m, significantly lower than 34–36 mN/m reported in the literature for whole-tear fluid [5]. The same equilibrium surface tension values were also found in the current study for human tear lipids extracted from Schirmer strips. This low value is also in agreement with surface tensions reported for condensed phospholipid layers and lung surfactants [19,31]. It corresponds to a surface pressure of 50 mN/m, which is 10–15 mN/m higher than the maximum pressure reported for Meibum-lipid films in studies where a Langmuir trough was used as an instrument for iso-cycles recording [9,11–13].

We have shown [18,33] that our sessile-bubble technique provides new essential information that is more representative and relevant to in vivo ocular lipid films than results obtained by studying monolayers. This is important because under ocular conditions tear lipids in the form of monolayers are typically not observed. The importance of lipid-film thickness and high surface pressures achieved in expansion-contraction iso-cycles of thick films become even more obvious when the results from human and animal Meibum monolayers studies [11] are compared with our results from thick multilayered tear lipid films. As reported previously

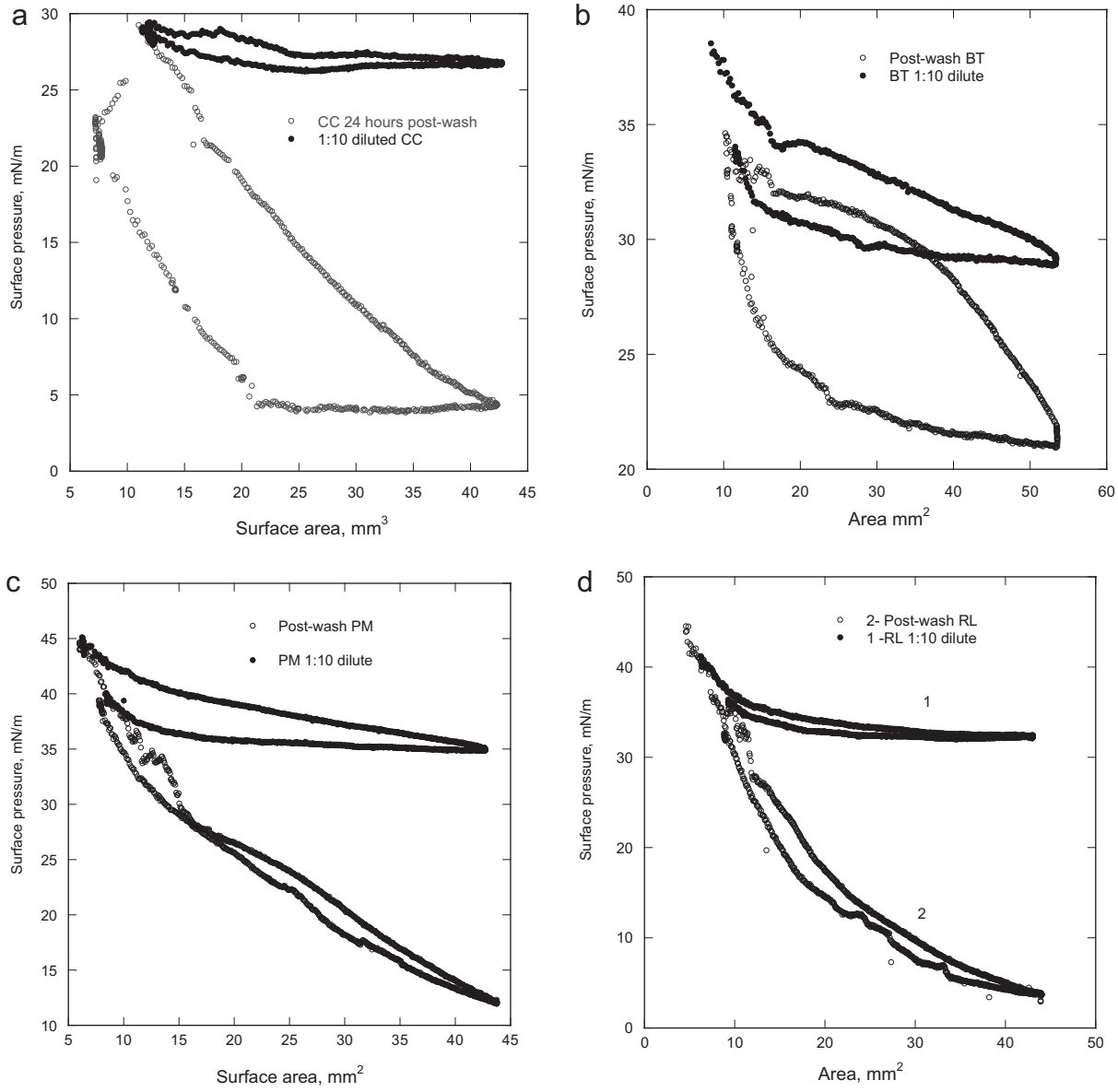

Fig. 4. (a) Surface pressure vs surface area iso-cycles for diluted CC and post-wash CC. (b) Surface pressure vs surface area iso-cycles for diluted BT and post-wash BT. (c) Surface pressure vs surface area iso-cycles for diluted PM and post-wash PM. (d) Surface pressure vs surface area iso-cycles for diluted RL and post-wash RL.

[11], the isotherms of human and animal Meibum were qualitatively similar within a surface pressure interval of 0–30 mN/m. These iso-cycles exhibited little hysteresis between expansion and compression cycles. This trend is in agreement with our findings regarding dynamic interfacial behavior of human tear lipids collected from different subjects within a 0–30 mN/m surface-pressure range (Fig. 3). The difference between these lipid samples becomes evident only at higher surface pressures corresponding to film thicknesses of ~20 nm and greater. It has been shown that the addition of polar lipids such as sphingomyelin and dipalmitoylphosphatidyl choline affected the shape of Meibomian-lipid iso-cycles and increased the maximum attainable surface pressure from 35 to ~50–55 mN/m [31]. We assume that the differences in iso-cycles at high surface pressure are related to variations in polar lipid content or composition among the samples collected from different subjects.

It has been recently reported that the integrity of Meibomian-lipid monolayers could be compromised by penetration of surface-active preservative (e.g., benzalkonium chloride, BAC) found in ophthalmic formulations (e.g., Travatan) [16]. However, no substantial evidence of long-term effects of this particular preservative or Travatan formulation on Meibomian-lipid properties was reported. These authors did not examine effects of surface-active components typically present in LCS. The effect of LCSs (e.g., OptiFree Express and OptiFree Replenish) on human tear-lipid interfacial properties was first described in our previous work [17].

We focused our studies on the properties of thick multilayered human tear lipids (as opposed to Meibomian-lipid monolayers [11–13,16]) and on both short-term as well as long-term effects of diluted LCS, e.g., reversibility of the changes caused by LCS-exposure. Polymeric surfactants present in these LCS are more likely to adsorb irreversibly at water–air or water–oil interface. Previous investigations [19] have shown that polymeric surfactant Pluronic is able to adsorb at the interface in the presence of double-tailed surfactant Aerosol OT™ (AOT, bis-ethylhexyl sulfosuccinate, sodium salt). The chemical structure of AOT is similar to that of phospholipids, except for polar head group. We hypothesize that adsorption of Pluronics at air–water interface is also taking place in the presence of lipids. We have also shown [17]

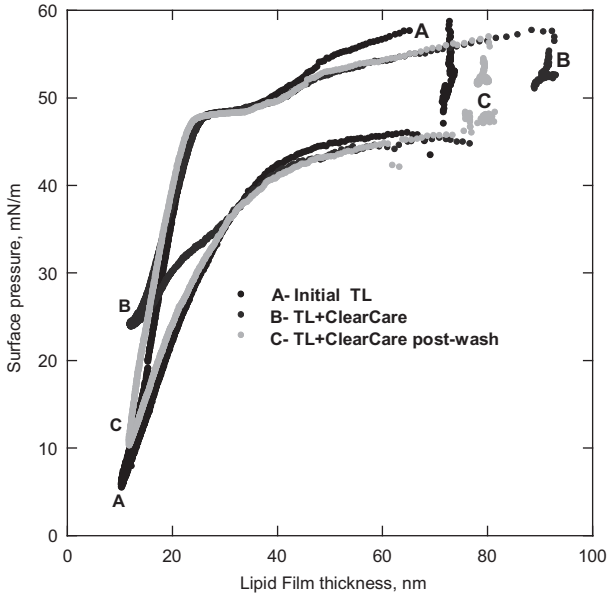

Fig. 5. Surface pressure vs film thickness iso-cycles for initial human tear lipids, treated with CC and post-CC-wash.

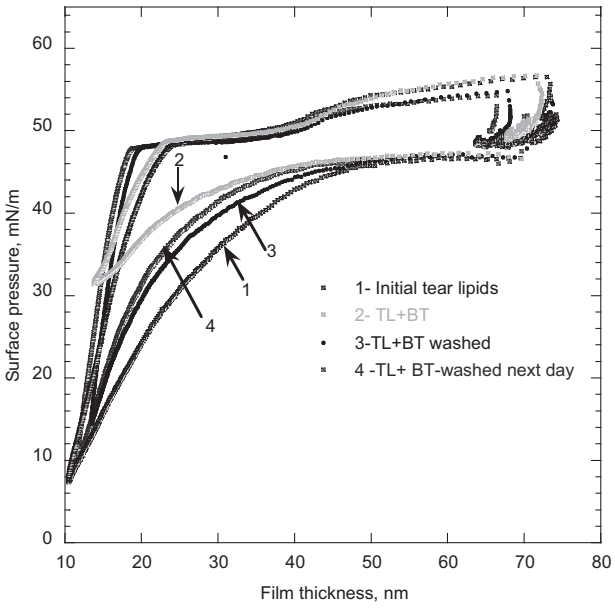

Fig. 6. Surface pressure vs film thickness iso-cycles for initial human tear lipids, treated with BT and 2-h and 24 h post-BT-wash.

that some components of LCS, for instance, Tetronic 1304 present in OptiFree Express and Replenish, were bound irreversibly and thus significantly altered the rheological properties of ex vivo soft-lens-extracted lipid films. Indeed, the results reported in Fig. 4a–c clearly show that components of diluted LCS were adsorbed irreversibly to air–water interface. They remained bound to air–water interface or to lipid films even after total removal of LCS from bulk solution during washout. Table 2 demonstrates that rheological parameters of thick human tear-lipid films changed in the presence of LCS but to a different extent. The most pronounced effect was produced by diluted PM and RL solutions, whereas the effects of CC and BT on elasticity modulus and relaxation time were less significant. The same trend was observed for the lipid films exposed to diluted LCS when we studied dynamic interfacial properties and recorded surface pressure vs film thickness iso-cycles. As seen from Figs. 5–8, at low surface pressure and for thinner films, the shape of the iso-cycles changes very dramatically as an outcome of LCS exposure. Those alterations are to some extent similar to the transformations observed in other study [16] with Meibum monolayer exposed to BAC-containing eye drops Travatan. On the other hand, these changes are not persistent and practically fade away after CC and BT were displaced by a surfactants-free model tear electrolytes solution. That was not the case for the films exposed to PM and RL solutions, for which after the washout procedure, the changes in iso-cycles shape became less pronounced but did not disappear.

At elevated pressures, the alterations of the iso-cycles are less evident; nevertheless, the changes are significant as revealed by the film-compressibility data summarized in Table 3. The

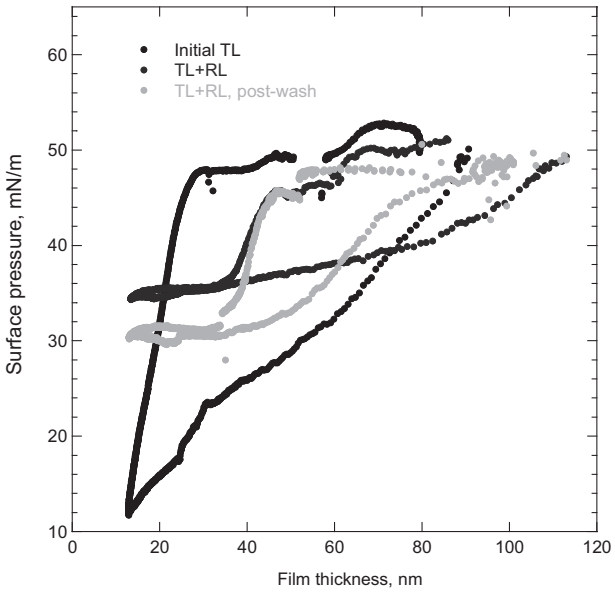

Fig. 7. Surface pressure vs film thickness iso-cycles for initial human tear lipids, treated with RL and 2-h post-RL-wash.

Table 3
Compressibility parameters for human tear lipids and mixed TL + LCS films.

LCS	π range, mN/m for thickness 60–90 nm	Compressibility, $d\pi/dh$	π_{max}, mN/m	π_{min}, mN/m	Compressibility ratio TL + LCS/TL
None – TL alone	48–52	0.02–0.11	52	12	NA
ClearCare	48–50	0.126	52	20	6.3
ClearCare washed	47–50	0.13	52	11	6.5
Biotrue	46–50	0.24	50	28	12
Biotrue washed	43–50	0.18	50	33	9
Revitalens	46–52	0.8	52	33	40
Revitalens washed	46–50	0.24	50	28	12
PureMoist	48–54	3.2	52	28	32
PureMoist washed	46–50	0.14	50	29	28

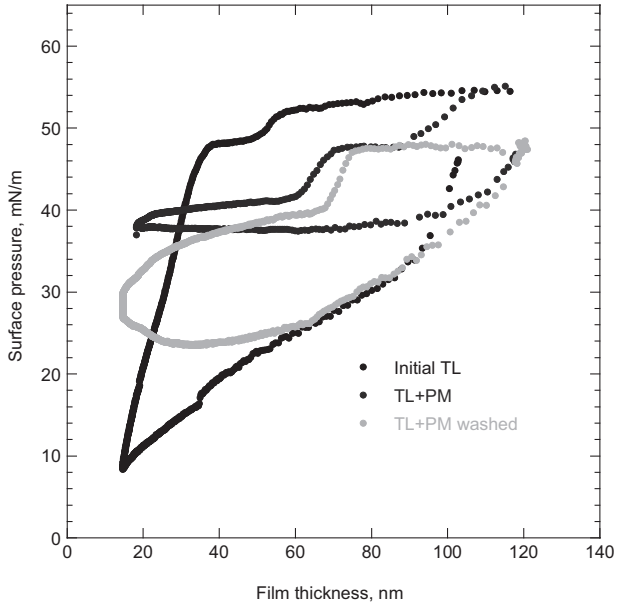

Fig. 8. Surface pressure vs film thickness iso-cycles for initial human tear lipids, treated with PM and 2-h post-PM-wash.

compressibility of initial lipid films is lower than that of the films exposed to LCS, both before and after washout. Note that exposure to all LCS does not significantly change maximum surface pressure of lipid films. This fact indicates that surface-active components of LCS are not able to significantly displace human tear lipids when the films are exposed to LCS. The extent of compressibility changes correlates with surface activity of LCS as gauged by equilibrium surface tension or surface pressure of their diluted solutions. The higher LCS surface pressure correlates with more pronounced alterations in dynamic properties of human tear lipids. For LCS studied, the maximum surface pressure and potency to modify interfacial dynamics increases in the following order: CC < BT < RL < PM.

The clinical implications of our results remain to be determined. However, these results lay down the foundation for future investigations to determine the role of tear-lipid dynamic interfacial behavior on tear-film stability. The risk of prolonged exposure to different LCS that might be causally related to dry eye symptoms has to be taken into consideration.

5. Conclusions

We demonstrated that the sessile captive-bubble technique is a proficient technique suitable for investigations of dynamic and equilibrium interfacial properties of thick films formed using ultra-small amounts of human tear lipids. The exposure of human tear-lipid films to 10-times diluted LCS causes significant alterations in interfacial dynamic and rheological properties; in most cases these changes are irreversible. Surface-active components of LCS adsorb and irreversibly bind to thick human tear-lipid films. The interactions of these substances with lipids change lipid-film interfacial dynamics significantly. After exposure to LCS, gel-like human tear-lipid films become less viscous and less elastic and behave similar to condensed liquid films. Clinical implications of these findings suggest the possibility of human tear-film destabilization upon prolonged exposure to LCS.

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Conflict of interest

None declared.

References

[1] Holly F, Lemp MA. Formation and rupture of the tear film. Exp Eye Res 1973;15:515–25.
[2] Holly FJ. Physical-chemistry of the normal and disordered tear film. Trans Ophthal Soc 1985;104:374–80.
[3] Craig JP, Tomlinson A. Importance of the lipid layer in the human tear film stability and evaporation. Optom Vis Sci 1997;74:8–13.
[4] Rieger G. The importance of the precorneal tear film for the quality of optical imaging. Br J Ophthalmol 1992;76:157–8.
[5] Nagoyova B, Tiffany JM. Components responsible for the surface tension of human tears. Curr Eye Res 1999;19:4–11.
[6] McCulley JP, Shine WE. A compositional based model for the tear film lipid layer. Trans Am Ophthalmol Soc 1997;XCV:79–93.
[7] McCulley JP, Shine WE. The lipid layer: the outer surface of the ocular surface tear film. Biosci Rep 2002;21:407–18.
[8] Green-Church KB, Butovich I, Willcox M, Borchman D, Paulsen F, Barabino S, et al. The international workshop on Meibomian gland dysfunction: report of the subcommittee on tear film lipids and lipid–protein interaction in health and disease. IOVS 2011;52:1979–93.
[9] Millar TJ, Tragoulias ST, Anderton PJ. The surface activity of purified ocular mucin at the air–liquid interface and interactions with Meibomian lipids. Cornea 2006;25:91–100.
[10] Nishimure SY, Magana GM, Ketelson HA, Fuller GG. Effect of lysozyme adsorption on the interfacial rheology of DPPC and cholesteryl myristate films. Langmuir 2008;24:11728–33.
[11] Leiske DL, Raju SR, Ketelson HA, Millar TJ, Fuller GG. The interfacial viscoelastic properties and structures of human and animal Meibomian lipids. Exp Eye Res 2010;90:598–604.
[12] Rosenfeld L, Fuller GG. Consequences of interfacial viscoelasticity on thin film stability. Langmuir 2012;28:14238–44.
[13] Leiske DL, Miller CE, Rosenfeld L, Cerretani C, Ayzner A, Lin B, et al. Molecular structure of interfacial human Meibum films. Langmuir 2012;28:11858–65.
[14] Bergmanson IPG, Barbeito R. Clinical assessment of ocular response to a multipurpose contact lens care solution. Ophthalmic Physiol Opt 1995;15(6):535–44.
[15] Carnt N, Jalbert I, Stretton S, Naduvilath T, Papas E. Solution toxicity in soft contact lens daily wear is associated with corneal inflammation. Optom Vis Sci 2007;24:309–15.
[16] Georgiev GA, Yokoi N, Ivanova S, Krastev R, Lalchev Z. Surface chemistry study of the interactions of pharmaceutical ingredients with human Meibum films. IOVS 2012;53:4605–15.
[17] Svitova TF, Lin MC. Interfacial rheological properties of ex vivo human-tear lipids: the effect of lysozyme and multi-purpose lens care solutions. Optom Vis Sci 2010;87:10–20.
[18] Dumortie G, Chaumeil JC. Lachrymal determinations: methods and updates on biopharmaceutical and clinical applications. Ophthalmic Res 2004;36:183–94.
[19] Serrano AG, Perez-Gil J. Protein–lipid interactions and surface activity in the pulmonary surfactant systems. Chem Phys Lipids 2006;141:105–18.
[20] Svitova TF, Radke CJ. AOT and Pluronic F68 co-adsorption at fluid/fluid interface: a continuous flow tensiometry study. Ind Eng Chem Res 2005;44:1129–38.
[21] Guzmán E, Ritacco H, Ortega F, Svitova T, Radke CJ, Rubio RG. Adsorption kinetics and mechanical properties of ultrathin polyelectrolyte multilayers: liquid-supported versus solid-supported films. J Phys Chem B 2009;113:7128–37.
[22] Freer EM, Svitova T, Radke CJ. The role of interfacial rheology in reservoir mixed wettability. J Petrol Sci Eng 2003;39:137–45.
[23] Vollhard D, Fainerman VB. Progress in characterization of Langmuir monolayers by consideration of compressibility. Adv Colloid Interface Sci 2006;127:83–97.
[24] Wojtowicz JC, Butovich IA, McCulley JP. Historical brief on composition of human Meibum lipids. Ocul Surf 2009;7:145–53.
[25] Butovich IA. On the lipid composition of human Meibum and tears: comparative analysis of nonpolar lipids. IOVS 2008;49:3779–89.
[26] Butovich IA, Millar TJ, Ham BM. Understanding and analyzing Meibomian lipids – a review. Curr Eye Res 2008;33:405–20.
[27] Kulovesi P, Telenius J, Koivuniemi A, Brezesinski G, Rantamaki A, Viitala T, et al. Molecular organization of the tear fluid lipid layer. Biophys J 2010;99:2559–67.
[28] Kulovesi P, Telenius J, Koivuniemi A, Brezesinski G, Vattulainen I, Holopainen JM. The impact of lipids composition on the stability of the tear fluid lipid layers. Soft Matter 2012;8:5826–34.
[29] Rantamaki AH, Seppanen-Laakso T, Oresic M, Holopainen JM. Human tear fluid lipidome: from composition to function. PLoS ONE 2011;6:1–7, e19553.
[30] Dean AW, Glasgow BJ. Mass spectrometric identification of phospholipids in human tears and tear lipocalin. IOVS 2012;53:1773–82.

[31] He Q, Zhang Y, Lu G, Miller R, Mohwald H, Li JB. Dynamic adsorption and characterization of phospholipids and mixed phospholipids/protein layers at liquid/liquid interfaces. Adv Colloid Interface Sci 2008;140:67–76.
[32] Georgiev GA, Kutsarova E, Jordanov A, Krastev R, Lalcheva Z. Interactions of Meibomian gland secretion with polar lipids in Langmuir monolayers. Colloids Surf B 2010;78:317–27.
[33] Svitova TF, Lin MC. Racial variations in interfacial behavior of lipids extracted from worn SCL. Optom Vis Sci 2013;90:1361–9.
[34] Brown SHJ, Kunnen CME, Duchoslav E, Dolla NK, Kelso MJ, Papas EB, et al. A comparison of patient matched Meibum and tear lipidomes. IOVS 2013;54:7417–24.