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ORIGINAL ARTICLE

Racial Variations in Interfacial Behavior of Lipids Extracted From Worn Soft Contact Lenses

Tatyana F. Svitova* and Meng C. Lin†

ABSTRACT

Purpose. To explore interfacial behaviors and effects of temperature and dilatation on dynamic properties of multilayered human tear lipids extracted from silicone hydrogel (SiH) lenses worn by asymptomatic Asian and white subjects.

Methods. Interfacial properties of lipids extracted from Focus N&D lenses worn by 14 subjects continuously for 1 month were studied. The lipids were deposited on an air bubble immersed in a model tear electrolyte (MTE) solution to form 100 ± 20–nm–thick films. Surface pressure was recorded during slow expansion/contraction cycles to evaluate compressibility and hysteresis of lipid films. Films were also subjected to fast step-strain dilatations at temperatures of 22 to 45°C for their viscoelastic property assessment.

Results. Isocycles for Asian and white lipids were similar at low surface pressures but had distinctly different compressibility and hysteresis at dynamic pressures exceeding 30 mN/m. Rheological parameters of reconstituted lipids were also dissimilar between Asian and white. The elastic modulus E_∞ for white lipids was 1.5 times higher than that for Asian lipids, whereas relaxation time (t) was on average 1.3 times higher for Asian. No significant changes were observed in rheological properties of both Asian and white lipids when temperature increased from 22.0 to 36.5°C. However, for white lipids, E_∞ reduced considerably at temperatures higher than 42.0°C, whereas t remained unchanged. For Asian lipids, both E_∞ and t started to decline as temperature increased to 38°C and higher.

Conclusions. Higher elastic modulus of white lipids and elasticity threshold at certain deformations indicate stronger structure and intermolecular interactions as compared with more viscous Asian lipids. The differences in interfacial behaviors between Asian and white lipids may be associated with the differences in their chemical compositions.
(Optom Vis Sci 2013;90:1361–1369)

Key Words: tear lipids, meibum lipids, tear breakup, ethnicity, race, lipid film rheology, diffusion-relaxation mechanism temperature

The tear film has a complex multilayered structure. Its outermost layer is formed by an intricate mixture of oily substances known as tear lipids, which play a vital role in maintaining ocular surface homeostasis by facilitating spreading of the tear-aqueous fluid.[1–7] The interactions between the lipid layer and aqueous fluid constituents (e.g., proteins) are also essential for maintaining tear film stability.[5–7] It is generally accepted that the physical and mechanical properties (such as elasticity and viscosity) of thin liquid films are the key factors determining thin film stability.[8–10]

In our previous study, we reported a novel technique to study multilayered 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. Thick lipid films exhibited viscoelastic behaviors when subjected to small and uniform radial dilatational deformations. We also showed that the interfacial rheological characteristics of these films were altered by interactions of the lipids with the model protein lysozyme.[11] These findings were obtained by using reconstituted lipids extracted from worn contact lenses. Although it has been reported that lipids deposited on worn lenses contained most of the lipid classes/groups found in human meibum lipids (MLs), the question of how well these contact lens–deposited lipids represent actual tear lipids or human ML remains largely unexplored.[12–14]

Recently, analytical results for ML compositions of normal subjects and subjects with Meibomian gland dysfunction (MGD) were independently published for different ethnic study cohorts.[15–21] The findings of these articles show divergent trends: for white with MGD, ML were depleted of polar lipid (lower polar/nonpolar

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ratio).[18–20] However, the amounts of polar lipids, namely, phosphatidylcholines, and triglycerides in ML were higher in Asians with dry eye symptoms compared with those in asymptomatic Asian subjects.[21] From these findings, it is not obvious whether these differences were caused by disease or were inherent differences between Asian and white tear-lipid compositions. Given that average precorneal and prelens tear film stabilities, as gauged by noninvasive breakup time (NI-BUT), are lower for asymptomatic Asian than for white,[22] the logical approach is to examine rheological behavior of thick-lipid films (100 ± 20 nm) that is comparable in thickness to human ocular lipid films using samples collected from subjects of different ethnicity. We hypothesize that there might be dissimilarities in the compositions and mechanical viscoelastic properties of tear lipids collected from normal subjects of different ethnicities.

In this pilot study, our primary aim was to investigate tear-lipid samples from subjects of Asian and white descents and analyze dynamic behavior and interfacial rheological characteristics of tear lipids, with the emphasis on the effects of temperature and amplitude of interfacial deformation on these rheological characteristics.

MATERIALS AND METHODS

Distilled and deionized water from a MilliQ filter system (Millipore Co., Bedford, WA) was used for solution preparations. The aqueous phase in all experiments was a buffered model tear electrolyte (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^+ and Ca^{2+} and Mg^{2+} phosphate and bicarbonate).[11] The pH value of MTE solution was adjusted to 7.3 by addition of small amounts (0.5 to 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.

Lipid Collection and Extraction Procedures

Human tear lipids were extracted from lotrafilcon A contact lenses (Focus Night & Day [FND]; CIBA Vision Corp., Duluth, GA) that had been worn continuously for 1 month. Neophytes without contact lens experience or former lens wearers with no lens wear for at least 1 year before the study were recruited from the campus of the University of California at Berkeley. Each participant first adapted to daily wear for at least 1 week and then commenced 1 month of continuous wear. 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.

Focus Night & Day lenses were chosen for this study because they are US Food and Drug Administration approved for continuous overnight wear. The FND lenses were inserted onto subjects' eyes and then collected by clinicians wearing powder-free examination gloves. The clinical examinations were conducted before and after lens removal to ensure that none of the subjects had shown any adverse consequences of continuous lens wear. The lenses were then rinsed in deionized distilled water, and excess water was removed with a filter paper. Each lens was placed into separate glass vials containing 4 mL of toluene + isopropanol (5:1, vol/vol) solvent, sonicated for 15 minutes at medium power level; then a lens was removed, and solvent was evaporated under vacuum at ambient temperature. The details of the lipid extraction procedure have been described elsewhere.[11] Dry lipid extracts were stored in a freezer at –20°C. The samples were redissolved in 100 μL of the same solvent before their deposition on the air-water interface for tensiometric and interfacial rheological measurements.

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 *ex vivo* tear lipids. This method has been used in studies of surface activity and protein-lipid interactions in pulmonary-surfactant systems.[23] Detailed descriptions of the sessile bubble apparatus and technique have been presented elsewhere.[11,24–26] The major advantage of this method over the widely used Langmuir trough technique is very small interfacial areas (10 to 15 mm^2), which take only approximately 1 μm^3 of lipids to coat the surface area with 100-nm-thick multilayered film.

A Ramé-Hart tensiometer (Ramé-Hart Instrument Co., Netcong, NJ) with DropImage Advanced software, version 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,[11] enough to provide the initial film thickness of ~100 nm) of reconstituted lipid solution were deposited onto the surface of the air bubble from underneath using a 5-μL high-precision syringe (Hamilton Co., Reno, NV). The aqueous phase in the optical cell 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 250 mL of MTE solution at a flow rate of 2 to 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 to 24 hours (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 rheological properties were measured after 17 to 24 hours of equilibration.

Surface pressure versus film thickness isocycles were recorded for film thicknesses from 2 to approximately 120 nm and pressures ranging from 10 to 50 ± 2 mN/m to evaluate the compressibility, reversibility, and degree of compression-expansion hysteresis in this wide range of lipid film thicknesses. Analogously to the bulk compressibility, the compressibility of monolayers is defined as:

$$C = -1/A(dA/d\Pi) \quad (1)$$

where A is the area per molecule. Thus, the compressibility properties of Langmuir monolayers can be determined in a simple way from the slope of the Π-A isotherms.[27] In our case of thick

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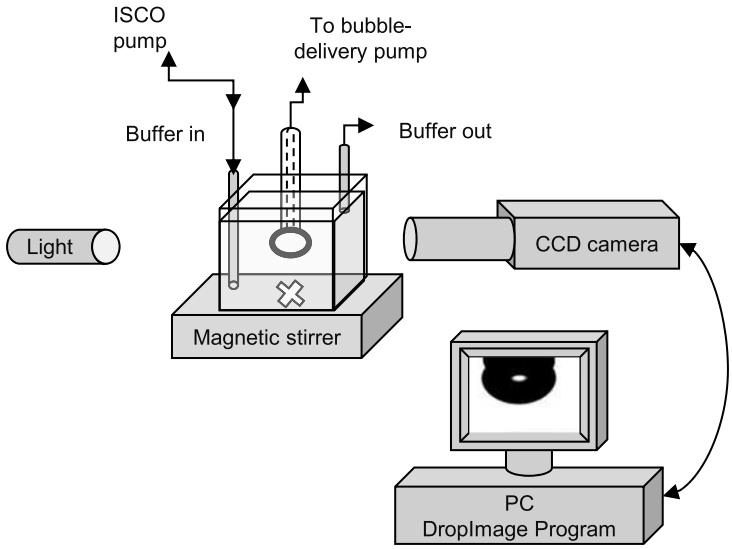

FIGURE 1.
Sessile bubble tensiometer–schematics.

multilayered films, we characterized compressibility C as the slope of $\Pi - h$ surface pressure versus film thickness (h) isotherms.

Interfacial Rheology

A dilation step-strain technique was used to study the interfacial dilatational viscoelastic properties of lipid layers. The air bubble, previously coated with lipids and equilibrated for 17 to 24 hours, was expanded or contracted very fast, within 0.2 seconds, so that its change in surface area (ΔA) was 5 to 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 minutes. 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:

$$E(t) = A_0 \Delta\gamma(t)/\Delta A \quad (2)$$

where A_0 is the initial bubble surface area (in mm^2), and $\Delta\gamma(t)$ (in mN/m) is the change in surface tension induced by the change in surface area.[11,24–26] Lipids layers studied in this project were approximately 50 times thicker than a monolayer and 5 to 8 times thicker than the reconstituted lipid layers we studied in our previous publication.[11] To quantify their response to dilatational perturbations, these thick layers required an approach different from that used before single-exponential decay fits. Here, we adopted a combined Maxwell viscoelastic and diffusion-relaxation model used earlier[24,26] to describe dilatational behavior of mixed polymers and surfactants thick layers:

$$E(t) = E_\infty + A_M \exp(-t/\tau_M) + A_D \exp(2t/\tau_D) erfc\sqrt{2t/\tau_D} \quad (3)$$

where the first two terms on the right account for viscoelastic 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 other direction when the film is expanded. τ_M and τ_D represent the characteristic times for viscoelastic 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 viscoelastic and diffusion mechanisms into transient elastic modulus $E(t)$, respectively.

Temperature was kept constant (22 ± 0.5°C) during the experiments conducted with varying dilatational amplitudes. Measurements of the effects of temperature on interfacial rheology were conducted at constant deformation amplitude (5 ± 2.0%) and temperatures of 22, 30, and 36 to 46°C, with temperatures between 36 and 46°C changed in 2 ± 0.5°C increments. During the heating, cooling, and washout procedures, the aqueous phase in the optical cell was stirred using a magnetic stirrer (EchoTherm HS50, Torrey Pines Scientific, Inc., Carlsbad, CA) at a rate of 400 rpm.

RESULTS

A total of 14 subjects completed the study; five Asian (three men, two women; age [mean ± SD], 20 ± 1 years) and nine whites (white Europeans, 5 men and 4 women; age [mean ± SD], 22 ± 3 years) based on self-reported ethnicity. Gravimetric analysis had shown that the total amount of dry lipid extracts (mean ± SD) was 125 ± 17 μg/lens for white and 140 ± 57 μg/lens for Asian participants. The amount of dry remnants extracted from blank unworn FND lenses (mean ± SD), as measured by ellipsometry, was 5.4 ± 2 μg/lens, less than 5% of the total material extracted from worn lenses. These dry remnants did not exhibit any interfacial activity when deposited at the air-water interface, as shown in our previous study.[11]

In our previous publication,[11] it was shown that the equilibrium surface tension of reconstituted lipid films is a function of film thickness. For a monolayer of approximately 2 nm thickness, surface tension was found to be 32 to 33 mN/m. Equilibrium surface tension decreased with increasing film thickness and reached a constant value of 21.5 ± 1.2 mN/m (surface pressure, approximately 50 mN/m) for the films with thicknesses

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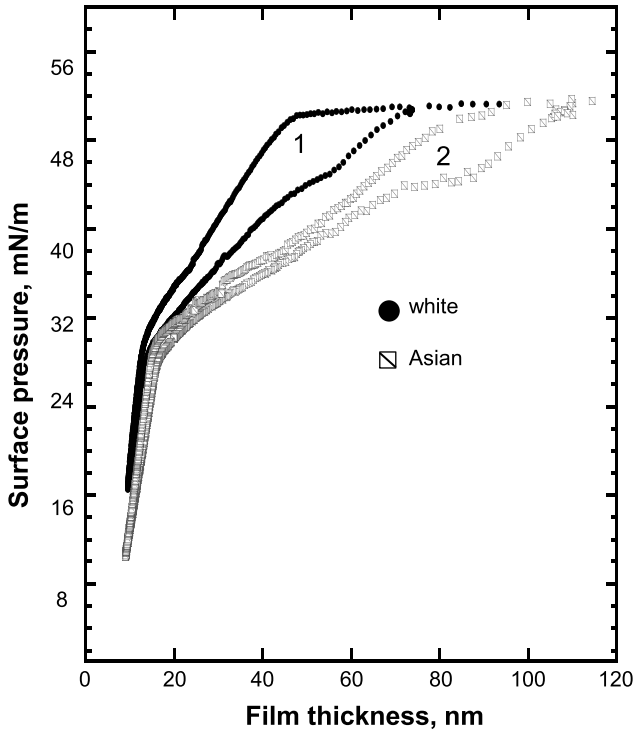

FIGURE 2.
Dynamic interfacial pressure as a function of lipid film thickness.

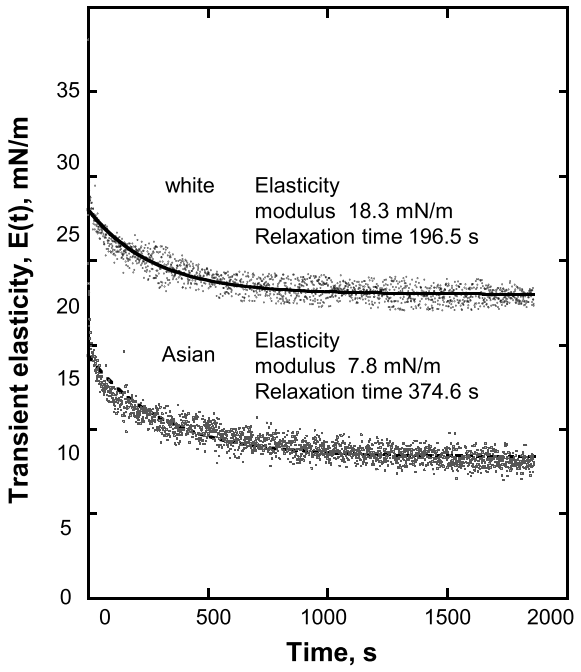

FIGURE 3.
Transient elasticity as a function of time for Asian and white lipids.

more than 20 nm, which were composed of approximately 10 monolayers. In Fig. 2, we report the examples of dynamic surface pressure as a function of film thickness curves or isocycles measured while slowly expanding lipid-coated bubble from its original area of approximately 5 mm^2 to the area of approximately 50 mm^2, with the rate 0.07 mm^2/s and then contracting it back to initial size with the same speed. Curve 1 corresponds to the lipid sample from white subjects, whereas curve 2 is for the lipids from Asians. The diverging of these curves becomes quite significant at surface pressures greater than 30 mN/m; these curves will be analyzed and described in detail in the Discussion.

In the current study, we find that a minimum equilibrium surface tension of 21.5 ± 2 mN/m, or a maximum surface pressure of 50 ± 2 mN/m, was independent of the subjects' ethnicity. Despite the invariance of equilibrium surface tension between ethnic groups, the dynamic behavior and viscoelastic properties of the lipid films were distinctly different between lipids collected from Asian and white subjects (Table 1). Fig. 3 illustrates that the Asian lipid film relaxed more slowly than the film formed by white lipids, and that the final estimate of the elasticity modulus for Asian lipids was lower than that for white lipids. Fig. 3 and Table 1 show the lower elasticity modulus for Asian on average as a group.

Fig. 4 illustrates an example of the elasticity modulus as a function of degree of deformation. It demonstrates that, for Asian lipids, the interfacial elasticity decreases only slightly with growing deformation amplitude compared with white lipids.

Examples of elasticity modulus and interfacial viscosity versus temperature dependencies for Asian and white lipids are presented in Fig. 5. The increase in temperature from 23°C to a physiologically normal 36°C did not alter the interfacial rheological parameters of the lipid films for either Asian or white subjects; however, at higher temperatures, noticeable changes were observed.

DISCUSSION

The most current data regarding the structure and chemical composition of the Meibomian and tear lipids were recently summarized in several reviews.[15–17] It is generally recognized that tear lipid films are at least 20 molecules thick. The polar double-tailed phospholipids detected in tear lipids[20,28] are one of the most surface-active compounds found in nature; they reduce the surface tension of water down to 21 to 22 mN/m

TABLE 1.
Compressibility (mN*109) of *ex vivo* human tear lipids at different states

	State				
	Liquid expanded		Liquid condensed		Semisolid gel
Branch	Compression	Expansion	Compression	Expansion	Compression
Asians	3.28 ± 0.5	2.97 ± 0.5	0.65 ± 0.3	0.21 ± 0.08	0.03 ± 0.009
Whites	5.9 ± 0.8	4.5 ± 0.7	1.55 ± 0.5	1.02 ± 0.5	0.183 ± 0.08

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when spread as a densely packed layer at the air-aqueous interface.[29] Mass spectrometric studies of human ML have shown substantial differences in phospholipid compositions in samples collected from healthy and diseased eyes.[21] Other authors reported that changes in the polar lipid concentrations might lead to instability of the outer tear lipid layer and the subsequent development of dry eye syndrome.[19,20] Rheology of the tear film is also different between normal and aqueous tear-deficient eyes.[30]

Investigators generally accept that human tear-lipid films are multilayered and are from 40 to 100 nm thick, yet published studies of physical characteristics of ML films have examined only monolayers (i.e., single-molecule layers ~2 nm thick). The interfacial pressure of ML monolayers has been explored,[9,10,31–35] these monolayers were designated to model the tear-lipid layer and the interaction of a lipid layer with model tear proteins. Only the expanded monolayers of ML with relatively low surface pressures up to 30 to 35 mN/m and low surface coverage were explored. Proteins were shown to penetrate into the lipid monolayer and reduce surface pressure.[31] In contrast, our work focused on the interfacial properties of approximately 100–nm–thick condensed lipid multilayers, which exerted significantly higher surface pressures of 50 ± 2 mN/m. We therefore inferred that thick reconstituted *ex vivo* human tear lipid films are better models to investigate the behavior of human ocular lipid layers than previously studied ML monolayers.[31–35]

Questions of interest and importance, namely, how well the films composed of contact lens–deposited lipids represent actual human tear lipid films and how they are similar to the films formed by ML remain unclear. An *ad hoc* video-microscopic experiment provided images of thick films formed using ML and tear lipids extracted from worn lenses (details of the instrument setup have been described elsewhere).[36] The approximately

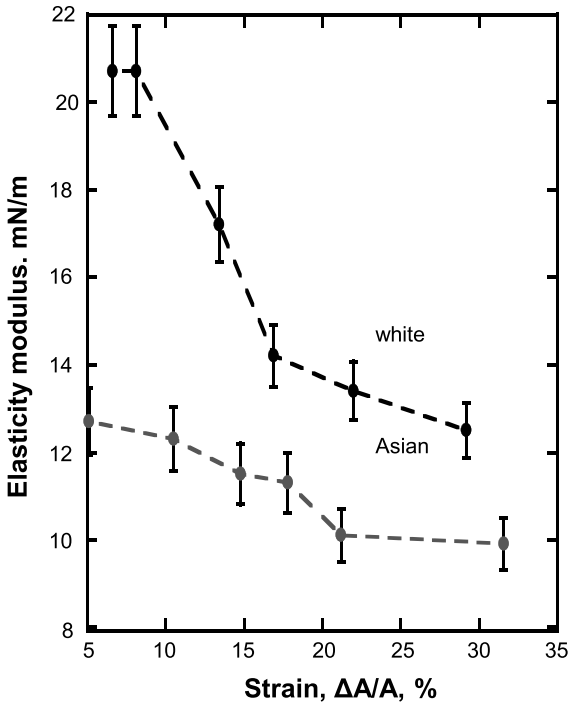

FIGURE 4.
Elasticity modulus as a function of strain.

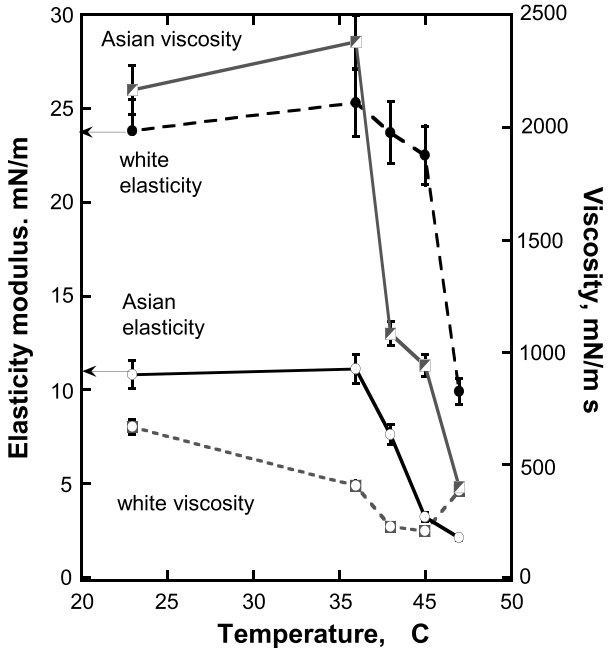

FIGURE 5.
Rheological parameters as functions of temperature.

100–nm–thick lipid films were spread on the surface of the MTE solution in minitrough with a surface area of 45 cm^2 at an ambient temperature of 22°C. Fig. 6 shows images of thick films formed by cow (Fig. 6A) and human meibum (Fig. 6B) (courtesy of Dr. C. Cerretani, UC Berkeley, Chemical Engineering Department); Fig. 6C shows the film formed using pooled lipid samples extracted from 10 lenses worn by Asian subjects. These images demonstrate substantial differences in appearance and uniformity in the films constituted from MLs and contact lens–extracted *ex vivo* human tear lipids. Images of ML films display intricately shaped areas with rainbow-like coloration and some dark spots, all together indicating uneven film thickness. Reconstituted lipid film looked much more uniform in color and thus in thickness. Note that these reconstituted tear lipid films were stable for more than 6 hours and exhibited neither dark spots indicating film thinning and rupture nor a combination of thicker islands formed by condensed liquid with some de-wet areas; whereas ML films did form island-like structures and eventually break up. The differences in stability of these thick films may imply that contact lens–extracted *ex vivo* human tear lipids may better represent healthy *in vivo* tear film lipid films than ML films, which eventually showed instabilities and breakup. These distinctions observed for the films formed using ML and *ex vivo* tear lipids may be related to a substantially higher level of amphiphilic lipids such as phospholipids, sphingolipids, and OAHFA found in tear lipids as compared with meibum.[19–21,28] Saville et al.[37] have pointed out that the phospholipids deposited on worn contact lenses show a molecular profile similar to that in tear lipids; however, the relative abundance of polar lipids might be higher in lens extracts.

Our previous study[11] had shown that equilibrium surface tension is a function of *ex vivo* lipid layer thickness. The transition from the expanded state to the condensed state was reached at a lipid film thickness of between 15 and 25 nm. Little

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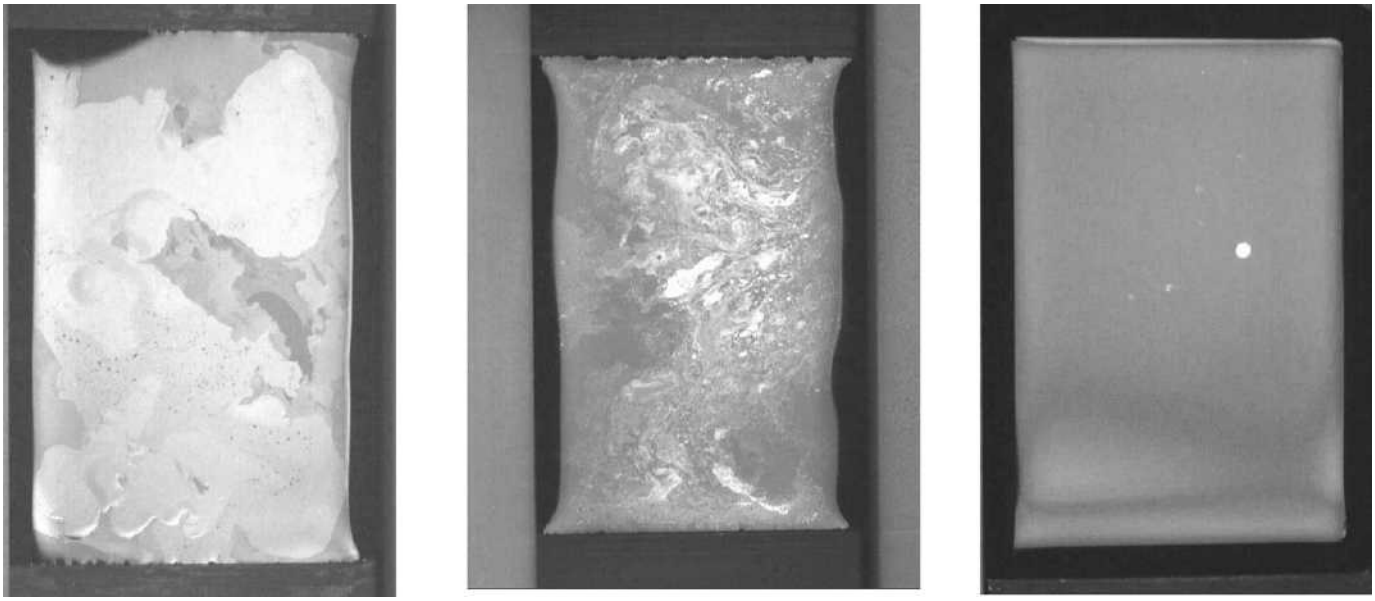

FIGURE 6.
Images of cow meibum, human meibum, and FND-extracted lipid films.

intersubject and intrasubject variability (SD, 1.2 mN/m for intersubject; SD, 0.5 mN/m for intrasubject) in equilibrium surface tension was observed for these 15- to 20-nm-thick multilayers. This value of 22 ± 1 mN/m is significantly lower than the surface tension of 34 to 36 mN/m reported in the literature for whole-tear fluid.[4]

The same minimum equilibrium surface tension values were found in the current study for both Asian and white lipids, which is in agreement with surface tensions reported for condensed phospholipid layers and lung surfactants.[23,29] The tension of 22 mN/m corresponds to the surface pressure of 50 mN/m, and it is 10 to 15 mN/m higher than maximum pressure reported for ML films in all studies published to date, where Langmuir trough (surface area, ~50 cm^2) was used as a device for isocycle recording.[8,10,31,32]

Sessile bubble technique used in our studies has significant advantage as compared with Langmuir trough, that is to say, the 1000 times smaller initial surface area of a bubble. This smaller initial area allows film thicknesses close to ocular lipid films to be obtained by deposition of very small (0.5 to 1 μg) amounts of lipids. The other noteworthy distinction of sessile bubble technique from Langmuir trough is that, in our novel sessile bubble method, the lipid films were initially deposited as approximately 100–nm–thick multilayers and were kept at rest for equilibration and structure formation in a maximum compressed state for prolonged (from 2 up to 24 hours) periods. In contrast, for the Langmuir trough method, films are initially deposited in a maximum extended submonolayer state with a surface pressure close to zero and are typically equilibrated for a much shorter period, 10 to 60 minutes.

We stipulate that the sessile bubble technique, permitting the examination of thick lipid films with well-developed structures, provides new essential information more representative and relevant to *in vivo* ocular lipid films than results obtained by studying monolayers. Note that tear lipids in the form of monolayers are typically not present under ocular conditions. The effect and importance of lipid film thickness and high surface pressures in expansion-contraction isocycles becomes evident when one compares the results for human and animal meibum monolayers[10] with the results we obtained for thick multilayered *ex vivo* lipid films.

As it was reported in Leiske et al.,[10] the isotherms of human and animal meibum were qualitatively similar, with little hysteresis between expansion and compression cycles for surface pressure intervals from 0 to 30 mN/m. These results are in general agreement with our findings regarding similar behavior of Asian and white *ex vivo* lipids in this low–surface pressure range. The difference between Asian and white lipids becomes evident only at higher surface pressures corresponding to film thicknesses approximately 20 nm and greater. As seen from Fig. 2, the isocycles for Asian and white lipids almost completely coincide from the lowest pressure up to a surface pressure of about 28 mN/m, and then they start to diverge.

At elevated pressures, white lipids exhibit greater slopes in both compression (upper) and expansion (lower) branches of these cycles (Fig. 2) and reach 50 mN/m maximum pressure at film thicknesses approximately 50 nm, whereas the Asian lipid film has to be compressed to almost 70 nm thick to attain the same high surface pressure. Note that the line slopes change along the branches with clearly detectable inflection points in compression branch of both Asian and white lipid films at analogous surface pressures approximately 26 and 50 mN/m; however, for Asian lipids, the second inflection takes place at almost two times higher film thickness. These inflection points signify phase transitions from liquid-expanded to liquid-condensed and then from liquid to semisolid or gellike condensed states. Fig. 7 demonstrates that

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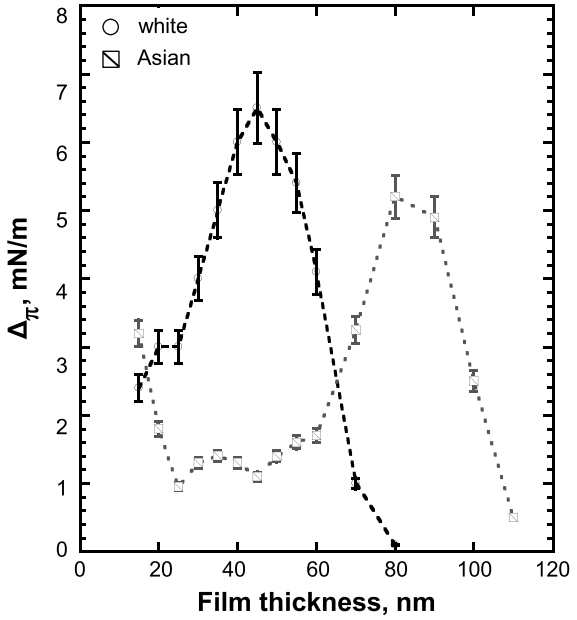

FIGURE 7.
Interfacial pressure hysteresis as a function of lipid film thickness.

the hysteresis between expansion and compression branches determined as[27]:

$$(\Delta\Pi)_h = (\Pi_c - \Pi_e)_{h=const} \quad (4)$$

between compression and expansion branches of the isocycles is not constant but grows as film thickness rises, reaching its maximum when the surface pressure reaches the maximum value in compression branch and then decreases as the branches draw closer. For Asian lipids, hysteresis reaches maximum at approximately two times higher film thicknesses than that for white lipids.

The compressibility values determined according to Equation 2 and averaged for Asian and white lipids are summarized in Table 1 for liquid expanded, liquid condensed, and gel states on expansion and compression branches separately. Note that gellike phase compressibility was determined on compression branches only. One can see from Table 1 that the compressibility of white lipids is, in general, higher than that of Asian lipids for all states and branches (analysis of variance [ANOVA] $p < 0.05$).

The dissimilarities in dynamic behavior of Asian and white lipids at relatively slow deformation rates are confirmed and explained by the rheological step-strain experiments. All rheological experiments were conducted applying practically instantaneous (<0.3 seconds) stepwise deformation to approximately 100 nm thick film, with an initial surface pressure of 50 ± 2 mN/m, that corresponds to the region of isocycles where surface pressure is only slightly dependent on film thickness. These experiments demonstrated that Asian and white lipids have significantly different interfacial rheological properties. On average, as one can see from Table 2, Asian lipids have a lower elasticity modulus, higher interfacial viscosity, and longer relaxation time, and the differences are statistically significant (ANOVA $p < 0.05$). Moreover, the response of the thick lipid films to variations in interfacial dilatation amplitude was dissimilar between Asian and white subjects. A five-fold increase (Fig. 4) in dilatation amplitude caused a two-fold decline in the elasticity modulus for white lipids; whereas for Asian lipids, the same increase in dilatation amplitude affected the elasticity modulus only slightly. Also, the interfacial viscosity of the lipid layers was independent of dilatation amplitude for subjects of both geneses.

We hypothesize that, when high deformations are applied to the lipid multilayers, the hydrophobic bonds between the hydrocarbon chains of adjacent lipid molecules first weaken and gradually deteriorate until the deformation becomes high enough for these bonds to be completely severed. The observed differences in stress versus deformation dependencies for Asian and white lipids suggest that films formed by Asian lipids have initially weaker bonds between adjacent lipid molecules than films formed by white lipids. Comparing interfacial rheological properties of the thick lipid layers, we found that Asian lipids are more viscous and noticeably less elastic than white lipids. In recently published articles describing the results of infrared-spectroscopic studies of human ML from normal subjects and from subjects with MGD, it was shown that dysfunctional lipids have a higher bulk viscosity than normal lipids, which correlates with a reduced tear breakup time for subjects with MGD when compared with that in healthy subjects.[38]

One of the clinical parameters measured in this study was NI-BUT of prelens tear film assessed by Medmont E300 Corneal Topographer (Medmont Pty Ltd, Victoria, Australia). The average Asian NI-BUT of prelens tear film was 3 ± 3 seconds and 5 ± 4 seconds for whites (ANOVA $p < 0.05$), confirming the implication of dissimilarities in interfacial rheological properties of *ex vivo* lipids between subjects of Asian and white ethnicities. Recent data also confirmed a lower tear film stability for Asians compared with that in whites.[39] The same numbers of Asian and white subjects were recruited for extended wear with FND lenses; however, a dropout rate was much higher among Asians because of lower tolerance of extended wear and a higher incidence of dry eye complaints. Thus, only five Asians, compared with nine whites, were able to complete the study, suggesting that lens-wearing comfort might have been affected by prelens tear quality that was in turn influenced by tear lipid rheology. Further investigation is warranted to confirm these findings.

Temperature is found to have an effect on interfacial behavior of human meibum monolayers, which show signs of melting close or slightly higher than ocular temperature.[10,40]

TABLE 2.
Summary of parameters for diffusion-relaxation fits, 22°C

	E_0, mN/m	E_∞, mN/m	τ_{rel}, s	A_{rel}, mN/m	τ_{dif}, s	A_{dif}, mN/m
Asians	37.1 ± 7.3	13.9 ± 3.2	323 ± 43	5.2 ± 2.4	12.7 ± 4.6	28.5 ± 9.4
Whites	35.9 ± 4.6	19.7 ± 2.6	88.3 ± 19.7	4.7 ± 1.3	25.7 ± 8.8	27.4 ± 8.6

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Our study of the effects of temperature on the rheological properties of *ex vivo* lipids has provided another substantiation of the distinctions between Asian and white lipids (Fig. 6).

The reduction in elasticity modulus at elevated temperatures indicates that lipid films go through a transition from a gellike to a liquid-like state.[25,26] These results imply that lipid films made of samples collected from healthy white subjects have a higher melting temperature than lipid films from Asian subjects. It has been reported that the phase transition temperature of human meibum decreased by 4°C over an age range of 4 to 90 years because of age-related compositional changes in the meibum.[41] Analogously, we deduce that the observed differences in interfacial properties of Asian and white tear lipids are caused by a disparity in their respective chemical compositions.

As mentioned previously, the samples for this study were collected from healthy young individuals of both ethnicities. There are distinct dissimilarities in interfacial relaxation mechanisms for these lipid films. Specifically, at small dilatational deformation, relaxation is predominantly a viscous process for Asian lipids, whereas, for white lipids, it is dominated by film elastic properties. Because rheological properties of thin liquid films are linked to film stability, we hypothesize that the rheological parameters of lipids collected from individuals with dry eye symptoms might be dissimilar from that of healthy subjects and also might vary among subjects of different ethnicities. If future studies show that this is indeed the case, it could have clinical implications for the treatment of dry eye in different ethnic populations. Our studies form the basis for future investigation to determine the role of interfacial rheological behavior of tear lipids in overall tear film stability for genetically diverse normal healthy individuals and for those with different degrees of dry eye manifestation.

CONCLUSIONS

Sessile-captive bubble technique is shown to be particularly suitable for investigation of the dynamic interfacial properties of microgram-sized biological samples, in particular, *ex vivo* human lipids. It was shown that there are distinct differences in dynamic interfacial and rheological behavior between human lipids collected from healthy subjects of Asian and white genesis. White lipids exhibited higher elasticity modulus and elasticity limit with phase transition at larger deformations. These observations indicate strong intermolecular interactions within an interfacial layer; these lipids behave like cross-linked gels when exposed to uniform radial deformation. In contrast, the rheological behavior of more viscous/less elastic Asian lipids is more typical for weakly structured gels. Our hypothesis that these differences stem from variations in chemical composition will be tested in further studies.

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