The TFOS International Workshop on Contact Lens Discomfort: Report of the Contact Lens Interactions With the Tear Film Subcommittee

Jennifer P. Craig,1 Mark D. P. Willcox,2 Pablo Argüeso,3 Cecile Maissa,4 Ulrike Stahl,5
Alan Tomlinson,6 Jianhua Wang,7 Norihiko Yokoi,8 Fiona Stapleton,2 and the members of TFOS International Workshop on Contact Lens Discomfort

1Department of Ophthalmology, New Zealand National Eye Centre, University of Auckland, Auckland, New Zealand
2School of Optometry and Vision Science, University of New South Wales, Sydney, New South Wales, Australia
3Schepens Eye Research Institute and Massachusetts Eye and Ear, Harvard Medical School, Boston, Massachusetts
4Optometric Technology Group Research & Consultancy, London, United Kingdom
5Centre for Contact Lens Research, School of Optometry and Vision Sciences, University of Waterloo, Waterloo, Ontario, Canada
6Glasgow Caledonian University, Glasgow, United Kingdom
7University of Miami, Miller School of Medicine Department of Ophthalmology, Bascom Palmer Eye Institute, Miami, Florida
8Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Correspondence: Fiona Stapleton, School of Optometry and Vision Science, University of New South Wales, Rupert Myers Building, Barker St, Sydney, 2052, New South Wales, Australia; f.stapleton@unsw.edu.au.

See the tables in the Introduction for the members of the TFOS International Workshop on Contact Lens Discomfort.

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The aim of the subcommittee report was to review published evidence describing tear film changes secondary to contact lens wear and to examine the evidence for associations between tear film changes and contact lens–related discomfort (CLD) in order to identify potential etiologies for CLD and strategies for the optimization of comfort.

The report comprises two main sections; the first describes biophysical interactions between the contact lens and the tear film, and the second deals with biochemical changes to the tear film associated with contact lens wear.

In first considering the tear film structure, recent tomographic, interferometric, and reflectance spectral techniques indicate central corneal tear film thickness values around 3 μm,1–3 aligning closely with earlier measurements.4,5

The thin outermost lipid layer of the tear film, on the order of 50 to 100 nm in thickness,6–7 forms the barrier between the environment and the eye. The lipid secretions arise mainly from the meibomian glands through orifices located at the mucocutaneous lid margin junctions, and are combined with a lesser lipid contribution from the eyelid glands of Moll and Zeiss.8 Spread over the tear film surface by blinking, the lipid layer comprises a thin inner polar layer, overlaid by a thicker outer nonpolar layer.9,10 In addition to preventing overspill of tear fluid onto the eyelids and contamination of the tear film by skin lipids,9,11 the most significant role of the lipid envelope is considered to be in retarding evaporation from the ocular surface.12,13

The aqueous phase of the tear film forms the bulk of the tear film thickness.8 It arises primarily from the main lacrimal gland and accessory lacrimal glands of Krause and Wolfring,14 with additional fluid and electrolytes secreted by the ocular surface epithelial cells. The tear flow rate varies according to the level of sensory stimulation, in response to the demands of the external environment. The overnight tear production rate is significantly lower than that during the day.15

The role of the aqueous phase is to nurture and protect the epithelia by providing a medium for the transfer of oxygen and nutrients to the avascular corneal tissue; conveying signals between the structures bathed in aqueous; and flushing away epithelial debris, toxins, and foreign bodies.16 The electrolytes within the aqueous phase dictate the osmolarity of the tear fluid, as well as playing a role in regulating pH and maintaining epithelial integrity.16 Hyposmolarity, which reflects an increased electrolyte concentration, is recognized to damage the ocular surface.17 Aqueous layer proteins contribute to ocular surface defense18 and maintenance of tear film stability.9 The proportions of plasma-derived and conjunctiva-derived proteins relative to lacrimal gland proteins within the tear film are dependent upon the tear flow rate and the level of ocular surface stimulation. As well as electrolytes and proteins, the tear film contains antioxidants to scavenge free radicals and growth factors important in epithelial regeneration and wound healing.19 Inflammation causes changes in the tear film constituents with release of inflammatory markers precipitating an escalating cycle of inflammation with ocular surface...
irritation, tear film instability, epithelial cell dysfunction, and apoptosis, which ultimately affects corneal epithelial barrier function. 

The aqueous phase of the tear film contains soluble, gel-forming mucins, produced by the conjunctival goblet cells. These mucins are important for removing pathogens and debris from the ocular surface, smoothing the ocular surface, and for protecting the surface, through lubrication, from the blink and from environmental insult. Anchored to the apical plasma membrane of the corneal and conjunctival epithelial cells are the transmembrane mucins, which contribute to forming the glycocalyx. These molecules can interact with multiple proteins through both the extracellular and intracellular domains. Carbohydrate structures present on the highly glycosylated extracellular region allow interaction with carbohydrate-binding proteins, such as galectin-3, to promote barrier function on the most apical epithelial cell layer.

In situ, contact lenses (CLs) divide the tear film into pre- and postlens films (Fig. 1). This compartmentalization impacts the tear film in a number of ways, affecting both the biophysical and biochemical properties of the tear film. Contact lens wearers are recognized to exhibit significantly more ocular symptoms than nonwearers. In an attempt to determine the relevance of tear film changes to CLD, each of the tear film parameters is described for the non–CL-wearing eye and CL-wearing eye. Wherever possible, associations between the tear film changes and reported discomfort in CL wearers are discussed.

**CHANGES IN THE BIOPHYSICAL PROPERTIES OF THE TEARS WITH CONTACT LENS WEAR AND THEIR EFFECT ON COMFORT**

**Blink Impact on Precorneal and Pre-Lens Tear Film Spread and Volume**

The integrity of the tear film and of the superficial layer of ocular surface epithelium is codependent. Therefore, if abnormalities are present in either one, a cycle of damage may be triggered at the ocular surface. For example, abnormalities in the tear film (presenting as an unstable tear film) can induce abnormalities in the ocular surface epithelium (presenting as decreased wettability and decreased barrier function) and vice versa, such that a suboptimal interaction between the tear film and the ocular surface epithelium ensues and is maintained in a cyclical fashion. Such possible interrelated vicious cycle mechanisms are presented here in the context of the tear film–CL interactions.

To help maintain clear vision and ocular surface health, eye blinks occur to distribute natural tears over the ocular surface, especially the corneal surface. Two major types of blink can be distinguished, complete and incomplete (partial), in which the eyelid covers more or less than 67% of the cornea.

It is reported that in healthy subjects, the proportion of incomplete blinking, for an unspecified vision task, can reach up to 20% of the total blinks. The insertion of a CL onto the ocular surface might not modify the overall blinking frequency immediately; however, the following findings are repeatedly reported:

1. There is a higher percentage of incomplete blinks in rigid CL wearers.
2. Although no clear difference is observed in the frequency of incomplete blinks between soft CL wearers and control subjects, the correlation between the percentage of incomplete blinks and the grade of corneal fluorescein staining is much stronger in the eyes of subjects who wear soft CLs. Moreover, subjects with incomplete blinks reportedly suffer more from discomfort and dryness, and more lens deposits.

The ratio between the tear film breakup time (TBUT) and the interblink interval (IBI) defines the Ocular Protection Index (OPI). The ocular surface is considered to be protected when the TBUT matches or exceeds the IBI (OPI ≥ 1). In the case of a blink rate of 12 per minute (mean IBI, 5 seconds) and a TBUT...
Report on Interactions With Tear Film

Comparison to compositions instilled as eye drops, much longer residence time at the ocular surface in the presence of tear-deficient dry eye induced due to CL wear or other reasons, the extent of lipid layer spread decreases and the viscous contribution to its spread becomes dominant. Indeed, lipid layer spread becomes much slower after 8 hours of soft CL wear (measured as a 4-fold increase of the exponential time constant describing the spread kinetics). The impaired lipid spreading is thought to correlate with the thinner aqueous layer formed over the soft CL surface, particularly over the surface of silicone hydrogel CLs, in which the nonwettable hydrophobic silicone moieties might reorient themselves toward the lens surface after an initial breakup event. This dramatic delay of lipid layer spread is somewhat indicative of the deterioration of the CL surface experienced in the course of daily use, and reflects the decreased aqueous tear volume observed during daily CL wear.

Tear Film Stability

Qualitative changes in the lipid layer appearance manifest clinically as alterations in the tear film integrity, described as the stability of the precorneal tear film, a clinical index of which is the tear breakup time. In the non-CL-wearing eye, thinner lipid layers have been associated with shorter tear breakup time measurements, and thicker layers with increased breakup times.

Tear film stability does not remain constant throughout the day. Decreases in TBUT have been observed in non-CL wearers immediately after awakening and also toward the end of the day, with the latter observation proposed to contribute toward increased end-of-day discomfort reported by office workers and CL wearers. Over the longer term, TBUT has been shown by a number of investigators to reduce with age while others have observed no difference with age. Sex also appears to have an effect on tear film stability, with females reported to exhibit reduced TBUTs relative to age-matched males. Conflicting effects of low ambient relative humidity on noninvasive tear film breakup in the non-lens-wearing eye have been reported.

Contact lenses disrupt the TFL and reduce tear film thickness. The disruption is most marked with rigid lens wear, where typically no pre-lens lipid layer is visible clinically, and tear breakup occurs within 2 to 3 seconds in contrast to values around 5 to 6 seconds over a soft CL. Larger, less mobile soft CLs have greater potential to support a pre-lens TFL, but this lipid layer tends to be thinner and consequently susceptible to more rapid breakup than the non-CL tear film, irrespective of lens material. Overall tear film thinning has been shown to be significantly faster on the surface of a CL than on the corneal surface. This instability may be related to a thinner pre-lens film, but it has been proposed that even where the pre-lens and precorneal tear films are similar in thickness, the pre-lens tear film is still considerably less stable. The location of tear film breakup is also influenced by the presence of a CL, with the locus of tear film breakup of the pre-lens film most often being central, while that of the non-lens-wearing eye is more frequently paramesial. Although CL dehydration has been implicated as a major factor in the development of CL-related dry eye in
high water content soft lens wearers, more recent evidence suggests that dehydration plays a less significant role; and the mechanism involved in the thinned lipid layer and reduced stability is related more to alterations in the lipid layer structure, possibly due to the affinity of the polar components of the lipid layer to the lens surface, resulting in increased tear evaporation and lens surface dewetting.

Comparison of the precorneal tear film before and after CL wear highlights significant decreases in breakup time initially but, over the longer term, precorneal tear film breakup (without a CL in situ) appears to be largely unaffected by CL wear, irrespective of the material or wear regimen, with similar effects observed for continuous and daily wear.

In CL wearers, reduced pre-lens breakup times have been associated with increased symptoms of discomfort, both in hydrogel and silicone hydrogel lens wearers. Based on the differences in pre-lens breakup time between symptomatic and asymptomatic individuals, Hom and Bruce suggested a cutoff TBUT value of 3 seconds as a suitable criterion for identifying tear film dysfunction likely to cause dryness symptoms in CL wearers.

With respect to tear film stability, consistent comfort differences relating to lens material have not been established, but environmental conditions have been described as further affecting symptoms of dryness and stability of the pre-lens tear film. Maruyama and colleagues found reduced tear film thickness and breakup times in conditions of low relative humidity (20%), suggesting that increased evaporation plays a role in this process. In the presence of a soft CL, this was associated with increased symptoms of discomfort. Higher lens water content was found to correlate with increasing dryness symptoms, but not to tear film breakup.

Significant differences in NIBUT have been reported between CL wearers described as either tolerant or intolerant on the basis of their ability to tolerate lens wear for a period of at least 6 hours. Tolerant wearers averaged a NIBUT of around 20 seconds in comparison to 13 seconds for intolerant wearers. Interestingly, the pattern of pre-lens tear film drying on the CL surface was shown to vary with tolerance to CL wear, with all intolerant CL wearers exhibiting a streak pattern of breakup in comparison to tolerant wearers, in whom more spot breakup patterns were observed. Stepwise discriminant function analysis, used to predict tolerance or otherwise to ≥6 hours of lens wear, indicated that, of the broad range of tests performed, tear film stability indices (NIBUT and drying pattern) were the most highly predictive measures of tolerance. Others, too, have conceded from a range of tests that NIBUT, combined with lid parallel conjunctival folds (LIPCOF) and Ocular Surface Disease Index (OSDI) score, provided the best predictive power (positive predictive value 87%, accuracy 91%) for symptom development in new CL wearers.

In a subsequent study by the same group, the effect of 6 hours of soft CL wear on a similarly large range of tear film evaporation rates described in the literature have been attributed to subject selection, measurement techniques, and instrumentation. Published studies reporting evaporation rates in the non–CL-wearing eye are summarized in Table 1.

The rate of tear film evaporation has been demonstrated to increase with a CL in situ. It is generally accepted that the physical presence of a CL disrupts the normal tear film structure, and in particular the lipid layer, facilitating a more rapid loss of tear fluid by evaporation. This is supported by research describing decreased tear film stability in the presence of a CL. Under constant environmental conditions, researchers have failed to demonstrate consistent differences in the tear evaporation rate with different lens materials, even between rigid and soft lenses. As with non–CL-wearing eyes, there is significant variation in tear evapora-
tion rate values between research groups for CL wearers; however, overall, the literature shows that CLs typically result in a 1.2 to 2.6 increase in the rate of tear evaporation relative to the non–lens-wearing eye, with no clear pattern relating to either lens form or water content (Table 2). It does, however, appear possible to differentiate lens types under adverse environmental conditions. Kojima and colleagues found significant increases in evaporation rate in wearers of hydrogel lenses but not silicone hydrogel lens wearers following exposure to a controlled adverse environment chamber that was significantly drier at 18% relative humidity (RH) than the ambient environment (30%–40% RH).

Increased tear evaporation rates lead to dryness and discomfort symptoms in CL wearers. Kojima and colleagues noted a relationship between increased tear evaporation and ocular discomfort in nonadapted CL wearers fitted with hydrogel lenses (etafilcon A) and exposed to a controlled adverse chamber environment with RH of 18%. Interestingly, however, no differences in either symptoms or evaporation rate were observed for neophytes fitted with silicone hydrogel lenses (narafilcon A) under the same conditions.

### Table 1. Evaporation Rates Reported for the Non–Contact Lens–Wearing Eye

<table>
<thead>
<tr>
<th>Authors</th>
<th>TER ± SD, $\times 10^{-7}$ g/cm²/s</th>
<th>TER Measurement Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamano and colleagues (1980)</td>
<td>26.9</td>
<td>Pressure gradient, open chamber (in contact with tears)</td>
</tr>
<tr>
<td>Tomlinson and Cedarstaff (1982)</td>
<td>109.2 ± 49.3, at 70%</td>
<td>Resistance hygrometry</td>
</tr>
<tr>
<td>Cedarstaff and Tomlinson (1983)</td>
<td>119.8 ± 39.9, at 50% RH</td>
<td>Resistance hygrometry</td>
</tr>
<tr>
<td>Rolando and Refojo (1985)</td>
<td>4.1 ± 0.4, at 29.5% RH</td>
<td>Change in relative humidity measured within closed chamber filled with dry air</td>
</tr>
<tr>
<td>Herold (1987)</td>
<td>68.9 ± 18.9</td>
<td>Vapor pressure</td>
</tr>
<tr>
<td>Tomlinson and colleagues (1991)</td>
<td>12.5 ± 6.9</td>
<td>Vapor pressure</td>
</tr>
<tr>
<td>Tomlinson and Cedarstaff (1992)</td>
<td>166.7 ± 5.0, at 70%</td>
<td>Resistance hygrometry</td>
</tr>
<tr>
<td>Tsubota and Yamada (1992)</td>
<td>15.6 ± 3.8, at 40% RH</td>
<td>Vapor pressure</td>
</tr>
<tr>
<td>Tomlinson and Giesbrecht (1994)</td>
<td>10.6 ± 6.6</td>
<td>Vapor pressure</td>
</tr>
<tr>
<td>Mathers and colleagues (1993)</td>
<td>14.7 ± 6.4, at 30% RH</td>
<td>Change in relative humidity measured within closed chamber filled with dry air</td>
</tr>
<tr>
<td>Craig and Tomlinson (1997)</td>
<td>0.39 ± 0.37, at 48% RH</td>
<td>Vapor pressure</td>
</tr>
<tr>
<td>Goto and colleagues (2003)</td>
<td>4.1 ± 1.4</td>
<td>Change in relative humidity measured in ventilated chamber system with air flow</td>
</tr>
<tr>
<td>Thai and colleagues (2004)</td>
<td>10.8 ± 5.3</td>
<td>Vapor pressure</td>
</tr>
<tr>
<td>Guillon and Maissa (2010)</td>
<td>16.6, median 15.9, at 30% RH</td>
<td>Change in relative humidity measured within closed chamber filled with dry air</td>
</tr>
<tr>
<td>Mathers and colleagues (2009)</td>
<td>15.7, median 11.4, at 40% RH</td>
<td>Change in relative humidity measured within closed chamber filled with dry air</td>
</tr>
<tr>
<td>Khanal and colleagues (2011)</td>
<td>5.8 ± 2.8</td>
<td>Vapor pressure</td>
</tr>
<tr>
<td>Dogru and colleagues (2011)</td>
<td>4.1 ± 0.5, at 30%–50% RH</td>
<td>Quartz crystal humidity sensor</td>
</tr>
<tr>
<td>Arciniega and colleagues (2011)</td>
<td>5.5 ± 2.0, at 30% RH</td>
<td>Change in relative humidity measured within closed chamber filled with dry air</td>
</tr>
<tr>
<td>Kimball and colleagues (2010)</td>
<td>3.8 ± 1.3, at 40% RH</td>
<td>Extrapolated from spectral interferometry*</td>
</tr>
<tr>
<td>Petznick and colleagues (2013)</td>
<td>26.8 ± 2.4</td>
<td>Extrapolated from infrared thermography</td>
</tr>
</tbody>
</table>

**TER, tear evaporation rate.**

* Includes four dry eye patients.

### Table 2. Summary of Studies Reporting Tear Evaporation Rates in Contact Lens Wearers

<table>
<thead>
<tr>
<th>Authors</th>
<th>% Increase in TER During Lens Wear</th>
<th>Lens Type</th>
<th>Lens WC, %</th>
<th>RH, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomlinson and Cedarstaff (1982)</td>
<td>216</td>
<td>PMMA</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>216</td>
<td>Paragon-18 (PMMA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>191</td>
<td>Silicone elastomer: Silsoft (Bausch and Lomb, Rochester, NY)</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>187</td>
<td>Hydrogel Sauflon (Sauflon Pharmaceuticals Ltd., Twickenham, UK)</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Cedarstaff and Tomlinson (1983)</td>
<td>258</td>
<td>Hydrogel Cibasoft (Alcon, Fort Worth, TX)</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>Hydrogel 70%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>Hydrogel 55%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>155</td>
<td>Hydrogel 38%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thai and colleagues (2004)</td>
<td>127</td>
<td>Hydrogel: polymacon</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>123</td>
<td>Hydrogel: omafilcon A</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>142</td>
<td>Hydrogel: phenfilcon A</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>Silicone hydrogel: balafilcon A</td>
<td>36</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>140</td>
<td>Hydrogel: etafilcon A</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Guillon and Maissa (2008)</td>
<td>156</td>
<td>Hydrogel</td>
<td>NS</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>167</td>
<td>Hydrogel</td>
<td>NS</td>
<td>40</td>
</tr>
</tbody>
</table>

NS, not stated; PMMA, Polymethyl methacrylate; WC, water content.
Tear Film Temperature

The temperature of the normal ocular surface, and thus the adjacent tear film, is lower than core body temperature on account of its exposed location, somewhere on the order of 32°C to 36°C.131 Mean ocular surface temperature in dry eyes is reported to be similar to132 or slightly higher than that in normal eyes.134,135 Increased ocular surface temperatures measured in uveitis136 suggest that the increased temperature in dry eye is likely attributable to ocular surface inflammation, a key element in dry eye.17,134

Technological advances, resulting in the advent of noncontact infrared thermography, have enabled measurement of ocular temperature with significantly improved sensitivity as well as spatial and temporal resolution.153,134,137,138 This has led to the observation by most researchers that the ocular surface temperature varies across the exposed surface, with the normal cornea being warmest at the limbus and coolest centrally.134,139 With the exception of one study,135 this temperature differential between the limbus and corneal center has been shown to be greater in dry eye, such that the central cornea is significantly cooler, relative to the limbus, in dry eyes than in normal eyes.100,134,135,140 The faster rate of cooling observed postblink in dry eyes has been attributed to a more rapid rate of tear film evaporation.135

The relationship recognized to exist between tear film stability and ocular surface temperature46,100,141 suggests that ocular surface thermography is an indirect measure of tear film stability.142 More recently, thermography has also become recognized as a surrogate for evaporation rate measurement.113,128

Variations in participant characteristics, study design, and methodology between published studies evaluating the effect of CLs on tear film temperature preclude direct comparison of their results. Participant age and health status, as well as the environmental conditions and the precise measurement site on the eye, can affect the results. The invasive nature of some earlier techniques, in contrast to the noncontact techniques reported more recently, further compounds this issue.

In one of the earliest reports, with thermistors embedded in scleral lenses, Hill and Leighton143 observed insignificant temperature differences in the presence of the CLs, although temperatures increased significantly during eye closure. Hamano,144 too, observed only small differences (<0.5°C) between eyes with and without rigid CLs using a noncontact radiometer. Conversely, Fatt and Chaston,145 with a noncontact bolometer, recorded a more significant difference in ocular surface temperature, of 0.5 to 1.5°C, between hard CL wearers and non–lens-wearing eyes. They found the ocular surface temperature in soft CL wearers to differ no more than 0.5°C from the naked eye temperature. Also evaluating soft CLs, Martin and Fatt146 detected insignificant differences in temperature beneath hydrogel lenses, using a technique with thermistors sandwiched between thin hydrogel CLs, although again they were able to observe significant temperature increases during eye closure. Montoro and colleagues,147 as one of the first groups to use the current standard technique of infrared thermography with customized analysis software, identified irregular thermal patterns in a group of 19 CL wearers.

More sensitive current techniques indicate that the temperature of the pre-lens tear film in soft lens wearers is cooler than that of the non–CL-wearing eye.140 while the temperature of the postlens tear film beneath the CL is higher.131 Lens materials with high water content and a correspondingly rapid rate of water loss show lower lens surface temperatures in situ than those with low water content.140 This difference between lens materials is reflected in ocular surface temperatures (postlens tear film), which increase beneath all CL materials,140 but more so with silicone hydrogel lenses than with hydrogel lenses.131 This is attributed to the higher bound-to-free water ratio, which results in a lower rate of water loss from silicone hydrogel lenses.131

Few studies have directly evaluated comfort and ocular surface temperature in CL wearers. Hill and Leighton149 experienced some success in correlating temperature-related sensations described by subjects to corneal temperatures assessed by thermistor units embedded in scleral CLs. Some predictive value could be assigned to specific descriptors, and it was concluded that tear film temperature could reflect the neural contributions that influence subjective experience.149 However, such a study has not been performed with the high-resolution thermographic technology available now. Lowering the ocular surface temperature with cooled (4°C) artificial tears reduces ocular surface sensitivity and improves comfort.150 This might suggest that, if ocular surface temperature is raised in CL wear, the concept of reducing ocular surface temperature could be advantageous. However, this should be viewed in conjunction with the hypothesis put forward that cold neuroreceptors in the corneal and lid margin may be partly responsible for CLD (see Report by the Subcommittee on Neurobiology).

Tear Film Thickness

The precorneal tear film (PCTF) is regarded as an important layer in keeping ocular surface wet and smooth so that epithelial integrity and sharp vision can be maintained. The thickness of the PCTF is a key parameter that relates to tear secretion, spreading, evaporation, and drainage. Previously no consensus could be reached on tear film thickness, mainly on account of the difficulty in measuring a fluid layer that is highly dynamic in nature.155 However, it is generally accepted that the total tear film thickness is around 5 μm.141

Each blink alters the tear distribution, resulting in variation of the tear film thickness.151,152 During each blinking cycle, the tear film thickness varies within a couple of microns.151 If the blinking is delayed (by the subject’s consciously refraining from blinking),151 the tear film thickness increases to approximately 7 μm due to reflex tearing. Recently, ultra-high-resolution optical coherence tomography (OCT) has been used to corroborate these measurements.153–155 The tear film begins to thin during the open-eye period due to redistribution and evaporation.1,72,151

Nichols and King-Smith64 found that the pre-lens tear film (PLTF) was approximately 2 μm with interferometry, which was about the same as measured at 3 minutes after lens insertion using ultra-high-resolution OCT.153 When the measurement was taken at the time of lens insertion, the PLTF was higher, at around 6 μm, due to reflex tearing or surplus lens wetting solution.153 The PLTF thickness can be altered by adding drops onto the lens; however, the increase of the PLTF is transient (approximately 10 minutes) (Fig. 2).153 Interferometry measurements reveal that PLTF thickness might be approximately 1 μm thinner compared to the 3.5-μm-thick PCTF and that the PLTF thinning rate is higher compared to that of PCTF (which in turn leads to a shorter TBUF of the PLTF).155,152,156

The postlens tear film (PoLTF) may play an important role in the interactions with the ocular surface and may impact lens movement and ocular comfort.147,152 Depletion of the PoLTF may also cause lens adherence160 and surface staining,145,159 which have to do with CL-related complications144,161 and discontinuation.28,162 The thickness of the PoLTF at the center location of the cornea is approximately 1 to 3 μm (Fig. 3),136,64,153,156 in agreement with other studies. In contrast,
Lin and colleagues\textsuperscript{163} found the central PoLTF to be approximately 11.5 \( \mu \)m using optical pachymetry. It may be slightly thicker at the time of lens insertion, and rapid thinning is evident.\textsuperscript{64,153} The PoLTF remains thin irrespective of the instillation of artificial tears.\textsuperscript{153}

**Tear Production/Turnover**

Quantification of tear production during CL wear has received limited attention, in part because of the technical difficulty of measurement. The earliest attempts by Hamano and colleagues (1983)\textsuperscript{119} employed a wetting measure, the phenol red thread test, to overcome the problems of repeatability and consistency with the Schirmer test. Thread wetting was not found to increase with CL wear. Sorensen and colleagues (1980)\textsuperscript{164} were the first to use tear clearance as a measure of tear flow in 14 individuals before and after 1 month of adaptation to a Soflens CL (Bausch & Lomb, Rochester, NY). They used a gamma camera to assess the rate of new tear production through observation of the elimination of a radioactive tracer, technetium, from the conjunctival sac. The fractional turnover rate with a soft CL presoaked in the technetium solution was similar to the rate with a solution instilled directly into the eye.\textsuperscript{164}

Other investigators have observed the elimination of a fluorescein dye from the eye to measure tear production during CL wear.\textsuperscript{165–168} Puffer and colleagues (1980)\textsuperscript{165} studied 51 normal subjects with a simple method that permitted measurement of the rate of fluorescein loss from the central PTCF. No statistically significant correlations were found between tear elimination coefficient and sex, eye color, or CL wear.\textsuperscript{165} Occhipinti and colleagues (1988)\textsuperscript{166} were the first to employ the automated scanning fluorophotometer (Fluorotron; OcuMetrics, Mountain View, CA) and found no significant difference in tear turnover rate (TTR) in CL wearers compared with nonwearers.

The use of either an automated scanning fluorophotometer or slit lamp–mounted fluorophotometer offers potentially the most accurate measure of TTR in CL wear without the cost and inherent restrictions of the gamma camera. The use of small molecular weight fluorescent tracers, however, confounds measurement due to dye penetration into soft CLs.\textsuperscript{168,169,170} Notwithstanding this difficulty, the TTR appears to decrease significantly in CL wear compared with the non–CL-wearing eye.\textsuperscript{167} An average TTR of 15.5%/minute is typical of normal young subjects without lenses.\textsuperscript{167,171–175} Further experiments with a more likely nonpenetrating tracer, 70-kDa fluorescein-isothiocyanate (FITC) dextran, were carried out on a group of 20 habitual wearers.\textsuperscript{168} The measures with a conventional hydrogel lens, etafilcon A (Acuvue 2; Johnson & Johnson Vision Care, Inc., Jacksonville, FL), and silicone hydrogel lens, balafilcon A (PureVision; Bausch & Lomb), and with no lens showed TTRs of 12.4%/minute, 13.2%/minute, and 16.4%/minute, respectively. Therefore fluorophotometric measurements of TTR with this tracer showed no statistically significant difference in the presence of a CL, consistent with the consensus from previous studies.

In an attempt to relate tear production in CL wear to the discomfort experienced by some CL wearers, Tomlinson and colleagues\textsuperscript{176} compared tear physiology in symptomatic and asymptomatic wearers. Subjects with symptoms of CL dry eye (CLDE) had a significantly lower basal TTR (in the absence of a lens) than asymptomatic subjects.\textsuperscript{90} TTR in this study was measured with the Fluorotron (OcuMetrics) immediately after CL removal. This finding is in accord with the speculation of Glasson and colleagues\textsuperscript{80} of reduced tear flow in intolerant wearers. Thai\textsuperscript{177} had previously shown that values on CL removal were consistent with the normal basal tear flow rate. No significant differences between the groups were found for tear evaporation, osmolality, or tear breakup time. The greater basal tear production facility in asymptomatic patients may offset the loss of tear fluid due to the increased tear evaporation rate induced by CL wear.\textsuperscript{104,120,178}

**Tear Volume**

Tears are secreted by the lacrimal gland and approximately 4.5 \( \mu \)l is distributed into the cul-de-sac, approximately 2.9 \( \mu \)l into
In contrast, the tear meniscus volume immediately increased compared with the precorneal tear film (PCTF, post hoc test, \( P < 0.05 \)). The PoLTF decreased continuously for the next 8 minutes (post hoc test, \( P < 0.05 \)), and the PLTF decreased in a similar fashion \((P < 0.05)\). \(A\) After lens insertion without prior application of a drop of artificial tears to the concave surface, the PLTF was immediately thicker than the PCTF at baseline \((P < 0.05)\). After 5 minutes of lens wear, both the PLTF and PoLTF decreased significantly compared with the moment of lens insertion \((P < 0.05)\). When 35\(\mu\)L of artificial tears was instilled on the lens, the PLTF increased significantly and then decreased gradually in the following 8 minutes \((P < 0.05)\). However, the PoLTF did not increase immediately after drop instillation and also did not change in the following 10 minutes (Repeated measures ANOVA, \( P > 0.05 \)). Reproduced from Chen et al. Ultrahigh-resolution measurement by optical coherence tomography of dynamic tear film changes on contact lens. IOVS. 2010:51:1988–1993.\(^{155}\)

In summary, there is no direct evidence based on published studies showing the relationship between tear film thickness (pre- or post tear film) and ocular discomfort in CL wearers. However, decreased tear meniscus volumes appear to be related to ocular discomfort at the end of the day.\(^{50}\) Similar findings with intolerant CL wearers have been reported by Glasson and colleagues.\(^{76}\)
Tear Film Profile at the Edge of a Soft CL

Soft CLs cover a portion of conjunctiva, which is soft tissue. The conjunctiva appears to distort at the lens edge. The interaction between the lens edge and conjunctiva may occur because of different pressure profiles that are produced across the ocular surface underneath each lens. It may be possible to have a tear meniscus around the lens edge at the point of lens insertion or on instillation of artificial tears. However, the tear meniscus around the soft CL edge appears much smaller than that around the hard lens edge. With excessive tears from tearing or instillation of artificial tears, the tear film can be augmented around the periphery of the lens, with the thickest layers at the inferior portion of the lens due to gravity.

Figure 5. Total tear volume during normal and delayed blinks in 21 subjects. The upper tear meniscus volume (UTMV), tear film volume (TFV), and lower tear meniscus volume (LTMV) were estimated during normal (A) and delayed (B) blinks. The tear volume was greater during delayed blinking than during normal blinking ($P < 0.01$). Most of the change was due to increases in the LTMV (B). Both UTMV and LTMV were higher ($P < 0.001$) during delayed blinking (B) compared with normal blinking (A). The UTMV and LTMV increased significantly at the end of the eye-opening period compared with the beginning during delayed blinking ($P < 0.05$). Reproduced from Palakuru et al. Effect of blinking on tear dynamics. IOVS. 2007;48:3032–3037.

Figure 6. Ocular surface comfort ratings (A) and UTMV (B), LTMV (C), and TTMV (D) during 10 hours of contact lens wear. Group 1, experienced contact lens wearers with dry eye complaints; group 2, experienced contact lens wearers without dry eye complaints; group 3, inexperienced contact lens wearers without dry eye complaints. Reproduced from Chen et al. Tear menisci and ocular discomfort in symptomatic wearers. IOVS. 2011;52:2175–2180.
Evaluation of the PoLTF peripherally may reveal more information on lens fitting tightness and matching between lens design and ocular surface. Using ultrahigh-resolution OCT, two gaps beneath the lens, filled with PoLTF, can be visualized. One type of PoLTF can be found at the peripheral cornea, and the other one can be found at the limbal junction area. The thickness of the PoLTF beneath the lens edge ranges from several micrometers up to approximately 60 μm. It appears that the PoLTF may vary depending on lens design and material.

Tear Exchange

From mathematical formulas, Weissman inferred that flexure of a ~3.0-diopter (D) lens should exchange approximately 0.01 μL of fluid per diopter. Lubrication theory predicted 10% to 20% tear exchange at each blink for a normal blink with the usual tear film thickness. Using fluorophotometry and a nonpenetrating tracer, the measured T95 (time to deplete 95% of a fluorescent dye from beneath a CL) was 27.3 minutes, and the tear exchange turnover rate was calculated to be 9.0%/minute. In another study conducted by McNamara and colleagues, the mean tear mixing rate was 1.82%/minute with a 12-mm-diameter CL, 1.61%/minute with a 12.5-mm-diameter CL, 1.54%/minute with a 13-mm-diameter CL, and 1.24%/minute with a 13.5-mm-diameter CL. Ocular surface OCT has been used to track tear mixing beneath the CL edge (Wang J, et al. IOVS 2011;52:ARVO E-Abstract 3628). In a small sample of five eyes, tear mixing was evident. Preliminary data showed that the 95% decay time was approximately 10 to 20 minutes (Wang J, et al. IOVS 2011;52:ARVO E-Abstract 3628).

Osmolarity

Tear osmolarity or the saltiness of tears can be regarded as an indicator of the balance between the production of tears and their elimination via evaporation, drainage, and absorption. The main contributors to tear film osmolarity are the electrolytes of the aqueous phase, principally the cations sodium and potassium and the anions chloride and bicarbonate; proteins and sugars play a minor role only. Mean tear film osmolarity measurements in the normal eye range between 283 and 318 mmol/kg, with an average value of approximately 302 mmol/kg. It must be noted that osmolarity is commonly determined for tears from the lower meniscus, and it has been speculated that the osmolarity across the ocular surface might be significantly higher due to the variable effects of evaporation. Generally, the sex of an individual and the hormonal cycle do not affect tear osmolarity, but increased osmolarity can be observed throughout the day and with increasing age. Dehydration and increased tear evaporation also lead to increased tear film osmolarity.
osmolarity, measurements on patients with epiphora remain equivocal.\textsuperscript{210–215} Tear osmolarity measurements find their most frequent application in the diagnosis of dry eye. The measurement of tear osmolarity has been suggested as a gold standard in the diagnosis of dry eye, as the often observed elevated levels (hyperosmolarity) are considered a core mechanism in symptoms and ocular surface damage in this condition.\textsuperscript{17,65,214} A comprehensive summary of causes and effects of tear film hyperosmolarity in dry eye is provided in the 2007 TFOS Report of the Dry Eye Workshop.\textsuperscript{17}

During CL wear, tear film osmolarity undergoes a series of changes. Initially, the insertion of a CL results in a reduction of tear film osmolarity, potentially caused by some reflex tearing during the early adaption to the lens.\textsuperscript{215–217} This initial reduction has been considered as a cause for PoLTF depletion with subsequent lens adherence and a contributor to corneal swelling, although all osmolarity measurements at the current time are limited to the tear meniscus. A subsequent increase in osmolarity is often observed.\textsuperscript{216–218} However, there remains some debate as to the level of tear osmolarity over time, the effect of lens type and wear modality, and the effect on ocular comfort. Some authors have reported that tear film osmolarity will return to or remain at its pre-CL insertion level,\textsuperscript{217–219} while others report an increased level, postremoval, compared to baseline.\textsuperscript{216,220,221} A summary of tear osmolarity values during CL wear is given in Table 3. Farris\textsuperscript{222} demonstrated that wear of soft CLs on an extended-wear basis and hard lenses on a daily-wear basis significantly increased tear osmolarity, but such effect was not observed with soft lenses worn on a daily-wear basis. In contrast, other authors have shown significantly increased tear film osmolarity with soft daily-wear lenses.\textsuperscript{221} So far, no differences in tear osmolarity have been demonstrated between hydrogel and silicone hydrogel CLs.\textsuperscript{215–218,223,224}

Until recently, most studies assessing tear film osmolarity required a large tear volume and consequently the collection of large amounts or dilution of the sample with subsequent recalculation. It must be considered that these requirements may have hindered the observation of subtle differences between lens types. Increased tear film osmolarity during CL wear has been attributed to two main factors: reduced tear production due to reduced corneal sensitivity, and excessive evaporation due to a disrupted tear film and reduced tear film stability.\textsuperscript{214,225} Considering that these mechanisms are similar to those in dry eye, there has been some interest in the impact of tear osmolarity in CL wear on ocular comfort. Nichols and Sinnott\textsuperscript{226} demonstrated significantly higher osmolarity values in participants with CL-induced dryness. Glasson and colleagues\textsuperscript{227} found that symptomatic CL wearers tended to display a high tear film osmolarity even without CLs. However, in a study by Stahl and colleagues,\textsuperscript{221} an association between tear osmolarity and ocular comfort during CL wear could not be shown.

Ferning

Tear ferning refers to the distinct crystallization pattern that appears when tears are allowed to air dry on a glass slide. This image of fern-like crystals is most commonly assessed, under white light microscopy, on a simple qualitative grading scale from I (complete, uninterrupted ferning pattern with no spaces between ferns) to IV (total absence of ferning).\textsuperscript{220–258} Although other quantitative methods, such as area assessment through counting the number of micrometer lattice squares,\textsuperscript{250–252} or digital image analysis have been applied,\textsuperscript{241,242} As outlined by Golding and Brennan,\textsuperscript{243} as well as Pearce and Tomlinson,\textsuperscript{244} there remains some discussion as to which components of the tear film are responsible for the successful development of tear ferns. However, there seems to be agreement that it is not the level of a single component but rather the ratio between the organic salts and macromolecules that will determine the quality of the ferning pattern.\textsuperscript{243–245} The majority of individuals display a tear ferning pattern of grade I or II.\textsuperscript{221} An increase in the tear ferning grade, reflecting abnormal tear functionality, can be seen with age.\textsuperscript{234} CL wear,\textsuperscript{229} in the morning,\textsuperscript{235} and in conditions such as keratoconjunctivitis sicca,\textsuperscript{251,252,256} Sjögren’s syndrome,\textsuperscript{247} Down syndrome,\textsuperscript{248} and cystic fibrosis.\textsuperscript{249}

The potential benefits of tear ferning in predicting CL tolerance were first described by Koghe and Lioret.\textsuperscript{250} Besides being able to predict discomfort, it was also possible to identify individuals with excessive protein deposition, as they would display a grade I ferning pattern but with subtle differences such as big and very closely branched ferns, with the branches being significantly more curved. Defining CL intolerance as cessation of CL wear due to ocular symptoms, deposition, or ocular health issues, Ravazzoni and colleagues\textsuperscript{251} found that grades I and II before the first lens fit can be used as predictors for CL tolerance with a sensitivity of 57.9% and a specificity of 88.5%. With a more strict approach using only grade I as predictive of tolerance, a sensitivity of 78.95% and specificity of 78.35% were achieved. The sensitivity and specificity of the prediction could even be improved further if tear ferning was performed after 1 month of CL wear. Using tear ferning in a group of established CL wearers and nonwearers, Evans and colleagues\textsuperscript{229} demonstrated a significantly higher tear ferning grade in lens wearers. However, the authors were not able to show a significant correlation to ocular symptoms, assessed via the Ocular Comfort Index questionnaire, or to demonstrate a difference in tear ferning patterns between symptomatic and asymptomatic lens wearers. The authors concluded that tear ferning provided good accuracy for discriminating between lens wearers and non-lens wearers but that the prediction of dry eye symptoms was rather poor. However, a negative predictive value of 86% indicated that normal tear ferning grades could be considered a good predictor for good ocular comfort during CL wear.

Tear ferning is an indication of tear functionality, and only limited information is available about tear ferning in CL wearers or the relationship to ocular comfort. To draw valuable conclusions about tear ferning in CL wear and the association to ocular comfort, more controlled studies are needed, including studies that assess the correlation between tear ferning in neophytes and ocular comfort during lens wear assessed via questionnaires, the impact of lens type, or the impact of length of lens wear on tear ferning. Currently, Rolando’s grading scale is the most commonly used method to assess tear ferning.\textsuperscript{252} Although Pensyl and Dillehay\textsuperscript{252} showed good intra- and interobserver repeatabilities when assessing proportions of tear ferning samples, Rolando’s method is based on a subjective grading, and Norn\textsuperscript{250,240} demonstrated poor repeatability using this latter system.

pH Measurement

Different methodological approaches have estimated the pH of the normal tear film to be within the 6.5 to 7.8 range.\textsuperscript{253–258} The tear film pH varies throughout the day, shifting from acid to alkaline, but such variations are contained within fairly narrow limits, usually a range of approximately 0.6 of a pH unit.\textsuperscript{254–256} Stimulation of tear secretion and blinking lead to acidification, whereas eyelid opening leads to alkalization by equilibration with the partial pressure of the CO\textsubscript{2} in the surrounding air.\textsuperscript{257}

The tear film has been shown to be more acidic in CL wearers, decreasing between 0.27 and 0.53 pH units.\textsuperscript{255,258} This decrease has been observed in the tear fluid behind the
**Table 3. Summary of Tear Film Osmolality Findings During CL Wear**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Subject Group</th>
<th>Tear Osmolarity, mmol/kg</th>
<th>Type of Osmometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarac and colleagues</td>
<td>Hydrogel</td>
<td>295.0 ± 1.4</td>
<td>In situ tear osmolarity system</td>
</tr>
<tr>
<td></td>
<td>Silicone hydrogel</td>
<td>298.8 ± 7.2</td>
<td>In situ tear osmolarity system</td>
</tr>
<tr>
<td>Stahl and colleagues</td>
<td>Baseline</td>
<td>314.4 ± 13.9</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td></td>
<td>Hydrogel</td>
<td>323.1 ± 13.3</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td>Glasson and colleagues</td>
<td>Baseline</td>
<td>322.4 ± 16.7</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td></td>
<td>Hydrogel</td>
<td>318.1 ± 12.8</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td>Nichols and Sinnott</td>
<td>Subjects with CL-induced dry eye</td>
<td>307.7 ± 52.4</td>
<td>Freezing point depression osmometer</td>
</tr>
<tr>
<td>Miller and colleagues</td>
<td>Control, non-CL wear</td>
<td>305 ± 21</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td></td>
<td>Hydrogel daily wear</td>
<td>319 ± 30</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td></td>
<td>Silicone hydrogel continuous</td>
<td>319 ± 32</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td></td>
<td>RGP</td>
<td>324 ± 25</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td>Iskeleni and colleagues</td>
<td>Hydrogel daily wear, 55% H2O</td>
<td>312.2 ± 16.0</td>
<td>Freezing point depression osmometer</td>
</tr>
<tr>
<td></td>
<td>Hydrogel daily wear, 38.6% H2O</td>
<td>316.5 ± 12.1</td>
<td>Freezing point depression osmometer</td>
</tr>
<tr>
<td></td>
<td>RGP, 90 Dk</td>
<td>313.1 ± 9.7</td>
<td>Freezing point depression osmometer</td>
</tr>
<tr>
<td></td>
<td>RGP, 52 Dk</td>
<td>316.4 ± 11.6</td>
<td>Freezing point depression osmometer</td>
</tr>
<tr>
<td>Dabney and colleagues</td>
<td>Control, non-CL wear</td>
<td>309.0 ± 17.0</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td></td>
<td>Hydrogel</td>
<td>313.7 ± 28.5</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td></td>
<td>Silicone hydrogel</td>
<td>524.3 ± 41.7</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td></td>
<td>RGP</td>
<td>317.0 ± 15.0</td>
<td>Vapor pressure osmometer</td>
</tr>
<tr>
<td>Martin</td>
<td>Baseline</td>
<td>316 ± 30</td>
<td>Freezing point depression osmometer</td>
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<tr>
<td></td>
<td>Hydrogel lens eye</td>
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<td>Contralateral eye</td>
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<tr>
<td>Farris</td>
<td>Aphakic nonwear control</td>
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<td>Freezing point depression osmometer</td>
</tr>
<tr>
<td></td>
<td>Aphakic extended wear</td>
<td>318 ± 7</td>
<td>Freezing point depression osmometer</td>
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<tr>
<td></td>
<td>Phakic RGP daily wear</td>
<td>316 ± 6</td>
<td>Freezing point depression osmometer</td>
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<tr>
<td></td>
<td>Phakic hydrogel daily wear</td>
<td>309 ± 8</td>
<td>Freezing point depression osmometer</td>
</tr>
<tr>
<td></td>
<td>Phakic hydrogel extended wear</td>
<td>318 ± 7</td>
<td>Freezing point depression osmometer</td>
</tr>
</tbody>
</table>

Dk, oxygen permeability. Defined as the amount of oxygen passing through a contact lens material over a defined period of time and pressure difference, unit is 10⁻¹¹ (cm³ O₂ cm)/(cm³ sec mmHg). Although studies have used different osmometers and therefore units varied, for simplicity, all units are considered as mmol/kg. Adapted from Stahl U, Willcox M, Stapleton F. Osmolality and tear film dynamics. *Cin Exp Optom.* 2012;95:3–11. Copyright 2012 John Wiley & Sons, Inc. 228

CL, both in gas-permeable and impermeable lenses, and has been attributed to the lens preventing CO₂ loss from the eye.255 There is limited evidence to support the notion that alteration in tear film pH affects CLD. It has been suggested that acidic changes in pH during CL wear could contribute to tight lens syndrome, based on data demonstrating decreased hydration of soft CLs induced by acidification.256,260

**Viscosity**

Natural tears display non-Newtonian behavior, dependent upon the shear rate.261 This shear-thinning behavior with higher-viscosity fluids at low shear rates, as occurs during the open-eye state, is necessary for the tears to contribute to the lubrication of the ocular surface without damage during a high shear rate situation such as blinking. Viscosity values of normal human tears have been reported in the range of 1 mPa·s at high shear rate (~120 s⁻¹) to up to 10 mPa·s at lowest shear rate (~0 s⁻¹).261–264 The underlying mechanism is not well understood, and the components involved are still questioned. It was originally thought that soluble mucins were the main components contributing to the viscosity of tears,261,262,265 but more recently the involvement of tear proteins and lipids has been suggested.262–264 Loss of shear-thinning behavior has been reported if the tear lipids are removed, but artificial mixtures of proteins containing lysozyme or lactoferrin can also exhibit shear-thinning behavior.254,261 Subsequently, Gouveia and Tiffany264 have proposed an interactive role between the proteins and lipids with protein-protein and protein-lipid interactions responsible for the viscous properties of tears.263 Slight differences in viscosity have been reported with dry eye disease265; however, the effect of CL wear on tear film viscosity is currently unknown, and it is not known whether there is a change in viscosity with CLD.

**Surface Tension**

The stability and spreading of the tear film is governed by the balance of the interfacial forces acting at the air-tear film, tear film-cornea, and cornea-air interfaces. A negative correlation has been shown between surface tension measures of the tear film and the rate of tear film breakup; that is, the higher the surface tension, the quicker the tear film breakup.266 The tear film surface tension is approximately two-thirds that of water267 or saline.268 Therefore, evaluation of the equilibrium surface tension (pressure) at the air-tear film interface is important for understanding the stability of tear film and its ability to spread.269 At one time it was believed that mucin in tears was a major contributor to surface tension,270 but more recent evidence suggests that the concentration of mucin needed (0.5% or 5 mg/mL)268,270 exceeds that present in normal tears (estimated as 32 ng/ml. Mucin 5AC a protein encoded by the gene *MUC5AC*).271 Another possible confounder of previous results was the use of nonocular mucin (often bovine submaxillary mucin or porcine gastric mucin) in place of ocular mucin. Tests have shown that purified bovine ocular mucin has no surface activity even at concentrations 100 times higher than those normally occurring in tears272 and that purified rabbit ocular
mucin has only weak surface activity.273 Furthermore, the initial assays demonstrating that removal of tears and mucin from the cornea left a hydrophobic surface274-275 have been questioned, as the treatments were harsh. Using more gentle removal of mucin has been shown to leave behind a wettability deficit.276-281 The tear film lipids are likely to be the most important contributors to the surface tension of tears, as delipidating tears increases their surface tension, and adding back the lipids restores this to its previous value.273 The polar components of the tear film with their amphipathic nature are likely to be key contributors to the spread of the lipid layer upon the aqueous component of tears.

There are several methods available to measure surface tension. These include a Wilhelmy plate used together with a Langmuir trough for tension measurements at a planar air-lipid layer upon the aqueous component of tears.\textregistered\ *

Langmuir trough for tension measurements at a planar air-lipid layer upon the aqueous component of tears.

There are several methods available to measure surface tension. These include a Wilhelmy plate used together with a Langmuir trough for tension measurements at a planar air-lipid layer upon the aqueous component of tears.274-275,281 An axisymmetric drop/bubble shape analysis for tension determination at curved surfaces of pendant drops284,285 or sessile bubbles.286-288 Using a capillary tube and determining the pressure needed to flatten a meniscus of tears, the surface tension of reflex or basal tears collected by capillary tubes is 42 to 46 mN/m275,280 and is 46.6 ± 3.8 mN/m using a Wilhelmy balance.280

Data using an artificial TFLL290 have shown that during increases in surface pressures (as would be seen during blinking),291 the area/molecule may be too small to accommodate all lipids (polar and nonpolar) at the air-aqueous interface, and it is most likely that the nonpolar lipids deposit upon the polar lipids. These in vitro data also demonstrated that at all pressures the lipid layer was most likely inhomogeneous, with condensed domains of nonpolar lipids above a layer of polar lipids.290 Results of other in vitro experiments, performed by adding saturated or unsaturated cholesterol or wax esters to lipid films made of human meibum, have been taken to indicate that the bulk nonpolar layer of the tear film contains liquid crystals of cholesterol esters interacting with wax esters. To date, work on the polar tear film lipids has focused on the role of phospholipids. Phosphatidylcholine or sphingomyelin can restore the surface tension of delipidated tears273 in a capillary meniscus model, and dipalmitoylphosphatidylcholine can interact strongly with meibum lipids.292

Although the role of mucin in the surface tension of tears has been largely discredited (see above), there may be a role for tear film proteins. Several investigations using monolayers of meibomian gland lipids have shown that proteins or mucins can penetrate them, and some proteins change the associated surface pressure.272,283,295,295,294 Lipocalins in tears have been shown to have some surface activity,285 and adding back a mimic of tear film lipocalin (bovine beta-lactoglobulin) with lipids can improve the surface tension of saline.272 Millar and colleagues295 concluded that the effect of lipocalin on the TFLL was complex and depended on the types of lipids present in the lipid film and adsorbed to lipocalin. Proteins that can be isolated by high-performance liquid chromatography (HPLC) in the 23-minute fraction (likely to be lipophilins) from rabbit tears are surface active and can decrease tear surface tension. They are associated with increases in tear breakup time in vitro.290 The presence of divalent cations in rabbit tears may also influence tear film stability, as chelators of these can decrease the tear film surface pressure and decrease the breakup time of rabbit tears on eye, but these ions do not appear to play the same role in human tears.297

Most of the studies discussed above have used monolayers of lipids, but lipids derived from the meibomian glands form multilayers, not monolayers,292 and have a thickness of approximately 20 molecules.2,10,208,301 Svitova and Lim263 used lipids extracted from the surface of lactoferrin A silicone hydrogel CLs (using toluene/isopropyl alcohol) and demonstrated that thick multilayers of these extracted lipids exhibited a low surface tension (using sessile bubble apparatus) of 32 to 22.5 mN/m (depending on the thickness of the film) compared to monolayers (40 mN/m).272,285,294 Addition of lysozyme to these thick lipid layers did not alter their surface tension, but there was evidence that lysozyme could adsorb irreversibly to the lipid layer and increase the relaxation time of the layer.269

There is a paucity of information on the effect of CL wear on the surface tension of tears, or indeed on the components that may influence the surface tension of tears. One study has examined the role of different lipids on the ability of an artificial tear fluid (ATF) to wet the surface of a teloflon A Hydroxyethyl methacrylate (HEMA)-based soft CL. Addition of various phospholipids, in particular phosphatidylinositol, was able to improve the ability of the ATF wet the lens surface,291 although surface tension of the ATF with or without phospholipids was not measured.

Dry eye tears have increased surface tension, reported as 44 to 55 mN/m compared to 42 to 46 mN/m.275 Using the capillary tube method or 52.9 ± 7.4 mN/m compared to 46.6 ± 3.8 mN/m using a Wilhelmy balance. As yet there appear to be no studies examining whether any form of CL wear changes the surface tension of tears, or evidence of a link with discomfort associated with CL wear.

Since the CL divides the tear film and creates new interfaces, it is critical that the PLTF components be able to spread over the anterior CL surface. Most hydrophobic tear lipids are unable to spread over the aqueous, and polar lipids are required to attain more favorable spreading conditions. In contrast to the non-CL situation, where tear aqueous components are able to spread over the cornea, in the presence of a CL, tear film aqueous spreading over a surface that is potentially already coated by tear lipids is compromised.303

Summary of Biophysical Changes to the Tear Film With CL Wear and Their Influence on Comfort

The physical presence of a CL in situ divides the tear film into a pre- and a postlens tear film and creates new interfaces with and within the ocular environment. This partition and new interaction has been shown to lead to biophysical changes of the tear film properties, including a decrease in tear film stability, pre-lens lipid layer thickness, and tear volume as well as an increase in evaporation rate, as summarized in Table 4. To date, the effect on comfort of many of these biophysical properties is unknown or inconclusive. However, evidence points toward a link between decreased stability, increased evaporation, reduced tear turnover, and ferning and CLD. Further evidence is required to establish the associations of tear volume, surface tension, osmolarity, pH, and ocular temperature with CLD.

Changes in Tear Composition With Contact Lens Wear and Their Effect on Comfort

Biochemistry

Tear Types. Challenges facing analysis of the tear film proteome include the volume and type of tears that can be collected. Tears have been classified into four types: basal, reflex, emotional, and closed-eye tears. Basal315 (sometimes also referred to as open-eye) tears bathe the mucous membranes of the eye during the day and have a turnover rate between 3.4 µL/min314 and approximately 1 µL/min,315 and a volume of approximately 7 µL.314 Reflex tears are produced upon stimulation of the lacrimal reflex by irritant substances or foreign particles. Emotional tears are produced as a result of various emotions, such as sadness. Closed-eye
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range in Precorneal Tear Film</th>
<th>Range in CL Wear, Pre-Lens Tear Film</th>
<th>Evidence of Relation to Contact Lens Discomfort</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blink</strong></td>
<td>Interblink interval in healthy eye is 5–15 s. Most of the blinks are complete blinks. In cases of people working on display or exposed to other dry eye factors, interblink time and percentage of incomplete blink increase.25,35,45</td>
<td>Prolonged CL wear results in increased percentage of incomplete blinks (for rigid CL) and stronger association between tear film instability and percentage of incomplete blinks (for soft CL). CL wear frequently reduces blink frequency. CL wetting solutions can maintain normal blink frequency.25,27–31,34,35</td>
<td>Reduced blink frequency or increased percentage of incomplete blink resulted in CL discomfort.2,27–30,47,64,72,156</td>
</tr>
<tr>
<td><strong>Lipid layer integrity</strong></td>
<td>TFLL spreading and integrity can be analyzed to evaluate the viscoelasticity of the lipid layer. Elasticity prevails in healthy eyes, while the contribution of viscosity increases in dry eyes.9,11,42,44,304</td>
<td>TFLL spread depends on the CL material. Prolonged wear of CL frequently delays the kinetics of TFLL spread and worsens the TFLL integrity.2,9,46,49,53,305,306</td>
<td>Impaired TFLL integrity and spread correlates with CL discomfort.9,44,45,50,53,307</td>
</tr>
<tr>
<td><strong>Tear film stability</strong></td>
<td>NIBUT 4.6–60 s,51,54,56,57, 50,70,308–511</td>
<td>SOFT: 5–10 s,153,66–70 ( \pm 2 ) s</td>
<td>Decreased tear film stability associated with CL discomfort.70,75,78–86</td>
</tr>
<tr>
<td><strong>Evaporation</strong></td>
<td>Range 0.4–16.7 g/m²/h 12,81,96,101,103,109,111,114, 119–128</td>
<td>1.2–2.6% ( \pm 0.1 ) in ( 0^\circ )C evaporation98,120,121,126</td>
<td>Discomfort associated with increased tear evaporation in neophytes fitted with hydrogel CL (but not SiHy) at 18% relative humidity.130</td>
</tr>
<tr>
<td><strong>Ocular surface temperature profile</strong></td>
<td>52°C–36°C151</td>
<td>Pre-lens tear film: cooler than without CL148</td>
<td>No clear relationship demonstrated between tear film temperature and discomfort in CL wear, although artificially lowering the ocular surface temperature, with cooled (4°C) artificial tears, reduces ocular surface sensitivity and improves comfort.150</td>
</tr>
<tr>
<td><strong>Tear film thickness</strong></td>
<td>1–7 ( \mu )m,3,72,151,154,158,306</td>
<td>Pre-lens: 1–7 ( \mu )m3,72,153,154,158,163,306</td>
<td>No evidence showing a link between tear film thickness and discomfort.</td>
</tr>
<tr>
<td><strong>Tear turnover rate</strong></td>
<td>16.9 ± 6.8167 16.2 ± 5.1 ( \pm 1 )01</td>
<td>15.6 ± 5.9167</td>
<td>Symptomatic wearers 20.6 ± 6.0 Range = 16–36</td>
</tr>
<tr>
<td><strong>Tear volume</strong></td>
<td>2–4 ( \mu )L50,151,152,185,188</td>
<td>1–2 ( \mu )L185,188</td>
<td>Asymptomatic wearers 33.8 ± 8.8 Range = 27–42175†</td>
</tr>
<tr>
<td><strong>Tear exchange</strong></td>
<td>10%–20% per blink195</td>
<td></td>
<td>Lower tear volume has a weak but significant relation to discomfort in CL wear.50,76</td>
</tr>
<tr>
<td><strong>Osmolality/electrolytes</strong></td>
<td>280–318197,200</td>
<td></td>
<td>No link between tear exchange and ocular discomfort.</td>
</tr>
<tr>
<td></td>
<td>297–331185,216,219,221–224,226,227</td>
<td></td>
<td>No association between tear film osmolality and ocular comfort has been established.221 although tendency toward higher tear film osmolality in patients with CL discomfort.55,76</td>
</tr>
</tbody>
</table>
tears are those tears that bathe the eye during sleep. The protein component of these tear types is known to be different; for example, the levels of secretory immunoglobulin-A (sIgA) decreases in concentration from closed-eye to basal to reflex tears.\textsuperscript{313,316–320} Other tear proteins such as lactoferrin, lipocalin-1, and lysozyme do not appreciably change their concentration in closed-eye, basal, and reflex tears.\textsuperscript{315,319} These findings led to the classification of different proteins in the tear fluid into constitutive (i.e., those that have a constant level of production and so their concentration decreases during increases in tear fluid production, e.g., sIgA),\textsuperscript{315} regulated (i.e., those that have changes in production during changes in amount of tears, e.g., lysozyme, lactoferrin, and lipocalin-1),\textsuperscript{315} and serum derived (which also decrease during emotional tears).\textsuperscript{313,317} Emotional tears may differ from reflex tears by containing chemo signals (pheromones) that affect behavior\textsuperscript{321} and having a slightly higher total protein concentration of 6 mg/mL compared to 4 mg/mL.\textsuperscript{322} Table 5 lists the major tear proteins and their changes during CL wear.

**Tear Collection Methods.** There are essentially three methods for collecting tears: using a microcapillary tube to draw tears into the lumen of the glass tube (it is believed that this causes minimal change to the ocular surface and minimal reflex tearing), using a Schirmer strip placed into the lower fornix to adsorb tears, and using a sponge placed within the fornix to adsorb tears. It is well established that tears collected by capillary tube. Due to the low volume of tears, even with normal eyes (approximately 7 μL per eye), some researchers have used a flush method to help collect tears. This involves instilling a volume of buffered saline onto the ocular surface, allowing that to interact, and collecting it usually using capillary tubes.\textsuperscript{324} While this method does dilute the tear sample, it may have advantages where the tear volume is low or it is difficult to collect sufficient tears for biochemical analysis.

**Lipidome**

The tear lipids are primarily secreted from the meibomian glands and form the outermost bilayer of the tear film.\textsuperscript{274,300} The TFLL has an outer layer of nonpolar lipids at the air interface to retard water evaporation from the tear film and to protect from external contaminants and an inner layer of polar lipids that creates an interface with the aqueous layer to help the spreading of the outer layer and increase its stability.\textsuperscript{9,12,13,106} The major lipid components of the meibomian gland secretion are nonpolar wax esters, cholesterol esters, diesters, and triacylglycerol, with smaller concentrations of cholesterol, fatty acids, and other polar lipids. Polar lipids account for 5% to 15% of the total lipids and are suggested to include phospholipids (phosphatidylcholine, phosphatidylethanolamine, sphingomyelin), ceramides, and cerebroside\textsuperscript{329,330,331} and more recently long chain (O-acetyl)-omega-hydroxy fatty acid (OAHFA).\textsuperscript{327,328} The presence of phospholipids in meibum remains controversial,\textsuperscript{329–331} and OAHFAs have been suggested as being responsible for creating the interface between the aqueous and the nonpolar lipid layer instead of phospholipids.\textsuperscript{326,327} Limited information is available on the compositional analyses of tear film lipids. Recent research has shown that the lipid composition of tears is possibly more complex than that of meibum.\textsuperscript{352} Nonpolar lipids in tears differ from those found in meibomian secretions.\textsuperscript{332–355} Also, in contrast to meibomian secretion lipids, several researchers have confirmed the presence of phospholipids in tears.\textsuperscript{335–340}

The clinical appearance, thickness, and stability of the pre-lens TFLL have been shown to be disrupted by the presence of a CL (refer to section Lipid Layer Interferometry), and lipids are known to deposit on CLs (see TFOs Report from the Contact Lens Materials, Design and Care Subcommittee). Changes in the tear film lipid composition associated with CL wear could be expected; however, compositional analyses of tear film lipids during CL wear are very limited. Young and Hill\textsuperscript{341,342} measured cholesterol levels in normal subjects and subjects with CL problems; the cholesterol levels in the subjects with CL problems ranged from 190 to 203 mg/100 mL. In normal subjects, tear cholesterol levels were measured for normal subjects ranged from 186 to 190 mg/100 mL. In normal subjects, tear cholesterol levels were initially decreased after rigid CL fitting but returned to prefitted levels once adaptation was complete.\textsuperscript{342} More recently, another study in soft CL wearers reported a negative association between the level of cholesterol esters in tears and the thickness of the lipid layer as well as a positive association between the level of cholesterol esters and dryness symptoms, with higher levels associated with increased dryness symptoms.\textsuperscript{343} Yamada and colleagues\textsuperscript{345} reported concentrations of phospholipids of 186 ± 39 and 162 ± 33 μg/mL in tears of subjects wearing polymacon (group I) and etafilcon A (group IV) CLs, respectively, the latter being significantly lower than for the same subjects when not wearing CLs (220 ± 35 μg/mL, \( P = 0.0023 \)). These findings are

---

**Table 4.** Continued

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range in Precorneal Tear Film</th>
<th>Range in CL Wear, Pre-Lens Tear Film</th>
<th>Evidence of Relation to Contact Lens Discomfort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferning</td>
<td>Grades I to IV\textsuperscript{231,232,235,259}</td>
<td>Grades I-IV\textsuperscript{251} Mean 2.02 ± 0.60\textsuperscript{229} 0.87 mm\textsuperscript{2} to 1.24 mm\textsuperscript{2} ( P = 0.0023 ). These findings are</td>
<td>No correlation to comfort assessed via Ocular Comfort Index, but Grades I and II are good predictors for good ocular comfort.\textsuperscript{228} Grades I and II are predictors for CL tolerance.\textsuperscript{351} Limited evidence to support link between pH and discomfort.\textsuperscript{256,260}</td>
</tr>
<tr>
<td>pH</td>
<td>6.5–7.8\textsuperscript{254–259}</td>
<td>≤ in CL wear\textsuperscript{255,256}</td>
<td>No evidence linking tear viscosity with contact lens discomfort.</td>
</tr>
<tr>
<td>Viscosity</td>
<td>High shear rate 1 mPa·s Low shear rate 10 mPa·s\textsuperscript{262–265}</td>
<td>No data</td>
<td>No evidence linking tear viscosity with contact lens discomfort.</td>
</tr>
</tbody>
</table>

\( \text{SilHy, silicone hydrogel lenses.} \)  
\( ^* \) With high molecular weight FITC dextran tracer; \( \dagger \) ex vivo immediately on CL removal.
## TABLE 5. Concentration of Some of the Major Proteins in Tears and the Effect of CL Wear

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Reflex</th>
<th>Basal*</th>
<th>Closed Eye</th>
<th>Lens Wear†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein, mg/mL</td>
<td>6.0⁵¹⁹</td>
<td>9.0⁵¹⁹</td>
<td>18.0⁵¹⁹</td>
<td>5.4 ± 0.4⁵²⁷</td>
</tr>
<tr>
<td></td>
<td>3.9–5.0³¹⁷</td>
<td>7.3³¹⁷</td>
<td>15.5 ± 8.4³⁵³</td>
<td>11.9 ± 2.0⁵⁷⁰</td>
</tr>
<tr>
<td></td>
<td>4.6 ± 0.2⁵³²</td>
<td>5.4 ± 1.5³⁵³</td>
<td>5.6–6.6¹⁴⁰</td>
<td>7.2 ± 2.3³⁵³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.4 ± 3.0³⁴⁰</td>
<td>7.2 ± 2.3³⁵³</td>
<td>5.2 ± 1.5³⁰</td>
</tr>
<tr>
<td>Lysozyme, mg/mL</td>
<td>1.6³¹⁹</td>
<td>2.0³¹⁹</td>
<td>1.8³¹⁹</td>
<td>4.0 ± 0.6³⁵³</td>
</tr>
<tr>
<td></td>
<td>1.3–1.6³¹⁷</td>
<td>2.1 ± 0.2³¹⁷</td>
<td>3.0³¹⁷</td>
<td>2.9, closed eye, OK RGP³⁵⁵</td>
</tr>
<tr>
<td></td>
<td>1.5 ± 1.1²²²</td>
<td>0.7 ± 0.6²²²</td>
<td>2.5³⁵⁷</td>
<td>3.3 ± 0.4³⁵³</td>
</tr>
<tr>
<td></td>
<td>2.7 ± 0.5³⁵³</td>
<td>3.0 ± 0.3³⁵³</td>
<td>1.4–1.9³⁶⁷</td>
<td>1.1 ± 0.5, RGP‡; 1.2 ± 0.4, high water soft; 0.8 ± 0.2, low water soft ²²⁶</td>
</tr>
<tr>
<td>Lactoferrin, mg/mL</td>
<td>1.8³¹⁹</td>
<td>2.6³¹⁹</td>
<td>1.8³¹⁹</td>
<td>3.3 ± 0.4³⁵³</td>
</tr>
<tr>
<td></td>
<td>1.3–1.5³¹⁷</td>
<td>1.6 ± 0.1³¹⁷</td>
<td>5.5³⁷⁵</td>
<td>3.3, closed eye, OK RGP³⁷⁵</td>
</tr>
<tr>
<td></td>
<td>4.0 ± 3.1²²²</td>
<td>2.9 ± 0.2³¹⁷</td>
<td>4.6³⁵⁵</td>
<td>1.1 ± 0.2³⁵³</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.4³⁵³</td>
<td>2.5 ± 0.9³⁵³</td>
<td>3 ± 2³⁸³</td>
<td>1.6 ± 0.3³⁵³</td>
</tr>
<tr>
<td>Lipocalin-1, mg/mL</td>
<td>1.9³¹⁹</td>
<td>1.3³¹⁹</td>
<td>1.7³¹⁹</td>
<td>3.2, closed eye, OK RGP³⁷⁵</td>
</tr>
<tr>
<td></td>
<td>1.1–1.3³¹⁷</td>
<td>1.6 ± 0.1³¹⁷</td>
<td>3.3³⁵⁷</td>
<td>3.2, closed eye, OK RGP³⁷⁵</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5³⁵⁷</td>
<td>1.5 ± 0.2³⁵³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5 ± 0.9³⁵³</td>
<td>1.5 ± 0.2³⁵³</td>
<td>1.5 ± 0.6³⁸³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1 ± 0³³⁷</td>
<td>1.2 ± 0.2, DW RGP; 0.7 ± 0.1, DW soft; 0.6 ± 0.1, EW soft³⁵¹</td>
<td>1 ± 1, closed eye³⁵³</td>
</tr>
<tr>
<td>slgA, mg/mL</td>
<td>0.1³¹⁹</td>
<td>2.6³¹⁹</td>
<td>10.0³¹⁹</td>
<td>0.1 ± 0.1³⁰⁰</td>
</tr>
<tr>
<td></td>
<td>0.1–0.2³¹⁸</td>
<td>0.2–0.9³¹⁸</td>
<td>2.3–8.4³¹⁸</td>
<td>5.0, closed eye, OK RGP³⁷⁵</td>
</tr>
<tr>
<td></td>
<td>0.1–0.4³¹⁷</td>
<td>0.9 ± 0.1³¹⁷</td>
<td>4.6³⁵⁵</td>
<td>1.1 ± 0.5³⁵³</td>
</tr>
<tr>
<td></td>
<td>0.1 ± 0.¹³⁸⁰</td>
<td>2.8³⁵⁷</td>
<td>0.8 ± 0.3³⁰⁰</td>
<td>1.6 ± 0.5³⁶⁷</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8 ± 0.3³⁵³</td>
<td>3 ± 2³⁸³</td>
<td>1.5 ± 0.6³⁸³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.7 ± 0.7³⁵³</td>
<td>1.2 ± 0.2, DW RGP; 0.7 ± 0.1, DW soft; 0.6 ± 0.1, EW soft³⁵¹</td>
<td>1 ± 1, closed eye³⁵³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.6 ± 1.8³⁵³</td>
<td>1.1 ± 1.6³⁴³</td>
<td>0.7³⁵³‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9 ± 0.1³⁸³</td>
<td>0.7³⁵³‡</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3 ± 1.0³⁴³</td>
<td>1 ± 1, closed eye³⁵³</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.4³⁵⁷</td>
<td>1 ± 1, closed eye³⁵³</td>
<td>1 ± 1, closed eye³⁵³</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9 ± 0.1³⁷⁶</td>
<td>1 ± 1, closed eye³⁵³</td>
<td></td>
</tr>
<tr>
<td>Specific slgA, units</td>
<td>2.1 ± 2.7 (S. epidermidis)³⁸⁰</td>
<td>82 ± 15 (P. aeruginosa)³⁸³</td>
<td>1 ± 1, closed eye³⁵³</td>
<td>1.3 ± 2.0 (S. epidermidis; reflex tears)³⁸⁰</td>
</tr>
<tr>
<td></td>
<td>9.0 ± 12.2 (E. coli)³⁸⁰</td>
<td>100% (P. aeruginosa)³⁸⁵</td>
<td>3.7 ± 3.4 (E. coli; reflex tears)³⁸⁰</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.0 ± 12.3 (H. influenzae)³⁸³</td>
<td>2.7 ± 5.8 (H. influenzae; reflex tears)³⁸³</td>
<td>27 ± 7.1, DW RGP; 52 ± 9, DW soft; 38 ± 6, EW soft (P. aeruginosa)³⁸³</td>
<td></td>
</tr>
<tr>
<td>Albumin, µg/mL</td>
<td>20³¹⁹</td>
<td>100³¹⁹</td>
<td>110³¹⁹</td>
<td>540, closed eye, OK RGP³⁷⁵‡</td>
</tr>
<tr>
<td></td>
<td>3.0–8.4³¹⁷</td>
<td>42.0 ± 4.7³¹⁷</td>
<td>760–1100³¹⁸</td>
<td>10.3–24.1³⁷⁴</td>
</tr>
<tr>
<td></td>
<td>1830 ± 2440³¹⁹</td>
<td>380 ± 640³¹⁹</td>
<td>200³⁷⁵</td>
<td>49.8 ± 57.1³⁸⁰</td>
</tr>
<tr>
<td></td>
<td>10–20³¹⁸</td>
<td>20³⁷⁵</td>
<td>1100³¹⁸</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72.3 ± 71.2³⁸⁰</td>
<td>14.6 ± 8.6³⁶⁰</td>
<td>20³⁷⁵</td>
<td>10.2 ± 3.0³⁶⁷</td>
</tr>
</tbody>
</table>

OK RGP, rigid gas-permeable lenses designed for orthokeratology.
* Used as default if tear type not mentioned.
† Compared to basal tears unless otherwise stated; soft lenses unless otherwise stated.
‡ Significant effect of contact lens wear.
§ Tears collected using flush method.
in agreement with another study showing an increase in the ratio of nonpolar to polar lipids in the tears of CL wearers. A low level of phospholipids/polar lipids in tears could conceivably be a contributing factor to the dryness symptoms and discomfort reported during soft CL wear.

When the lipid layer is compromised and no longer able to supply full coverage over the aeous layer, the tear film stability is significantly decreased and evaporation increased. Degradation of lipid components by autoxidation (or photooxidation), enzymatic oxidation, or enzymatic lysis is expected to have a deleterious effect on the layer.

The presence of one or more double bonds (unsaturation) in the structure of certain lipids makes them susceptible to oxidation due to the availability of the allylic hydrogen. Hence, mono- or di-unsaturated fatty acids or esters present in the tears and, in particular, polyunsaturated fatty acids possibly originating from vascular leakage into the tear layer, are susceptible to autoxidation under the effect of light, atmospheric oxygen, and so on. Primary oxidation products, hydroperoxides, can then be further converted into secondary peroxidation products such as hydrocarbons, aldehydes, hydroxyaldehydes, and epoxides. Two aldehydes commonly used as oxidative stress markers are 4-hydroxy-2(E) nonenal (4-HNE), a peroxidation product of linoleic acid (18:2 n-6), and malondialdehyde, an end product of the degradation of linolenic acid (18:3).

Phospholipases are lipolytic enzymes found in tears, contributing to their antibacterial properties. That are able to degrade phospholipids into diacylglycerols and lysophospholipids; group II phospholipase A2 (sPLA2 GI) is the most abundant in tears and is secreted by both the acinar and ductal cells of the lacrimal gland. There have been several reports of PLA2 in tears of CL wearers as well as deposited on hydrogel CL materials as well as without consensus on the effect of CL wear, with both no change and a reduction in concentration being reported. Group II phospholipase A2 hydrolyses the ester bond at the sn-2 position of phospholipids, producing a lysophospholipid and a free fatty acid, often arachidonic acid, a precursor in the production of eicosanoids resulting in the production of prostaglandins and leukotrienes and known to be involved in ocular surface inflammation. The suspected role of lipolytic enzymes in modulating tear film lipid composition. In blepharitis patients, PLA2 activity has been shown to be enhanced and is hypothesized to cause the disruption of tear film phospholipids, compromising the function of the polar lipid layer and contributing to a breakdown of tear film structure. Intolerant wearers unable to wear their CLs for longer than 6 hours during the day were found to have a decreased level of secretory phospholipase A2 (sPLA2) in the tears (1.86 P = 0.08 ng/mL), as well as increased concentration of secretory phospholipase (sPLA2) in the tears (1.86 P = 0.08 ng/mL). However, the exact role of sPLA2 in tear film stability is not yet clearly understood.

Tear lipid chemistry is likely to be affected by CL wear. This effect will depend upon the characteristics of the CL but also on the individual patient tear composition. Studies of the tear lipid chemistry investigating nonpooled, individual samples are required to further understand the potential role of lipids in CL discomfort.

### Proteome

The Tear Film Proteome. The tear film proteome (defined as all the proteins and peptides that can be identified from tears) has not yet been definitively described, although researchers have been examining the proteins of tears for many decades. Various techniques have been used to investigate the tear film proteome. These include one-dimensional polyacrylamide gel electrophoresis (PAGE) and Western blotting to identify and quantify the separated proteins; chromatography and mass-spectrometry (MS) techniques with additions such as isobaric tag for relative and absolute quantitation to enable quantification of proteins in the original sample; and enzyme-linked immunosorbent assays using specific antibodies to quantify proteins in a sample without prior separation of the proteins. The numbers of proteins in the tear proteome have been reported to vary widely. Using sodium dodecyl sulfate (SDS)-PAGE-MS to separate proteins and reverse-phase (RP) capillary HPLC and matrix-assisted laser desorption/ionization (MALDI)-MS to identify peptides, Funke and colleagues identified 267 proteins in tears collected from experienced soft CL wearers. Using nano-HPLC-MS/MS, de Souza and colleagues identified 491 proteins in the tear film of one individual. Zhou and colleagues, using various fractions of tears and nano-RP HPLC-MS/MS, identified 1543 proteins in tears collected from four healthy non-CL wearers, with 714 proteins being present in all samples. It has been estimated that the tear film proteome contains approximately 35% of proteins in common with the proteome of plasma, 25% of proteins in common with those found in saliva, and 24% of proteins in common with those found in urine. These findings indicate that tears contain many unique proteins as well as a smaller fraction of proteins in common with other human body fluids.

Table 5 gives details of the major proteins found in the tear film. The basal tear film contains 3.5 to 9.5 mg/mL total protein (as with all protein analyses, differences are partly due to collection methods, techniques to quantify proteins [i.e., Lowry, Bradford, bicinchoninic acid or fluorescence-based protein assays], and use of different standards [usually albumin but occasionally, e.g., IgG or soybean trypsin inhibitor]). The total protein concentration does not change significantly in reflex tears (Table 5), but does increase during sleep in closed-eye tears to approximately 16 to 18 mg/mL (Table 1). The level of the regulated major tear proteins lysozyme (0.7–3.0 mg/mL), lactoferrin (0.7–4.0 mg/mL), and lipocalin-1 (0.5–3.5 mg/mL) does not change in reflex, basal, or closed-eye tears. The constitutive protein slgA changes from a low concentration in reflex tears, of 0.06 to 0.38 mg/mL, to 0.84 to 2.8 mg/mL in basal tears and to 3 to 10 mg/mL in closed-eye tears. slgA has shown that it is in tears in rats is slgA. Similarly, the serum-derived proteins albumin, complement C3, complement C4, and complement factor B also increase in concentration from reflex to basal to closed-eye tears.

The tear film can create compositional changes detrimental to tear film stability. Tear lipid chemistry is likely to be affected by CL wear. This effect will depend upon the characteristics of the CL but also on the individual patient tear composition. Studies of the tear lipid chemistry investigating nonpooled, individual samples are required to further understand the potential role of lipids in CL discomfort.
Inflammatory Mediators in Tears. Tears have been shown to contain a variety of inflammatory mediators, including complement (Table 6), arachidonic acid metabolites (e.g., leukotriene B₄ and prostaglandin E₂), and a range of different cytokines (Table 6). The range of cytokines differs depending on the study methodology, but the most commonly observed cytokines in tears are interferon (IFN-β), interleukin (IL-1α, IL-1β), IL-4, IL-6, IL-8, IL-10, and IL-12(p70); and tumor necrosis factor (TNF-α). One of the reasons for differences in the descriptions of the cytokines present in tears may be that not all tear samples contain all cytokines. For example, Enriquez-de-Salamanca and colleagues found that epidermal growth factor, CX3CL1, interleukin-1 receptor agonist and CXCL10 were detected in 100% of basal tear samples; IL-8/CXCL8 and vascular endothelial growth factor were detected in >93% of samples; IL-6 in 65%, IL-10 in 48%, IFN-γ in 30%, IL-1β in 30%, IL-17 in 13%, and IL-13 in 9%; GM-ganglioside, monocyte colony stimulating factor in 7%; and TNF-α in 2% of samples. These inflammatory mediators appear to be tightly regulated, as tears also contain inhibitors of the complement cascade including lactoferrin, decay accelerating factor, and CD59; soluble receptors of cytokines and growth factors, for example, EGFR, IL-2R, IL-6R, and TNFRI2; and the IL-1 antagonist IL-1Ra, as well as certain cytokines (such as IL-10) that are themselves anti-inflammatory.

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Reflex</th>
<th>Basal*</th>
<th>Closed Eye</th>
<th>Lens Wear†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibronectin, ng/mL</td>
<td>21 ± 25</td>
<td>27.4 ± 47.5</td>
<td>106.5 ± 84.5</td>
<td>120 ± 160</td>
</tr>
<tr>
<td>Complement C3, μg/mL</td>
<td>4.0 ± 5.6</td>
<td>4.4 ± 2.1</td>
<td>5.6 ± 5.1</td>
<td>5.3 ± 1.7</td>
</tr>
<tr>
<td>Complement C4, μg/mL</td>
<td>0.2 ± 0.4</td>
<td>1.7 ± 3.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Complement factor B, μg/mL</td>
<td>0.1 ± 0.1</td>
<td>4.0 ± 5.0</td>
<td>20.8 ± 8.1</td>
<td>5.0–5.1</td>
</tr>
<tr>
<td>sPLA2, ng/μL</td>
<td>1.8 ± 0.1</td>
<td>5.0 ± 0.7</td>
<td>147 ± 112</td>
<td>3.6, RGP lenses</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>~70</td>
<td>2.2</td>
<td>148 ± 103</td>
<td>935.3, OK RGP</td>
</tr>
<tr>
<td>IL-8, pg/mL</td>
<td>~1200</td>
<td>601.5</td>
<td>2000.7</td>
<td>412.6 ± 104.1</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>1.8</td>
<td>698.1</td>
<td>2000.7</td>
<td>572.6–438.2</td>
</tr>
<tr>
<td>EGF, pg/mL</td>
<td>1.8</td>
<td>698.1</td>
<td>2000.7</td>
<td>1277</td>
</tr>
<tr>
<td>MMP-9, ng/mL</td>
<td>9.8 ± 14.3</td>
<td>6.1</td>
<td>12</td>
<td>9.3 RGP lenses</td>
</tr>
<tr>
<td>TIMP-1, ng/mL</td>
<td>27.8 ± 282.3</td>
<td>10.5</td>
<td>32, neophyte†; 229 ± 175, adapted</td>
<td>37.4</td>
</tr>
<tr>
<td>NGAL, ng/mL</td>
<td>680.8 ± 523.3</td>
<td>13.9</td>
<td>32, neophyte‡; 218 ± 45, adapted</td>
<td>1084.3</td>
</tr>
</tbody>
</table>

NGAL, Neutrophil gelatinase-associated lipocalin.
* Used as default if tear type not mentioned.
† Compared to basal tears unless otherwise stated; soft lenses unless otherwise stated.
‡ Significant effect of contact lens wear.
§ Tears collected using flush method.
Table 6 details the changes that occur to some of these inflammatory mediators in different tear types and in CL wear. The concentration of IL-6 and IL-8 decreases in reflex tears compared to basal tears, whereas the concentration of VEGF, Fas ligand (FasL), and monocyte chemotactic protein (MCP-1) does not change, whereas the level of its inhibitor TIMP-1 increases only 3-fold.373 However, the level of protease active forms.415,416 increases during sleep as its level increases by approximately 200-fold,363 partly because, for IL-8, its level is already high in basal tears.

**Proteases in Tears.** The major proteinase activities of tears are gelatinolytic and collagenolytic.388 Tears contain high levels of cathepsin-C activity, but also cathepsin-B, trypsin-like, and urokinase activity.407 The proteolytic study by de Souza and colleagues365 showed large numbers of proteases (such as matrix metalloproteinase [MMP]-8, MMP-9, leukocyte elastase, plasminogen, cathepsins, and aminopeptidases) as well as antiproteases (such as α2-macroglobulin, α1-microglobulin, cystatins, α1-antitrypsin, α1-antichymotrypsin, leukocyte elastase inhibitor, plasminogen activator inhibitor-2, thrombospondin-1, secretory leukocyte protease inhibitor, and tissue inhibitor of matrix metalloproteinase [TIMP]-1).318

Research has tended to focus on the presence of MMPs and their inhibitors (such as TIMPs) in tears. Tears have been shown to contain MMP-1 (also known as interstitial collagenase), MMP-2 (gelatinase A), MMP-3 (stromelysin-1), MMP-8 (neutrophil collagenase), MMP-9 (gelatinase B), MMP-10 (stromelysin-2), MMP-13 (collagenase 3), TIMP-1, TIMP-2, and TIMP-4 (Table 6).387,388,395,408-411 However, often the active forms of the MMPs have not been demonstrated, and often tears contain only inactive pro-forms387,412-414 or low levels of active forms.415,416

Plasmin activity in tears increases during sleep,520,417 as does the level of pro-MMP-9.373 However, the level of protease inhibitors such as α1-antiprotease, α1-antichymotrypsin, α2-macroglobulin, secretory leukocyte protease inhibitor, and cystatin C also increases in closed-eye tears between levels 2 to 23 times those in reflex tears.318,575 In the case of MMP-9 and TIMP-1, there is the potential for activation of MMP-9 during sleep as its level increases by approximately 200-fold whereas the level of its inhibitor TIMP-1 increases only 3-fold.575

**Other Types of Inflammatory Mediators.** Tears contain histamine (N-methyl histamine), and its concentration increases from reflex (80 ± 110 pg/mL) to basal (200 ± 140 pg/mL) to closed-eye (840 ± 1150 pg/mL) tears. Its concentration in tears during asymptomatic CL wear (370 ± 80 pg/mL) is not different from that in basal tears of non-lens wearers.419 Tears also contain the neuromediators: substance P, calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY), vasoactive intestinal peptide, and nerve growth factor (see report from the Sub-committee on Neurobiology for more details).386,419-421

**Effect of CL Types and Wear Schedules on the Tear Film Proteome.** It is possible that different CLs or the disinfecting/cleaning solutions used with them, as well as the wear schedule on which lenses are worn, can affect the tear film proteome, although there is a general lack of information on the effects of CL wear on the tear film proteome. Tables 5 and 6 outline the publications that have examined the effect of CL wear on the tear film proteome.

Initial studies on total proteins in tears by Hill and Uniacke422 and Gallender and Morrison423 showed that, during adaptation to hard CLs, protein concentration decreased, but returned to normal after the first 7 days of lens wear.423 However, overall there does not appear to be a change in the total protein content or the concentration of lysozyme, lactoferrin, or lysozyme during lens wear (Table 1)90,525,340,561,367,368,570,372,374-376,378,422-424 (with the excep-
tions that one study showed a significant decrease in antibody intensity of lysozyme in PAGE from tears of silicone hydrogel and rigid gas-permeable [RGP] lens wearers compared to nonwearers,423 and one other study showed that the level of lysozyme increased in tears from RGP [1.1 ± 0.5 mg/mL] or soft lens wearers [0.2 ± 0.4 mg/mL] compared to non lens wear [0.8 ± 0.4 mg/mL] or wear of low water content soft lenses [0.8 ± 0.2 mg/mL]).426

In contrast, the effect of CL wear on the concentration of sIgA in tears is more controversial. Six studies have reported no effect of lens wear,367,370,375,380,381,427 However, Kijlstra and colleagues428 reported that there was a decrease in the concentration of sIgA in tears during the first 3 months of RGP daily lens wear compared to non-lens wear, by approximately 27%, but the concentration returned to normal 1 year after lens fitting. Others have shown a similar effect of a decrease in sIgA concentration in tears of CL wearers (type of lens, wear schedule, or length of wear was not given)431 in a mixed group of CL wearers (lens type not given; mixed DW and EW; average 8 years of wear),427 in a group of EW soft lens wearers (at least 6 months lens wear),432 and in the closed-eye tears collected from a group of either daily wear or extended wear soft lens wearers (average length of wear 4 years).383 In addition, there appears to be a reduction in the concentration of sIgA specific for *Pseudomonas aeruginosa* or *Escherichia coli* but not for *Staphylococcus epidermidis* or *Haemophilus influenzae* in tears.380,381,385

The concentration of albumin in basal tears does not change during relatively long-term wear of RGP367 or soft lenses574 but is increased in closed-eye tears during wear of RGP lenses for orthokeratology.575 The concentration of complement proteins C3 or C4 does not change with DW or EW of soft lenses.570 The concentration of fibronectin in basal tears is increased during EW of soft lenses (average wearing time of 3 months).429 Most studies (with the exception of one measuring the concentration of IL-6 in tears of RGP lens wearers, another measuring the level of IL-6, IL-8, or MMP-9 in the tears of long-term lotrafilcon A silicone hydrogel lens wearers, and one measuring IL-6 in tears after 2 weeks of silicone hydrogel lens wear92,430,431 have found that CL wear in general increases the concentration of IL-6, IL-8, TNF-α, EGF, and MMP-9 in tears,430-435 and moreover there appears to be an effect of length of lens wear.435

After collecting tears using the flush method and two-dimensional differential gel electrophoresis (2D-DIGE), Markoulli and colleagues436 found a significant decrease in the level of Zn-alpha2-glycoprotein in the tears of people who had worn silicone hydrogel lenses (lotrafilcon B) on a DW basis compared to their tear film collected prior to lens wear. Kramann and colleagues,425 using wearers of RGP or silicone hydrogel lenses and a semi-quantitative analysis, found that there was a significant increase in the concentration of protein S100 A8 in the tears of both lens-wearing groups and a significant decrease in concentration of secretoglobin but increase in cystatin in the RGP lens wearers compared to silicone hydrogel or non-lens wearers. There is an increase in plasmin activity in tears during CL wear (soft lens wearers) acknowledged by most437-439 but not all studies.417

**Association of CL Discomfort With the Tear Proteome.** There has been very little research on whether the tear film proteome changes with CL discomfort (by any definition). No significant difference was found in the concentration of total protein, lysozyme, lactoferrin, or sIgA between tears of tolerant or intolerant CL wearers in the absence of lens wear compared to soft lens wear during 1 day.76,90 However, there is an apparent association between the levels of lipocalin-1 or sPLA2 in tears and intolerance to lens wear, with intolerant individuals in the absence of CL wear having increases in both...
these proteins (2.40 ± 1.5 vs. 0.45 ± 0.85, P < 0.001; 1.86 ± 0.95 vs. 1.80 ± 0.08, P = 0.047, respectively) compared to tolerant lens wearers.359

Nichols and Green-Church360 analyzed the tears of normal CL wearers and CL wearers classified, using the Contact Lens Dry Eye Questionnaire, as having CL-related dry eye symptoms during lens wear (all wore galyfilcon A silicone hydrogel CLs). Using combinations of SDS-PAGE, 2D-DIGE, and nano-LC-MS/MS, they found that the total protein of tears was significantly reduced in the CL-related dry eye group (P = 0.02). Furthermore, the concentrations in tear secretions of β-2-microglobulin, proline-rich protein-4, lacritin, and secretoglobin 1D1 were found to be decreased, whereas the concentrations of secretoglobin 2A2, albumin, deleted in malignant brain tumor (DMBT)-1, and prolactin-inducible protein were increased in the tears of the CLDE group compared to the normal CL group.

Mucins and Glycocalyx

Mucins are a family of high molecular weight, heavily glycosylated proteins that form the protective biofilm on the surface of epithelial cells. They are characterized by the presence of multiple tandem repeats of amino acids, rich in serine and threonine, in the central domain of the mucin core peptide; these tandem repeats provide sites for O-glycosylation.340,441 Epithelial mucins can be divided into two different classes, transmembrane (or cell surface associated) and secreted. The glycosylated regions of these molecules are hydrophilic and contribute to the prevention of ocular surface desiccation by binding water. On the apical glycocalyx, transmembrane mucins and their O-glycans prevent adhesion and maintain epithelial barrier function through interactions with galectins.39 Other O-glycan-containing glycoproteins, such as lubricin, also promote boundary lubrication between the cornea, conjunctiva, and CL-like materials.442

The normal human tear film contains MUC5AC, a secreted mucin produced by goblet cells within the conjunctival epithelium. The stratified corneal and conjunctival epithelium produce three transmembrane mucins: MUC1, MUC4, and MUC16. Transmembrane mucins are concentrated on the tips of the apical cells’ microvilli, forming a dense glycocalyx at the epithelial-tear film interface, but they can also be shed from the cell surface and consequently are found in the tear film.345

Several studies have demonstrated a decrease in the amount of secreted mucin at the ocular surface of CL wearers. MUC5AC messenger ribonucleic acid (mRNA) in the conjunctiva and MUC5AC protein in tears are significantly reduced in subjects wearing both soft and rigid CL.82,444–446 Also, levels of sialic acid, a terminal carbohydrate in glycoproteins, is reduced in the tears of CL wearers.447 Studies evaluating transmembrane mucins during CL wear have generated more variable results. Binding of the CA 19-9 antibody to a sialic acid on MUC1 in tear samples decreased significantly during CL wear.448 Conversely, exposing tear film from CL wearers to immortalized human corneal epithelial cells has resulted in MUC1 upregulation.449 This variability could be ascribed to the use of different experimental approaches, methods, or CL types in these studies. For instance, use of CLs with different water contents has been shown to differentially influence the levels of MUC1 mRNA.450

Contact lens wear is commonly associated with damage to the ocular surface glycocalyx, including physical changes in the form of thinning or compression and signs of biochemical changes reflected as an increase in the number of carbohydrate receptors.351 Multipurpose CL solutions further contribute to disruption of the integrity of the glycocalyx, affecting the shedding of MUC16 from the cell surface and reducing MUC1 and MUC16 mucin gene expression.352,453

Mechanical interaction of the CL with the epithelial surface and the blinking forces of the lid are also involved in formation of so-called mucin balls.344 This is a common but innocuous phenomenon that appears to cause spherical indentations in the corneal epithelium after lens removal.355–358 Histology shows that mucin balls are negative for lipids and bacteria, but are periodic acid Schiff positive, indicating that glycoproteins constitute a major component of their content.359 The development of mucin balls does not depend on the CL type worn, but lens type does influence the degree of mucin ball formation.451 There does not appear to be a link between CLD and mucin ball formation.

A limited number of studies have attempted to correlate mucin expression during CL wear with comfort. Protein analyses have shown that CL wearers with symptoms of discomfort, as measured using the Contact Lens Dry Eye Questionnaire, have decreased levels of MUC5AC in the tear film.448 Additional analyses in asymptomatic CL wearers, on the other hand, have produced conflicting results. MUC5AC content in conjunctival goblet cells is low in CL wearers with no subjective symptoms or clinical signs of intolerance compared to healthy controls.445 However, data from additional studies have shown no significant changes in the levels of transmembrane or secreted mucins, or in the content of glycosidic residues in non-goblet epithelial cell vesicles in tolerant CL wearers.460,461 These discrepancies in mucin expression in asymptomatic wearers could be attributed to long-term differential inflammatory responses, known to affect mucin biosynthesis.460 More recently, it has been proposed that the pattern of mucin degradation during CL wear could also affect comfort, since mucin fragmentation in response to a new material has been observed in asymptomatic, but not symptomatic, CL wearers.462

Other Tear Film Components

Tears have antioxidant activity463,464 and contain several antioxidant components, including gamma-glutamyl transpeptidase that protects against oxidative stress via glutathione recapture.421 Cysteine, ascorbic acid/ascorbate, glutathione, uric acid/urate and tyrosine,465,466 and superoxide dismutase.19 Ascorbate and lactate dehydrogenase, but not urate, increase in concentration in tears from basal to closed eye.755 While CL wear increases the level of the antioxidant tyrosine in tears,66 it does not increase the concentration of ascorbic acid or the total antioxidant activity.467 Wearing a RGP orthokeratology lens for one night significantly increases the concentration of ascorbate and lactate dehydrogenase in tears.755 And lactate dehydrogenase increases with extended wear of highly oxygen-permeable soft or RGP lenses.224,468 The magnitude of lactate dehydrogenase increase is dependent on the type of CL and especially on the oxygen permeability of the lens.469,470 Tears contain nucleotides and dinucleotides that have a function in controlling teariness and ocular surface wound healing.771 but the effect of CL wear on the concentration of these in tears is not known.

There is no published information on the relationship between antioxidants or nucleotides on the comfort response during CL wear.

Cellular Content of Tears (PMNs)

The earliest demonstration of white blood cells in tears was by Norm,772 who observed a relative leukocytosis, first thing in the morning, in tears collected from the conjunctival sac. Subsequently, others have shown that during sleep the tear film and
ocular surface are infiltrated by large numbers of polymorphonuclear leukocytes/neutrophils (PMN).318, 435, 473–475 This recruitment is likely to be mediated by the increased concentrations of chemokines, such as IL-8 and leukotriene B\(_4\),305, 476 that are found in closed-eye tears.

Using neophytes to CL wear and placing a CL in one eye only, Wilson and colleagues\(^{477}\) demonstrated that there were >6000 leukocytes that could be washed from the ocular surface after sleep and that lens wear did not affect this number. On the other hand, in a study of three separate groups of subjects (non-lens wearers, neophytes to lens wear, and adapted CL wearers), numbers of PMNs were significantly higher from tears/ocular surface wash of neophyte compared to non-lens wearers, but adapted lens wearers had fewer PMNs recovered.\(^{435}\) The number of PMNs recovered from the two CL-wearing groups was also significantly different. These changes were at least partly the result of changes to chemokine levels in tears of the three groups.\(^{455}\) Similarly, Stapleton and colleagues\(^{475}\) demonstrated that there was a significant reduction in the numbers of PMNs washed from the corneal surfaces of experienced (adapted) daily-wear soft lens wearers following sleeping in their lenses.

There have been no studies relating the role of PMN recruitment onto the ocular surface during sleep to CLD.

**External Components**

Multipurpose disinfecting solutions (MPDS) used to clean and disinfect soft CLs overnight often contain surface-active ingredients (e.g., Tetronic, Pluronic),\(^{477}\) added to improve cleaning efficiency and CL wettability and to maximize comfort. Surface-active agents have the capacity to emulsify the lipid layer and destabilize the tear film.\(^{274}\) These surfactants are introduced in the tear film upon CL insertion, after overnight soaking in MPDS, and can further destabilize the tear film.\(^{50, 269, 274}\) Svitova and Lin\(^{269}\) have reported some effect of surfactant-containing lens care solutions on the rheological properties of mixed lipids-lysozyme films in vitro. Further, any uptake into the CL material during overnight storage will create a slow release of the surface-active substance during wear.\(^{269, 478}\) No information is currently available on the effect in vivo of MPDS on the tear film.

Eye cosmetics, even though applied externally, have been shown to migrate onto the ocular surface and through the tear film\(^{479}\) and deposit onto CLs; cosmetic products include a variety of ingredients (oils, waxes, pigments, powder, stearates, surfactants, diluents, preservatives) that can have a potential destabilizing effect on the tear film.\(^{480}\) One ingredient commonly found in eye cosmetics, to prevent bacterial growth during storage, is the preservative benzalkonium chloride, which has been associated with a decreased TBUT and dry eye symptoms.\(^{304, 481, 482}\) While deposition of cosmetics on the CL surface is recognized to affect CL comfort,\(^{122}\) no information on cosmetics within the tear film, specifically, has been linked to CL-induced discomfort.

**Future Directions**

It is clear from the preceding report that there remain significant gaps in our understanding of the extent to which tear film changes in CL wear might be responsible for inducing symptoms of discomfort in CL wearers. A number of areas in which further research is indicated and should be prioritized to help address the identified shortfalls are described below.

To understand the relationship between CLD and tear film dynamics and composition, possible major directions of research are as follows:

1. Examining associations between biochemical parameters in the tear film with CLD using a consistent definition of comfort, particularly in establishing parameters that may be predictive in neophyte wearers and understanding changes over time.

2. Refining the selection of wetting agents that can be included in CL care solutions to help maintain long-term wettability of the CL surface (e.g., in addition to poloxamer and Tetronic molecules incorporated in current formulations, many other hydrophilic polymers and block copolymer wetting agents require further exploration).

3. Development of novel CL materials that can resist evaporation of water content or can maintain a highly wettable surface after a prolonged wearing time.

In terms of lipid layer integrity, major priorities for future research include the following:

1. Elucidating the mechanism of lipid/CL and protein/CL interactions responsible for deposit formation to explore the effect of CL surface charge, roughness, and effect of lens surface modification with phospholipid or polymer coatings, and so on.

2. Designing nonadhesive CL surfaces with long-term resistance when worn or to develop lens care formulations improving the CL wettability.

3. Gaining an understanding of how wetting agents can modify the spread and the quality of the TFLL over the CL surface.

With regard to PLTF stability, future research should be directed toward development of lens materials, designs, and surfaces, with or without the aid of care products that promote biocompatibility, to a level where the tear film can remain stable over the surface. Current evidence leads us to believe that more biocompatible CL surfaces could promote more physiological tear film structure in at least those deemed tolerant of CL wear.\(^{90}\)

Whether the ocular surface temperature in CL wearers directly impacts comfort has not been established. However, cooled artificial tears have been found, subjectively, to improve comfort in normal non-lens-wearing eyes, suggesting that this area is deserving of further exploration. The close relationship between ocular surface temperature, tear film stability, and tear evaporation would suggest that interventions that modify one aspect will have influence on all.

Contemporary high-resolution technologies such as OCT, allowing detailed observation of the tear film profile during lens wear, have confirmed the significant physical impact of CLs, and particularly rigid CLs, on the tear film. This approach has benefit in optimizing the fitting relationship and edge characteristics of the lens as related to CLD.

Osmolarity is recognized as a key property of the tear film, but its assessment in CL wear has been limited to date, in part by the need for large tear volumes for analysis. The design of osmometers that require only minute amounts of tears may help in more accurately defining location-specific tear film osmolarity changes in the pre- and post-CL tear film, particularly in those suffering from CL-induced dry eye. Although a variety of studies have investigated the effect of CLs on tear film osmolarity, there is limited information on the impact of the osmotic level on ocular comfort, unlike the situation with dry eye disease. Of particular relevance would be studies that not only compare the osmolarity of symptomatic and asymptomatic lens wearers but also assess its correlation to ocular comfort indices in order to improve our understanding of the impact of tear film osmolarity on CL-
induced dryness. Also, if osmolarity of tears does affect comfort, then investigations on the biochemical/chemical changes that occur may provide insight into methods of alleviating the discomfort.

Tests such as tear ferning have shown some potential to discriminate between lens wearers and non-lens wearers and perhaps even predict ocular comfort during CL wear. Availability of digital image analysis may allow for more accurate and objective tear ferning quantification in the future and lend support to the investigation of the relationship between tear ferning and ocular comfort during CL wear.

Clinical investigations have yielded some limited data indicating that CL wear induces a modest decrease in tear film pH. Additional evidence-based data are required to support any mechanistic link between reduced pH in the tear film and CL discomfort.

With regard to the surface tension of tears, there is a lack of data specifically addressing the questions of whether CL wear alters the surface tension of tears, whether this can be related to discomfort during lens wear, and what might be the underlying biochemical and physical changes to the tears that manifest as changes to surface tension. Furthermore, experiments specifically addressing the issue of whether changes to the surface tension of tears are related to the wettability of CLs during wear are lacking.

With the advent of newer proteomic,439 440 441 glycomic,442 443 and lipidomic techniques, reexamination of the role of the proteins, glycoproteins, mucins, and lipids, as well as nonbiological components of the tears in tear film surface tension and effects of CLs, should be undertaken. In recent years the polar lipids OAHFAs and their esters in meibum have been discovered,444,445 and their concentration is correlated more strongly than that of phospholipids to dry eye severity446; therefore an examination of the effect of these lipids and other polar lipids on the spreading behavior of the tear film, surface tension of tears, changes during CL wear, and discomfort is warranted, as well as further evaluation of the effect of tear lipid degradation.

In relation to effect of CLs on tear composition, it is clear that there is probably no effect of CLs on the concentration of total protein, lysozyme, and lactoferrin (at least with soft lens wear). Research needs to be conducted on the effect of lens wear on the concentration of lipocalin-1 in tears, especially given its potential change in tears in intolerant compared to tolerant lens wearers.359 The effect of CL wear on the concentration of total or specific sIgA requires further investigation. It should be noted that while substantial progress has been made in defining the tear proteome, this has not been taken into account the changes that are known to occur between the different tear types; this should be addressed in future work.

Concerning the inflammatory mediators in tears, further research on the potential for CL wear to affect arachidonic acid metabolites, neuropeptides, histamine, and other inflammatory mediators would be beneficial. While lens wear does appear to generally increase the level of several cytokines in tears, which if any of these relate to CL discomfort is unknown at present. An effort to relate changes of these mediators, proteases, and all the potential inhibitory factors for inflammatory mediators and proteases in tears with comfort during CL wear is urgently needed. Aside from these major directions, the very large differences in the amount of cytokines reported in tears (see Table 6, IL-6 and IL-8 as examples) also merit investigation. It seems unlikely that these large differences are physiological but are perhaps related to methodological differences,360 and further research is required for clarification.

Clinical investigations have yielded some supportive data indicating that symptomatic CL wear might be associated with decreased levels of secreted mucin in tears. However, there is not complete agreement on whether reduced mucin content contributes to ocular surface discomfort, due to some degree of variability in methods across studies. Contact lens wear is clearly associated with physical and biochemical changes to the epithelial glycocalyx. Future investigations on the integrity of this carbohydrate-rich zone on the cell surface could offer potential new information on the mechanisms leading to discomfort during CL wear. At present, there are insufficient molecular data to demonstrate accurately the presence of either transmembrane or secreted mucins within “mucin balls.”

**Conclusion**

Numerous opportunities exist for further research to be conducted in this area. Answers to these research questions will foster a better understanding of the impact of tear film changes secondary to CL wear on ocular comfort, in order that we might strive to reduce the effects and optimize CL comfort. Evidence suggests that the biophysical properties of the tear film are interrelated, and it is likely that no single component can be isolated as responsible for CL discomfort. This theory is supported by the demonstration that the feature with the strongest link to ocular comfort during CL wear is tear film stability, a property recognized to reflect myriad tear film components and interactions.

**Acknowledgments**

Disclosure: Each workshop participant’s disclosure data can be found in the Appendix of the Introduction.

**References**


104. Nichols JJ. *Evaporative Tear Film and Contact Lens Factors Associated with Dry Eye Symptoms in Contact Lens Wearers*. Columbus, OH: Ohio State University; 2004.


