EVAPORATION IS THE PRIMARY MECHANISM OF TEAR FILM THINNING

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Vision Science in the Graduate School of The Ohio State University

By

Samuel H. Kimball, B.S.

Graduate Program in Vision Science

The Ohio State University

2009

Thesis Committee:

Jason J. Nichols, OD, MPH, PhD, Advisor
Kelly Nichols, OD, MPH, PhD
P. Ewen King-Smith, PhD
ABSTRACT

The purpose of this study was to determine the contribution of evaporation in the thinning of the pre-corneal tear film. The human tear film is essential to the optical and physiological function of the eye. A malfunctioning tear film can be visually disruptive as well as cause damage to the ocular surface. Dry eye disease is an ocular surface disorder that is essentially a manifestation of a faulty tear film. Dry eye disease represents a significant public health concern and a better understanding of the mechanisms involved in the eradication of the tear film will be needed in order to better treat and manage this significant disease. There are three possible mechanisms of tear film thinning and they include absorption (inward flow), tangential flow, and evaporation (outward flow) of the tears. Previous research, designed to establish the contribution of each of the three proposed mechanisms of tear film thinning, has led to disagreement as to the significance of evaporation or outward flow in the thinning of the human tear film. This study was designed to discover the contribution of evaporation in the thinning of the pre-corneal tear film.

Tear thickness values and tear film thinning rates were gathered using spectral interferometry from the right eye of 39 subjects with a mean age of 30.0 ± 9.5 years. Tear
film data was gathered under two differing conditions for each subject: open-air and airtight goggles. Two separate recordings of the tear film were first made given the open-air condition then two recordings were made for subjects wearing the airtight goggles. Each subject also completed an Ocular Surface Disease Index (OSDI) questionnaire.

Data analysis revealed that the mean initial thickness for subjects under open-air conditions was $3.46 \pm 0.83 \, \mu m$ compared to $3.54 \pm 0.83 \, \mu m$ for subjects wearing goggles ($p = 0.53$). The mean tear film thinning rate for subjects in open-air was $3.53 \pm 4.12 \, \mu m/min$ and $-0.16 \pm 1.78 \, \mu m/min$ for the same subjects wearing airtight goggles. The mean OSDI score was $10.8 \pm 7.1$, with four subjects being classified as dry eye (OSDI $>22$).

A significant reduction in the tear film thinning rate is seen when evaporation is controlled with airtight swimming goggles. In fact on average the tear film thinning rate is reduced to nearly zero when simulating a non-evaporative environment. This suggests that evaporation is the primary means by which the tear film thins. The reason for the contradictory evidence put forth in the literature concerning the contributions of the three proposed mechanisms of tear film thinning may be explained by the difference in testing methodology. It appears conclusive from this current study data that evaporation is the primary mechanism of tear film thinning.
DEDICATION

To my wife, Christine, and my children, for their untiring support of my career
ACKNOWLEDGEMENTS

I want to thank Jason Nichols for his guidance, patience, and dedication during the entire process of researching, writing, and defending this thesis. His oversight has allowed me to grow as a student and as an individual and I have gained a deep appreciation for what it takes to be a successful researcher. Thanks to his efforts this thesis has been possible.

I thank Ewen King-Smith for his support and insight, and for his example of a love for research. He always freely gave of his time in order to see my efforts come to fruition. His kindness and compassion as an educator have made a significant impact in my career.

I thank Kelly Nichols for her perspective, advice, and support in editing and refining this thesis.

This research was supported by a Beta Sigma Kappa International Optometric Honor Society research grant.

Thanks to Vistakon for sponsoring my Academy Travel Fellowship, and to the American Academy of Optometry for allowing me to present my research at the annual meeting.
VITA

May 2005..........................................................B.S. Biology
Brigham Young University

September 2005-Present.................................Doctorate Student of Optometry
The Ohio State University College of Optometry

September 2006-Present...............................Graduate Student of Vision Science
The Ohio State University College of Optometry

FIELDS OF STUDY

Major Field: Vision Science
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CHAPTER 1: INTRODUCTION

1.1 Current View of Ocular Evaporation

There is currently disagreement concerning the contribution of evaporation in the thinning of the pre-corneal, or ocular surface, tear film. The current literature describes the process of ocular evaporation as too slow to account for the amount of tear film thinning that is observable [1-14]. These reports of slow ocular evaporation rates conclude that there must be some other mechanism, or combination of mechanisms involved in the thinning of the tear film, with evaporation only playing a minor role [1, 15-17]. Contrary to these findings of slow ocular evaporation rates are other reports with implications that evaporation plays a substantial role in the thinning of the tear film and can even lead to dry eye disease [18-22].

1.2 Proposed Mechanisms of Tear Film Thinning

There are three possible mechanisms of tear film thinning and they include absorption (inward flow), tangential flow, and evaporation (outward flow) of the tears from the surface of the eye [23].
1.2.1 Absorption (Inward Flow)

Nichols et al. studied the possibility of inward flow of the tears into the cornea [17, 23]. The authors used simultaneous interferometric measures of pre-corneal tear film thinning and corneal epithelium thickness to explore the possibility of inward fluid flow. Their measurements showed no obvious increase in the thickness of the corneal epithelium between blinks. The authors concluded that because there was no change in the thickness of the corneal epithelium in the presence of the tear film thinning then absorption, or inward flow, played little to no part in the thinning of the tear film.

1.2.2 Tangential Flow

There are a number of reports of tangential tear flow in the literature. Tangential flow is parallel movement of the tear film along the ocular surface. Two types of this flow, pressure-gradient and surface-tension-gradient (Marangoni), have been described and shown to lead to tear film break-up [23-26]. A recent study gave evidence to suggest that tangential flow is too slow to account for the thinning seen with the pre-corneal tear film [27]. It is thought that tangential flow is likely to be a contributing factor in the overall thinning rate of the tear film though [17, 27]. The proportion of tangential flow in the overall thinning or the tear film is still in question. King-Smith et al. concluded that tangential flow is perhaps more important when considering special cases of tear film break-up, and that evaporation is likely to be a more important mechanism when considering tear film thinning [17].
1.2.3 Evaporation

There is some evidence to suggest that dry eye patients have increased tear film evaporation rates [18-20]. However there are also a number of reports indicating that evaporation, or outward flow, only accounts for about 10-36% of the total thinning of the tear film in normal subjects and only as much as 55% in dry eye [16]. King-Smith et al. reported that, based on the reported evaporation rates and tear film thinning rates provided in the literature, evaporation only accounted for about 20-25% of the precorneal tear film thinning [17]. Additional reports also discount the role of evaporation in the thinning of the tear film and suggest that other mechanisms, such as absorption (inward flow) and tangential flow, must play a more significant role than previously thought [1-4, 15]. In his text on the tear film, Korb concludes that based on previous research, only a “small amount of tear fluid is lost passively by evaporation [15].”

Table 1 is a summary of the evaporation rates for the eye as reported in the literature [3-10, 12-14, 18, 28]. The evaporation rates range from as low as 0.004 \( \mu \text{m/min} \) (Craig, 2000) to as high as 1.94 \( \mu \text{m/min} \) (Yamada, 1990). The average evaporation rate of these studies is 0.82 \( \mu \text{m/min} \). These reported rates suggest that evaporation is too slow of a process to account for the amount of tear film thinning typically seen. Assuming a tear film thickness of 3 \( \mu \text{m} \), and a thinning rate of about 1 \( \mu \text{m/min} \), then it would take approximately 3 minutes for the tear film to break-up (assuming break-up at zero thickness) [29]. Holly concludes that in order for evaporation to completely thin the tear film, given a certain thickness, it would take at least 5 minutes [1]. Three to five minutes
of thinning is inconsistent with reported tear break-up times (TBUT) and noninvasive tear break-up times (NITBUT) and with what can be seen clinically [30-32].

<table>
<thead>
<tr>
<th>Investigator, Year</th>
<th>Evaporation Rate (µm/min)</th>
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<tr>
<td>Yamada, 1990</td>
<td>1.94</td>
</tr>
<tr>
<td>Hamano, 1980</td>
<td>1.61</td>
</tr>
<tr>
<td>Tomlinson, 1991</td>
<td>1.20</td>
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<tr>
<td>Tsubota, 1992</td>
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<td>Shimazaki, 1995</td>
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<td>Mathers, 1996</td>
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<tr>
<td>Mathers, 1993</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Goto, 2003</td>
<td>0.25</td>
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<tr>
<td>Rolando, 1983</td>
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</tr>
<tr>
<td>Craig, 1997</td>
<td>0.09</td>
</tr>
<tr>
<td>Craig, 2000</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 1. Summary of Reported Evaporation Rates
1.3 Study Purpose

The proportion each of the three proposed mechanisms (absorption, tangential flow, and evaporation) has in the thinning of the tear film remains unclear. Given the current literature, each mechanism individually appears to contribute very little to the overall thinning. Considering the current dilemma, this project was designed to evaluate the contribution of evaporation in the thinning of the human pre-corneal tear film.
2.1 Human Pre-Corneal Tear Film

2.1.1 Function

A thin layer of tears covers the corneal surface of the human eye. This thin film of tears functions to enhance the optics of the eye, serve as a protective barrier, provide nourishment to the cornea, and act as a lubricant. The tears are the initial refracting surface of the eye. Maintaining a smooth optical surface is important for optimal visual function [33, 34]. The tears also maintain an important physical barrier to external forces such as temperature variations, chemicals, foreign material and a variety of microbial invaders [35]. In addition to its barrier function, the tears contain proteins and molecules that provide protection in the form of immunity and osmolarity [36]. The tear film also transports oxygen and some nutrients to the avascular cornea as well as removes metabolic waste products [35]. The tear film lubricates the corneal surface and protects it and the conjunctiva from mechanical damage due to the blink [37]. The variety of functions of the tear film all work towards maintaining the health and functionality of the ocular surface. If these several functions were not performed, visual performance and ocular integrity would be compromised. A malfunctioning tear film therefore may disturb the vision and health of the eye.
2.1.2 Structure

The human pre-corneal tear film has classically been described as a three-layered structure consisting of an outer lipid layer, a middle aqueous layer, and an inner mucous layer [38]. There is some thought that the tear film may not actually exist in three distinct layers but rather as an outer lipid layer overtop a gradient mixture of the aqueous and mucous layers [39, 40]. In addition there is evidence of a glycocalyx that protrudes from the epithelial surface of the cornea into the tear film [40].

2.1.3 Composition

The main lacrimal gland and the accessory glands of Krause and Wolfring secrete the aqueous portion of the tear film. Aqueous consists of proteins, water, and electrolytes [35]. The proteins contained within the aqueous are varied and numerous and are mostly protective in nature. Aqueous secretion is mainly regulated by the parasympathetic nervous system but does also contain sensory and sympathetic innervation [35]. Many factors can influence the secretion of aqueous including hormonal fluctuations and systemic inflammatory diseases.

Mucins are glycosylated glycoproteins that make up the mucous portion of the tear film. There is a number of different mucin types that differ based on their core protein structure. Mucins are secreted by conjunctival epithelial goblet cells and can be either soluble or membrane bound. The lacrimal gland also secretes some soluble mucin types [35, 40]. The membrane bound mucins attached to the epithelium of the cornea
make up the glycocalyx. Goblet cell secretion is regulated by parasympathetic, sympathetic and sensory neurons [35]. The mucins contained in tears serve as an additional barrier to microbial invasion as well as protection from chemical and mechanical trauma. Mucins are highly hydrophilic and provide valuable surface wettability to the cornea [41, 42]. It is also thought that mucins are integral to tear film stability by preventing dry spots from forming between blinks [36].

The lipid layer is secreted by meibomian glands located along the margin of the upper and lower eyelids. Meibomian gland innervation is predominantly parasympathetic but does have sympathetic and sensory neurons as well [35]. Lipid composition is complex and has been the subject of much study. Some of the notable molecules contained in lipids are hydrocarbons, esters, triglycerides, sterols, fatty acids, and polar lipids [35, 41]. Lipid secretions along the eyelid margins inhibit damage to the lid margin by preventing spillover of the tears. Traditionally lipids have been thought to be a deterrent to tear film evaporation [35, 38].

2.2 Dry eye

2.2.1 Definition

According to the Dry Eye Workshop dry eye is defined as an ocular surface disease that has a number of causes [22]. Dry eye can produce discomfort, visual disturbance and tear film instability. Two hallmarks of dry eye disease are increased osmolarity and inflammation, both of which can lead to damage to the ocular surface.
2.2.2 Epidemiology

Dry eye disease represents a growing health concern throughout the world. In the United States alone studies have shown that 4.91 million Americans 50 years and older have moderate to severe dry eye with an estimated tens of millions more suffering from milder forms that may go undiagnosed [21]. Dry eye also has a high prevalence among older individuals and projected population statistics point to an aging population worldwide. Some parts of the world could see their elderly populations, 65 years and older, as much as triple by the year 2030 [43]. Additionally the proportion of elderly 65 years and older in the United States will increase from 12.6 % in 2000 to 20.3 % in 2030 [43]. Over the next 20-30 years eye care professionals will see an increasing number of dry eye patients as well as an increase in the amount of at-risk patients. A better understanding of dry eye and its treatment will be needed to meet the demands placed on practitioners.

2.2.3 Classification

Objective clinical measures of dry eye disease can be highly variable and at times inconclusive [37]. Additionally objective measures do not always correlate well with patient symptoms and dry eye often has a highly symptom based diagnosis [37]. A dual classification scheme, based on the known etiopathogenesis of dry eye disease, was developed from the International Dry Eye Workshop in 2007 [22]. According to this scheme dry eye can be classified as either aqueous deficient or evaporative. Aqueous deficiency implies an insufficiency of the lacrimal system in producing the aqueous
component of tears. Evaporative dry eye is disproportionate water loss with normal lacrimation. Both classifications represent numerous forms of dry eye but offer two distinct pathways of the cause and development of the disease. The advantage of this classification scheme is that it helps point clinicians towards the correct diagnosis of dry eye and then guides them towards the appropriate treatment.

2.2.4 Mechanisms

Both classifications of dry eye, aqueous deficient and evaporative, can lead to hyperosmolarity and tear film break-up [37]. Hyperosmolarity of the tear film occurs when the ratio of solute to solution increases due to low tear flow, excessive evaporation or any combination of these. When there is a decrease in the aqueous portion of the tears, there will be an increase in the osmolarity. Additionally high amounts of evaporation lead to a decrease in solution and a subsequent increase in osmolarity. An increase in osmolarity has been reported to be fundamental to the damage, inflammation and symptoms involved in the dry eye process [22, 44]. Aqueous deficiency and evaporation are common in dry eye and are thought to be central to dry eye disease [22].

2.2.5 Treatment

In order to better diagnose and treat dry eye it is important to understand how the tear film is produced, how it maintains its composition, and how it is eliminated from the ocular surface. There are therapeutic agents currently in use, such as Restasis (Allergan) and many others seeking approval, that target ocular tissue with the intention of returning
the tear film to its natural state by affecting tear film production [45, 46]. Manipulation of tear film composition is also being used to treat and prevent dry eye disease. Some artificial tear formulations contain molecules intended to mimic the natural make-up of the pre-corneal tear film in an effort to supplement deficient tear films [37]. Allergan claims that the carboxymethylcellulose molecule found in their Refresh eye drops works to bind moisture in a similar fashion to mucins [47]. Soothe XP emollient eye drops (Bausch & Lomb) are formulated to contain lipids in order to retain moisture [48]. Tear film production and composition represent an expanding proportion of ocular research due to the implications of helping individuals who suffer from the discomforts of a malfunctioning tear film. Manipulating the way in which the tear film is eliminated from the ocular surface is another way to manage dry eye symptoms. Ocular devices such as moisture chambers are designed to restrict airflow around the ocular surface and minimize tear evaporation [49]. An important measure of how well these several dry eye treatments work is by evaluating the thinning, or elimination, of the tear film.

2.3 Measuring Tear Film Thinning

2.3.1 Methods of measurement

Tear film thinning and break-up is an observable phenomenon. It can be viewed clinically with fluorescein and a cobalt blue filter and is termed tear break-up time (TBUT). Although TBUT can be useful it remains an indirect means of measuring tear film thinning. A more direct method of measuring tear film thinning is spectral interferometry. The validity and repeatability of spectral interferometry have been
previously reported [50-52]. Interference effects at different wavelengths are recorded and analyzed as light is reflected back from the ocular surface to a spectrophotometer. The data from the recorded reflection spectra can then be used to calculate tear film thickness over a given time course.

2.3.2 Interferometer

Figure 1 is a schematic of an interferometry system. The interferometer consists of a point source of light, lenses (L₁, L₂, L₃), an aperture stop, and a beam splitter to focus the light on the eye. Reflections from the surface of the eye are recorded over a spectrum of wavelengths using a spectrophotometer. The subject is placed in front of the apparatus and the subject’s eye is aligned in three-dimensions to the appropriate focal plane. Eye movements are monitored using a mounted video camera and display. A small cone of light, at normal incidence, is focused at the center of the aligned cornea. The spot being sampled measures approximately 25 x 33 µm. Light is reflected back from the front surface of the eye and the intensity of the reflections is recorded, at different wavelengths, over a period of time using the Spectrophotometer. The Spectrophotometer used in this study was calibrated to detect wavelengths in the region of 562 to 1030 nm.
2.3.3 Principles of Interferometry

Figure 2 is a diagram of the principles used to design a wavelength-dependent interferometer. Reflections of light at the interface between air and the tear film (R₁) will be altered by light reflected from the deeper surface interface of tear film and cornea (R₂). When the two reflections of one particular wavelength are in phase with each other, constructive interference or a maximum intensity will be produced as seen in Figure 2a. Figure 2b shows what happens when the thickness of the layer (e.g. tear film) remains the
same but a different wavelength is used. The reflections are out of phase with each other and destructive interference creates a minimum intensity.

Figure 2. Principles of Interferometry

The pattern of maxima and minima of reflected intensity, at different wavelengths, is described as spectral oscillations. The thickness of the tear film is proportional to the frequency of these spectral oscillations seen from reflected light and thus tear film thickness can be measured. In addition these oscillations can be compared to each other over time to determine tear film thinning.
2.3.4 Advantages of Interferometry

Interferometry offers a non-invasive method of measuring tear film thickness. The retinal irradiance produced by the light source is below the maximum permissible exposure level for both thermal and photochemical effects. Calculations based on the amount of incoming radiation indicate that the tear film would warm by less than 0.3°C. Additionally interferometry has the ability to offer very good resolution [29].
CHAPTER 3: METHODS

3.1 Subject Sample

The Ohio State University Institutional Review Board approved the recruitment of subjects and the research protocol for this study. The criteria for acceptance into the study stated that the subject must be 18 years of age or older, must not be pregnant or breast feeding, and must currently not be a contact lens wearer. Pregnant or breast-feeding individuals were excluded on the basis that hormone fluctuations might affect tear film stability. Moreover contact lens wear is thought to disrupt the dynamics of the tear film so individuals currently wearing contact lenses were excluded as well. All qualifying subjects were given a copy of the consent form and asked to sign the consent.

Calculations of sample size for paired data analyses suggested that a minimum of 30 subjects would be needed to show what might be considered a clinically meaningful effect. To ensure that the study was appropriately powered, we enrolled 39 subjects.

3.2 Study Design

3.2.1 Data Collection

This current study employed spectral interferometry to record tear thickness values over time for each subject’s right eye only. Recordings were made under two
separate conditions: open-air and airtight goggles. Two trials were made for each condition totaling four interferometric recordings for each subject. The first trial would commence and the subjects were asked to blink and hold their eyes open for a period of about 19 seconds. The subjects were first measured for the open-air condition and two separate trials under this condition were made. In between recordings each subject was given a three-minute resting interval to allow the tear film to re-stabilize. Following the second open-air recording the subjects put on a pair of airtight swimming goggles and waited three minutes for the humidity inside the goggle to equilibrate. During this equilibration period with the goggles on the subjects completed an Ocular Surface Disease Index (OSDI) questionnaire to determine their dry eye status. Subsequently another two measurements trials, with a three-minute resting period in between, were captured under the airtight goggle condition.

3.2.2 Goggle fogging

Preliminary testing with the goggles revealed that the reflections from the tear film of certain individuals were obscured by condensation on the inner surface of the testing goggles. Not all individuals seemed to be susceptible to this fogging but in an attempt to prevent condensation the goggles were warmed in a heating pad to bring the temperature closer to that of the human body before each interferometry recording. In addition to warming the goggles, an anti-fog compound by the name of Tyr® Anti-Fog, was applied to the right goggle lens. This agent is reported by the manufacturer to reduce the amount of fogging in swimming goggles [53]. The combination of warming the
goggles to body temperature and the anti-fog compound before each recording made it possible to obtain reflectance readings through the goggles consistently.

### 3.3 Data Processing

Study data were processed using Fourier analysis plus least squares fit to determine thickness and thinning rates for each of the four trials. A computer program was used to make the calculations based on recorded spectral oscillations. The calculations employed by the program have previously been described in detail [29]. The two measurements for the open-air condition were averaged and compared to the average of the two measurements with goggle wear. Once the data were processed, a committee reviewed each of the subject’s measurements to determine the validity of the measurements. Predetermined criteria based on past experience with spectral interferometry were applied to each subject’s data. These criteria took into account factors such as subject alignment, inadvertent blinking, stray light and goggle fogging. Of the 39 subjects originally enrolled, one subject had no acceptable measurements and one other had no acceptable measurements with goggles in place, leaving 37 subjects with appropriate data for analysis.

### 3.4 OSDI

The Ocular Surface Disease Index (OSDI) questionnaire, developed by the Outcomes Research Group at Allergan Inc (Irvine, CA), was administered to subjects in
order to distinguish those patients with dry eye disease from normal subjects. It has been reported that this 12 item questionnaire has good reliability and correlates well with some clinical assessments [54]. In addition the OSDI has been shown to have good correlation with other instruments for identifying dry eye such as the McMonnies Dry Eye Questionnaire that was designed to discriminate dry eye patients within the normal population [54]. All OSDI scores for individual patients were computed using the published OSDI scoring guidelines. Scores were assessed on a scale of 0 to 100, with higher scores indicating increased disability. The cut off for classification of dry eye in this study was ≥ 22, indicating at least mild dry eye.

3.5 Statistical Analysis

Statistical analysis was performed using SPSS statistical software. Tear film thickness and thinning rates do not follow a normal distribution so non-parametric statistics were used to evaluate the collected tear film data. In particular the Wilcoxon signed ranks test and $R^2$ correlation were used to identify significance among the tear film data. A p value < 0.05 was considered to be statistically significant.
CHAPTER 4: RESULTS

4.1 Demographics and Baseline Data

Data were analyzed from 37 subjects, 14 females, with a mean age of $30.0 \pm 9.5$ years. The mean room temperature was $20.9 \pm 0.5^\circ\text{C}$ and the mean relative humidity of the examination room was $34.2 \pm 9.9\%$. The mean OSDI score for subjects in this study was $10.8 \pm 7.1$. Four of the subjects were classified as dry eye by OSDI standards with a score greater than 22 and were included in the data analysis. It was decided that the dry eye subjects should be included in the study data because the intent of the study was to study the effect of evaporation on the thinning of the tear film. If evaporation plays a role in the thinning of the tear film there should be a change in the thinning rate of normal subjects and dry eye subjects alike.

4.2 Open-Air vs. Goggles

4.2.1 Illustration of the Effect

Figure 3 is data collected from one subject for the two testing conditions set forth by the study protocol and demonstrates the type of data collected from each subject. This represents a very rapid open-air thinning rate and serves as an example of the effect seen
in this study. A linear plot of reflectance from the ocular surface is shown at the bottom of each graph in Figure 3 and is a function of time. Large relative drops in reflectance mark the subject’s blinks. The subject in Figure 3a had two blinks under the no-goggles, or open-air, condition. One blink is at 2 seconds and the other at 18 seconds. The scatter plot shows thickness values of the tear film over the given time course. An observable transient phase of upward drift of the tear film is seen immediately after the subject blinks. This transient drift is associated with movement of the lipid layer and is seen on the graph as a thickening of the tear film [34]. In order to avoid biasing the thickness and thinning rate measurements, data is analyzed two seconds after the subject’s first recorded blink and includes all values of thickness recorded before the start of the second blink. The black circles therefore indicate tear film thickness data included in the analysis. Linear extrapolation on Figure 3a shows where the tear film thickness would have reached zero thickness. This is the point where tear film break-up is thought to occur.

Figure 3a shows data from one of two no-goggle, or open-air, measurements taken for this particular subject. Figure 3b is data from the same subject wearing airtight swimming goggles. Analysis of the data for this subject with no-goggles showed an initial thickness value, 2 seconds after the blink, of 4.10 µm, with a mean thickness of 2.73 µm, and a thinning rate of 19.74 µm/min. When the subject wore goggles, as seen in Figure 3b, the initial thickness value was recorded as 4.31 µm, with a mean thickness of 4.07 µm, and a thinning rate of 1.78 µm/min. Note that for this subject the initial
thickness was relatively unchanged with goggle wear but the thinning rate was substantially reduced compared to the no-goggle condition.

![Figure 3. Example of Study data for one subject](image)

4.2.2 Summary of Study Data

Table 2 is a summary of the mean study data including mean initial thickness, mean thickness, and mean thinning rates for the 37 subjects under conditions of no-goggle and goggles wear. The mean initial thickness for subjects was found to be similar regardless of the testing condition. The mean initial thickness for the open-air condition two seconds after the blink was $3.46 \pm 0.83 \, \mu m$ while it was $3.54 \pm 0.83 \, \mu m$ under non-evaporative conditions ($p = 0.53$). The difference in mean thickness for the duration of the recording was significantly different between the open-air condition and the goggle-wear, with mean thicknesses of $3.13 \pm 0.87 \, \mu m$ and $3.56 \pm 0.96 \, \mu m$ ($p = 0.004$)
respectively. The mean thinning rate was 3.53 ± 4.12 µm/min without goggles, while it was -0.16 ± 1.78 µm/min under non-evaporative conditions and this was found to be statistically significant (p<0.0001).

<table>
<thead>
<tr>
<th></th>
<th>No-goggles</th>
<th>Goggles</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Initial Thickness (µm)</td>
<td>3.46 ± 0.83</td>
<td>3.54 ± 0.83</td>
<td>p = 0.53</td>
</tr>
<tr>
<td>Mean Thickness (µm)</td>
<td>3.13 ± 0.87</td>
<td>3.56 ± 0.96</td>
<td>p = 0.004</td>
</tr>
<tr>
<td>Mean Thinning Rate (µm/min)</td>
<td>3.53 ± 4.12</td>
<td>-0.16 ± 1.78</td>
<td>P &lt; 0.0001</td>
</tr>
</tbody>
</table>

Table 2. Summary of Mean Study Data.

Figure 4 is a plot of goggle-wear thinning rates (y-axis) as a function of no-goggles, or open-air, thinning rates (x-axis). The mean thinning rate for the no-goggle condition has a large standard deviation of ± 4.12 µm/min. Subjects have a wide variety of thinning rates under normal open-air conditions and thus results in a large standard deviation. The fastest thinning rate for open-air recorded in this study was 18.47 µm/min and the slowest was -1.97 µm/min. For the goggle-wear condition the standard deviation is significantly reduced to ± 1.78 µm/min. Figure 4 shows a visible clustering of thinning rates right around zero, with a mean thinning rate of -0.16 µm/min. The linear regression is shown in Figure 4 as the solid black line and has a slightly upward slope (R² = 0.12). The dashed line represents what the graph would look like if the no-goggle thinning rate
equaled the goggle thinning rate. The three data points situated to the left of the dashed line represent subjects that showed an increase in the thinning rate while wearing goggles. Therefore 34 out of the 37 subjects show a decreased thinning rate with goggle wear, which is a significant result (binomial test, \( p < 0.001 \)).

![Plot of Thinning Rates: No-goggles vs. goggles](image)

**Figure 4.** Plot of Thinning Rates: No-goggles vs. goggles

### 4.3 Open-Air (No-goggle) Data

#### 4.3.1 Open-Air Thinning Rate Distributions

Figure 5 shows a histogram of both trials of open-air thinning rates for each subject. The open-air thinning rates are not normally distributed. The distribution is represented by the solid curve seen in Figure 5. The overall distribution however can be
represented by the sum of two individual normal, or Gaussian distributions, depicted by the dashed lines. The far right point on the graph where the two distributions cross is 3.17 µm/min. If the distribution of open-air thinning rates is thought of as a combination of two separate distributions fitted to Gaussian curves, then the 3.17 µm/min could represent the boundary between what may be considered slow thinning versus rapid thinning. According to this model the slow thinning is represented by the narrow gaussian distribution and has a mean rate of 1.77 µm/min. Using 3.17 µm/min as the upper limit for slow thinning, 59% of the current study data would fall into this category. Rapid thinning then is represented by the much wider distribution and has a mean rate of 5.61 µm/min.

Figure 5. Distribution of Open-Air Thinning Rates.
4.3.2 Open-Air Thinning Rate Repeatability

Open-air thinning rates tend to be highly variable and therefore repeatability can be poor. Parametric statistics yield a moderately significant result in repeatability ($r^2 = 0.37$, $P < 0.001$). However, as was previously discussed, the distribution of thinning rates is not normally distributed so non-parametric statistics would be more appropriate. The Spearman correlation is much poorer and suggests reduced repeatability among open-air thinning rates ($R^2 = 0.046$, $P = 0.21$). Inter-subject repeatability improves however if the two-distribution model, seen in Figure 5, is applied to the open-air thinning rate data. Figure 6 is a plot of the open-air thinning rates for each of the two trials for each subject. It is plotted as second trial thinning rate versus first trial thinning rate. The solid horizontal and vertical lines are drawn at 3.17 $\mu$m/min and represent the boundaries between “slow” and “rapid” rates. The number shown in each quadrant represents the number of data points for each quadrant. According to this model, of the 27 subjects who had a slow thinning rate on the first trial, 24 had a slow thinning rate on the second trial (specificity of 89%). Similarly of the 9 subjects with rapid thinning on the first trial, 7 showed rapid thinning in the second trial (sensitivity of 78%). Using the criterion of two separate rate distributions, open-air thinning rates are significantly repeatable ($p < 0.001$, Fisher Exact Test).
Figure 6. Open-Air Thinning Rate Repeatability.
4.4 OSDI vs. Thinning Rate

Figure 7 shows a plot of mean open-air thinning rates (y-axis) as a function of OSDI score (x-axis). The dashed line represents the regression line ($R^2 = 0.023$). There is a slight upward trend but it is not statistically significant ($p = 0.37$). The solid vertical line on the right side of the graph (OSDI score $\geq 22$) represents the cut-off for dry eye and the four subjects with dry eye can be seen to the right of the line. The thinning rates...
for subjects classified as having dry eye in this study varied widely from 0.26 µm/min to 18.47 µm/min. One of the subjects demonstrate thickening of the tear film with a mean thinning rate of -1.97 µm/min and an OSDI score of 11.4.

4.4 Tear Film Thickening

Tear film thickening, or a negative thinning rate, was observed in both the open-air and the goggle measurements. A mean thickening was observed in one subject under open-air conditions and in 16 of the 37 subjects while wearing goggles. These negative thinning rates can be seen in Figures 4, 6, and 7. The mean thinning rates from subjects wearing goggles was -0.16 µm/min (see Table 2), and is not statistically significant from zero (p = 0.56).
CHAPTER 5: DISCUSSION

5.1 Contribution of Evaporation in Tear Film Thinning

Three possible mechanisms for tear film thinning have been proposed including inward, tangential, or outward flow of the tears from the surface of the eye. The results from the current study suggest that evaporation, or outward flow, is the main contributor in tear film thinning. By simulating a non-evaporative environment for subjects, using airtight swimming goggles, thinning rates were significantly reduced compared to open-air, or habitual, thinning rates (see Table 2). In fact the mean thinning rate for subjects wearing the goggles was -0.16 µm/min, which represents a slight thickening of the tear film. Figure 4 also presents a compelling illustration of the conclusion that evaporation plays a significant role in tear film thinning. If evaporation was an insignificant part of the overall thinning of the tear film, then there should have been a measurable decrease in tear thickness over time while wearing the goggles. Instead there is a visible clustering of evaporation rates right around zero thinning with goggles in place. Of the three possible mechanisms responsible for tear film thinning, the only one being affected by the goggle wear should be outward flow, or evaporation. Inward flow and tangential flow should
remain relatively unaffected by the goggle wear, thus evaporation appears to be the key mechanism in the thinning of the tear film.

The linear nature of the thinning also suggests that the mechanisms responsible for tear thinning don’t wax and wane but work at a constant rate. Thus contributing mechanisms to tear film thinning would need to be present throughout the process. Evaporation likely isn’t contributing only when there is compromise to the lipid layer for example. Absorption into the cornea, if present, may reach a saturation point and a drop plateau in thinning would be seen. Perhaps only tangential flow with its acceleration phase, as seen after the blink, plays into the mechanism responsible for tear thinning.

The conclusion that evaporation is the primary mechanism in tear film thinning is contrary to some previous studies [1-14]. As previously described, reported ocular evaporation rates are too slow to account for the relatively rapid thinning seen clinically in the human tears. Three to Five minutes of thinning before reaching a zero thickness is too long of a process to account for tear thinning [32]. This inconsistency may be explained by the difference in the methods used by previous studies compared to the current study in order to measure the evaporation of the tear film.

5.2 Devices Used to Measure Ocular Evaporation

5.2.1 Closed Chamber

Closed chamber systems employ a swim-type goggle placed over the eye [19]. The air inside the chamber is conditioned to a desired baseline and changes in water content are monitored by an evaporimeter attached to the goggle and recorded as
evaporation. These closed chamber systems do not allow free exchange of air over the surface of the eye thereby artificially reducing evaporation from the ocular surface. Therefore the true evaporation rate from the ocular surface would actually be much higher than in the humid environment of the closed chamber evaporimeter. Hamano reported an evaporation rate from a simple closed chamber system of 1.61 µm/min. Rolando modified the closed chamber system slightly and reported an ocular evaporation rate of 0.24 µm/min. Yamada and Tsubota enhanced the closed chamber system in order to monitor relative humidity and temperature and reported an evaporation rate of 1.94 µm/min. Mathers found normal evaporation to be approximately 0.88 µm/min using a closed chamber system.

5.2.2 Ventilated Chamber

Ventilated chamber systems use an eye cup with air streamed across the ocular surface [12]. Water content of the air coming in is compared to the water content of the air coming out. Goto and Tsubota measured an evaporation rate of 0.25 µm/min using a ventilated system. While this system has the advantage of dynamic evaporation measurements it too may be artificially reducing evaporation rates by surrounding the ocular surface with the eye cup.

5.2.3 Open-Air System

Interferometry is an open-air system and therefore may be capable of more accurately assessing the natural evaporation rate of the tear film. In addition to measuring
the tear film in a more natural setting it also allows a direct comparison of open-air and of non-evaporative conditions such as with airtight goggles. Evaporation rates measured by this system are greater than the other systems. The mean evaporation rate, assuming evaporation is the primary contributor in tear film thinning in open-air for this study, was 3.53 µm/min. This is nearly twice the evaporation rate reported by Yamada using a closed chamber system and 14 times the rate found by Goto’s ventilated chamber.

While interferometry offers the advantage of non-invasive measurements of the tear film, along with being an open system with good resolution, it may be limited in some aspects. Open-air measurements can be highly variable and repeatability is only moderately significant unless special considerations are made (see section 4.3.2). Additionally the small sampling area used by interferometry, approximately 25 x 30 µm in size, may not be representative of the entire tear film. Certain factors that influence the tear film in general, but to an insignificant degree in thinning, may appear as though they are acting in a profound way. Tangential flow may be an example of this. The small spot being sampled appears to change more rapidly because a small region of the tear film is experiencing a transient change.

5.3 Two-Distributions Model of Thinning

A two-distribution model of tear film thinning rates, as seen in Figure 5, allows the data to follow a more Gaussian distribution. Slow thinning would follow a more narrow distribution and have a much smaller standard deviation. Rapid thinning would be a much wider distribution with a larger range of variability and would include thickening
of the tear film as well as thinning. This might also help explain some of the repeatability
issues found with open-air thinning rates. Perhaps rapid thinning rates have a different
proportion of thinning mechanisms at play compared to slow thinning rates. This has
implications for further study such as how the different distributions might correlate with
dry eye symptoms or with tear film composition.

5.4 Thickening of the Tear Film

Thickening of the tear film can be seen at times and is still not completely
understood (see section 4.4). Figure 4 shows the thinning rates of subjects with and
without goggles. Examples of a mean thickening can be seen in two subjects for the
open-air condition and many of the subjects had mean thickening with goggle wear
(Figure 4). The greatest amount of thickening seen by one subject was -1.97 µm/min.

5.5 Reasons for Thickening

5.5.1 Anatomical

The human tear film is not uniform in thickness across the ocular surface. There
are areas where the tear film is thicker, such as along the margins of the eyelids. During
interferometry measurements, subjects are asked to hold their eye open without blinking.
A subject’s blinks can be monitored with reflection data (see Figure 3). However if a
subject squints, no blink will be seen, and a thickening of the tear film centrally is possible due to compression of the remaining tear film.

5.5.2 Tangential Flow

Interferometry uses a small spot size to record reflections from the tear film. The spot size is approximately 25 x 33 µm and it represents only a small region of the overall tear film (see discussion section 5.2.3). Regional changes in the tear film thickness will therefore have an influence on the recorded thickness. It is possible that tangential tear flow could result in a thickening of the tear film. The small area of measurement may experience a transient phase of thickening due to an increase in the volume of tears in that one particular region.

5.5.3 Tearing

Another explanation of thickening may be due to tearing. If downward flow of the naturally produced tears onto the ocular surface reaches the area of measurement then a thickening of the tear film might be seen. Additionally if there is reflex tearing as a result of delayed blinking then the increase in tear fluid would produce thickening of the tear film. A better understanding of the basal production rate of tears and the rate of tear production due to reflex tearing would be valuable in understanding tear film thinning dynamics.
Tear film evaporation rates can be influenced by the amount of the aqueous portion present in the tears. Some reports have suggested that watery tear films thin at a faster rate [5, 11]. Having excess tearing may compromise the balance of tear film composition and may cause not only regional thickening but also perhaps a higher rate of evaporation.

In individuals that show tear film thickening or minimal thinning, there may be an argument for the other mechanisms of tear film thinning at play. Perhaps when evaporation is well controlled then the other mechanisms of thinning become more conspicuous. It is still important to note that individuals with very minimal tear thinning rates will still exhibit a reduction in that rate when wearing goggles. This suggests that evaporation continues to be a critical factor in controlling tear film thickness.

5.6 TBUT and Interferometry

It is uncertain how evaporation rate and tear film break-up time (TBUT) relate. The normal tear film begins to break-up between blinks after 10-12 seconds. If we assume an average thinning rate of 3.5 um/min and a tear film thickness of 3 um, then it should take approximately one minute for the tear film to thin completely. It would seem then that break up and complete thinning are not the same thing. Further research is certainly warranted in order to understand how thinning rate and TBUT relate. It stands to reason however that individuals with high rates of thinning might be more likely to experience dry eye, similar to those with fast TBUTs.
CHAPTER 6: CONCLUSION

Tear film evaporation is the primary mechanism responsible for the thinning of the pre-corneal tear film. When ocular evaporation is controlled, as with airtight goggle wear, tear film thinning is essentially reduced to zero. Controlling tear film evaporation has the potential for being an intricate part of dry eye therapy. The most accurate method for measuring tear film thinning is one that uses an open-air system that will not artificially alter the ocular evaporation rate. Questions still remain concerning how tear film thinning and tear film break-up relate. Also a better understanding of basal tear film production as well as reflex tear production would be beneficial. It is also still unclear how tear film thinning relates to dry eye disease. These questions warrant further investigation.
Bibliography


