Simultaneous Measurement of Tear Film Dynamics Using Wavefront Sensor and Optical Coherence Tomography

Shizuka Koh, Cynthia Tung, James Aquavella, Rabul Yadav, James Zavislan, and Geunyoung Yoon

PURPOSE. To investigate tear film dynamics using simultaneous measurements of ocular aberrations and lower tear meniscus.

METHODS. Simultaneous measurements of wavefront aberration and lower tear meniscus were performed for 11 normal eyes and 7 eyes with short tear film break-up time (SBUT) dry eye, with a tear film break-up time shorter than 5 seconds, using a wavefront sensor and an anterior segment optical coherence tomography (OCT). During the measurement, the subjects were instructed to blink every 6 seconds for a total of 30 seconds. From the measured aberration, root mean square (RMS) wavefront error and volume modulation transfer function (vMTF) induced by changes in tear film dynamics were calculated for a 5-mm pupil. Lower tear meniscus height (TMH) and area (TMA) were estimated from the cross-sectional OCT images of lower tear meniscus.

RESULTS. There was a positive correlation between RMS and tear meniscus dimensions and a negative correlation between vMTF and tear meniscus in both groups. There were moderate negative correlations between the postblink initial RMS change and baseline TMH (R = 0.61) and TMA (R = 0.54) in SBUT dry eyes that were stronger than in normal eyes (R = 0.37, R = 0.38).

CONCLUSIONS. Tear meniscus dimensions increase with RMS over time, and tear quantity before blink has a significant role in maintaining initial optical integrity, especially in SBUT dry eye. Simultaneous measurement of optical quality and tear meniscus has the potential to improve understanding of tear stability in normal eyes and dry eyes. (Invest Ophthalmol Vis Sci. 2010;51:3441–3448) DOI:10.1167/iovs.09-4430

Tear film has different phases in the tear cycle. After the blink occurs, a tear meniscus forms and spreads over the entire ocular surface to form a uniform tear film, which then thins and breaks up, leaving dry spots until the next blink. The changing nature of the tear film has caused evaluation of tear film dynamics to be difficult. However, recent application of noninvasive technologies for evaluation of the tear film has made it possible to measure the temporal changes of tear film characteristics in real time.

Measurement of wavefront aberration with a Shack-Hartmann wavefront sensor is one of the common methods used to evaluate optical quality. Wavefront changes are measured using a lenslet array. Refractive error, irregular astigmatisms, and higher-order aberrations of the entire eye can then be determined quantitatively with wavefront analysis. According to the previous studies, 5-9 wavefront sensors are sensitive enough to monitor the optical changes associated with tear film after a blink in normal eyes and dry eyes continuously. It may provide insight into the two-dimensional relationship between change in the wavefront aberration and the ocular surface.

Optical coherence tomography (OCT) is a noninvasive, high-resolution imaging technique that is based on low-coherence interferometry. It can provide in vivo cross-sectional images of tissue structure. 10 Although OCT has been used predominantly for imaging of the posterior segment of the eye, recently it has been used to image the anterior segment as well. Evaluation of the tear film using OCT allows us to quantify tear meniscus dimensions, and it has a potential to measure tear film thickness. 11,12

Previous studies have had certain limitations. Some studies have used eyedrops such as topical anesthesia and dilation drops for wavefront measurement 7,8 and artificial eyedrops for tear film imaging with OCT. 11,12 These may lead to disruption of the tear film, and results may be subject to related changes. In addition, because tear film varies during the tear cycle, the static image taken just before the blink may not accurately represent what happens between blinks. It is not ideal to synchronize the data from multiple devices if they do not correspond in time. This has presented a challenge to investigators.

To address these issues, we have developed a new method to investigate real-time tear dynamics naturally using noninvasive, simultaneous measurements of the tear meniscus and wavefront aberrations. Specifically, we are interested in exploring the relationship between tear volume (tear meniscus dimensions) and optical quality in evaporative dry eyes compared with normal eyes.

METHODS

This study was approved by the institutional review board of the University of Rochester. The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects after the potential consequences of the study were explained fully.

From the 1Eye Institute and the 2Institute of Optics, University of Rochester, Rochester, New York.


Supported in part by a research grant from Finger Lakes Eye & Tissue Bank, by a research grant from Japan Eye Bank Association, and by a grant from Research to Prevent Blindness.

Submitted for publication August 5, 2009; revised December 17, 2009; accepted January 24, 2010.

Disclosure: S. Koh, None; C. Tung, None; J. Aquavella, None; R. Yadav, None; J. Zavislan, None; G. Yoon, None.

Corresponding author: Shizuka Koh, University of Rochester Eye Institute, 601 Elmwood Avenue, Box 314, Rochester, NY 14642; skoh@cvs.rochester.edu.
Inclusion criteria for the SBUT dry eye group were as follows: tear deficiency (5 minutes without anesthesia) (24.4 ± 7.4 mm). Between these groups, there was no significant difference in age or Schirmer test values. BUT was significantly decreased in the SBUT dry eye group compared with the normal group (P < 0.001, ttest). Exclusion criteria for both the normal group and the SBUT dry eye included other ocular disease or previous ocular surgery, systemic disease, or a history of drug use that would alter the ocular surface. One eye of each subject was chosen randomly for the measurement. To avoid the effects of other tests on the wavefront measurement, tear function tests and slit-lamp examinations were conducted on a separate day before the measurements. The measurements were conducted in a room in which the temperature was maintained at 20°C to 25°C and the humidity was 35% to 45%.

Subjects
Eleven eyes of 11 healthy volunteers (8 women, 3 men; average age, 26.6 ± 3.6 years), and 7 eyes of 7 patients with short tear film break-up time (SBUT) dry eye (5 women, 2 men; average age, 34.2 ± 12.2 years) were enrolled in this study at the University of Rochester Eye Institute. Inclusion criteria for the SBUT dry eye group were as follows: tear film break-up time (BUT) shorter than 5 seconds (average of 3 values evaluated with fluorescein), dry eye symptoms, absence of fluorescein staining of the ocular surface, and Schirmer I values that showed no tear deficiency (5 minutes without anesthesia) (24.4 ± 7.4 mm). Between these groups, there was no significant difference in age or Schirmer test values. BUT was significantly decreased in the SBUT dry eye group compared with the normal group (P < 0.001, ttest). Exclusion criteria for both the normal group and the SBUT dry eye included other ocular disease or previous ocular surgery, systemic disease, or a history of drug use that would alter the ocular surface. One eye of each subject was chosen randomly for the measurement. To avoid the effects of other tests on the wavefront measurement, tear function tests and slit-lamp examinations were conducted on a separate day before the measurements. The measurements were conducted in a room in which the temperature was maintained at 20°C to 25°C and the humidity was 35% to 45%.

Measurements
A custom-developed high-resolution Shack-Hartmann wavefront sensor and a commercial anterior segment OCT (Visante, Zeiss, Germany) were combined (Fig. 1) and used to measure wavefront aberrations and obtain cross-sectional OCT images simultaneously. Light coming from the two instruments had different wavelengths (850 nm for wavefront sensor, 1310 nm for OCT). A custom-made dichroic beam splitter was used on the optical path to separate the two light sources so that there was almost no loss of light for either wavelength. Light sources were infrared so that they did not induce reflex tearing or blinks from stimulated vision. The wavefront sensor lenslet array had a lenslet diameter of 150 μm, and the focal length was 3.5 mm, allowing detection of local refractive error changes caused by the tear film. Measurements were obtained in a dark room through a natural pupil without use of dilating drugs. Cross-sectional OCT images of the lower tear meniscus were recorded continuously across the central cornea with desktop screen capture software (AmaRecCo, version 1.21). The OCT display window was limited to image one tear meniscus at higher resolution; therefore, we chose to measure the lower tear meniscus in this study.

Subjects were asked to rest their heads on the chin-rest of the OCT and were instructed to blink every 6 seconds with a metronome for a total of 30 seconds. They were instructed to keep their eyes open and to gaze at the built-in target of the OCT between each blink. The blinking interval used in this study was longer than the average blink interval for a person in a relaxed state but shorter than the blink interval during activities that require prolonged gazing. A uniform blink interval was used because variations in the blink interval during consecutive measurements would make statistical analysis difficult. Measurements were performed three times for each subject, and each subject was given at least a 10-minute rest between measurement sessions. Sequential wavefront aberration was measured and recorded at 4 frames per second, and continuous OCT imaging was simultaneously recorded at 2 frames per second.

Data Analysis
Thirty-second measurements were taken consecutively from one eye of each subject; each measurement consisted of five 6-second post-blink intervals. The images obtained during actual blinking were not analyzed.

For the wavefront data analysis, the aberration changes induced by the tear film after blink were calculated using the zonal-based Fourier transform reconstruction algorithm, in accordance with the procedure used in an earlier study. The previous studies found that the Zernike polynomial-based reconstruction algorithm may not fully represent the high spatial frequency changes in wavefront aberrations caused by tear film break-up. Moreover, the fact that most of the ocular aberration is static refractive error (defocus and astigmatism) makes it difficult to detect relatively small changes in the high spatial frequency wavefront features. Therefore, we first reconstructed wavefronts for each frame using the Fourier algorithm and used the Zernike method to compute defocus and astigmatism. We then subtracted the lower-order aberration from the raw wavefront, resulting in the residual wavefront representing the tear film–induced aberration and computed root mean square (RMS) values from the residual wavefront as the square root of the variance of the residual wavefront.
RMS wavefront error and volume modulation transfer function (vMTF) induced by changes in tear film dynamics were calculated for a 5-mm pupil and were used to evaluate the temporal change of the tear film.

All OCT images were processed by a single trained observer using custom software. Lower tear meniscus height (TMH) and lower tear area (TMA) were calculated from the cross-sectional OCT images of the lower tear meniscus (Fig. 2). In the entire captured images, 1 pixel corresponded to 12.27 μm. Repeatability of the OCT data analysis was tested by analyzing the same set of data multiple times and confirmed that the standard deviations of TMH and TMA between the sessions were 22.7 μm and 4111 μm².

Multiple (10–15) blink intervals were averaged for each subject. After investigating the sequential time-dependent tear film–induced changes in wavefront data and OCT data, the correlation between wavefront data (RMS and vMTF) and tear meniscus variables (TMH and TMA) were analyzed to investigate the relationship between the wavefront data and OCT data between blinks. Statistical analysis for the sequential time-dependent tear film–induced changes in wavefront data and OCT data were performed based on data averaged across subjects in each group. Friedman repeated-measures ANOVA on ranks was used to analyze the sequential changes in both groups except for OCT data in the SBUT dry eye group, which used one-way repeated-measures ANOVA. The appropriate post hoc Dunnett correction for multiple comparisons was used. P < 0.05 was considered significant for all analyses.

RESULTS

Figure 3 shows the synchronized sequential color-coded wavefront maps and lower tear meniscus images obtained by simultaneous wavefront and tear meniscus measurements obtained from one subject with SBUT dry eye. Tear film–induced aberrations increased with increasing lower tear meniscus parameters after the blink. Note that the changes in optical quality between consecutive wavefront maps before 2 seconds were more remarkable than after 2 seconds.

Time-Dependent Tear Film–Induced Changes in Wavefront Data

Figure 4 shows the wavefront data from 11 normal eyes and 7 SBUT dry eyes and the average wavefront data for all subjects in each group. Each colored curve represents an individual eye. In normal eyes, there was no significant difference in tear film–induced changes of wavefront RMS until 2.75 seconds after the blink compared with the first data point at 0.5 seconds. RMS continued to be significantly increased after 2.75 seconds (Fig. 4A) (P < 0.001). In SBUT dry eyes, there was no significant change in wavefront RMS until 2.25 seconds after the blink compared with 0.5 seconds (P < 0.001). In both groups, tear film–induced changes of vMTF were significantly decreased at 2.25 seconds after the blink compared with 0.5 seconds (P < 0.001) (Fig. 4B). Although variability was found in both groups, optical quality generally degraded with time, and the changes in optical quality before 2 seconds were more significant than after 2 seconds. Interestingly, RMS of the normal eyes primarily starts around 0.1 μm, whereas that of the SBUT dry eyes have wide range. A similar trend was seen with vMTF.

Time-Dependent Tear Film–Induced Changes in OCT Data

Figure 5 show the sequential changes in lower meniscus dimensions for 11 normal eyes and 7 SBUT dry eyes and the average tear meniscus data for all subjects in each group. In both groups, there was no significant increase in TMH until 4 seconds after blink compared with the first data point at 0.5 seconds. TMH continued to be significantly increased after 4 seconds.
seconds (normal, \( P = 0.006 \); SBUT dry eye, \( P = 0.014 \)) (Fig. 5A). In normal eyes, TMA was significantly increased at 4.5 seconds after the blink when compared with 0.5 seconds (\( P = 0.028 \)). In the SBUT dry eye group, TMA was significantly increased at 2.5 seconds (\( P = 0.006 \)) (Fig. 5B).

**Relationship between Optical Quality and Tear Meniscus Dimensions**

The correlation between wavefront data (wavefront RMS and vMTF) and lower tear meniscus parameters (TMH and TMA) between blinks was calculated for each subject (Table 1). Although there was variability among subjects, a positive correlation was found between wavefront RMS and tear meniscus, and a negative correlation was found between vMTF and tear meniscus in both groups.

**Correlation between Baseline Tear Meniscus and Postblink Initial Optical Quality Changes**

Changes in wavefront RMS and vMTF were seen primarily in the early postblink phase, after which they stabilized. To see how the initial postblink optical quality was affected by the tear meniscus just before the blink, the changes in wavefront RMS and vMTF during the first second after the blink were calculated over time, and these time-dependent parameters were correlated with the tear meniscus parameters just before the blink. Calculations were made for each subject. There was a moderate negative correlation between postblink initial wavefront RMS change and both baseline TMH (\( R = -0.61 \)) and TMA (\( R = -0.54 \)) in the SBUT dry eye group and a weaker correlation in the group with normal eyes (Fig. 6).

**DISCUSSION**

In the present study, we found that tear meniscus dimensions increased with wavefront RMS over time by measuring the dynamic tear film changes using wavefront sensor and OCT. Another interesting finding was that baseline tear meniscus just before the blink correlated with initial optical integrity, especially in SBUT dry eye. To the best of our knowledge, this is the first study to image the tear film using simultaneous real-time measurements of wavefront sensor and OCT. Moreover, the present study may use a more natural condition of the eye than previous studies, in which additional eyedrops were used.

The time-course change in wavefront RMS data showed a significantly increasing trend between blinks that was consistent with findings of the previous studies, which also reported increasing wavefront RMS in normal eyes and SBUT dry eyes. It is important to keep in mind, however, that the previous studies calculated ocular higher-order aberrations based on Zernike polynomials, which are less sensitive in detecting the high frequency changes.

The change in volume MTF over time in subjects with normal eyes was similar to that found in a previous study. It is interesting that the temporal variabilities in RMS and vMTF were higher in the group with SBUT dry eye than in the group with normal eyes. A similar tendency was reported in dry eye, though there was no description about dry eye inclusion criteria. Even though the lower-order aberrations (defocus and astigmatism) were subtracted, we cannot exclude the possibility that the reported tear film-induced aberrations might include other sources, such as accommodation, pupil decentration during measurement, and variability in gaze fixation.
However, we believe that our measurements were more sensitive than Zernike modes, especially for detecting the wave-front error induced by sequential tear film dynamics, because we could detect high frequency changes by using the zonal-based Fourier transform reconstructor in representing the tear film–induced aberrations.\(^7,17\)

Lower TMH and lower TMA increased over time after the blink in both normal eyes and SBUT dry eyes. Johnson and Murphy\(^21\) captured en face images of lower TMH using fluorescein and reported that lower TMH increased with time for 10 seconds after the blink. They showed that the growth rate of TMH was most rapid after the blink and slowed exponentially with time; however, there was no such pattern in either group in our present study. By using anterior segment OCT, Parakuru et al.\(^1,12\) reported that lower TMH decreased just after the blink and increased during prolonged eye opening (19.1 seconds), but continuous measurements were not performed. In normal blink conditions, there seemed to be no significant change in the tear meniscus, though normal blink was not defined in their study. Interestingly, if we compared the TMH value just after the blink obtained in normal eyes in this study with the values reported in the literature, the TMH value in this study (331 ± 85 \(\mu\)m, mean TMH for normal eyes) seemed higher than values in previous studies.\(^1,12,22–25\) In addition, the initial postblink TMH value in our study was higher than the reported TMH value with normal blinking (297 ± 79 \(\mu\)m) and lower than the reported TMH value with delayed blinking (493 ± 294 \(\mu\)m).\(^12\) Disagreement in the results between previous studies and the present study may be explained in part by the difference in measurement devices and blinking conditions. Further studies using Fourier domain anterior segment OCT will be needed to improve image resolution and to image the upper and lower tear meniscus simultaneously.

Several previous studies\(^22,26–28\) have also investigated the relationship between tear meniscus and BUT. Mainstone et al.\(^26\) reported the significant positive correlations between the

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<th>Table 1. Correlation between Optical Quality and Tear Meniscus Parameters over Time between Blinks.</th>
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<td><strong>R</strong> Value</td>
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Values are mean ± SD; ranges are in parentheses.
TMH and fluorescein BUT, and Wang et al. used a custom-made OCT for meniscus imaging and a tear interference device (Tearscope; Keeler, Windsor, UK) to measure noninvasive BUT and found that TMH and TMA had a weak but significant correlation. In contrast, Yokoi et al. measured the lower tear meniscus radius with custom meniscometry as an index of total tear volume and found no correlation between lower tear meniscus radius and fluorescein BUT. Using commercial posterior segment OCT, Savini et al. reported there was no relationship between TMH and fluorescein BUT. These studies all evaluated planar and cross-sectional aspects of the tear film using measurements of BUT and tear meniscus at just one point in time after a blink.

The wavefront sensor has also been used to evaluate noninvasive BUT and tear film disruption over time. Although tear film-induced aberrations calculated in the present study do not give specific values of BUT, two-dimensional changes in the tear film can be represented by RMS and vMTF, and the relationship between two-dimensional and cross-sectional parameters over time can be determined. Results obtained by simultaneous measurements with wavefront sensor and OCT showed that both lower tear meniscus parameters and wavefront aberrations increase over time between blinks. This relationship was more strongly correlated in the SBUT dry eye group. Although certain trends were found between wavefront aberrations and lower tear meniscus parameters, variability among subjects was large because of the small number of subjects. To verify the conclusions, the sample size should be increased in future studies. In this regard, factors such as subtle eye movements, accommodation, and slight differences in environmental conditions may also be considered. Further studies with greater numbers of patients, including those with aqueous tear-deficient dry eye, or with a controlled-environment chamber will help to further characterize the dynamics of the tear film.

We found that larger tear meniscus before the blink was associated with less initial postblink RMS change. There was a stronger correlation in the SBUT dry eyes than in the normal eyes, implying that the dynamics of tear film change might be accelerated in SBUT dry eye during the initial postblink period. It has been reported that SBUT dry eye might not be able to maintain stable optical quality after the blink when blinking is
suppressed.\textsuperscript{9,35} Kaido et al.\textsuperscript{35} reported that the increase in tear volume after punctal occlusion is helpful for maintaining functional visual acuity in patients with SBUT dry eye. Our results are consistent with the previous results, though there may not be a remarkable difference in the tear meniscus dimensions among our subjects when they are compared with the differences found in patients who have received punctal plugs. SBUT dry eye is dry eye in which tear volume is sufficient but tear stability is impaired. It seems reasonable that the quantity of tears before the blink may have the potential to slow the initial optical degradation after the blink in SBUT dry eye. Some patients who have received punctal occlusion may develop visual impairment because of the excessive retention of tears\textsuperscript{9,35,36}, hence, simultaneous measurements might have some potential to determine whether there is an optimal balance between optical quality and tear volume.

Although a moderately negative correlation was found between the initial postblink change in RMS and the tear meniscus parameters, there was no correlation between vMTF and tear meniscus parameters. A previous study reported that RMS and vMTF could represent tear film-induced aberrations\textsuperscript{36} though the time-dependent change in each parameter was not investigated. It is unknown which optical metric is superior in detecting optical change associated with tear film dynamics.

Yokoi et al.\textsuperscript{35} using a Voigt rheological model, showed the relationship between tear lipid layer spread and tear volume and found that the initial velocity of the tear lipid layer spread after a blink increased with increasing tear volume. Goto and Tseng\textsuperscript{36} using the kinetic analysis of tear lipid layer interference images, found that tear lipid layer spreading time was longer in aqueous tear-deficient dry eyes (3.5 ± 1.8 seconds) than in normal eyes (0.3 ± 0.2 seconds) and was shortened in eyes that had received punctal occlusion. It seems reasonable to hypothesize that the faster degradation in optical quality observed in SBUT dry eyes may be the result of smaller tear meniscus before the blink with slower lipid layer spread. The dynamics of the tear lipid layer spread have been reported for aqueous tear-deficient dry eyes and normal eyes,\textsuperscript{35,36} but they have not been reported in patients with SBUT dry eye. The lipid layer may play an important role in maintaining optical quality during the initial postblink period. The correlation between tear lipid layer spread and tear film-induced aberration in SBUT dry eye should be investigated because there are no studies at present that report this relationship.

In clinical examination of patients with dry eye or tear film abnormalities, precise understanding of the spatial relationships and dimensions of tear film is essential for determining the appropriate course of management. Simultaneous measurements with wavefront sensor and OCT eliminate any influence of sampling order that might be introduced by making separate measurements.

There are some limitations to this study. First, the images captured by our commercial time domain anterior segment OCT had limited resolution. In some images, it was difficult to detect the tear film boundary where the cross-points of the tear film, eyelid, and cornea were faint.\textsuperscript{23,25} It will be important to use higher-resolution OCT to make precise measurements of the tear film thickness because this is crucial to understanding tear film dynamics over the ocular surface. Second, the 6-second blink interval used in this study does not reflect the average spontaneous blinking in daily life. We succeeded in avoiding use of all invasive factors such as instillation of topical anesthesia, use of dilating drops, and prolonged eye-opening time, but the use of conditions that more closely resemble natural conditions should be emphasized more in future studies. Third, it is important to be aware that reflex tearing may occur under experimental conditions even with noninvasive techniques,\textsuperscript{37} though none of the subjects in the present study exhibited reflex tearing.

In conclusion, noninvasive, simultaneous measurement of wavefront aberration and tear meniscus parameters can help us obtain more accurate time-dependent measurements, further develop our understanding of the tear film, and provide us with better methods to define dry eye.

References


