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ORIGINAL ARTICLE

Ocular Surface Cooling Corresponds to Tear Film Thinning and Breakup

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ABSTRACT

Purpose. To investigate the relationship between ocular surface temperature (OST) and tear film thinning and breakup.

Methods. Simultaneous imaging of OST and fluorescein tear thinning and breakup (FTBU) was performed on 20 subjects. Subjects were asked to open their eyes and refrain from blinking for as long as they could during testing. Ocular surface temperature was measured using an infrared thermographic camera (FLIR A655sc) and rates of ocular surface cooling (OSC) were analyzed using commercially available software. A method was developed to quantify the rate of FTBU formation using image-processing software.

Results. Areas of FTBU and regions of OSC were observed to be colocalized, with localized cooling preceding the formation of FTBU. The rates of OSC and FTBU formation were positively correlated ($r = 0.74$). A second-order polynomial model accurately describes the physiological relationship between the area of FTBU and OST ($p < 0.001$). A linear approximation provides a more clinically interpretable rate of FTBU formation with decreasing OST ($p < 0.001$), while still retaining high R^2.

Conclusions. The results suggest a direct relationship between FTBU formation and OSC. That cooling of the ocular surface precedes FTBU formation implies a process of evaporation contributing to tear film thinning and breakup. Our study suggests that measuring the OSC rate could be an indirect assessment of tear evaporation and could contribute to the management of evaporative dry eye.

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Key Words: tear film stability, tear breakup time, ocular surface cooling, dry eye disease, dry eye, evaporative dry eye, thermography, ocular surface temperature, infrared, evaporation, race

The International Dry Eye Workshop in 2007 identified aqueous evaporation as the leading cause of dry eye (DE) and evaporative dry eye (EDE) as the most prevalent type of this widespread disease.[1] Although EDE has a high prevalence and creates a significant economic burden, there is still a limited understanding of how evaporation of the tear film contributes to the disease process.[2–5] The etiology of EDE has been investigated through mathematical models, but there has been a general lack of clinical work done to confirm the models because of limitations in the tools available to clinically assess tear evaporation.[6,7] This gap in knowledge has served as an impetus for clinical investigators to develop new ways to assess tear film evaporation; one promising approach is the use of a thermographic camera (TC) to measure ocular surface temperature (OST).[8–11]

Mapstone[12,13] first adapted the TC to measure the temperature of the ocular surface, and other researchers later implemented the technique to investigate the role of OST in DE. Recent research has focused on using the TC to measure tear film evaporation indirectly, based on the theory that when tear evaporation occurs, the phase change from liquid to gas is associated with heat transfer to the surrounding environment and thus a cooling of the ocular surface.[8,9,14–17] It is known that liquids with a higher rate of evaporation evince a greater rate of surface cooling; as an example, when ethanol and water are applied to the skin, the area treated with ethanol will decrease in surface temperature more rapidly than the area with water applied, because the rate of evaporation is higher for ethanol.[18,19] It is reasonable to hypothesize that tear evaporation leading to tear film thinning and breakup should be

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associated with concomitant ocular surface cooling (OSC).[8,9,20,21] Furthermore, the OSC rate should reflect the rate of tear evaporation and thus be directly related to the rate of tear thinning and breakup.[17–19] These relationships, however, have not been conclusively demonstrated to date, as some studies have reported an association between OST and tear film stability, whereas others have found no such association.[14,16,22–25] A possible reason for the conflicting results is that most studies have measured OST and tear film stability separately—a significant issue given the highly variable and dynamic nature of the tear film.[9,14,15,22,26–28]

In the current study, our aim is to develop a methodology to investigate the OSC during the interblink period concomitantly with a quantitative measure of fluorescein tear breakup (FTBU). We will develop a statistical model describing the increase in the area of the ocular surface exhibiting FTBU as a function of the decrease in OST. We will also present a linear approximation to this model that will provide clinicians with an easily interpretable rate of FTBU formation as a function of OST. In addition to improving our understanding of the physiology of tear thinning and breakup, such an indirect measure of tear evaporation that can be performed concomitantly with standard imaging of FTBU could be a useful clinical tool in pharmacological management of DE. Finally, it has been suggested that FTBU and OSC play a central role in the etiology of symptoms associated with EDE, and this study may help us to better understand this relationship.[29,30]

METHODS

Subjects

Subjects were recruited from the University of California, Berkeley, School of Optometry. Subjects taking systemic or ocular medication or those with a history of ocular disease or surgery were excluded from the study. Subjects were instructed to refrain from using any eyelid makeup or eye drops on the day of the visit. Informed consent, with a complete description of the goals, risks, benefits, and procedures of the study, was obtained from all participants. This study observed the tenets of the Declaration of Helsinki and was approved by the University of California, Berkeley, Committee for Protection of Human Subjects. A full slit lamp examination was performed at the beginning of the visit to ensure that no sign of ocular surface disease was present.

Instrumentation and Procedures

Ocular surface temperature was measured using the FLIR A655sc (FLIR Systems, Inc, Wilsonville, OR), an uncooled microbolometer TC with a 640 × 480 video resolution, 17 μm pixel size, and 0.1°C thermal sensitivity. A digital video camera (DXC390 3CCD Exwave HAD, Sony Electronics, Inc, Tokyo, Japan) attached to a slit lamp (SL 120, Carl Zeiss Meditec AG, Jena, Germany) was used to record FTBU. The TC, mounted on a tripod, was placed behind the slit lamp at a distance of 16 to 18 inches from the eye, aligned about 15 degrees off-axis temporally from the geometric center of the cornea, whereas the optical system of the slit lamp and digital video camera was aligned about 15 degrees off-axis nasally (Fig. 1). Fluorescein tear breakup was assessed under cobalt blue illumination and viewed through a 530-nm yellow barrier filter.

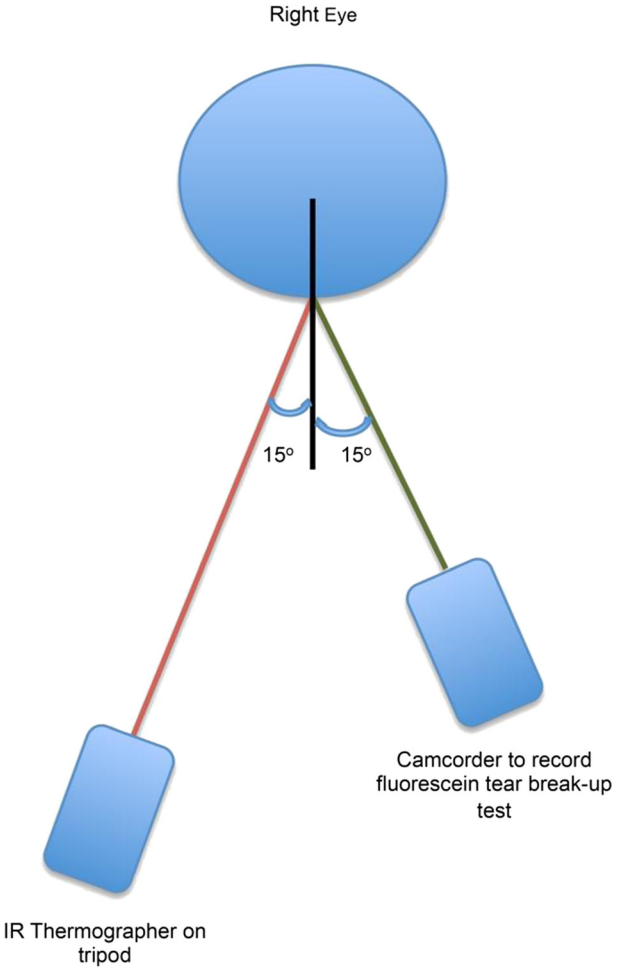

FIGURE 1.
Placement of the TC and the slit lamp relative to the subject's eye. A color version of this figure is available online at www.optvissci.com.

Subjects were asked to complete an Ocular Surface Disease Index (OSDI) questionnaire before measurements. All measurements were taken in an examination room and subjects were acclimated to the ambient environment for a minimum of 10 minutes before testing.[23] A micropipette was used to instill 4 μL of 2% sodium fluorescein dye onto the superior bulbar conjunctiva and subjects were instructed to close and roll their eyes to evenly distribute the dye. Subjects were then positioned at the slit lamp, and the slit lamp and the TC were focused on the right eye. Subjects were instructed to blink five times and then to refrain from blinking or moving their eyes for as long as possible, to maximize the observation of FTBU and OSC, while the digital video camera and TC simultaneously imaged the ocular surface. Three such trials were conducted in sequence, each trial separated by 20 seconds of eye closure to allow the tear film to recover.

The video sequences from the OST and FTBU recordings were synchronized using Final Cut Pro X (Apple Inc, Cupertino, CA). The FLIR+ Tools software suite was used by an experienced observer (WL) to specify a user-defined region of interest in the TC images corresponding to the cornea; the region represented 4000 to 7000 measurement points (depending on anatomical variation), with the mean value of the points interpreted as the average OST.[8] Image-processing software (NI LabVIEW Vision Assistant 2012, National Instruments Corp, Austin, TX) was used

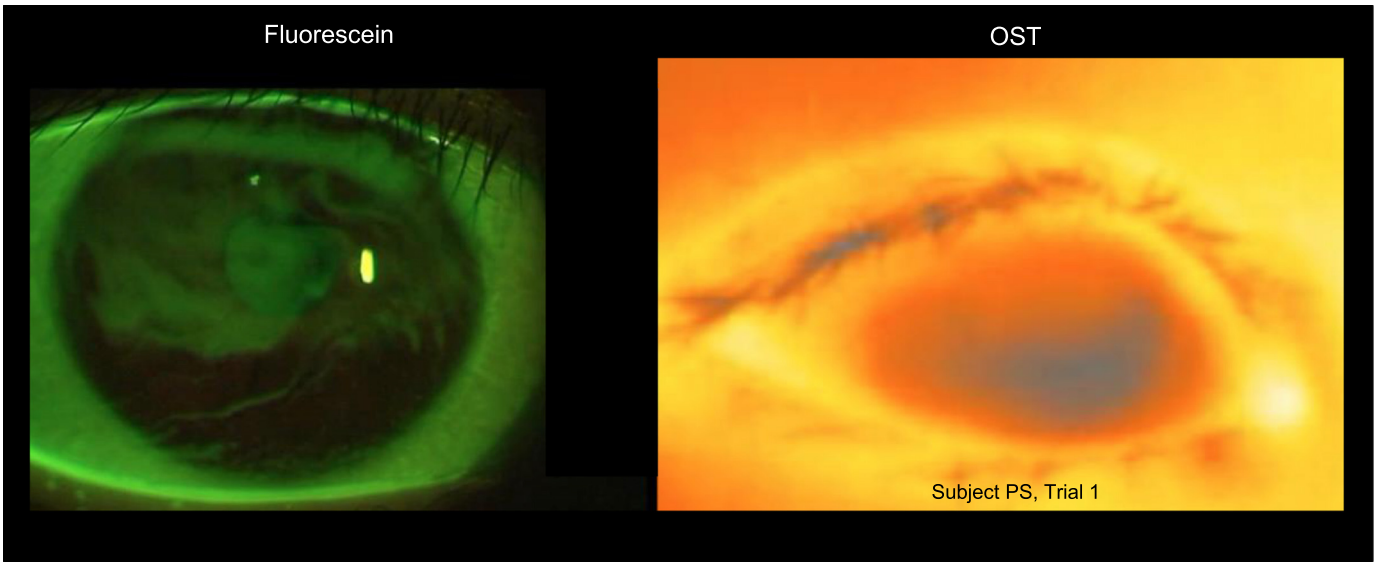

FIGURE 2.

Screen captures taken from synchronized OST and FTBU video recordings shortly before subjects blinked. Areas of OSC (blue regions) appeared to coincide with regions of FTBU when examined at the same time point. The video recording can be seen in Supplementary Video 1, available at http://links.lww.com/OPX/A218.

to isolate the imaged area of the cornea, after which the full-color videos were split into the red, green, and blue channels. The green channel, which provided the best imaging of FTBU, was then converted to 8-bit gray scale with 256 levels of luminance.

In this study, we used a fluorescein video sequence to capture the process of FTBU, which began with some areas losing fluorescence and beginning to darken and in some cases culminated in complete disruption of the tear film. Our method set a pixel threshold luminance value above which a pixel was considered "bright" and represented an intact area of tear film and below which was considered "dark" and represented an area of tear film that was undergoing FTBU, presumably through

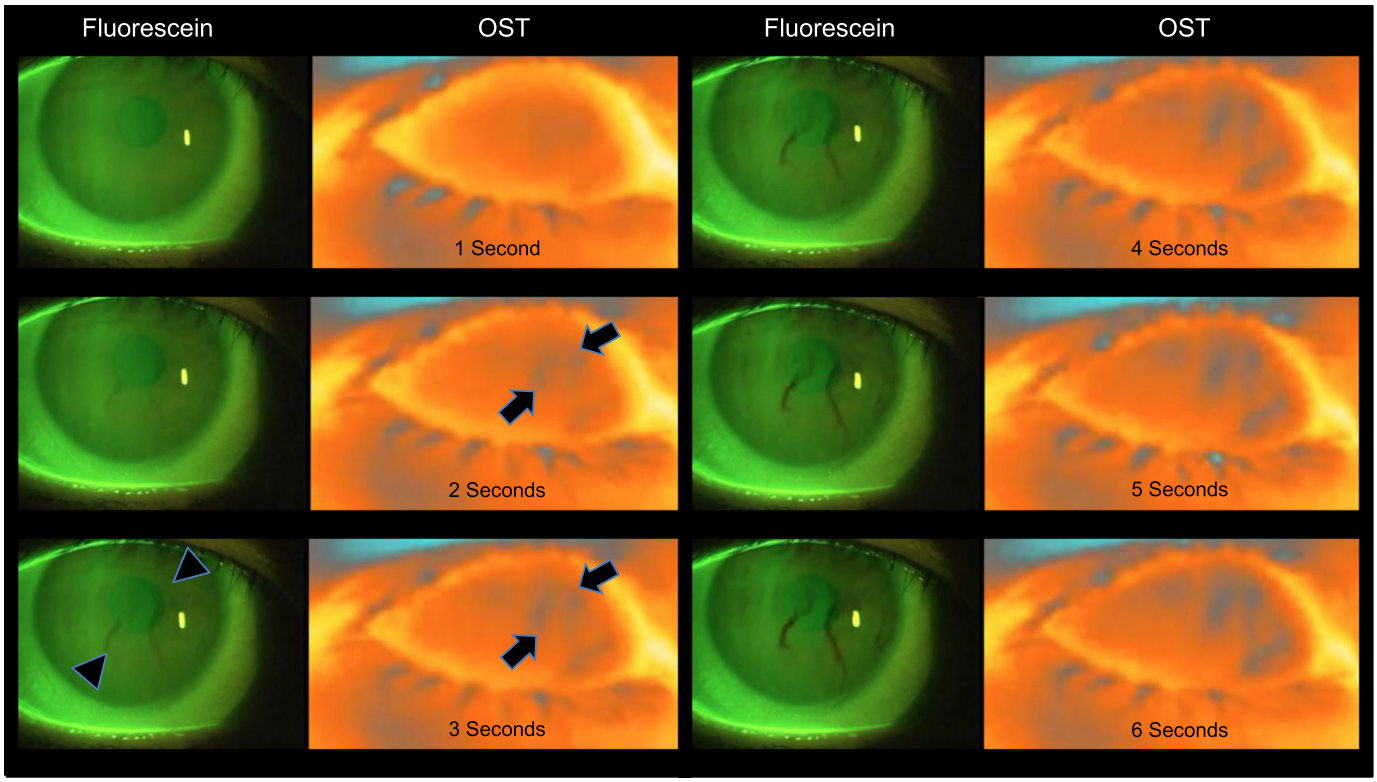

FIGURE 3.

Time-lapse sequence of a subject's synchronized recordings. Areas of cooling (arrows) were identified at 2 seconds, whereas corresponding areas of FTBU formation (arrowhead) appeared about 1 second later. The video recording can be seen in Supplementary Video 2, available at http://links.lww.com/OPX/A219.

evaporative thinning.[28,31] The principles for FTBU quantification used in this study were drawn from previous research in which a threshold luminance value was subjectively chosen that most closely approximated the pattern of FTBU formation observed.[32] To mitigate the subjective nature of the assessment, the threshold values we determined for all subjects were averaged to obtain a mean threshold value used uniformly for FTBU quantification in all subjects. The quantification provided the proportion of the corneal image exhibiting FTBU (i.e., the proportion of "dark" pixels) over the time course of each trial, which was then compared with the mean OST from the TC recording, over the same, synchronized time sequence.

Statistical Methods

For each subject, it was first verified that FTBU (as represented by the proportion of subluminance threshold pixels) increased and OST decreased over the time course of the trial while the subject refrained from blinking and that their respective rates of change were directly correlated. As it turned out, for reasons detailed below, 20 of 25 subjects showed a clear trend of decreasing OST and increasing FTBU over time. A type of mixed-effects repeated-measures model, referred to as a "random intercepts model," was fit to the aggregate data from all 20 subjects who exhibited FTBU and OST changes over time. The most physiologically accurate model of FTBU was determined to be a second-order polynomial function of OST in the fixed effects, with each subject having an individual random offset to the population average estimated intercept. This type of model has an advantage for this study in that the threshold luminance we used to distinguish dark from bright pixels was a group average, resulting in the first image frames for all subjects having somewhat different starting proportions of dark pixels; such individual variation in starting values was reflected in the random intercept estimates. We also fit linear approximations to these quadratic models, because the linear regression slope is a more clinically interpretable measure of the rate of FTBU with decreasing temperature, and these models did retain acceptable fit statistics (e.g., high R^2).

RESULTS

Twenty-five subjects (22 women and 3 men), with a mean (SD) age of 21.2 (2.4) years (range, 18 to 27 years), completed the study. Five subjects were unable to provide usable data: two subjects (1 Asian woman and 1 Asian man) were unable to hold their eyes steady and open without blinking and could not provide usable images; one subject (white woman) had a partial blink in the middle of the measurement period; one subject (Latino woman) was unable to open the eye wide enough to prevent the eyelashes from producing artifacts in the images; one subject's (Asian woman) images suffered from low exposure, possibly because of insufficient

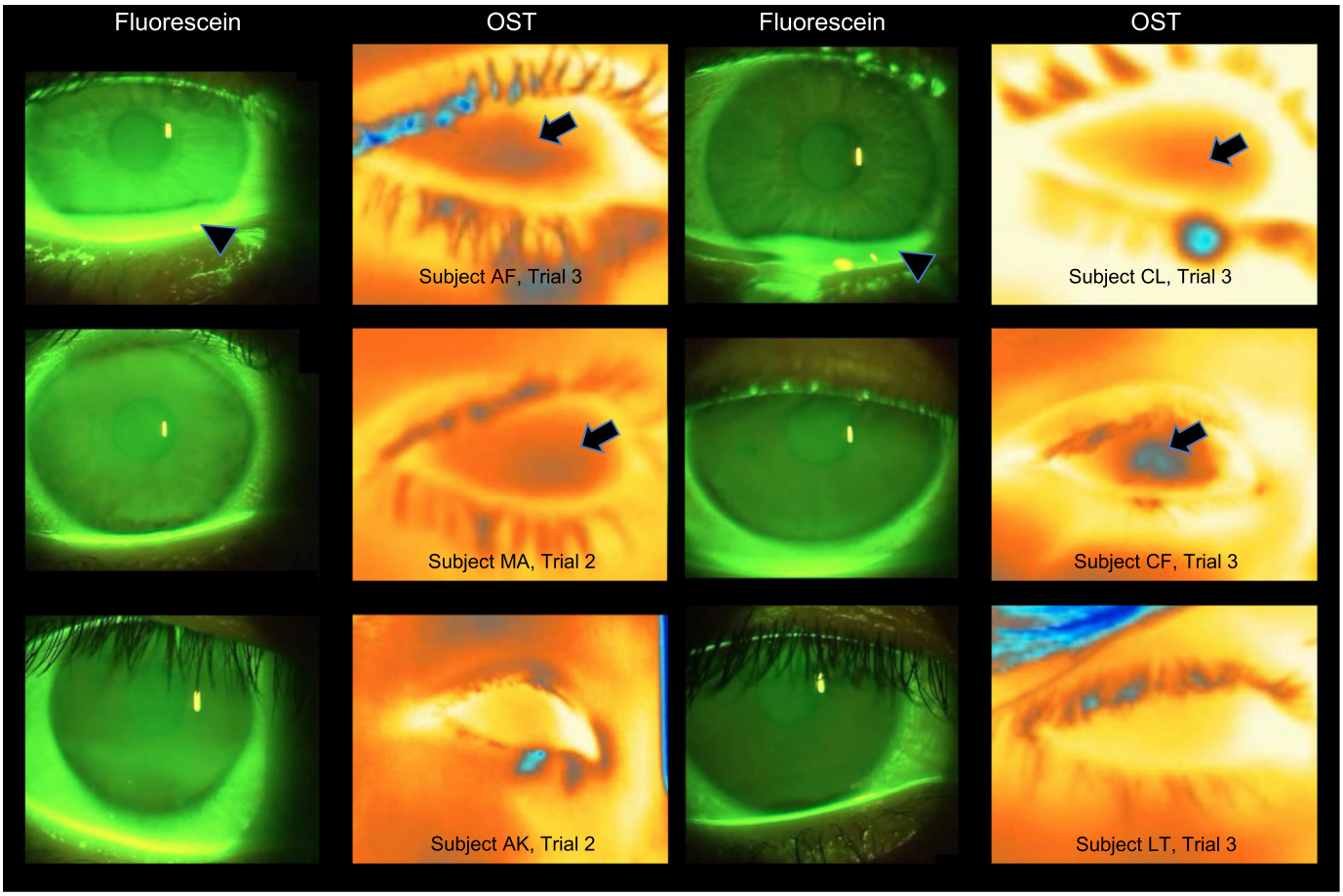

FIGURE 4.

Subjects AF, CL, MA, and CF exhibited OSC (arrows) without any obvious FTBU formation. Subjects AF and CL had increased lacrimal tear lake height (arrowhead). Subjects AK and LT showed no evidence of OSC or FTBU formation. Although OSC could occur in such cases without FTBU, in no case did FTBU occur without OSC.

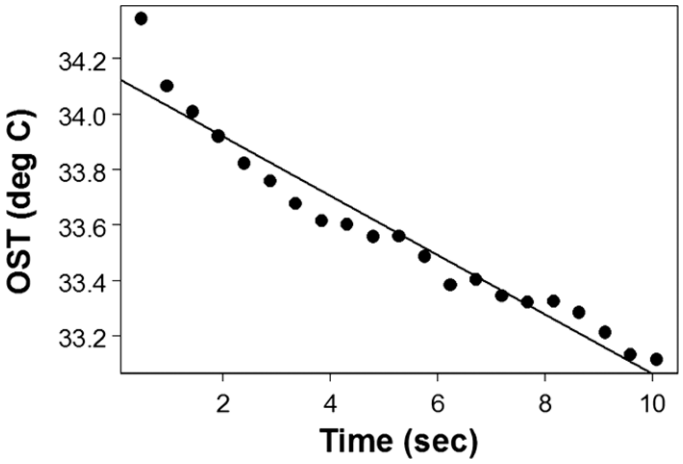

FIGURE 5.
Decrease in OST during one interblink period for a typical subject. Concomitant increase in FTBU for the same subject, same trial, shown in Fig. 6.

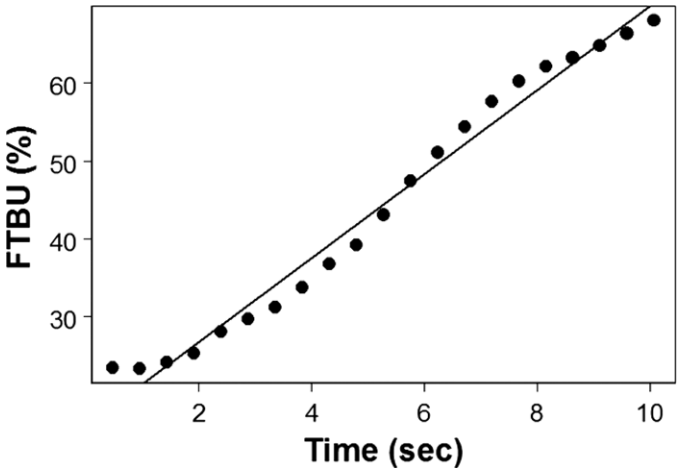

FIGURE 6.
Increase in percentage of image displaying FTBU during one interblink period for a typical subject. Concomitant decrease in OST for the same subject, same trial, shown in Fig. 5. Fluorescein tear breakup at time 0 is calculated as about 25% owing to the use of a group-averaged threshold luminance value to quantify FTBU and from image artifacts (e.g., eyelash) that were interpreted by the software as ''dark.''

fluorescein loading in the presence of reflex tearing. Twenty subjects successfully completed the study and provided data for analysis.

OSC and FTBU: Qualitative Observations

A review of the synchronized digital video recordings showed that a majority of subjects exhibited areas of OSC and FTBU formation that were located in the same region and presented with similar patterns (Fig. 2; Supplementary Videos 1, http://links.lww.com/OPX/A218 and 2, http://links.lww.com/OPX/A219). When regions of OSC and FTBU were colocalized, cooling was always noted 1 to 2 seconds before an observable area of FTBU; FTBU never occurred unless OSC preceded it (Fig. 3). The agreement between OSC and FTBU was most common during the first trial and became less common with each subsequent trial, which was usually associated with an increase in the height of the lacrimal tear lake and often increased reflex tearing (Fig. 4, Supplementary Video 3, http://links.lww.com/OPX/A220). For this reason, subsequent quantitative analyses focused on the first trial period only. Note that in trials in which OSC and FTBU failed to coincide, either only OSC was observed without FTBU or neither OSC nor FTBU were detected (Fig. 4).

OST and FTBU: Quantitative Analysis

Subjects were able to hold their eyes open without blinking for a mean (SD) time of 15.50 (10.27) seconds. During the first interblink period, subjects averaged a 33.6% increase in corneal surface area with FTBU (defined as the proportion of pixels in the fluorescein images that were below the luminance threshold, as described in detail above). The mean (SD) baseline OST after the first blink was 35.2°C (0.4°C), and during the first interblink period, OST decreased by a mean (SD) of 0.80°C (0.47°C). Fig. 5 shows the decrease in OST during the first interblink period for a typical subject, and Fig. 6 shows the increase in the corneal image area exhibiting FTBU for the same subject over the same period, both with regression lines shown.

The mean (SD) OSC rate was −0.057°C/s (0.036°C/s), and the mean FTBU formation rate, which describes the proportion of the image of the corneal surface with tear breakup or thinning developing over time, was 3.1%/s. The rates of OSC and FTBU formation showed a relatively strong positive correlation (r = 0.74), with a higher rate of cooling associated with a faster rate of FTBU formation (Fig. 7). Taking each subject's linear FTBU and OSC rates, we can see from the figure that a faster rate of cooling at the ocular surface is significantly related to faster FTBU formation ($p < 0.001$).

Fig. 8 shows the direct relationship between FTBU and OST, with data from all subjects modeled as second-order polynomials. Model fit statistics were uniformly good, with R^2 ranging from 0.77 to 0.99, with a median R^2 of 0.98 across all subjects. We examined various models of FTBU as a function of OST, using the aggregated data from all 20 subjects. We found the best-fitting model to be a random intercepts model, having fixed effects of:

$$\text{FTBU} = 82.201 - 4.195*\text{OST} + 0.053*\text{OST}^2$$

with p values for all three coefficients being less than 0.001. In this random intercepts model, each subject was considered to have an additional random offset from the population average estimated intercept. Fig. 8 supports this model, as it shows that subjects had about the same shape of upward-trending FTBU curve with decreasing OST, but there were individual variations in the initial proportion of pixels classified as subthreshold immediately upon opening the eye after the first blink. Starting proportions ranged from 21 to 44% of pixels, which was attributed, in part, to the use of a group-averaged threshold luminance value and, in part, to intersubject differences in palpebral aperture size and in the extent to which lashes created ''dark'' artifacts at the edges of the corneal images.

Although the second-order polynomial model was the most accurate in terms of the physiological process of evaporative tear thinning and breakup, we also found that linear approximations to these curves retained good fit statistics (e.g., R^2 ranging from 0.63 to 0.99, with a median R^2 of 0.88 across all subjects) and would be more clinically interpretable: the linear regression slope is an

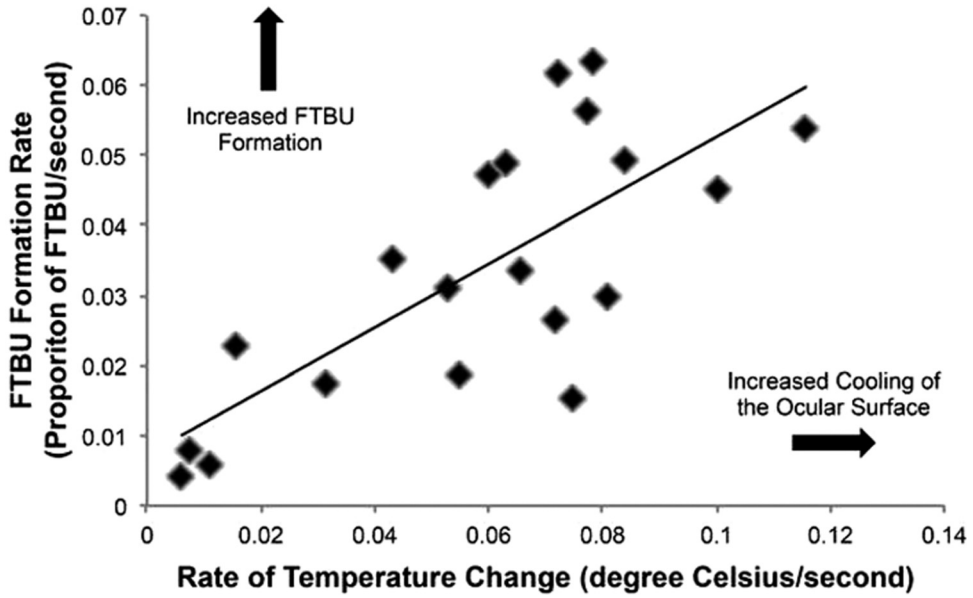

FIGURE 7.

Ocular surface cooling rate and FTBU rate from the synchronized thermographic and fluorescein recordings. A faster rate of evaporative surface cooling is associated with a faster formation of FTBU.

estimate of the rate of FTBU formation per unit OST decrease. The mean (SD) slope across all 20 subjects was 0.604 (0.222) and ranged from 0.201 to 1.122. The interpretation is that, on average, based on our study cohort and the methodology (e.g., volume of fluorescein instilled), the corneal area exhibiting FTBU increases about 60% per degree of OST decrease. The mean OST decrease during the first interblink period was 0.8°C, corresponding to an about 48% increase in the corneal area exhibiting FTBU. This number appears high by clinical standards because of the stress test nature of this experiment, in which subjects held their eyes open as long as possible, even after the onset of FTBU would normally have stimulated blinking. Further study is needed to determine the amount of OSC and FTBU (as quantified by this method) experienced by patients in a normal setting.

DISCUSSION

Cooling of the ocular surface has been attributed to evaporation of the tear film, convective heat transfer, and the emission of infrared radiation.[14,33–35] Fluorescein tear breakup has been attributed to lipid migration and dewetting of the cornea, rupture instability by Hamaker dispersion forces, surface-tension gradient

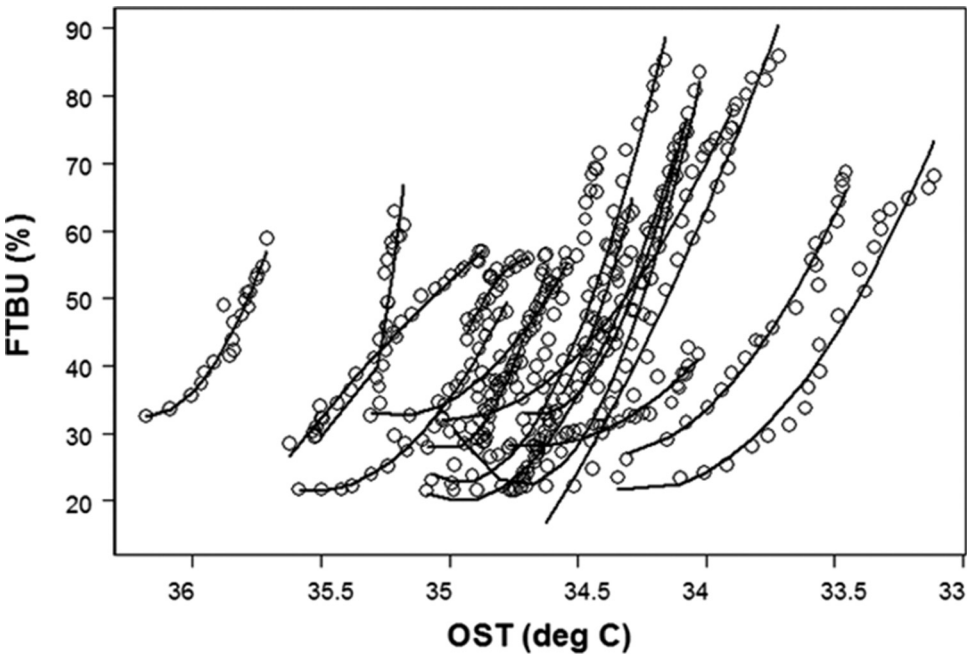

FIGURE 8.

Fluorescein tear breakup as a function of OST, with individual second-order polynomial mixed-effects model fits for each subject. An increased rate of OSC was associated with a higher rate of FTBU formation.

instability, tear rupture owing to mucin breakup, evaporation of the tear film, inflow of tears into the cornea, and tangential flow from the displacement of sodium fluorescein particles.[6,20,21,36] The common factor for OSC and FTBU appears to be the evaporation of the tear film.[6] This study provides evidence that OSC and FTBU are associated and suggests that a common physical force such as tear film evaporation is acting in both processes, which is in agreement with a previous study that also simultaneously assessed FTBU and OST.[25] Unlike the study by Su et al., which was specifically focused on demonstrating that areas of OSC and FTBU were colocalized, this study examined how OSC (as a proxy for evaporation) influenced FTBU formation over the time course of the interblink period.

Two subjects demonstrated signs of reflex tearing (likely because of ocular irritation from not blinking), evidenced by the significant increase in the lacrimal tear lake height seen during testing (Fig. 4; Supplementary Video 3, http://links.lww.com/OPX/A220). In these cases, FTBU was not observed but OSC was still detected, which suggests that these two measurements assess different aspects of tear film evaporation.[37,38] This argument is strengthened by the observation that OSC always preceded the formation of FTBU, implying that OST measurement was indirectly assessing the active process of tear film evaporation whereas FTBU was an end point resulting from a tear film that is thinned enough by evaporation to cause decreased fluorescence intensity or quenching.[21] Because evaporation of the tear film still occurs during reflex tearing, it would explain how OSC was observed without FTBU in these two subjects.

The results of this study are consistent with a mathematical model developed by Peng et al.,[6] which postulates a mechanism by which local rupture of the tear film lipid layer (TFLL) increases local tear evaporation rate leading to tear film rupture and FTBU. In this model, an intact, thick TFLL decreases tear evaporation rate. Thus, a prerequisite for FTBU formation is an area of tear lipid layer deficiency (i.e., rupture) that increases evaporation rate, which, in turn, is associated with OSC.[8,9,14–19] Local high evaporation rate drives a deepening rupture spot in the tear film, which, when sufficiently thin enough, can exhibit FTBU.[21] The time necessary for the tear rupture spot to evaporate toward FTBU explains the lag time observed between OSC and FTBU and is in agreement with the evaporative tear breakup model in which local tear cooling always occurs before areas of FTBU are observed.[6]

It is interesting to note that OSC and FTBU have both been linked to symptoms associated with EDE.[29,30] In a *post hoc* analysis, we found no association between the rates of OSC or FTBU formation and OSDI score; however, a borderline-significant association ($p = 0.060$) was found between OSDI score and the slopes of the linear approximations to the individual regression curves of FTBU on OST. Although not reaching statistical significance, this *post hoc* model suggests that subjects with a faster rate of FTBU formation per unit temperature decrease had, on average, higher OSDI scores. *Post hoc* power analysis based on statistical simulations was performed to examine how increasing the sample size would affect the statistical significance between OSDI and the rate of FTBU formation per unit temperature decrease. In our simulations, doubling the sample size to 40 subjects resulted in a statistical significance less than 0.05; however, it is difficult to make a definite statement about the association because of our small study cohort.

We also speculate that the slope of the regression curve of FTBU on OST (i.e., how fast the tear film thins and breaks up for a given amount of temperature decrease) could provide a measure of how sensitive a patient's tear film is to temperature change. It is conceivable that this measure could provide greater insight into the symptoms experienced by those with EDE than either the OSC rate or FTBU formation rate alone. Assuming that OSC represents the active process of evaporation, then the slope of the regression of FTBU on OST could provide information on the level of evaporative stress the tear film can withstand before breakup occurs. It is thought that subjects with EDE are more susceptible to evaporation of the tear film, possibly because of a deficient TFLL, which, in turn, is thought to cause hyperosmotic stress associated with EDE symptoms.[7,30,36,39,40] A subject with EDE may have a tear film that can withstand less evaporative stress before FTBU is noted (i.e., a steeper slope or faster rate of FTBU per unit temperature decrease) compared with a subject without EDE who may have a slower rate. Further investigation is warranted to test this hypothesis and to understand how the sensitivity of the tear film to evaporative stress varies among subjects with and without EDE.

Although this study suggests that there is potential in simultaneously examining OST and FTBU as a clinical tool, issues were also noted during the study that suggest that additional work is needed to refine the technique and to demonstrate its efficacy in the assessment of EDE. One issue was related to the volume of fluorescein instilled in the eye. Four microliters is within the commonly accepted range of volume reported in various clinical studies[41] but is on the high end of this range. This volume was selected to maximize the chances that sufficient fluorescein would be present during the second and third trials. Various studies have suggested that the volume of fluorescein instilled could alter tear film stability, which may have artificially influenced the rate of FTBU formation.[36,37] Future work should likely limit the fluorescein instilled to 1 to 2 μL to decrease the possible confounding influence of fluorescein volume on the rate of FTBU formation. Another issue arose in cases in which FTBU was not observed, such as the examples in Fig. 4. These cases were predominantly noted upon the second or third trial; hence, there is a possibility that the concentration of fluorescein decreased to a point where FTBU was difficult to see.[36] It should be noted that a relatively large volume of fluorescein was instilled into the eye to minimize the possibility of this occurring. Nevertheless, it is impossible to determine if a low fluorescein concentration or a lack of FTBU formation contributed to the occasional inability to observe FTBU.

In future work, we intend to explore possible methods for quantifying the extent to which colocalization occurs and to implement an automated algorithm for setting the luminance threshold. Finally, a larger sample size is warranted to determine the clinical value of assessing the rate of FTBU per unit temperature decrease and how this metric is associated with OSDI score by investigating how it differs in cohorts with and without EDE signs and symptoms.

CONCLUSIONS

By simultaneously assessing FTBU and OST, we were able to show that localized areas of OSC represent regions of elevated evaporation. For the foreseeable future, FTBU will be more commonly used in clinics to assess evaporation as it is more readily available and provides more spatial detail. Nevertheless,

measuring OST will likely become more common as it is more objective and more easily interpretable (as an OSC rate) and allows for evaporation assessment without disrupting the tear film. This holds important implications for the clinical evaluation of EDE owing to our current inability to accurately assess tear evaporation *in vivo*. The use of a TC may be the best method available today to indirectly measure tear evaporation rate, which may lead to improvements in the diagnosis, management, and treatment of EDE.

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SUPPLEMENTARY DIGITAL CONTENT

Supplementary Digital Content 1 and 2, videos showing synchronized OST and FTBU recording showing their geographic and chronologic relationship, are available at http://links.lww.com/OPX/A218 and http://links.lww.com/OPX/A219.

Supplementary Digital Content 3, a video showing a significant increase in the lacrimal tear lake (suggesting reflex tearing) during testing, is available at http://links.lww.com/OPX/A220. The video was edited to remove the twenty-second rest period between the inter-blink periods.

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