

Rheology of Tear Film Lipid Layer Spread in Normal and Aqueous Tear-Deficient Dry Eyes

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PURPOSE. To analyze the relationship between tear volume and tear film lipid layer (TFLL) spread.

METHODS. Twenty-nine eyes from 22 subjects, including normal eyes and eyes with aqueous tear-deficient dry eye, were enrolled in this study. In all eyes, the radius of curvature (R : mm) of the central lower tear meniscus was measured with a video-meniscometer, and interference images from the TFLL were recorded with a video-interferometer. Interference images were captured as still images every 0.05 second, and the relationship between the acquisition time for each image after a blink and the averaged heights of the spreading TFLL in the upstroke of the blink were calculated.

RESULTS. In all cases, the time-dependent changes in TFLL spread could be described by the expression $H(t) - H(0) = \rho[1 - \exp(-t/\lambda)]$, where $H(t)$ is the averaged height in millimeters at time t , $H(0)$ is the averaged height at $t = 0$, ρ is a constant, t is time in seconds, and λ is the characteristic time in seconds. A statistically significant correlation was found between those changes and the initial upward velocity of the spreading TFLL [$H'(0) = dH(0)/dt$] and R ($r = 0.573$; $P = 0.003$).

CONCLUSIONS. This study demonstrated that the time-dependent changes of TFLL spread are compatible with the Voigt model of viscoelasticity and that the initial velocity of TFLL spread after a blink decreased in proportion to the decrease of tear volume. There is potential interest in using this parameter to diagnose and evaluate the severity of aqueous tear deficiency. (*Invest Ophthalmol Vis Sci.* 2008;49:5319–5324) DOI:10.1167/iovs.07-1407

The aqueous layer of the tear film is covered by a thin lipid layer¹ that can be imaged noninvasively by interferometry.^{2–4} We have reported elsewhere that graded interference patterns can be used as a parameter to screen dry eye and evaluate its severity.⁵ Other studies have shown that after a blink, the tear film lipid layer (TFLL) spreads over the aqueous layer in a reproducible manner^{2,6–9} and that its dynamics can be observed using an interferometer. Such studies^{10,11} have

noted that the time taken for the interference pattern to stabilize after a blink (defined as spreading time) is longer in aqueous-deficient dry eyes than in aqueous-sufficient normal eyes. Goto and Tseng¹¹ noted that the spreading time was shortened in aqueous tear-deficient dry eyes after punctal occlusion and proposed that spreading is affected by aqueous tear volume. This semiquantitative method offers a novel direct approach to the study of lipid-layer kinetics. A related report has analyzed the kinetic behavior of particles embedded in the tear film, probably located at the level of the TFLL.^{12,13}

With the use of our interferometer, we found that in the upstroke of the blink, the speed of upward spread of the TFLL slows dramatically to reach a stable position in the normal eye, within approximately 1 second. This has also been observed by other authors.^{9,11,12} This effect can be seen more clearly in eyes with aqueous tear deficiency because the speed of spreading is slower.

We hypothesized that this time-dependent behavior might reflect a viscoelastic property of the tear film. With this in mind, we conducted a study of TFLL dynamics. In addition, we were interested in the relationship between the TFLL spread and tear volume.

SUBJECTS AND METHODS

Subjects

Twenty-nine eyes from 22 subjects (1 eye from 1 man and 28 eyes from 21 women) were enrolled in this study. The age of the subjects ranged from 42 to 87 years (64.6 ± 11.0 years; mean \pm SD). According to the diagnostic criteria given below, there were 5 normal eyes of 4 healthy subjects (54.8 ± 4.7 years) and 24 aqueous tear-deficient dry eyes of 18 patients (66.8 ± 10.9 years). Of the 24 aqueous tear-deficient dry eyes, 15 eyes were from 11 patients with Sjögren's-syndrome dry eye (SSDE), and 9 eyes were from 7 patients with non-Sjögren's-syndrome dry eye (NSDE).¹⁴

Tear tests were performed on all subjects before enrollment. These included the Schirmer I test¹⁵ (abnormal value, ≤ 5 mm/5 min), the measurement of fluorescein break-up time (BUT)¹⁶ (abnormal value, ≤ 5 seconds), and the grading of corneal staining with fluorescein¹⁷ and of ocular surface staining with rose Bengal.¹⁸ Abnormal scores for fluorescein were A1D1 and greater; A and D representing area and density, respectively, were graded from 0 to 1 (mild), 2 (moderate), and 3 (severe). An abnormal score for rose Bengal, based on the van Bijsterveld criteria, was ≥ 3 .

The aqueous tear-deficient dry eyes enrolled in the study met the inclusion criteria, among those being at least one abnormal Schirmer I test value and abnormal scores for either fluorescein or rose Bengal staining. All eyes also showed abnormal BUT values. Patients with aqueous tear-deficient dry eye were categorized as SSDE or NSDE, with the diagnosis of SSDE based on the criteria of Fox et al.¹⁹ Healthy eyes in this study met the following criteria: normal Schirmer I test value, normal scores for fluorescein and rose Bengal staining, and normal BUT values. Exclusion criteria were meibomian gland dysfunction, punctal-plug occlusion or surgical punctal occlusion, previous corneal surgery, previous or current corneal disease (excluding aqueous tear-deficient dry eye), and hard or soft contact lens wear by the subject.

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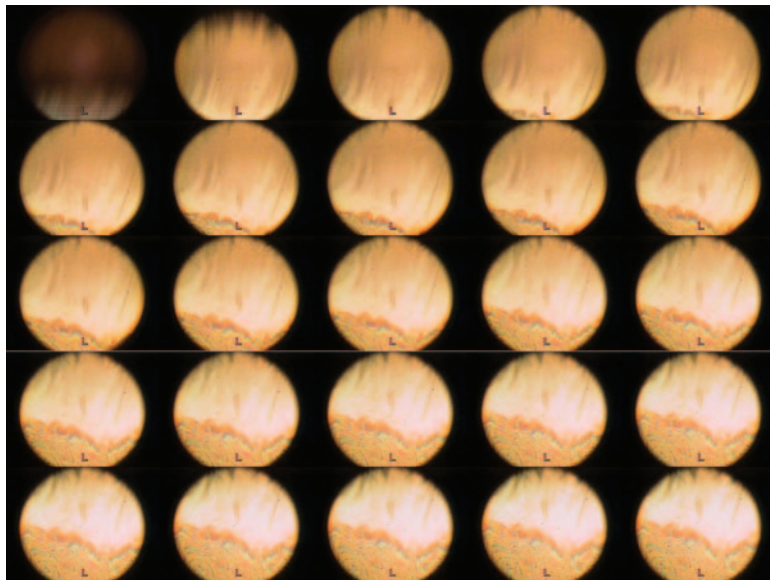


FIGURE 1. Representative sequential images of the spreading tear-film lipid layer, captured every 0.05 second after a blink (case 2).

In all subjects, reflected images from the central lower tear meniscus were first digitally recorded in the interblink period, during natural blinking with a video-meniscometer.²⁰⁻²⁵ Immediately after that, interference images from the TFLL were digitally recorded just after a blink with a video-interferometer (DR-1; Kowa, Tokyo, Japan).^{5,25}

This research was approved by the Committee for Ethical Issues on Human Research of Kyoto Prefectural University of Medicine (C-240) and followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all dry eye patients and healthy subjects after explanation of the nature and possible consequences of participation in the study.

Evaluation of the Radius of the Lower Tear Meniscus Using the Video-Meniscometer

The radius of curvature (R ; mm) of the tear meniscus at the central lower eyelid was measured by video-meniscometry.²⁰⁻²⁵ With this, a real-time reflected image of a target consisting of a pair of horizontal, black-and-white stripes was captured digitally, and R was calculated using the concave mirror formula.²⁰⁻²² In our video-meniscometry, the R of the lower tear meniscus is measured as the average of three consecutive measurements (Sugita J, et al. *IOVS* 2002;43 ARVO E-Abstract 95). In the model, R is assumed to be constant over the duration of the interblink, as demonstrated by Palakuri et al.²⁶ for the upper and lower tear meniscus using optical coherence tomography.

However, it should be noted that Johnson et al.,²⁷ using the video slit-lamp technique, reported an increase of R at the end of the interblink period. The tear meniscus is reportedly responsible for 75% to 90% of the total tear volume,²⁸ and the value of R reportedly reflects the total tear volume over the ocular surface.²³

Rheologic Analysis of Interference Images from the Precorneal Tear Film Lipid Layer by Use of the Video-Interferometer

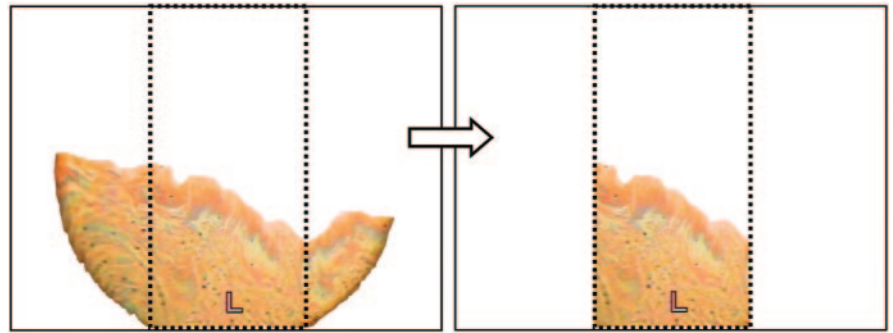
The video-interferometer (DR-1; Kowa) provides information about lipid layer thickness and lipid layer spread after a blink^{5,10,11,24,25} and is equipped with low- and high-magnification viewing modes that allow observation of 7-mm and 2-mm circular areas in the central cornea, respectively.²⁴ In this study, the low-magnification mode was the primary mode selected to obtain information regarding the behavior of the precorneal TFLL.

Interference images obtained by the video-interferometer were recorded noninvasively, in real time, using a digital video recorder. Images were sequentially captured into a computer as still images every 0.05 second (Fig. 1) with the use of specially developed software. Regions of the spreading lipid layer after a blink were clipped along the delineated border using graphic software (Photoshop CS, version 8.0.1; Adobe Systems, San Jose, CA; Fig. 2). Given that only



FIGURE 2. Regions of spreading lipid layer clipped along the border.

FIGURE 3. The central rectangular area (200 pixels horizontal \times 480 pixels vertical) was cropped from the clipped spreading lipid image as the representative region for the whole tear-film lipid layer over the cornea and the averaged height of spreading lipid within the area were calculated.



upward spreading was considered and not the associated widening of the TFLL within the viewing window, only the area of lipid film within a rectangle 200 pixels (horizontal) \times 480 pixels (vertical) was considered (Fig. 3). The averaged heights of each spreading lipid sheet within the rectangular areas were calculated using specially developed software that permitted averaging of the length of spreading lipid along the vertical y -direction at each pixel point along the horizontal x -direction within the rectangular area. Images of the spreading TFLLs with ill-delineated upper borders and images with no spreading pattern, possibly because of extremely severe aqueous tear deficiency, were excluded. After calculating the averaged heights of the spreading lipid, the relationship between the acquisition time for the images after a blink and the calculated averaged heights of the spreading lipid were plotted. Curve-fitting to the plots satisfied an exponential equation in keeping with the simple rheological model of Voigt²⁹ describing the behavior of a viscoelastic material (see Discussion).

In all 29 eyes studied, the relationship between the radius of the central lower tear meniscus (R : mm), which was proportional to the total tear volume over the ocular surface,²³ and the initial upward velocity of the lipid layer spread were analyzed. The Spearman correlation coefficient by rank test was performed, and $P \leq 0.05$ was considered statistically significant.

RESULTS

In all eyes, the time-dependent changes in H of lipid layer spread were found to conform to an exponential model of the form $H(t) - H(0) = \rho[1 - \exp(-t/\lambda)]$, where $H(t)$ is the averaged heights in millimeters at time t , $H(0)$ is the averaged heights at time $t = 0$, ρ is a constant, t is time in seconds, and λ is characteristic time in seconds.

One representative example (case 2, Table 1) of spreading behavior is shown in Figure 1. Curve-fitting to the representative three examples—including cases 1, 2, and 3 in Table 1—is shown in Figure 4. The constants of the exponential equations and corresponding R values calculated from the video-meniscometer-detected images (Fig. 5) from the central lower tear meniscus in three representative cases are given in Table 1.

The relationship between R and $H'(0)$ is shown in Figure 6. There was a statistically significant correlation between R and $H'(0)$ ($r = 0.573$; $P = 0.003$; Spearman correlation coefficient by rank test). The relationship between R and the other parameters, including $H(0)$, ρ , and λ , was also analyzed. As a

result, a similar and statistically significant correlation was found between R and ρ ($r = 0.573$; $P = 0.002$), and a weak, statistically insignificant correlation was found between R and $H(0)$ ($r = 0.318$; $P = 0.095$). However, there was no statistically significant correlation between R and λ ($r = -0.241$; $P = 0.194$).

DISCUSSION

In this study, images of the spreading TFLL were obtained noninvasively^{5,10,11,25} at intervals of 0.05 second, allowing the dynamics of the TFLL spread to be analyzed quantitatively. The time dependence of the averaged height of the spreading TFLL, as determined from the captured images, was successfully fitted to an exponential formula of the form $H(t) - H(0) = \rho[1 - \exp(-t/\lambda)]$, where $H(t)$ is the averaged height in millimeters at time t , $H(0)$ is the averaged height at time $t = 0$, ρ is a constant, t is time in seconds, and λ is characteristic time in seconds. For the averaged height, H is first measured when the TFLL first appears in the viewing window. True $H(0)$ is the point in time at which the TFLL front first crosses the lower boundary of the viewing window, so that $H(0)$ as recorded is not zero and has a positive value. The size of this depends on the rate of movement of the TFLL and is higher when the rate of TFLL movement is high rather than when it is low (Table 1).

One of the simplest models of linear viscoelasticity is the Kelvin-Voigt (commonly referred to as the Voigt) model.²⁹ The applied stress σ can be related to the shear γ through the sum of elastic and viscous forces such that

$$\sigma = \kappa\gamma + \eta d\gamma/dt \tag{1}$$

where κ is the coefficient of elasticity and η is the coefficient of viscosity. The elastic response can be represented by a

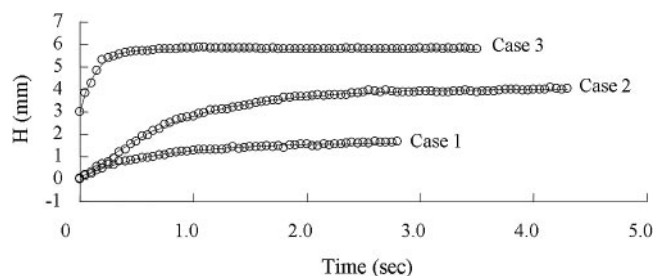


FIGURE 4. Rheological analysis of lipid layer spread in three representative cases. Each plot indicates averaged height in mm (H) of cropped spreading lipid layer, and individual exponential lines indicate the fitted curve on the basis of the Voigt model for the cases. The adapted formula was $H(t) = 0.08 - 1.54 \times \exp(-t/0.68)$ (mm) and $H'(0) = 2.25$ (mm/s) in case 1, $H(t) = 0.02 - 4.00 \times \exp(-t/0.79)$ (mm) and $H'(0) = 5.04$ (mm/s) in case 2, and $H(t) = 2.96 - 2.85 \times \exp(-t/0.14)$ (mm/s) and $H'(0) = 20.52$ (mm/s) in case 3.

TABLE 1. Constants of the Exponential Equations and the Corresponding R

Case	$H(0)$ (mm)	ρ (mm)	λ (s)	R (mm)	$H'(0)$ (mm/s)
1	0.08	1.54	0.68	0.09	2.25
2	0.02	4.00	0.79	0.14	5.04
3	2.96	2.85	0.14	0.45	20.52



FIGURE 5. Video-meniscometer-detected images from the central lower tear meniscus in three representative cases. $R = 0.09$ (mm) in case 1, $R = 0.14$ (mm) in case 2, and $R = 0.45$ (mm) in case 3.

spring and the viscous flow by a dashpot (i.e., a viscous damping system; Fig. 7). If a stress σ_1 is suddenly applied at time $t = 0$ and held constant thereafter, the linear differential equation can be solved to give

$$\gamma = (\sigma_1/\kappa)[1 - \exp(-t/\tau)] \tag{2}$$

where $\tau = \eta/\kappa$. That is, the shear does not rise instantaneously to a fixed value (as would occur with a purely elastic body) but rises asymptotically to this value. We can suppose that, in the case of the TFLL, the applied stress σ_1 is related to the surface tension forces pulling the lipid layer upward and that the resultant shear (or observed linear displacement) of the lipid front $H(t)$ corresponds to the shear parameter γ in the Voigt model. Hence, the resemblance between the exponential terms of the two expressions suggests a possible viscoelastic property of the tear film that will influence its spread. In relation to the above, Berger and Corrsin¹² analyzed the movement of particles in the tear film after a blink, taking them to be components of the TFLL. On this basis they concluded that immediately after the blink, the TFLL was displaced, like a spring being stretched, and that the spring (the TFLL) was then allowed to return to its equilibrium position. This approach gives rise to an exponential equation similar to the Voigt model but may better reflect what happens. In addition, it seems that there is no lipid layer above the boundary seen in Figure 1, but inspection of Figure 1 indicates that a thin lipid layer is in fact present. To explain this, it has been proposed that TFLL spread consists of two events: first, that in the upstroke of the blink, a polar (largely phospholipid) layer spreads over the aqueous subphase; and second, that the nonpolar lipids (chiefly cholesterol and sterol esters, the greater part of the meibomian secretion) spread over the phospholipid layer.^{30,31} Therefore, it is reasonable to conclude that a finite surface tension gradient causes the upward velocity of the TFLL rather than a discontinuity of surface tension at the border, which would theoretically cause infinite velocity at that point.

Owens and Phillips¹² also reported that the spreading velocity of particles on the tear film is adequately described by a logarithmic function. Because the particles they observed appeared to be located in the lipid film, their report may be taken to reflect the velocity of the spreading TFLL.

Curve fitting to the data, shown in Figure 4, indicates that for each of the cases studied, the relationship is of the form

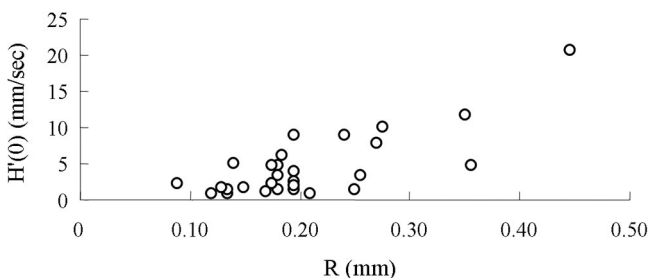


FIGURE 6. Relationship between the radius of the central lower tear meniscus (R) and the initial upward velocity of spreading TFLL, $H'(0)$, where $H'(0) = dH(0)/dt$. There was a statistically significant correlation between R and $H'(0)$ ($r = 0.573$; $P = 0.003$).

$H(t) - H(0) = \rho[1 - \exp(-t/\lambda)]$. Differentiation of this with respect to time gives $H'(t) = \rho/\lambda \times \exp(-t/\lambda)$. The velocity of particles observed by Owens and Phillips¹² can also be described in exponential form as (velocity) = $k_1 \times \exp(-k_2t)$, where k_1 and k_2 are constants. It can then readily be seen that this leads to a positional formula (position) = $\text{const.}[1 - \exp(-k_2t)]$ similar to ours and that the particle motion follows the same form as our TFLL spread. This strengthens the suggestion of Owens and Phillips¹² that the particles are embedded in and move with the lipid layer, though the sizes of these particles suggest that they may also project into the aqueous layer. Although this might help to explain our observed connection between TFLL spreading rate and tear volume, we did not notice such particles in the image samples of the TFLL images we studied.

Statistical analysis of the relationship between R and the other parameters, including $H(0)$, ρ , and λ , showed a strong and statistically significant relationship between R and ρ . However, there was no statistically significant correlation between R and λ , yet there was a weak, though statistically insignificant, correlation in the relationship between R and $H(0)$. It can theoretically be expected that the time constant, λ , correlates with the meniscus radius, R , and that one might expect equilibrium to occur more quickly when the tear film is thicker (greater meniscus radius), yet no correlation was found in the cases we studied. However, the correlation with statistical significance similar to that between R and $H'(0)$ was found between R and ρ . This is reasonable, taking the Voigt model (equation 2) into consideration, because λ in our empiric equation corresponds to τ (i.e., η/κ of equation 2, where both η and κ are material, intrinsic properties of the TFLL and are unrelated to R). Moreover, considering $H'(0) = \rho/\lambda$ and the significant correlation between $H'(0)$ and R obtained from our result, a significant correlation between R and ρ is also reasonably expected, where ρ is concerned with the equilibrium value for lipid spread and implies how high the lipid layer can spread along the y -direction; a lower height for lipid layer spread can be expected when the tear film is thinner (lower meniscus radius) because a thicker aqueous layer would be expected to allow the lipid layer to be carried higher.

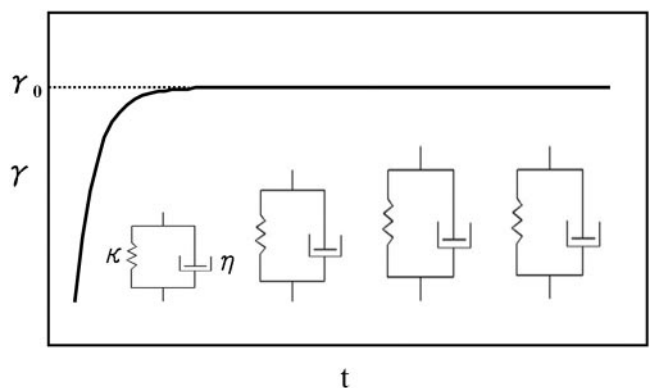


FIGURE 7. The Voigt model in rheology ($\gamma = \gamma_0[1 - \exp(-t/\lambda)]$), where $\gamma =$ extent of stretch, $\gamma_0 = \gamma$ at $t = \infty$, $t =$ time (s), $\lambda =$ retardation time (s) = η/κ , $\eta =$ viscosity, $\kappa =$ elasticity), consisting of a spring ($\kappa =$ elasticity) and a dashpot ($\eta =$ viscosity) in juxtaposition.

The findings of this study show that the Voigt model, a simple mechanistic rheological model, could successfully be adopted for analysis of the time-dependent TFLL spread in all cases studied. This suggests that the tear film is a viscoelastic material, in accordance with data showing that meibomian oil has shear-dependent viscosity and demonstrates shear thinning, which is characteristic of some viscoelastic fluids (Tiffany JM, unpublished observations, 2000).

A study by Goto and Tseng¹¹ of the speed of lipid layer spreading indicated that the time required for the lipid layer to stabilize after a blink was longer than normal in aqueous tear-deficient dry eyes but was shortened after punctal occlusion. This implied a normalization of the speed of spreading by the restoration of tear volume. However, the diagnosis of dry eye in that study was made by a Schirmer-based fluorescein clearance test, and tear volume was not estimated.

In a previous study we were able to show that the meniscus radius is directly related to the total tear volume over the ocular surface.²³ Furthermore, the formula given by Creech et al.³² suggests that the precorneal aqueous layer thickness is proportional to the meniscus radius. Our present study has shown a positive relationship between meniscus radius and initial velocity of the TFLL spread, which strongly supports the concept that this parameter is influenced by tear volume, particularly tear film thickness. Thus, it is likely that the slowed initial velocity of the TFLL spread in our patients with aqueous-deficient dry eye was related to reduced tear film thickness.

The relationship between the tear film thickness and the initial velocity of the TFLL can be explained based on the model by Berger and Corrsin.¹² Their analysis could be roughly described as a viscoelastic model in which the elastic component is provided by the TFLL, whereas the viscous component is attributed to the whole thickness of the tears, particularly the aqueous layer. They assume that surface tension is inversely related to the concentration of a surfactant (presumably related to lipid layer thickness); when the tear surface is stretched, the surfactant concentration is reduced and the surface tension increases. Thus, the lipid layer behaves like an elastic membrane whose tension increases when it is stretched. Moreover, according to their model, the force-per-unit area on the lipid layer is $dT/dx = \mu \cdot v/b$, where T is the surface tension, x is the vertical position on the cornea, μ is the viscosity of the (aqueous) tear film, v is the upward velocity of the lipid layer, and b is the thickness of the (aqueous) tears. Rearranged, $v = (b/\mu) \cdot dT/dx$, and upward velocity is thus proportional to tear film thickness.

If a low tear meniscus volume implies a low tear film thickness and a low tear film thickness is responsible for slow spreading of the TFLL, either the meniscus radius or the TFLL spreading rate could be used as an index of low aqueous volume and, therefore, of aqueous-deficient dry eye. In addition, because a low tear volume is considered to contribute to dry eye symptoms through a mechanism involving shear stress, which may increase friction during blinking,^{33,34} it will be of interest to study the relationship between TFLL spread and dry eye symptoms.

Although our method of analysis provides a useful quantitative approach, some limitations must be overcome in the future. Thus, we can only measure the area of the spreading TFLL if the uppermost border of the layer is well defined; cases with poorly defined borders were excluded from the present study. It has been noted that the interference image profile changes from a series of horizontal wave fronts in healthy subjects to a vertical disposition in patients with meibomian gland disease (MGD).¹⁰ Therefore, it could prove difficult to diagnose aqueous-deficient dry eye by such a method in the presence of MGD. In addition, analysis of the spreading using graphic software was time consuming. New image-processing

computer algorithms and analytical techniques are in development and should permit analysis of all interference patterns automatically in the near future.

In conclusion, our study has demonstrated that the time-dependent changes of the TFLL spread are consistent with a simple Voigt rheological model, implying viscoelastic properties of the tear film. Moreover, the initial velocity of the TFLL spread after a blink increases steadily with increase of the radius of the tear meniscus, suggesting that the rheological behavior of the TFLL is influenced by aqueous tear film thickness over the cornea. Therefore, it may be expected that the initial velocity of movement of the spreading TFLL could provide a noninvasive and quantitative parameter for the screening of aqueous tear-deficient dry eye, and the rheological analysis of the TFLL spread could potentially open a new field of research of the TFLL.

References

- Mishima S, Maurice DM. The oily layer of the tear film and evaporation from the corneal surface. *Exp Eye Res.* 1961;1:39-45.
- McDonald JE. Surface phenomena of the tear film. *Am J Ophthalmol.* 1969;67:56-64.
- Norn MS. Semiquantitative interference study of fatty layer of precorneal film. *Acta Ophthalmol (Copenh).* 1979;57:766-774.
- Doane MG. An instrument for in vivo tear film interferometry. *Optom Vis Sci.* 1989;66:383-388.
- Yokoi N, Takehisa Y, Kinoshita S. Correlation of tear lipid layer interference patterns with the diagnosis and severity of dry eye. *Am J Ophthalmol.* 1996;122:818-824.
- Brown SI, Dervichian DG. Hydrodynamics of blinking: in vitro study of the interaction of the superficial oily layer and the tears. *Arch Ophthalmol.* 1969;82:541-547.
- Holly FJ, Lemp MA. Tear physiology and dry eyes. *Surv Ophthalmol.* 1977;22:69-87.
- Wong H, Fatt II, Radke CJ. Deposition and thinning of the human tear film. *J Colloid Interface Sci.* 1996;184:44-51.
- Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. *Exp Eye Res.* 2004;78:347-360.
- Goto E, Tseng SC. Differentiation of lipid tear deficiency dry eye by kinetic analysis of tear interference images. *Arch Ophthalmol.* 2003;121:173-180.
- Goto E, Tseng SC. Kinetic analysis of tear interference images in aqueous tear deficiency dry eye before and after punctal occlusion. *Invest Ophthalmol Vis Sci.* 2003;44:1897-1905.
- Berger RE, Corrsin S. A surface tension gradient mechanism for driving the pre-corneal tear film after a blink. *J Biomech.* 1974;7:225-238.
- Owens H, Phillips J. Spreading of the tears after a blink: velocity and stabilization time in healthy eyes. *Cornea.* 2001;20:484-487.
- Bron AJ, Abelson MB, Ousler G, et al. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye Workshop (2007). *Ocul Surf.* 2007;5:108-152.
- Schirmer O. Studien zur Physiologie und Pathologie der Tränenabsonderung und Tränenabfuhr. *Albrecht von Graefes Arch Klin Exp Ophthalmol.* 1903;56:197-291.
- Lemp MA, Hamill JR Jr. Factors affecting tear film breakup in normal eyes. *Arch Ophthalmol.* 1973;89:103-105.
- Miyata K, Amano S, Sawa M, Nishida T. A novel grading method for superficial punctate keratopathy magnitude and its correlation with corneal epithelial permeability. *Arch Ophthalmol.* 2003;121:1537-1539.
- van Bijsterveld OP. Diagnostic tests in the sicca syndrome. *Arch Ophthalmol.* 1969;82:10-14.
- Fox RI, Robinson CA, Curd JG, Kozin F, Howell FV. Sjögren's syndrome: proposed criteria for classification. *Arthritis Rheum.* 1986;29:577-585.
- Yokoi N, Bron A, Tiffany J, Brown N, Hsuan J, Fowler C. Reflective meniscometry: a non-invasive method to measure tear meniscus curvature. *Br J Ophthalmol.* 1999;83:92-97.
- Yokoi N, Bron AJ, Tiffany JM, Kinoshita S. Reflective meniscometry: a new field of dry eye assessment. *Cornea.* 2000;19(suppl):S37-S43.

22. Oguz H, Yokoi N, Kinoshita S. The height and radius of the tear meniscus and methods for examining these parameters. *Cornea*. 2000;19:497-500.
23. Yokoi N, Bron AJ, Tiffany JM, Maruyama K, Komuro A, Kinoshita S. Relationship between tear volume and tear meniscus curvature. *Arch Ophthalmol*. 2004;122:1265-1269.
24. Maruyama K, Yokoi N, Takamata A, Kinoshita S. Effect of environmental conditions on tear dynamics in soft contact lens wearers. *Invest Ophthalmol Vis Sci*. 2004;45:2563-2568.
25. Yokoi N, Komuro A. Non-invasive methods of assessing the tear film. *Exp Eye Res*. 2004;78:399-407.
26. Palakuru JR, Wang J, Aquavella JV. Effect of blinking on tear dynamics. *Invest Ophthalmol Vis Sci*. 2007;48:3032-3037.
27. Johnson ME, Murphy PJ. Temporal changes in the tear menisci following a blink. *Exp Eye Res*. 2006;83:517-525.
28. Holly FJ. Physical chemistry of the normal and disordered tear film. *Trans Ophthalmol Soc U K*. 1985;104(pt 4):374-380.
29. Barnes HA. *A Handbook of Elementary Rheology*. Aberystwyth, Wales: Institute of Non-Newtonian Fluid Mechanics, University of Wales; 2000:81-106.
30. Holly FJ. Tear film physiology. *Int Ophthalmol Clin*. 1987;27:2-6.
31. McCulley JP, Shine WE. The lipid layer of tears: dependent on meibomian gland function. *Exp Eye Res*. 2004;78:361-365.
32. Creech JL, Do LT, Fatt I, Radke CJ. In vivo tear-film thickness determination and implications for tear-film stability. *Curr Eye Res*. 1998;17:1058-1066.
33. Cher I. Blink-related microtrauma: when the ocular surface harms itself. *Clin Exp Ophthalmol*. 2003;31:183-190.
34. Korb DR, Herman JP, Greiner JV, et al. Lid wiper epitheliopathy and dry eye symptoms. *Eye Contact Lens*. 2005;31:2-8.