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FOREFRONT REVIEW

Organizer: Mitsuru Sawa, MD, PhD, Kohji Nishida, MD, PhD

Tear-film-oriented diagnosis for dry eye

Norihiko Yokoi[1] · Georgi As Georgiev[2]

Abstract

Tear-film (TF) stability protects the ocular surface epithelium from desiccation and is ensured via cooperation between the ocular surface components including constituents of the TF and ocular surface epithelium. Thus, when those components are insufficient or impaired, the TF breakup that initiates dry eye occurs. Recently, new, commercially available eye drops have appeared in Japan that enable TF stabilization via targeted supplementation of deficient ocular surface components. Hence, a new *layer-by-layer* diagnosis and treatment concept for dry eye, termed *tear-film-oriented diagnosis* and *tear-film-oriented therapy* (TFOD and TFOT, respectively), have emerged and become widely accepted in Asian countries and beyond. TFOD is a diagnostic method for dry eye based on the TF dynamics and breakup patterns (BUPs), through which dry-eye subtypes, including aqueous-deficient dry eye, decreased-wettability dry eye, and increased-evaporation dry eye, are diagnosed. BUPs and/or each diagnosed dry-eye subtype can, in a layer-by-layer fashion, reveal the insufficient ocular surface components responsible for the TF breakup. Using these data, the optimal topical TFOT to treat dry eye can be proposed by addressing the TF breakup via the supplementation of the insufficient components. In Japan, TF breakup is now regarded as a visible core mechanism of dry eye, and abnormal breakup time (ie, ≤ 5 seconds) and symptoms are currently considered part of the diagnostic criteria for dry eye. In this review, the importance of TF instability as a core manifestation of dry eye, the molecular mechanism of TF breakup, the concept of TFOD, and the methods for implementing TFOD for TFOT are introduced.

Keywords Dry eye · Dry-eye subtype · Tear-film breakup pattern · Tear-film dynamics · Tear-film-oriented diagnosis

Introduction

The tear film (TF) is an important component of the ocular surface because it maintains the ocular surface health, and TF stability is one of the most important characteristics needed for protecting the ocular surface epithelium from desiccation [1, 2]. TF stability is reinforced via the cooperation of TF components such as the TF lipid layer (TFLL) [3] and the aqueous layer, which consists of aqueous tears [4]

Organizer: Mitsuru Sawa, MD, PhD, Kohji Nishida, MD, PhD.

Corresponding author: Norihiko Yokoi

✉ Norihiko Yokoi
  nyokoi@koto.kpu-m.ac.jp

1 Department of Ophthalmology, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Hirokoji-agaru, Kawaramachi-dori, Kamigyo-ku, Kyoto 602-0841, Japan

2 Department of Optics and Spectroscopy, Faculty of Physics, St. Kliment Ohridski University of Sofia, Sofia, Bulgaria

and secretory mucins [5, 6] that form a mucoaqueous-gel layer [1]. Therefore, when any TF components are quantitatively or qualitatively deficient, TF breakup will occur. Although TF stability is essential to maintain a healthy ocular surface epithelium, the ocular surface epithelium itself ensures TF stability by maintaining corneal-surface wettability via the glycocalyx layer that includes membrane-associated mucins [7]. Therefore, a close relationship exists between the TF and the ocular surface epithelium, and if either becomes dysfunctional, that relationship is broken and a vicious cycle between them is initiated [8].

In healthy eyes, it is expected that reflex tears secreted from the lacrimal glands in response to the initiation of the vicious cycle work to stop the vicious cycle and restore the healthy close relationship between the TF and ocular surface epithelium. However, in dry-eye cases with decreased reflex tearing, or even in DE cases in which the secretion is normal, the vicious cycle may not be repaired. TF breakup, which is a core manifestation of dry eye, results from the vicious cycle between the TF and the ocular surface epithelium, and

it is visualized invasively by use of fluorescein [9] or noninvasively by use of other methodologies such as interferometry [10], topography [11], and aberrometry [12].

In the clinical setting, fluorescein is a very useful and very visible dye that is routinely used for eye examination in combination with slit-lamp biomicroscopy. It is well known that the fluorescein breakup time (FBUT) of healthy eyes differs from that of dry eyes, and we can reasonably speculate that repeated TF breakup on the corneal surface may result in the superficial punctate keratopathy that is sometimes seen together in relation with fluorescein breakup in dry eyes

Previously, aqueous-deficient dry eye (ADDE), seen in such disorders as Sjögren syndrome, was thought to be the representative dry-eye type. However, short-BUT-type dry eye [13, 14], of which the positive manifestations are dry-eye symptoms and shorter FBUT [13, 14], ie, *unstable TF*, has become recognized as another important dry-eye subtype. According to our most recent previous version of the diagnostic criteria for dry eye in Japan [15], short-BUT-type dry eye was diagnosed as *probable dry eye*; however, the severity of symptoms was demonstrated to be equivalent to that of *definite dry eye* [16]. Therefore, since the importance of short-BUT-type dry eye has increasingly become recognized in Japan, more attention is paid to the unstable TF than to the tear volume or to superficial punctate keratopathy. Moreover, current advancements in the concept of dry eye in Japan have influenced the definition of and diagnostic criteria for dry eye in Japan, as well as in other Asian countries [17, 18], and the diagnosis of and therapy for dry eye has now shifted from superficial punctate keratopathy to BUT, and even to breakup patterns (BUPs) [8, 9]. Tear-film-oriented diagnosis (TFOD), a diagnostic method based on BUPs, is applicable in the clinical setting and is of great practical value to ophthalmologists. Therefore, the aim of this current review is to concisely present as separate sections the following points: (1) the paradigm shift in the diagnosis of and therapy for dry-eye patients in Japan; (2) the mechanism of TF formation in relation to blinking; (3) the mechanism of TF breakup in dry eye and BUPs in relation to blinking; and (4) important points regarding the implementation of TFOD.

Paradigm shift in the diagnosis of and therapy for dry-eye patients in Japan

In recent years, new eye drops have become commercially available in Japan that enable TF stabilization by targeted supplementation of the deficient components of the TF and the ocular surface epithelium in a layer-by-layer fashion [19–26]. Hence, not only the diagnosis and therapy, but also the understanding of the pathophysiology of dry eye, has greatly advanced, and a new concept of layer-by-layer diagnosis and treatment for DE has materialized, ie,

tear-film-oriented diagnosis and tear-film-oriented therapy (TFOD and TFOT, respectively) [8, 9, 17, 18]. These novel concepts are now becoming accepted and are spreading from Japan to other Asian countries [18].

TFOD is a diagnostic method for dry eye that is based on the TF dynamics and the BUPs after the eye is opened and when the eye is kept open. Using TFOD, not only the dysfunctional components of the ocular surface responsible for the TF breakup are identified, but also the necessary components needed to stabilize the TF can be discovered in a layer-by-layer fashion [8, 9, 18]. Therefore, on the basis of TFOD, we can diagnose dry-eye subtypes including ADDE, decreased-wettability dry (DWDE), and increased-evaporation dry eye (IEDE) and can propose the appropriate topical therapy as TFOT (Fig. 1) to treat dry eyes via the supplementation of the components necessary to treat TF breakup.

In Japan, TF breakup has been regarded as a visible core manifestation of dry eye [17], and dry eye is currently defined as a *multifactorial disease characterized by unstable TF causing a variety of symptoms and/or visual impairment, potentially accompanied by ocular surface damage*, and abnormal FBUT (≤ 5 seconds) and symptoms are used for the diagnosis of dry eye [17]. Therefore, TFOD and TFOT could be an ideal and practically useful pathway for clinicians to manage dry eye.

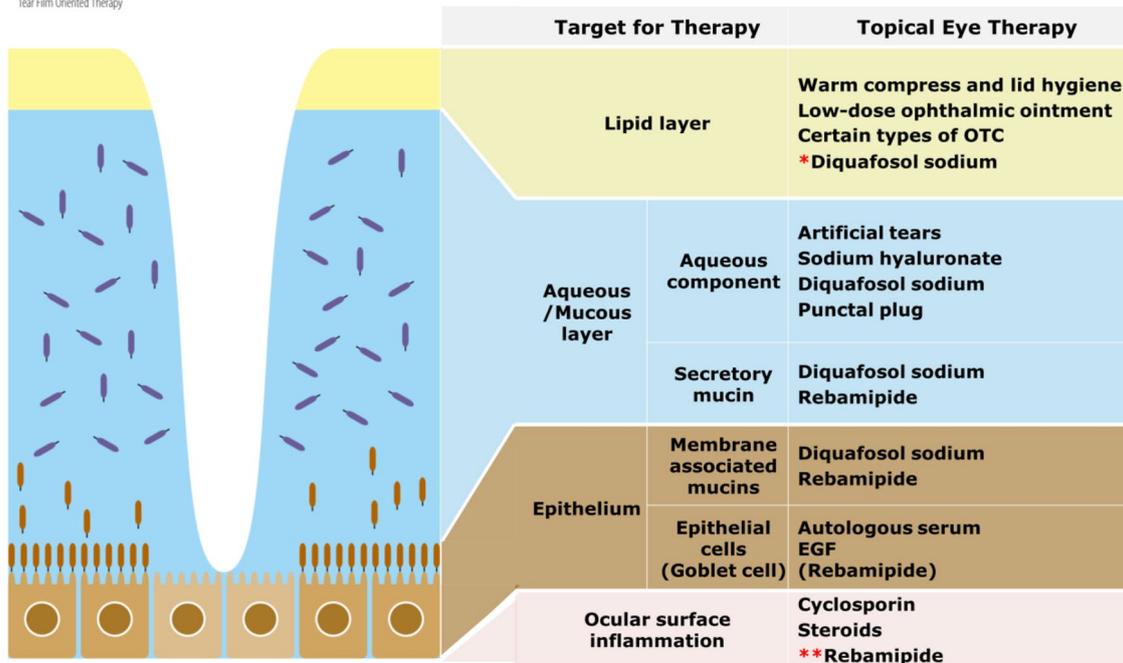## Mechanism of TF formation in relation to blinking

In this section, the (1) multiple factors that need to synchronize to ensure TF stability and (2) disturbances of the synchronicity resulting in TF breakup in relation to the precorneal TF in dry eye are reviewed.

When the upper-lid margin touches the lower-lid margin, the upper and lower menisci combine to form a single meniscus between the lid margins. As the upper lid rises, the combined meniscus separates into two: the upper one follows the rising lid and deposits an aqueous tear onto the ocular surface epithelium. The aqueous tear thickness (h) profile at deposition is determined by the balance between the suction pressure of the upper meniscus and the opposing downward force due to the viscous drag exerted on the tears by the OS epithelium. It is reasonably described by the following equation: $h = 1.338\ R(\eta U/\gamma)^{2/3}$ (γ: surface tension of the tears; R: radius of the meniscus; η: viscosity of the tears; U: velocity of the eyelid) [2, 26, 27]. Considering the variations in U during the upper eyelid movement, the equation predicts that immediately after deposition of aqueous tears and before spreading of the TFLL, the aqueous tear thickness (h) in the lower and central cornea will be higher than the TF thickness in the upper cornea, which is in reasonable agreement with previously reported in vivo observations [28, 29].

TFOT (Tear Film Oriented Therapy)

TFOT
Tear Film Oriented Therapy

Ver. 1

Target for Therapy			Topical Eye Therapy
Lipid layer			**Warm compress and lid hygiene** **Low-dose ophthalmic ointment** **Certain types of OTC** ***Diquafosol sodium**
Aqueous /Mucous layer	Aqueous component		**Artificial tears** **Sodium hyaluronate** **Diquafosol sodium** **Punctal plug**
	Secretory mucin		**Diquafosol sodium** **Rebamipide**
Epithelium	Membrane associated mucins		**Diquafosol sodium** **Rebamipide**
	Epithelial cells (Goblet cell)		**Autologous serum** **EGF** **(Rebamipide)**
Ocular surface inflammation			**Cyclosporin** **Steroids** ****Rebamipide**

*Diquafosol sodium may increase the function of the tear-film lipid layer by promoting spreading of the lipid layer through lipid and tear fluid secretion.
**Rebamipide may suppress the inflammation of the ocular surface in dry eye by its anti-inflammatory action.

Supervision: Dry Eye Society

Fig. 1 The concept of Tear Film Oriented Therapy (TFOT) version 1 produced by the Dry Eye Society of Japan. TFOT is the concept for dry-eye therapy by treating tear film (TF) breakup via the targeted supplementation of the insufficient components of the ocular surface (OS) that are responsible for the TF breakup. This scheme of TFOT illustrates the target of the OS components necessary to support TF stability and possible candidates for topical eye therapy currently available in Japan and elsewhere that can supplement the OS components that are insufficient in dry eye. This scheme can be cited from the homepage (http://www.dryeye.ne.jp/index.html) of the Dry Eye Society in Japan

Although it can be legitimately assumed [30–32] that a very thin, monomolecular, prespreading film of polar lipids is formed at the air/tear surface instantaneously with aqueous tear deposition, the bulk part (ie, the main portion of the lipid material) of the TFLL remains at the lower meniscus until the aqueous tear deposition is completed [30–33]. This is because lipids need an aqueous interface for spreading to occur, with the spreading rate being proportional to the aqueous tear thickness [31, 33]. Therefore, a surface tension gradient occurs between the lipid-deficient upper parts of the TF surface and the lower region of the TF covered by the TFLL. This gradient induces upward spreading (the so-called *Gibbs-Marangoni* effect) of the TFLL, which drags the underlying aqueous tear towards the upper cornea as well. The aqueous tear is thought to be thinner at the upper cornea immediately after deposition of the aqueous tear by the upper eyelid [31], and thus, the redistribution of the TF over the ocular surface associated with the TFLL spreading considerably changes the TF thickness contour [31, 32]. In the course of spreading, a characteristic transient interfacial profile, the so-called *dimple*, is thought to occur with a great increase of aqueous tear thickness below the leading-edge region of the spreading TFLL and a considerable decrease of aqueous tear thickness behind it [31, 32, 34, 35], so that the TF may rupture there if the corneal surface wettability becomes compromised. The in vivo changes in TF thickness during the 1- to 1.5-second duration of TFLL spreading, ie, its ~1-μm thinning over the central cornea and its thickening at the upper cornea, have been well documented [36, 37].

Apart from providing a matrix for TFLL spread, the aqueous tear deposition by the upper eyelid also distributes the secretory mucus layer over the corneal epithelium surface [38–40]. The secretory mucus layer acts both as a surface chemical trap for the capture and removal of hydrophobic contaminants (ie, lipids, dust particles, cell debris, and so forth) and as a surface chemical barrier preventing contamination of the epithelium [40, 41]. Owing to the presence of membrane-associated mucins (especially the longest MUC, MUC 16) [7] and other glycocalyx components, the surface of the corneal epithelium exhibits excellent wettability by

itself [41, 42]. However, if it becomes deprived of secretory mucus layer coverage, the attachment and accumulation of the contaminants on the epithelium become energetically possible, which can mask the hydrophilic nature of the cell glycocalyx [39, 40]. It has been clinically demonstrated that in the absence of goblet cells secreting the secretory mucin MUC5AC, the TF ruptures in 3 to 5 seconds after a blink [6]. Furthermore, after the post-blink TF redistribution is completed, owing to its shear thinning property, aqueous tears (the mucoaqueous layer) form a viscous (with a viscosity of 8–9 cP) gel-like structure [1] that renders mechanical stability to the TF (increasing its resistance to tangential flows and thinning) [43]. It was reported that altered glycosylation of secretory mucins (with otherwise normal aqueous tear volume) results in decreased TF stability [44]. Therefore, once the aqueous tear deposition and TFLL spreading are completed, the TF adopts its stable shape, ie, becoming perched between the black lines [45] produced by the lower and upper menisci-induced TF thinning [46], and can remain stable for 15 to 40 seconds before breakup appearing in healthy eyes [26, 27]. Currently, the exact role of the TFLL at this stage is under intensive investigation. It appears that the TFLL ensures the surface viscoelasticity of the air/tear interface (ie, ensuring rapid recovery of the TF structure between blinks) and may suppress aqueous tear evaporation [47, 48]. However, further research is necessary to clarify the functions of the TFLL. The functionalities of the TF layers are summarized in Table 1.

Numerous theoretical studies have focused on the mechanisms that may contribute to the breakup of the normal TF. Most probably, the TF becomes thinned by evaporation [49, 50]. When the aqueous tear thickness is sufficiently diminished, the diffusion of lipids from the TFLL to the secretory mucus layer, and subsequently to the underlying corneal epithelium surface, becomes possible. This disrupts the integrity of the secretory mucus layer (owing to adverse effects on the secretory mucus layer polarity and impact on

the Lifshitz-van der Waals forces that can destabilize the mucus layer) and will locally compromise the wettability of the underlying corneal surface, thus resulting in breakup [40, 41, 51]. However, the above-described mechanisms require extended time scales of 15 to 50 seconds (even if an evaporation rate close to that of the value for pure water is assumed) and, therefore, are not applicable to explain the rapid BUTs (ie, < 1–5 seconds) of the TF in dry eye.

Mechanism of TF breakup in dry eye and BUPs in relation to blinking

Considering the great difference in the stability of the TF between normal healthy eyes and dry eyes, different mechanisms of TF breakup are speculated to be operative in healthy eyes and diseased eyes [38–40], and they may also differ among the various types of dry eye [8, 9]. Therefore, an effort is made to identify which components of the OS are initially dysfunctional in each BUP and what types of abnormalities then develop in each TF layer. Such knowledge can assist clinicians in choosing the optimal treatment for dry eye.

Among other points, attention will be paid to (1) the moment of the appearance of the TF breakup and how it aligns with the other major events in TF formation (ie, the deposition of aqueous tears, upward spread of the TFLL, and so forth) and (2) the shape of the breakup spot. Previous studies [52–54] classified breakup spot shapes as *dots*, *streaks*, and *pools*, with the streaks corresponding to the shortest BUT, the fastest breakup expansion rate, and the shortest interblink intervals. From the theory of the stability of wetting films [55], it is well known that the breakup rate and morphology of breakup spots correspond to the chemical heterogeneity (in terms of wettability properties) of the film support (ie, of the corneal epithelial surface). Furthermore, it has been shown that the distribution of membrane-associated mucins, the major molecules rendering the

**Table 1** Functionalities of TF layers and moments of their manifestation

TF layer	Function	When it manifests during TF formation
AT	Matrix for TFLL spreading	During TF formation and immediately after (up to 1.5 seconds) eye opening
	Forms mucoaqueous gel that ensures high tear viscosity in the open eye and protects the underlying glycocalyx	Primarily at interblink, after TF is completely established
	Hydrates glycocalyx	Critically important from the very start of TF formation
TFLL	TFLL spreading (Gibbs-Marangoni effect) and redistribution of the AT over the corneal surface	During TF formation and immediately after (up to 1.5 seconds) eye opening
	Elastic stabilization of the air/tear surface and possibly suppression of TF evaporation	Primarily at interblink, after the TF is completely established
Corneal glycocalyx	Optimal wettability of the corneal surface	Critically important from the very start of TF formation. If compromised can result in instantaneous TF breakup

AT aqueous tears, *TFLL* tear film lipid layer

hydrophilic properties of the glycocalyx, loses its uniformity in dry eyes as compared with in healthy eyes [56, 57] and greatly differs between mature and undifferentiated corneal epithelial cell cultures [58]. (3) Data on the success of specific TFOT will be referenced when they become available [8].

As was previously described [2, 9, 26, 31, 33], the deposition of aqueous tears on the cornea by the upper eyelid is followed by the upward spread of the TFLL, which is accompanied by upward movement of the aqueous tears as visualized by fluorescein (Figs. 2, 3). Therefore, after the cessation of upward movement of fluorescein, the precorneal TF is completely established. The process of the initial aqueous tear deposition by the upper eyelid is so rapid that it cannot be observed even by fluorescein if there is no fluorescein breakup observed in this process (see the description of the *spot break* below). However, when aqueous tears are extremely diminished, such as in severe ADDE, then no, or quite limited, upward movement of fluorescein (ie, only within the inferior part of the cornea) can be observed after the eye is opened. Although the use of an interferometer [1, 9, 10, 33, 48] (DR-1; Kowa Company, Ltd) (Fig. 4) can illustrate whether in such a pathology aqueous tears are completely lacking on the cornea or whether they are deposited as an abnormally thinned layer, such information (ie, whether or not there are no aqueous tears in severe ADDE)

cannot be assessed by use of fluorescein. However, if there is no, or quite limited, upward movement of fluorescein after the eye is opened, it can reasonably be assumed that no complete TF can be established over the entire cornea, and we termed this BUP as the *area break* (AB) (Figs. 2a, 3A, 3a) [8, 9]. In contrast, another BUP can be observed in the phase of aqueous tear deposition by the upper eyelid during eye opening at the site where corneal surface wettability is impaired. We termed this BUP as the *spot break* (SB) (Figs. 2B, 3B, 3b) [8, 9]. One probable reason for the locally impaired corneal wettability is the deficiency/impairment of membrane-associated-mucin MUC16 [56–58]. MUC16 plays a key role in rendering the low corneal contact angle [41, 52], and its spatial distribution differs between healthy eyes and dry eyes [56, 57]. Alternatively, the glycocalyx may become contaminated with lipids, eg, owing to dimple formation below lipid "globs" (lipid particles that are supposed to precede the spreading of the major part of the TFLL), as suggested in other recent attempts at BUP classification [59]. Because they are not mutually exclusive, a synergistic action of both mechanisms is also possible. An interesting insight in relation to these points is illustrated by the impact of diquafosol sodium (P2Y2, a purinergic receptor agonist) eye drops [60–66]. They rapidly (ie, within 15 minutes of instillation) increase the aqueous tear volume in both healthy [64] and dry [65] human eyes, the secretory mucin content
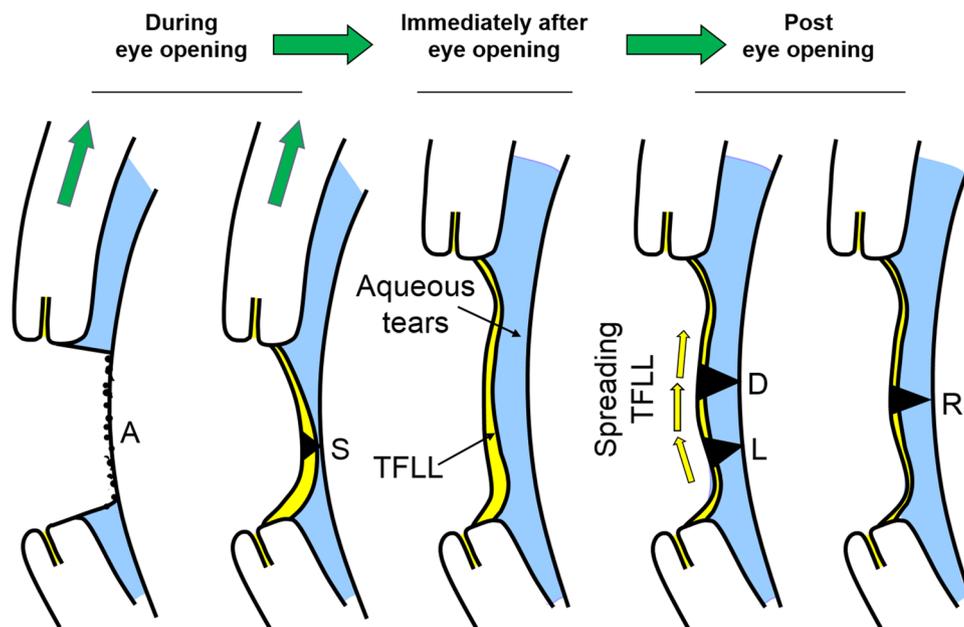
Fig. 2 Theoretical relationship between TF formation and TF breakup. The black dots and triangles show TF breakup. Related to this TF formation after the eye is opened and the eye is kept open, there are 5 types of TF breakup. **a** *Area break* occurs when the aqueous tear volume is extremely diminished. **b** *Spot break* occurs when the wettability of the corneal surface is impaired. **c** *Line break* results from the simultaneous action between the drag of the aqueous tear by the spreading TF lipid layer (TFLL) and the suction effect on the aqueous tear from the lower tear meniscus. **d** *Dimple break* is thought to result from the impaired wettability of the corneal surface. **e** *Random break* is related to the increased evaporation
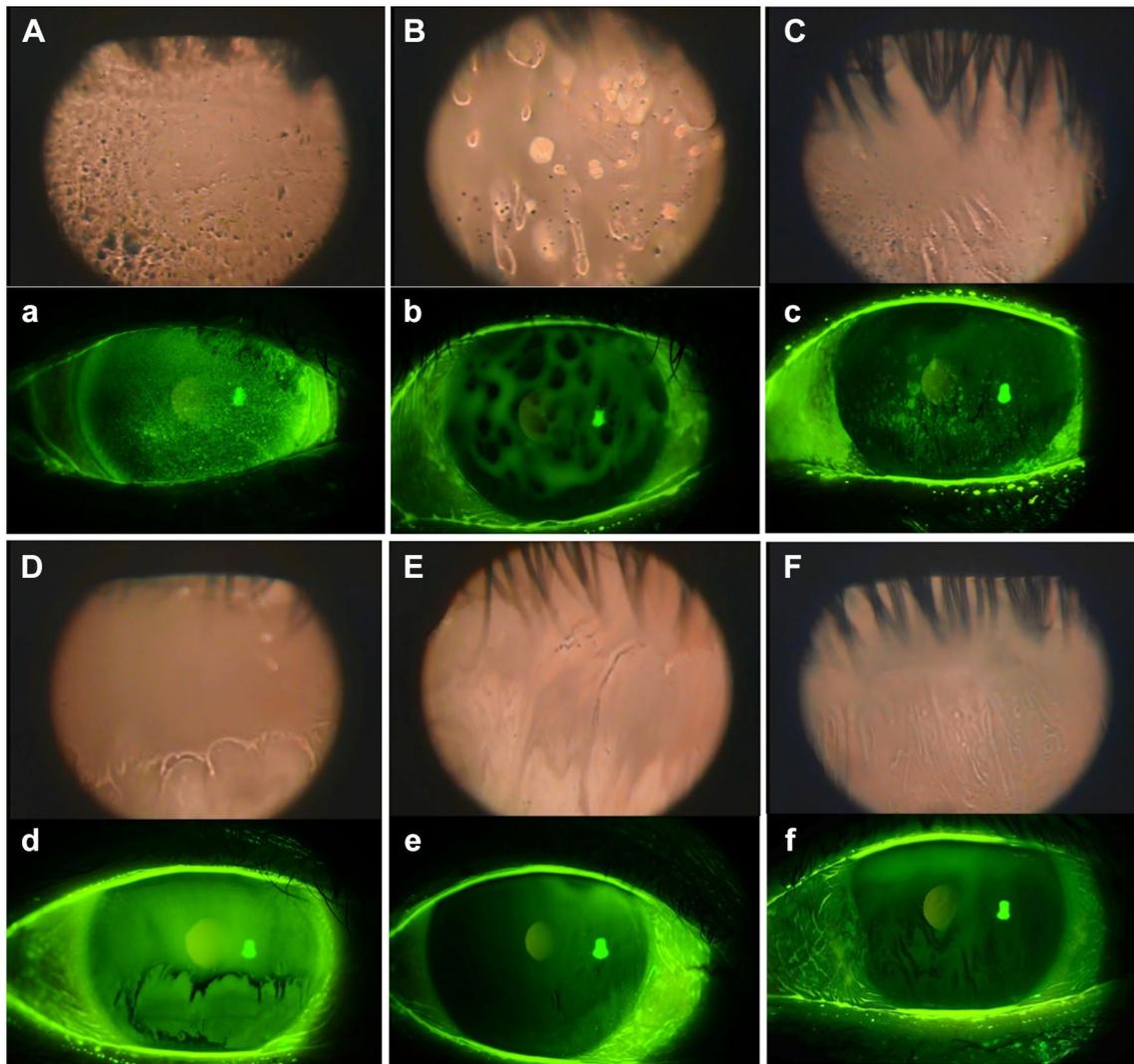
Fig. 3 Representative breakup patterns (BUPs) observed by a video-interferometer (DR-1; Kowa Company, Ltd) (**A–F**) and fluorescein (**a– f**). Each set of images (eg, **A** and **a**) was obtained from the same eye. **A** and **a**: Area break; **B** and **b**: Spot break; **C** and **c**: Line break; **D** and **d**: Dimple break; **E** and **e**: Random break; **F** and **f**: Line break with rapid expansion of the breakup regions. Area break and line break are thought to be associated with severe and less-severe aqueous-deficient dry eye, respectively. Spot break and dimple break are thought to be associated with decreased-wettability dry eye. Random break is thought to be associated with increased-evaporation dry eye. Line break with the rapid expansion of the breakup regions is thought to be associated with decreased-wettability dry eye

in healthy human eyes [66], and the gene expression of membrane-associated mucins (MUC1, 4, and 16) in cultured human corneal epithelial cells [60]. In the clinical setting, the use of 3% diquafosol sodium eye drops is an efficient treatment that gradually brings the SB pattern to normal after months of treatment [62], closely matching the MUC16 recovering action by diquafosol sodium [60]. The shape of the breakup areas corresponds to the *dots* type, ie, the small regions that did not expand after the breakup, which suggests discrete local impairment of the hydrophilic glycocalyx coating of the corneal epithelial surface. It should be noted that the normal glycocalyx covered with the secretory mucus layer should display a certain resistance to short-term contact with lipid aggregates [39, 40, 58]. This results in mobile and temporary thinning below the lipid glob without the occurrence of true fluorescein breakup pinning to the corneal surface. Such tentative SBs immediately after eye opening can also be observed via an interferometer (DR-1; Kowa Company, Ltd) (Fig. 5) [1, 9, 10, 33, 48] and are explained by the possible removal of deposited lipids from the corneal surface by the upwardly moving mucoaqueous fluid. In our classification, only cases with no disappearance of at least 1 SB until the cessation of the upward movement of aqueous tears were included. Moreover, in our previous DR-1-related studies, no lipid globs were observed in the eyes of most SB patients.

Fig. 4 Representative cases (each set of **a** and **A**, and **b** and **B** were obtained from 2 different cases of aqueous-deficient DE) in which no upward movement of aqueous tear or no upward spread of TFLL was observed after the eye was opened, respectively, by use of fluorescein (**a** and **b**) or by use of a video-interferometer (DR-1; Kowa Company, Ltd) (**A** and **B**). Each set of images (eg, **a** and **A**) was obtained from the same eye. With fluorescein, we were unable to report whether there were no aqueous tears on the surface of the cornea (**a** and **b**). However, via video-interferometry, we could tell that aqueous tears were completely lacking on the cornea in the left case (**A**) and that only deposited aqueous tears were present on the cornea in the right case (**B**)
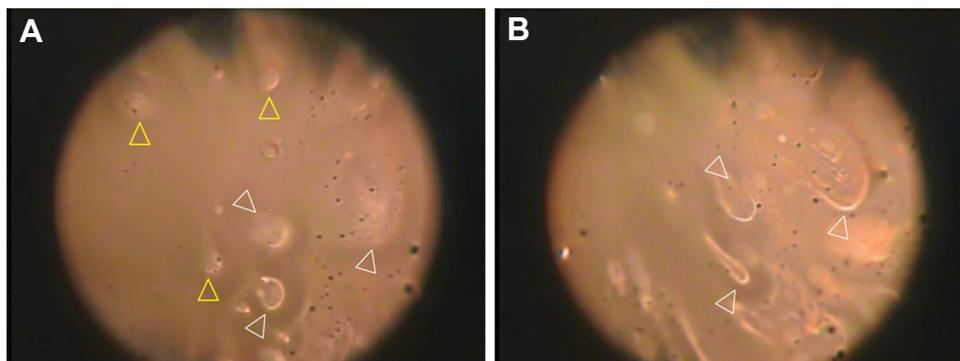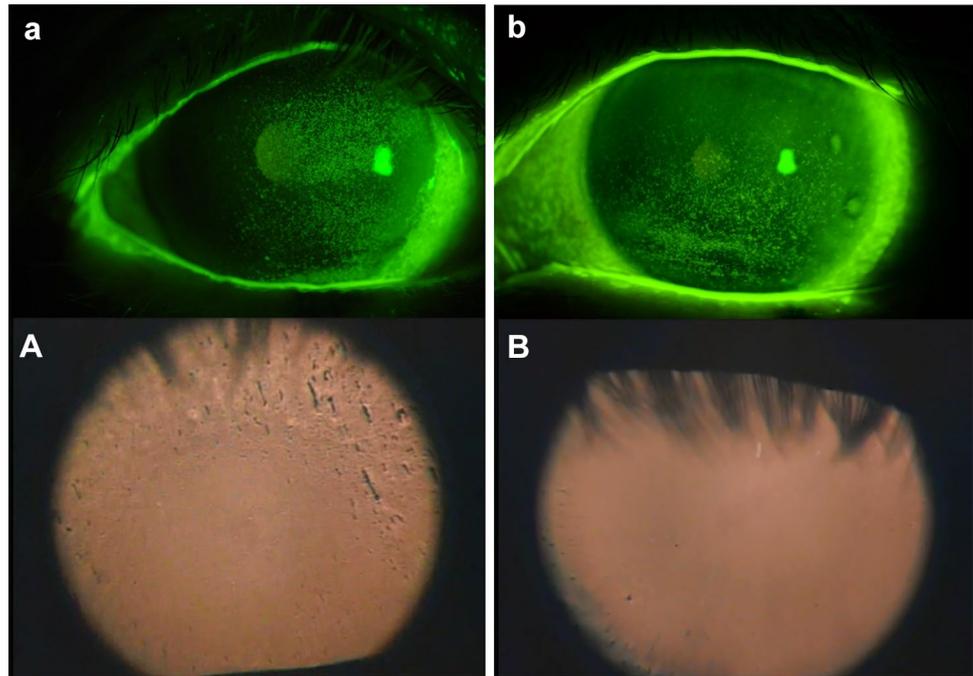
Fig. 5 Representative spot break (SB) observed with a video interferometer (DR-1;Kowa Company, Ltd) (**A**: immediately at eye opening; **B**: 4.0 seconds after the eye was kept open). SBs (white and yellow triangles) observed immediately after eye opening. However, some SBs (yellow triangles) were erased during the upward spread of the TFLL when the eye was kept open, while the other SBs (white triangles) led to the actual breakup. Such tentative SBs are explained by the possible removal of deposited lipids on the corneal surface by the upwardly moving mucoaqueous fluid triggered by the upward spread of the TFLL

On the other hand, in cases of relatively mild aqueous tear deficiency, the simultaneous thinning action on the aqueous tear due to (1) the upward drag of the aqueous tear by the upwardly spreading TFLL [31, 33] or (2) the suction effect of the lower tear meniscus [1, 46] results in thinner aqueous tears [1, 46] within the inferior part of the cornea, and therefore, TF breakup is likely to occur in that region. We termed this BUP as the *line break* (LB) (Figs. 2C, 3C, 3c) [8, 9]. In addition, when a dimple is being formed just behind the leading edge of the spreading TFLL [31, 32, 34, 35], if it passes over a region of the corneal surface with decreased wettability (eg, owing to a defective glycocalyx), especially at a relatively central part of the cornea,

then the breakup occurs via the contamination with lipids at the region with decreased wettability. We termed this BUP as the *dimple break* (DB) (Figs. 2D, 3D, 3d) [9]. Once the upward movement of fluorescein is completed and the TF structure is established, the breakup is thought to be caused by the facilitated evaporation and/or impaired viscoelasticity of the TFLL, and we termed this BUP as the *random break* (RB) (Figs. 2E, 3E, 3e) [8, 9]. The RB can be seen even in healthy eyes. However, in healthy eyes, the RB appears with longer FBUT (ie, > 10–15 seconds).

The different BUPs (Figs. 2, 3) appear to be based on pathophysiologically different mechanisms, probably owing to the dysfunction of different ocular surface components

supporting TF stability. Therefore, on the basis of the BUPs, the insufficient ocular surface components responsible for the TF breakup can be identified. From those insufficient components, 3 different dry-eye subtypes, ie, ADDE, DWDE, and IEDE, can be differentiated, and those DE subtypes are the essential part of TFOD. Accordingly, TFOD is a diagnostic pathway based on the observation of TF dynamics and BUPs through which insufficient components of the ocular surface responsible for the TF breakup, classification of the dry-eye subtype, and proposal of the optimal treatment via the stabilization of the TF can be implemented.

Important points in the implementation of TFOD

As was previously reported [9], to effectively diagnose BUPs, the following steps should be taken: (1) So as not to increase the tear volume, a less-invasive way of staining tears with a fluorescein strip being vigorously shaken and just touching the central top of the strip to the lower lid margin is essential. (2) After several blinks, the patient is verbally instructed to quickly open the eye after gently closing the eye as a kind of provocative test to discover the hidden breakup, and it should be observed whether rapid expansion of the breakup can be seen when the eye is kept open. In the classification of BUPs, reproducible BUPs must be considered as more important in relation to the pathophysiology. As previously described [9], the standard method for the application of fluorescein is to apply 2 drops of solution, such as saline, to a fluorescein test strip, vigorously shake the test strip, and then gently touch the central top of the test strip to the central lower lid margin. As a standard method, and because blinks may have an effect on FBUT [67], the patient is then verbally instructed to gently close the eye and to then quickly open the eye and keep the eye open, because this is a useful and effective way to obtain reproducible findings for classifying BUPs (data not shown).

The representative BUPs for ADDE involve the LB (Figs. 2C, 3C, 3c) and AB (Figs. 2A, 3A, 3a). The former is seen in relatively mild ADDE, and the latter, in the severest ADDE. However, it should be noted that in ADDE, together with the LB, there are cases where the SB and/or DB can also be seen. This is reasonably understood because LB occurs in ADDE with thinner aqueous tears, and in thinner aqueous tears, the epithelial surface is likely to be contaminated by lipids, and they are manifested as SB at the phase of deposition or the DB during the phases of TFLL spread. Therefore, it might be better to evaluate ADDE as LB with superficial punctate keratopathy at the inferior part of the cornea and as AB with wider distribution of superficial punctate keratopathy together.

The representative BUPs for DWDE involve the SB (Figs. 2B, 3B, 3b) and the DB (Figs. 2D, 3D, 3d), and those are combined in some cases. In addition, an important

finding diagnosed as DWDE, is the rapid expansion of the breakup, and this is seen in the LB and RB when the eye is kept open. Expansion of the breakup reflects the decreased wettability of the corneal surface [39–41], which is thought to be supported by the longest membrane-associated mucin MUC16 [7]. Notably, especially in cases with the LB showing rapid expansion despite minimal superficial punctate keratopathy at the inferior cornea and apparently normal tear volume, it may be better classified as DWDE than as ADDE, even if the LB can be seen (Fig. 3F, 3f). Likewise, even if an RB with shorter FBUT (eg, ≤ 5 seconds [7]) is observed, when the RB expands very rapidly, the appropriate diagnosis for the dry-eye subtype must be the combination of IEDE with DWED, which should be referenced when we consider TFOT.

The representative BUP for IEDE involves the RB (Figs. 2E, 3E, 3e), and this BUP can be seen after the cessation of the upward movement of fluorescein-stained aqueous tears, in other words, after the complete establishment of the TF, which is in contrast with the other BUPs in which BUPs are observed before or during the upward movement of fluorescein-stained aqueous tears. The RB can be seen even in healthy eyes, and therefore, RB in which FBUT is measured as ≤ 5 seconds should be diagnosed as abnormal according to the current diagnostic criteria for DE in Japan [17].

Considering the precorneal TF formation, the cause of the TF breakup is based on 3 different mechanisms. The first mechanism is the contamination of lipids on the corneal epithelial surface, such as those seen in the SB and DB. The second mechanism is the attachment of the TFLL to the corneal surface due to the thinning of the AT, such as those seen in the LB, RB, and rapid expansion of the breakup. The third mechanism is the incomplete formation of the TF seen in AB due to the inability of TFLL spread associated with severe aqueous-tear deficiency observed in severe ADDE. However, even in ADDE eyes, in relatively less severe cases, there are aqueous tears that can be deposited at the time of eye opening. However, even in such cases, not enough tears are present to allow TFLL spread. Whether such incompletely established TF should be considered breakup is a question that requires further discussion.

Conclusions and future directions

The new concept of TFOD and TFOT has opened another doorway for the diagnosis and therapy of dry eye, one that is based on the dynamics of the TF and BUPs. According to this concept, using fluorescein is enough for the implementation of TFOD, through which the information is given regarding the insufficient components of the OS responsible for the TF breakup, the classification of the dry-eye subtype, and the proposal of the optimal choice of the topical therapy through the improvement of TF stability via the

supplementation of the necessary components to the ocular surface to stabilize the TF. TFOD looks to be very practical and useful to clinicians, and therefore, this concept is now expanding from Japan to other Asian countries, together with the concept of TFOT. However, this concept still needs further evidence, the accumulation of which would help enhance the concept of TFOD. For example, in its current stage, TFOD is based on a number of subjective and objective clinical parameters, ie, subjective symptom evaluations, dynamics of TF, noninvasive and invasive assessments of tears, and fluorescein staining of the ocular surface [9]. However, as stated in the DEWS II report [68], not only TF instability, but also hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles in dry eye. Moreover, a recent report suggested a possible relationship between TFBUP and higher-order aberrations affecting the quality of vision [69]. Thus, the clinical estimates of the above parameters can be considered in further developments of TFOD, as they may complement the above-specified TF assessments and result in further enhancement of the TFOD and TFOT concept.

References

1. Yokoi N, Bron AJ, Georgiev GA. The precorneal tear film as a fluid shell: the effect of blinking and saccades on tear film distribution and dynamics. Ocul Surf. 2014;12:252–6.

2. King-Smith PE, Fink BA, Hill RM, Koelling K, Tiffany J. The thickness of the tear film. Curr Eye Res. 2004;29:357–68.

3. Craig JP, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. Optom Vis Sci. 1997;74:8–13.

4. Golding TR, Bruce AS, Mainstone JC. Relationship between tear-meniscus parameters and tear-film breakup. Cornea. 1997;16:649–61.

5. Gibson IK. Distribution of mucins at the ocular surface. Exp Eye Res. 2004;78:379–88.

6. Lemp MA, Dohlman CH, Holly FJ. Corneal desiccation despite normal tear volume. Ann Ophthalmol. 1970;284:258–61.

7. Argüeso P. Glycobiology of the ocular surface: mucins and lectins. Jpn J Ophthalmol. 2013;57:150–5.

8. Yokoi N, Georgiev GA. Tear-film-oriented diagnosis and therapy for dry eye. In: Yokoi N, editor. Dry eye syndrome: basic and clinical perspectives. London: Future Medicine; 2013. p. 96–108.

9. Yokoi N, Georgiev GA, Kato H, Komuro A, Sonomura Y, Sotozono C, et al. Classification of fluorescein breakup patterns: a novel method of differential diagnosis for dry eye. Am J Ophthalmol. 2017;180:72–85.

10. 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;122:818–24.

11. Goto E, Ishida R, Kaido M, Dogru M, Matsumoto Y, Kojima T, et al. Optical aberrations and visual disturbances associated with dry eye. Ocul Surf. 2006;4:207–13.

12. Koh S, Maeda N, Kuroda T, Hori Y, Watanabe H, Fujikado T, et al. Effect of tear film break-up on higher-order aberrations measured with wavefront sensor. Am J Ophthalmol. 2002;134:115–7.

13. Toda I, Shimazaki J, Tsubota K. Dry eye with only decreased tear break-up time is sometimes associated with allergic conjunctivitis. Ophthalmology. 1995;102:302–9.

14. Yamamoto Y, Yokoi N, Higashihara H, Inagaki K, Sonomura Y, Komuro A, et al. Clinical characteristics of short tear film breakup time (BUT)-type dry eye. Nippon Ganka Gakkai Zasshi. 2012;116:1137–43 (in Japanese).

15. Shimazaki J, Dry Eye Research Group. Definition and diagnosis of dry eye 2006. Atarashii Ganka. 2007;4:181–4 (in Japanese).

16. Yokoi N, Uchino M, Uchino Y, Dogru M, Kawashima M, Komuro A, et al. Importance of tear film instability in dry eye disease in office workers using visual display terminals: the Osaka study. Am J Ophthalmol. 2015;159:748–54.

17. Shimazaki J, Yokoi N, Watanabe H, Amano S, Ohashi Y, Kinoshita S, et al. Definition and diagnosis of dry eye in Japan, 2016. Atarashii Ganka. 2017;34:309–13 (in Japanese).

18. Tsubota K, Yokoi N, Shimazaki J, Watanabe H, Dogru M, Yamada M, et al. New perspectives on dry eye definition and diagnosis: a consensus report by the Asia Dry Eye Society. Ocul Surf. 2017;15:65–76.

19. Yokoi N, Kato H, Kinoshita S. Facilitation of tear fluid secretion by 3% diquafosol ophthalmic solution in normal human eyes. Am J Ophthalmol. 2014;157:85–92.

20. Yokoi N, Kato H, Kinoshita S. The increase of aqueous tear volume by diquafosol sodium in dry-eye patients with Sjögren's syndrome: a pilot study. Eye (Lond). 2016;30:857–64.

21. Shigeyasu C, Yamada M, Akune Y. Influence of ophthalmic solutions on tear components. Cornea. 2016;35(Suppl):S71–7.

22. Takaoka-Shichijo Y, Nakamura N. Stimulatory effect of diquafosol tetrasodium in the expression of membrane-binding mucin genes in cultured human corneal epithelial cells. Atarashii Ganka. 2011;28:425–9 (in Japanese).

23. Shimazaki-Den S, Iseda H, Dogru M, Shimazaki J. Effects of diquafosol sodium eye drops on tear film stability in short BUT type of dry eye. Cornea. 2013;32:1120–5.

24. Kase S, Shinohara T, Kase M. Effect of topical rebamipide on human conjunctival goblet cells. JAMA Ophthalmol. 2014;132:1021–2.

25. Itoh S, Itoh K, Shinohara H. Regulation of human corneal epithelial mucins by rebamipide. Curr Eye Res. 2014;39:133–41.

26. Wong H, Fatt I, Radke CJ. Deposition and thinning of the human tear film. J Colloid Interface Sci. 1996;184:44–51.

27. Sharma A, Tiwari S, Khanna R, Tiffany JM. Hydrodynamics of meniscus-induced thinning of the tear film. Adv Exp Med Biol. 1998;438:425–31.

28. Jones MB, Please CP, McElwain DL, Fulford GR, Roberts AP, Collins MJ. Dynamics of tear film deposition and draining. Math Med Biol. 2005;22:265–88.

29. Creech JL, Do LT, Fatt I, Radke CJ. In vivo tear-film thickness determination and implications for tear-film stability. Curr Eye Res. 1998;17:1058–66.

30. Afsar-Siddiqui AB, Luckham PF, Matar OK. The spreading of surfactant solutions on thin liquid films. Adv Colloid Interface Sci. 2003;106:183–236.

31. Berger RE, Corrsin S. A surface tension gradient mechanism for driving the pre-corneal tear film after a blink. J Biomech. 1974;7:225–38.

32. King-Smith PE, Reuter KS, Braun RJ, Nichols JJ, Nichols KK. Tear film breakup and structure studied by simultaneous video recording of fluorescence and tear film lipid layer images. Invest Ophthal Vis Sci. 2013;54:4900–9.

33. Yokoi N, Yamada H, Mizukusa Y, Bron AJ, Tiffany JM, Kato T, et al. Rheology of tear film lipid layer spread in normal and aqueous tear-deficient dry eyes. Invest Ophthalmol Vis Sci. 2008;49:5319–24.

34. Bull JL, Grotberg JB. Surfactant spreading on thin viscous films: film thickness evolution and periodic wall stretch. Exp Fluids. 2003;34:1–15.

35. Zhang YL, Matar OK, Craster RV. Analysis of tear film rupture: effect of non-Newtonian rheology. J Colloid Interface Sci. 2003;262:130–48.

36. King-Smith PE, Fink BA, Hill RM. Evaporation from the human tear film studied by interferometry. Adv Exp Med Biol. 2002;506(Pt A):425–9.

37. Benedetto DA, Clinch TE, Laibson PR. In vivo observation of tear dynamics using fluorophotometry. Arch Ophthalmol. 1984;102:410–2.

38. Yañez-Soto B, Mannis MJ, Schwab IR, Li JY, Leonard BC, Abbott NL, et al. Interfacial phenomena and the ocular surface. Ocul Surf. 2014;12:178–201.

39. Sharma A. Breakup and dewetting of the corneal mucus layer: an update. Adv Exp Med Biol. 1998;438:273–80.

40. Sharma A. Surface-chemical pathways of the tear film breakup: does corneal mucus have a role? Adv Exp Med Biol. 1998;438:361–70.

41. Tiffany JM. Measurement of wettability of the corneal epithelium. II. Contact angle method. Acta Ophthalmol (Copenh). 1990;68:182–7.

42. Shanker RM, Ahmed I, Bourassa PA, Carola KV. An in vitro technique for measuring contact angles on the corneal surface and its application to evaluate corneal wetting properties of water soluble polymers. Int J Pharm. 1995;119:149–63.

43. Tiffany JM. The viscosity of human tears. Int Ophthalmol. 1991;15:371–6.

44. Dilly PN. Structure and function of the tear film. Adv Exp Med Biol. 1994;350:239–47.

45. Miller KL, Polse KA, Radke CJ. Black-line formation and the "perched" human tear film. Curr Eye Res. 2002;25:155–62.

46. McDonald JE, Brubaker S. Meniscus-induced thinning of tear films. Am J Ophthalmol. 1971;72:139–46.

47. Willcox MDP, Argüeso P, Georgiev G, Holopainen J, Laurie G, Millar T, et al. TFOS DEWS II Tear Film Report. Ocul Surf. 2017;15:366–403.

48. Georgiev GA, Eftimov P, Yokoi N. Structure-function relationship of tear film lipid layer: a contemporary perspective. Exp Eye Res. 2017;163:17–28.

49. Tomlinson A, Doane MG, McFadyen A. Inputs and outputs of the lacrimal system: review of production and evaporative loss. Ocul Surf. 2009;7:186–98.

50. Peng C-C, Cerretani C, Braun RJ, Radke C. Evaporation-driven instability of the precorneal tear film. Adv Colloid Interface Sci. 2014;206:250–64.

51. Holly FJ. Basic aspects of tear film formation and stability. In: Velarde MG, editor. Physicochemical hydrodynamics. NATO ASI Series (Series B: Physics), vol. 174. Boston: Springer; 1988. p. 401–10.

52. Bitton E, Lovasik JV. Longitudinal analysis of precorneal tear film rupture patterns. Adv Exp Med Biol. 1998;438:381–9.

53. Rengstorff RH. The precorneal tear film: breakup time and location in normal subjects. Am J Optom Physiol Opt. 1974;51:765–9.

54. Liu H, Begley CG, Chalmers R, Wilson G, Srinivas SP, Wilkinson JA. Temporal progression and spatial repeatability of tear breakup. Optom Vis Sci. 2006;83:723–30.

55. Blossey R. Thin liquid films: dewetting and polymer flow. Dordrecht: Springer; 2012.

56. Danjo Y, Watanabe H, Tisdale AS, George M, Tsumura T, Abelson MB, et al. Alteration of mucin in human conjunctival epithelia in dry eye. Invest Ophthalmol Vis Sci. 1998;39:2602–9.

57. Liotet S, van Bijsterveld OP, Kogbe O, Laroche L. A new hypothesis on tear film stability. Ophthalmologica. 1987;195:119–24.

58. Yáñez-Soto B, Leonard BC, Raghunathan VK, Abbott NL, Murphy CJ. Effect of stratification on surface properties of corneal epithelial cells. Invest Ophthalmol Vis Sci. 2015;56:8340–8.

59. King-Smith PE, Begley CG, Braun RJ. Mechanisms, imaging and structure of tear film breakup. Ocul Surf. 2018;16:4–30.

60. Takaoka-Shichijo Y, Nakamura N. Stimulatory effect of diquafosol tetrasodium in the expression of membrane-binding mucin genes in cultured human corneal epithelial cells. Atarashii Ganka. 2011;28:425–9 (in Japanese).

61. Koh S, Ikeda C, Takai Y, Watanabe H, Maeda N, Nishida K. Long-term results of treatment with diquafosol ophthalmic solution for aqueous-deficient dry eye. Jpn J Ophthalmol. 2013;57:440–6.

62. Yokoi N, Sonomura Y, Kato H, Komuro A, Kinoshita S. Three percent diquafosol ophthalmic solution as an additional therapy to existing artificial tears with steroids for dry-eye patients with Sjögren's syndrome. Eye (Lond). 2015;29:1204–12.

63. Keating GM. Diquafosol ophthalmic solution 3%: a review of its use in dry eye. Drugs. 2015;75:911–22.

64. Yokoi N, Kato H, Kinoshita S. Facilitation of tear fluid secretion by 3% diquafosol ophthalmic solution in normal human eyes. Am J Ophthalmol. 2014;157:85–92.

65. Yokoi N, Kato H, Kinoshita S. The increase of aqueous tear volume by diquafosol sodium in dry-eye patients with Sjögren's syndrome: a pilot study. Eye (Lond). 2016;30:857–64.

66. Shigeyasu C, Hirano S, Akune Y, Yamada M. Diquafosol tetrasodium increases the concentration of mucin-like substances in tears of healthy human subjects. Curr Eye Res. 2015;40:878–83.

67. Himebaugh NL, Begley CG, Bradley A, Wilkinson JA. Blinking and tear break-up during four visual tasks. Optom Vis Sci. 2009;86:E106–14.

68. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK, et al. TFOS DEWS II definition and classification report. Ocul Surf. 2017;15:276–83.

69. Koh S, Tung CI, Inoue Y, Jhanji V. Effects of tear film dynamics on quality of vision. Br J Ophthalmol. 2018;102:1615–20. https://doi.org/10.1136/bjophthalmol-2018-312333.