Review

Tear film stability: A review

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A B S T R A C T
Tear film stability can be assessed via a number of tools designed for clinical as well as research purposes. These techniques can give us insights into the tear film, and allow assessment of conditions that can lead to dry eye symptoms, and in severe cases, to significant ocular surface damage and deterioration of vision. Understanding what drives tear film instability and its assessment is also crucial for evaluating existing and new therapies. This review examines various techniques that are used to assess tear film instability; evaluation of tear break-up time and non-invasive break-time; topographic and interferometric techniques; confocal microscopic methods; aberrometry; and visual function tests. It also describes possible contributions of different tear film components; namely meibomian lipids, ocular mucins and proteins, and factors such as age, contact lens wear, ocular surgery and environmental stimuli, that may influence tear film instability.

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1. Introduction

The ocular surface is a complex unit comprising various epithelial and glandular tissues (cornea, bulbar and palpebral conjunctiva, and lacrimal and accessory eyelid glands). These tissues secrete the tear film that coats and protects the ocular surface and allows clear vision (Holly, 1973; Stern et al., 1998; Tutt et al., 2000; Gipson, 2007). Its complexity is highlighted by observations that the composition of tears varies between open eye and closed eye (Sack et al., 2000), stimulated and non-stimulated (Fullard and Snyder, 1990), and in diseased versus normal (Li et al., 2010; Versura et al., 2010; Zhou et al., 2012) states. Therefore, deciphering the components of the tear film that are irregular and inadequate when it is unstable is a challenge for both scientists and clinicians, and the effects of a stable tear film on the ocular surface health is paramount. The purpose of this review is to bring to the forefront the current technologies and methods used to assess tear film stability, both in the clinical and laboratory setting, as well as critically revisit the literature on some of the concepts regarding tear film stability and what factors can influence tear film stability.

2. Measurement of tear film stability

In general, tear film stability is measured by its lack of stability, which is important clinically because it can be used for both diagnosis and assessment of treatments for dry eye states (Nichols et al., 2000; Bron, 2001). Its measurement is of importance to both clinicians and researchers. Clinicians are looking for evidence to support diagnosis of conditions that affect ocular health and patient comfort and quality of life, and to assess and monitor the effectiveness of treatments and interventions. Researchers are seeking techniques to better understand what drives tear film instability not only to guide the to guide development of effective therapies, but also to evaluate surface characteristics of new contact lenses materials.

A range of methods is now available to assess aspects of the tear film to provide insights into its “stability”. The direction of the development has been towards techniques that are non-invasive, assess a wide area of the ocular surface, and allow the dynamic nature and temporal instability of the tear film to be captured and analysed. As a consequence, many of the developing techniques are complex and unsuitable for routine clinical use. This section reviews the developments that have occurred.
2.1. Tear break-up time (TBUT)

TBUT was first introduced by Norm (1969) and remains the most frequently used diagnostic test to determine tear film instability (Smith et al., 2008; Korb, 2000). Currently, the technique involves instilling sodium fluorescein into the tear film using a moistened strip or a pipette and observing the tear film with a biomicroscope, cobalt blue light and a wratten 12 yellow barrier filter (Cho and Douthwaite, 1995). The patient avoids blinking and TBUT is the time interval between a complete blink and the appearance of the first break, discontinuity or dry spot observed in the tear film following a blink. Break-up occurs most frequently in the inferior or central cornea (data from 22 healthy subjects) and least frequently in the superior quadrant (Elliott et al., 1998). In normal eyes, the values for TBUT can range from 3s to 132s, with an average of 27s (Norm, 1969b). In contrast, TBUTs less than 10s suggest an abnormal tear film (Mengher et al., 1985a), with values of 5s to 10s, considered marginal, and less than 5s, indicative of dry eye symptoms (Plagfelder et al., 1996). According to Goto et al. (2004a), based on a sample of 80 eyes of 48 healthy subjects, the sensitivity and specificity of TBUT measurements were 75% and 60% respectively, for categorization of dry eye symptoms.

When using TBUT for diagnostic purposes, it is recognised that TBUT measurements have poor reproducibility (Vanley et al., 1977). Lee and Kee (1988) in a group of 30 normals and 20 dry eye patients reported the reproducibility to be 65% in normals and 95% in dry eye patients. BUT was determined on 4 visit occasions and the mean of the first and second were compared to the mean of the third and fourth visits to calculate reproducibility. BUT can be affected by clinician expertise, partial blinking, illumination techniques (Cho et al., 1992) uneven tear mixing (Vanley et al., 1977), and by the amount, concentration, pH, drop size, presence of preservatives and the type of fluorescein used (Mengher et al., 1985b).

Using a volume of fluorescein that exceeds the average tear volume of ~ 6 to 7 µl can affect tear film stability and increase TBUT artificially. Marquardt et al. (1986) found that pipetting 1 µl of 2% fluorescein solution into the tear film improved the repeatability of TBUT measurement. Similarly, Korb et al. (2001) showed that using strips that delivered 5 times less fluorescein than normal strips gave improved reproducibility. Pult and Riede-Pult (2012) reported that using a narrow (1 mm) fluorescein strip also improved repeatability. Abelson et al. (2012) using a reduced volume of fluorescein determined TBUT to be greater than 5s in normals (mean 7.1 (1.17) s) and less than 5s in dry eye patients (mean 2.2 (0.82) s).

Cho (1991) recommended that a mean of multiple measures is a more reliable indication of TBUT and later reported that if fluorescein TBUT were repeated, the first measurement was significantly different from the second; but the second and third were similar (Cho et al., 1998). Nichols et al. (2004) reported high repeatability (95% limits of agreement –5.71 to 5.83 s, upto ±8s difference between visits) when TBUT was measured on two occasions by a single examiner in a group of mild to moderate dry eye patients. The second measurement at each visit was significantly longer and interclass correlation coefficient for the average of two readings taken at a visit demonstrated better reliability than either the first or second TBUT measurement alone.

Papas’s (1999) analysis of Cho et al.’s data (1998) suggested that differences between first and subsequent measures of TBUT are unlikely to be of clinical significance. Sullivan et al. (2012) also reported on the clinical utility of objective tests for dry eye disease including BUT. Fifty-two subjects were monitored for 3 months in a longitudinal observational case series. Break-up times were determined using a slitlamp. Results of 3 consecutive measurements timed with a stopwatch were averaged. Results confirmed those of Lemp et al. (2011) illustrating that patients with mild/moderate dry eye have broadly distributed TBUT values making it difficult to differentiate these from normal subjects. In addition, during the therapeutic intervention arm of the Sullivan study, the variability or dynamic range in TBUT increased, suggesting either its resolution was insufficient to discern subtle changes or that it is a lagging indicator of ocular surface health and may need to be monitored for several months following cessation of chronic inflammation. These studies highlight the importance of both the use and reporting of standardized and detailed protocols to allow results from various studies to be compared.

Automation of the TBUT methodology has also been investigated. The technique involves location of different areas from a video of the tear film, determining regions of interest and measurement of BUT in these areas. Cebreiro et al. (2012) reported the automatic measurement values to be in the same range as that determined by a trained expert observer.

2.1.1. Tear film break-up dynamics (TBDUD)

To obtain more information about tear film break-up, the overall patterns have been examined. After instilling fluorescein, Begley et al. (2005) videotaped changes occurring after the first break in the tear film. By digitising individual frames and converting to grayscale, MATLAB was used to assess the total area of tear break-up (AB) of the exposed cornea along with TBUT and the maximum blink interval. Using a sample of 10 control and 10 dry eye subjects, TBDUs, which involves keeping the eye open for as long as possible, showed higher correlations (sensitivity and specificity) with symptoms compared to TBUT. For dry eye subjects, TBUT was faster and more extensive (24.5% vs 13.7% area) than for controls. The rate of tear break-up or dry area growth rate (DAGR) have been reported to be four times greater in dry eye subjects, who also demonstrate greater break-up in the central cornea than controls (Liu et al., 2006).

Similarly, using digital images, Ousler et al. (2005a) identified five distinct tear film break-up patterns (TFBUP) that occurred after instilling fluorescein into the tear film: amorphous blob (frequency 26%), linear (22%), spot (20%), fractured (20%) and wispy (12%). These TFBUPs were remarkably reproducible (93.8%) and the linear pattern was most frequently associated with dry eye.

2.2. Non-invasive break-up time (NIBUT)

Given the lack of reproducibility of TBUT numerous “non-invasive” techniques have been reported. However, it is important to define precisely what is non-invasive (Szczesna and Iskander, 2010). These techniques should not involve instillation of fluorescein, blinking should be natural not forced or suppressed and there should be no contact between the measuring instrument and the eye or eyelids. In addition, it is important that the methodology does not substantially alter the ocular environment such as by increased temperature from illumination systems. It has been noted that changes in meniscus curvature can be observed using non-invasive methods, indicating that some minor degree of reflex tearing is present (DEWS Diagnostic Methodology, 2007a).

Generally, non-invasive techniques involve the observation of an illuminated grid pattern reflected from the anterior tear surface. A regular image of the reflected target indicates a stable tear film and the time in seconds from the last blink to the appearance of the first discontinuity or break in the reflected image is recorded (Lamble et al., 1976; Holly, 1981; Little and Bruce, 1994a; Craig et al., 1995). Generally, comparisons of TBUT and NIBUT in the same group are poorly correlated with NIBUT being longer (Cho and Douthwaite, 1995; Nichols et al., 2002).

One advantage of using a reflected mire to examine tear stability is that events before break-up can be examined. One such
measurement is known as tear thinning time (TTT): the time after the complete blink until the first distortion of the keratometer mire (Patel et al., 1985). The mean values are ~ 16s for normal and 7s for dry eyes (Farrell et al., 1992; Little and Bruce, 1994a). Patel et al., 1985 compared TTT with TBUT and found, as expected, that TTT was shorter (18s vs 22.7s), and interestingly, the instillation of fluorescein reduced TTT by 3.6s on average, which is further evidence that instillation of fluorescein interferes with the measurement of TBUT.

In an attempt to refine the non-invasive methodology, Hirji et al. (1989) extended the keratometer method by adding a circular grid pattern, white grid on a black background, and recommended the mean of five measurements be recorded. It has been suggested that the fine grid pattern may allow easier detection of change/distortion than the standard mires (Craig et al., 1995). Results for 90 right eyes were 18.6s (1.2 SE); considerably lower than TBT of 30.6 (4.1 SE) (Vanley et al., 1977). Another refinement has been to look at a larger area of the ocular surface. Mengher et al. (1985a, 1985b) used a hemispherical bowl mounted on a biomicroscope with an illuminated rectangular grid pattern projected onto the corneal surface allowing assessment of NIBUT over the entire cornea. NIBUTs were similar to TBUTs with values for normals ranging from 4s to 214s, with a mean of 42s. Madden et al. (1994) compared the Mengher and the Hirji techniques and reported the different-day, same subject repeatability of each to be excellent, whilst the keratometer derived measurements were shorter (35.6 vs 44.7s for 45 normals).

This study again highlighted the impact of inter-observer differences.

2.2.1. Tear film particle assessment
Varikooty et al. (2012) developed an objective non-invasive measurement of tear film upward spread and tear film stability. Tear film particle velocity is measured as an assessment of tear hydrodynamics by tracking the movement of reflective particles in the tear film. Digital images of the central region of the ocular surface are collected for 10s when naturally seen particles are clearly visible in the tear film of subjects following a natural blink. Software determines the velocity of the particles as they traverse upwards in the tear film. This technique has been applied to the pre-contact lens tear film to differentiate the wetting characteristics of various contact lens materials, but has potential to also be a more precise and objective method of clinically assessing tear film stability.

2.3. Topographical analysis systems including videokeratoscopy
Corneal topography systems have replaced keratometers in clinical settings for measuring NIBUT. Liu and Pflugfelder (1999) used the TMS-1 corneal topography system to evaluate tear film stability and differentiate between 33 normal subjects and 42 eyes of 22 patients with aqueous tear deficiency. On the basis of dry eye subjects’ symptoms including foreign body sensation and dryness, Goto et al. (2004a) determined the sensitivity and specificity of the NIBUT measurements to be 97.5% and 62.5%, respectively.

Indices of the TMS-1 corneal topography instrument (Tomey Technology, Cambridge, MA), surface regularity index (SRI), surface asymmetry index (SAI) and topographic pattern have been used to evaluate corneal surface regularity and tear film stability. A series of measurements from placido disk images from the anterior ocular surface, allows changes in SRI following a blink to be used to determine of the time it takes the tear film to build-up (approximately 3–10s tear film build-up time) and reach its most regular state (Benedetto et al., 1984; Nemeth et al., 2002). The observed increase of the SRI and SAI toward the end of a 15s measurement period (in 55% and 64% of the subjects, respectively) may be an indicator of imminent tear film break-up and are comparable to those of Norn (1969) who reported a TBUt of less than 20s in 44% of normals. The TMS-1 topographic maps have been classified into round, oval, symmetric bow-tie, asymmetric bow-tie, and irregular patterns. With dry eye, a significantly lower percentage of symmetric bow-tie patterns and a greater percentage of irregular patterns were observed compared to normal eyes. An irregular topographic pattern was observed in 45% of dry eyes, which decreased significantly to 31% after the instillation of artificial tears.

These principles resulted in the in the commercially available Tear film Stability Analysis System software (Goto et al., 2003, 2004a) which records up to 10 consecutive topographic mire ring images every second and determines time-dependent changes in corneal topography to noninvasively assess tear stability. Eleven images are taken and break-up at each spot on the topographic map is calculated and used to produce one break-up map with the patient requested not to blink for 10s. Goto et al. (2003) reported significantly higher sensitivity with TSAS compared to TBUT. TSAS has been used with an anaesthetic to allow measurements to be taken without the patients blinking (Kojima et al., 2004) as well as under natural blinking conditions (Gumus et al., 2011). These corneal topography estimators however, are based on the assumption that the placido disk images are from a high quality tear film (Mejia-Barbosa and Malacara-Hernandez, 2001). Another limitation relates to problems of naturally occurring micro-fluctuations of the eye (Buchren et al., 2002), which have been corrected with an auto-alignment feature in a newer version of the software for the Tomey Auto Refractor—Keratometer resulting in sensitivity, as high as 82%, and specificity of 88% at the cut-off for unstable tear film (Gumus et al., 2011).

The Keratograph (Oculus, Wetzlar, Germany) with “Tear Film Scan” software allows for automated examiner independent assessment of tear film stability (Gumus et al., 2011). It has been evaluated to determine intra- and inter-observer differences (repeatability and reproducibility), measurement of objectively determined NIBUT, and its results compared with TBUT and TearScope NIBUT measurements (Pult and Riede-Pult, 2011; Best et al., 2012; Hong et al., 2013). The Keratograph NIBUT results were found to have acceptable sensitivity, specificity and repeatability. Results were correlated to TBUT, and Keratograph NIBUT measurements were significantly lower suggesting that it detects very early tear film changes.

Videokeratoscopy enables objective quantification through analysis of SRI and SAI of the quality of the tear film and its breakdown and also consequent effects on image quality (Montés-Micó et al., 2004; Tutt et al., 2000). It can be used both to evaluate tear film dynamically to assess temporal changes such as the build-up that occurs immediately after a blink, and deformation during the break-up phase if the eye remains open. It also has the advantage of providing large corneal coverage and precise estimators of tear film surface quality.

Introduction of high speed videokeratoscopy (Iskander and Collins, 2005) has allowed smaller time slices of the events surrounding tear film instability to be recorded and analysed. This technique uses the local disruption of reflected tear pattern to estimate tear break-up. It has been suggested that the technique measures micro changes in tear film stability (Kopf et al., 2008) compared with fluorescein TBUT that measures macro changes. Raw videokeratoscopy images under normal blinking conditions captured and processed, generally offline, overcome interferences from shadows of eyelashes or naturally occurring eye movements allowing an increased area of analysis and so producing more consistent and precise measurements of tear film surface quality (Alonso–Caneiro et al., 2009; Szczena et al., 2010). Improvements have been suggested by Szczena-Iskander and Iskander (2012) and
include incorporation of eye tracking. Dynamic-area HSV (high speed videokeratoscopy) has been used to examine tear film surface quality in normals (Szczesna et al., 2010), dry eye patients (Szczesna et al., 2011) and contact lens wearers (Kopf et al., 2008).

2.4. Using interferometry of the lipid layer for tear film stability and NIBUT

Coloured fringes occur from interference between light reflected from the surface of the lipid layer and from interface between that layer and the aequous layer of the tear film. McDonald (1968) suggested that these interference patterns could be used to observe the nature, thickness and rupture of the lipid layer. Interferometry, whilst commonly used to assess tear lipid layer thickness, can also allow measurement of NIBUT; the time between the last blink and the appearance of the first lipid layer discontinuity. Doane (1989) developed a tear film interferometer that could assess tear film break-up characteristics over time and more recently, other instruments and prototypes have been developed (Guillon, 1998; Nichols et al., 2002; Yokoi and Komuro, 2004).

The TearScope Plus is relatively common in clinical settings and has the advantage of using a cold light source, which minimizes evaporation of the tear film during the examination. In an NIBUT, the device is used directly in front of the eye or in conjunction with a slit-lamp biomicroscope to gain more magnification. A flexible grid insert is used to assess tear film stability by specular reflection (Guillon, 1998). It uses a black grid reflected onto a white background. NIBUT can be determined by two techniques: a direct non-invasive method by observing the break against the white background produced by the instrument; and indirectly by observing the deformation of rings or grid inserted within the illuminated inner surface of the instrument (Guillon, 2002). The NIBUT was found to vary significantly with lipid layer pattern with absent or abnormal colored fringe patterns being associated with tear film instability (Craig and Tomlinson, 1997). The instrument is however no longer commercially available, potentially because of its difficulty of use and subjectivity, although new software applications to aid its use in clarifying tear film lipid layer patterns are still being developed (Garcia-Resua et al., 2013).

Elliott et al. (1998) assessed the repeatability of various methods of assessing tear break-up time including the slitlamp with and without fluorescein, tearscope and videokeratoscope. For 22 subjects, they reported the videokeratoscope to be least repeatable while the tearscope was most repeatable (coefficient of repeatability 13.65 vs 8.99 vs 0.62 and intra-class correlation: 0.62 vs 0.83 vs 0.29 for videokeratoscope, tearscope and slitlamp respectively).

Lateral shearing interferometry is able to characterize up to five distinct phases of tear film kinetics over a substantial area of the ocular surface (Szczesna and Iskander, 2010). A series of raw interferometric images in natural blinking conditions are recorded at a frequency of 25 frames per second and processed offline to allow robust procedure for estimating tear film surface quality of either the cornea or contact lens (Szczesna and Iskander, 2009) and to detect tear film build up phase in 99% of cases. As a consequence, this technique has been reported to have better detection performance compared to dynamic-area high-speed videokeratoscope for dry eye under any blinking conditions (Szczesna et al., 2011).

For contact lens wearers, Hom and Bruce (2009) suggested use of specular reflection and a biomicroscope as an alternative to a specialist instrument to determine the time it takes for interference fringes to appear within an area of specular reflection to indicate pre-lens tear thinning time (PLTT). The median PLTT was determined to be 3.9s for asymptomatic contact lens wearers, and 2.2s for those with dryness symptoms. They suggested a PLTT of less than 3s as a suitable criterion for tear film dysfunction causing dryness symptoms because 75% of their symptomatic patients had an average PLTT of less than 3s.

2.5. Confocal microscopy

Confocal laser scanning microscopy allows high-resolution optical images with depth selectivity to be obtained. Images are acquired point-by-point and reconstructed with a computer, allowing three-dimensional reconstructions of topologically complex objects. It has been investigated as a tool to observe the tear film at high magnification, leading to the morphological representation of TBUT phenomena (Torens et al., 2000). A variant, Tandem Scanning Confocal Microscopy, has also been used to observe real time images of the tear film. Using an image intensified camera, reflected images of the human tear film including “dry spots” have been collected (Mathers and Daley, 1994). However, given the cost, maintenance and need for some level of user expertise, confocal microscopy is likely to be used to assess corneal and conjunctival changes related to dry eye and remain essentially a research tool for investigating tear film images (DEWS Diagnostic Methods, 2007b).

2.6. Visual acuity testing

Although dry-eye patients report decreased visual acuity when they engage in daily activities such as reading, driving, and watching TV (Tsujita and Nakamori, 1993; Goto et al., 2002), actual visual acuity testing does not support this claim. In “The Early Treatment Diabetic Retinopathy Study” (ETDRS), a protocol to test vision (Ferris et al., 1982) allowed measurement of the ability to resolve the fine spatial detail of a high contrast letter regardless of the patient’s acuity level. However, no significant differences between dry eye and normal patients in the best corrected visual acuity assessed by ETDRS have been demonstrated to date (Teson et al., 2009). This apparently anomalous result compared with the visual complaints of dry eye patients could be due to the loss of visual acuity being transitory and between blinks.

2.7. Functional visual acuity

Blink rates are higher in dry eye patients as a consequence of rapid break-up time and often reduced visual acuity (Miljanovic et al., 2007). Consequently, a fast and accurate functional visual acuity test has been developed to measure visual acuity during the inter-blink interval in patients with dry eye. Functional visual acuity (FVA) tests measure acuity during and after sustained eye opening to be more representative of impaired visual function during real-life daily activities. The decay in visual function during the blink interval is assessed. FVA is measured after sustained eye opening for 10–20s, as a simulation of visual function of daily acts of gazing, which is defined as looking at an object with involuntary blink suppression. Functional visual acuity did not change in normal controls, but decreased significantly in both non-Sjögren’s and Sjögren’s syndrome patients (Goto et al., 2002). To further improve FVA measurements, a new continuous functional visual acuity measurement system (FVAM, SSC-350®, NIDEK, Gamagori, Japan) was developed (Ishida et al., 2005). This device allows continuous monocular visual acuity measurement during a 30s blink-free period. The methodology has been useful for evaluating patients with tear instability (Kaido et al., 2008) and it has been reported that the assessments correlate with TBUT. Despite the criticism that patients are required to keep their eyes open longer than is normal, FVA tests have been widely used to assess visual disturbances in dry eye patients (Kaido et al., 2007).
2.8. Wavefront aberrometry

Aberrometry allows for the non-invasive assessment of the ocular surface and optical performance of the eye. Any local change in tear film thickness and regularity such as associated with tear break-up will introduce aberrations and subsequently reduce retinal image quality (Tutt et al., 2000). Tear film disruption consistently increases corneal and total higher order aberrations in both normal and dry eyes (Montés-Micó et al., 2004; Lin et al., 2005). Studies on 5 normal subjects demonstrated that aberrations occur as a consequence of the non-uniform tear film thinning and subsequent exposure of the rough epithelial surface (Himebaugh et al., 2012). The time course of increased aberration is accelerated in patients with an abnormal tear film (Montés-Micó et al., 2004).

Szczesna et al. (2011) found that whilst wavefront aberrometry exhibited some diagnostic ability in detecting dry eye under protocols when patients were instructed to refrain from blinking, dynamic-area high-speed videokeratoscopy and lateral shearing interferometry were superior. It was suggested that the increased variability (Szczesna et al., 2010) and potential to confound the interferometry were superior. It was suggested that the increased aberration is accelerated in patients with an abnormal tear film (Montés-Micó et al., 2004).

Kottaiyan et al. (2012) developed a four component multimodal tear imaging system integrated in a chamber in which individual environmental factors can be precisely varied to investigate their impact on tear parameters. This system includes a custom-built high-resolution wavefront sensor combined with an infrared placido disc tear surface topography to objectively detect spatial and temporal aspects of real time tear break-up and evaluate its impact on visual acuity.

2.9. Integrated multimodal metrology

Kottaiyan et al. (2012) developed a four component multimodal tear imaging system integrated in a chamber in which individual environmental factors can be precisely varied to investigate their impact on tear parameters. This system includes a custom-built high-resolution wavefront sensor combined with an infrared placido disc tear surface topography to objectively detect spatial and temporal aspects of real time tear break-up and evaluate its impact on visual acuity.

2.10. Summary

Evaluating tear stability ideally requires a non-invasive methodology that evaluates the temporal changes of the tear film in an interblink interval in an objective manner rather than a simple subjective endpoint such as TBUT. Automatic capturing of the natural dynamics of the tear film also allows the interaction between blinking and tear film stability to be further understood. Novel techniques that will, for example, allow simultaneous examination of tear film break-up and lipid layer or the spatial variability of the lipid layer and its detail during tear thinning are being developed (Arnold et al., 2010; King-Smith et al., 2011). These and the other automated dynamic assessment techniques require substantial processing and interpretation of the data and hence many are not yet available for routine clinical practice but confined to research laboratories. Clinically, TBUT is a simple and reliable method that remains the “gold standard” but it is imperative that standardised protocols be used and reported to allow comparison of results. There is a need to develop a “Rosetta Stone” by comparing the various techniques side by side for reliability and repeatability.

3. Factors controlling tear film stability

Instability is often regarded to be due to an excessive depletion of the aqueous component of tears and therefore, evaporation rates have often been a centrepiece of many studies into tear film stability. Mathers and Lane (1998) contended “... tear film stability actually refers primarily to evaporation rate; a stable tear film is one in which a minimum amount of tears evaporates.”

Fundamental to this idea is proving that the tear film thins due to evaporation rather than due to other mechanisms such as tangential flow. Kimball et al. (2010) used spectral interferometry to compare tear film thinning in the air with that occurring when wearing airtight goggles. From 37 subjects, they obtained a mean thinning value for the tear film of 3.22 μm/min in free air, compared with a mean thickening value of 0.16 μm/min in goggles. Although they concluded that on average, evaporation is the main cause for thinning of the tear film between blinks, some data were enigmatic. Surprising were that: the tear film thickness and thinning rates did not follow a normal distribution; in approximately 10% of the subjects wearing goggles, the tears became thinner at the same rate or more rapidly as in the free air; and that in two of the subjects, the tear film thickened in both the air and when wearing goggles.

Many authors have linked the evaporative rate to the quality of the lipid layer with the fundamental premise that the thicker the lipid layer, the less evaporation and more stable the tear film. Mathers and Lane (1998) have stated that “... tear film stability refers primarily to evaporation rate and that lipid layer is key to this problem.” In a thorough study (n = 161) by Craig and Tomlinson (1997), which covered characteristics including sex, age, and dry-eye and non-dry-eye subjects, they found that when there is an incomplete lipid layer, there is a significantly higher rate of evaporation (Craig and Tomlinson, 1997), but otherwise there was no correlation between evaporation and the thickness of the lipid layer. However, they found that on average, a thicker lipid layer does correlate with a more stable tear film based on NIBUTs. A blue-whitish amorphous lipid layer (80–90 nm thick) gave average NIBUTs of ~50s, and that tear films with thicker or thinner lipid layers than this ideal gave shorter break-up times on average. This general finding has been supported by Isreb et al. (2003) who showed a correlation coefficient of 0.76 between lipid layer thickness and fluorescent break-up time (FBUT) of the tear film. Mathers and Lane (1998) examined patients with obstructive meibomian gland disease (low lipid production), seborrheic MGD (excess lipid production), and normals and found no correlation between evaporation and lipid thickness, nor other measures used for evaluating tear film performance such as tear volume, tear flow, Schirmer test, meibomian viscosity or volume.

In a study by Creech et al. (1998), tear thickness (h) was estimated by using a formula that related tear thickness to the radius of the lower tear meniscus. They then related the estimated thickness to tear break-up pattern on subjects with and without contact lenses. The subjects were classified as no break-up, isolated non-expanding break-up spots, or expanding break-up spots. Calculated tear thicknesses varied from 24 μm to 42 μm in non-contact lens wearers and from 14 μm to 2.8 μm in contact lens wearers. It should be noted that more recent reflectance spectra from King-Smith et al. (2000) indicate the normal thickness of the tear film is close to 3 μm so the estimates of Creech et al. should be taken as a measure of relative thicknesses between subjects rather than absolute. As with other studies, there was a general trend that thinner films lead more readily to break-up, but it was not always the case. They also observed a tendency towards less stable films with contact lens wear. Over and above this, they placed a number of caveats on their findings such as possible different evaporative rates due to differences in meibum thickness.

3.1. Role of the meibomian lipid layer in tear film stability

It appears that the physicochemical properties of meibomian lipids are critical to the stabilisation of the tear film. Holly (1985) gave insight into this by discussing the tear film in relation to general principles of surface tension and interfacial parameters to outline the spread and collapse of thin films. The collapse of thin
films can also be regarded as dewetting of solid surfaces. In general, for very thin films, such as the tear film, the surface tensions are greater than the gravitational forces and so the aqueous tear film does not slump due to gravitational forces. In addition, there are critical surface tensions that enable the film to spread across a solid (corneal) surface. The surface tension of the cornea must be relatively high, which is achieved by wetting the surface with a mucin layer (Holly, 1974). Experimental evidence supports this because if the ocular surface is wiped removing the mucin layer, then dry spots occur in this area (Mishima, 1965; Holly, 1974). The surface tension of the aqueous surface must be also relatively low. Pure water has a surface tension of 72 mN/m, but this would be lowered by polar lipids at the air interface and by proteins in the sub-phase denaturing into the lipid layer at the surface. Holly dismissed evaporation as a reason for dewetting, estimating that under normal conditions dewetting due to evaporation would take about 10 min. Instead, Holly proposed a series of events to explain the spreading of the tear film after a blink and then the dewetting of the ocular surface at tear break-up. The spreading part of this model relies on there being practically a pure water surface at the top of the tear film during the upward phase of the blink. If proteins were involved at the surface, the proteins would lower the surface tension sufficiently to allow the lipids to spread over the aqueous surface, and if lipids were present, then there would not be a surface tension gradient at the surface, and hence lipids would not flow. The dewetting of the ocular surface assumes that polar lipids migrate from the lipid layer to the epithelial surface providing a hydrophobic region where the dewetting occurs. Holly (1973) proposed that the diffusion of meibomian lipids to the surface epithelium, which seeds the dewetting, is facilitated by local surface pressure differences at the lipid layer that promotes lateral flow of the lipids (Marangoni flow). This model is unlikely. Lin and Brenner (1981) argued that the Marangoni flow would cause a “convective diffusion opposing diffusion of lipids to the corneal surface to create a dry spot.” King-Smith et al. (2013) have recently directly compared the thickness of the lipid layer with break-up measured with fluorescein. It was found that tear break-up most often matched features in the lipid layer, the areas of break-up were not always thin areas of the lipid layer indicating that there are still unexplained complexities possibly due to thick but structurally deficient lipid layers or that the lipid layer is a poor barrier to evaporation. In terms of structural integrity, it is now known that the lipid layer appears to bunch up rather like a curtain and unfold on eye opening (PE King-Smith, personal communication), so Marangoni flow due to pressure differences is unlikely. The diffusion of lipids from the lipid layer to the epithelial surface is also unlikely because the polar lipids are predominantly long chain (O-acyl) α-hydroxy fatty acids, which would be firmly anchored in the lipid layer by their large hydrophobic acyl chains. Any smaller polar lipids such as fatty acids leaving the lipid layer would become bound to proteins such as the lipocalins which are specially designed for capturing lipids.

Some recent experiments (Rosenfeld and Fuller, 2012) point to the viscoelastic properties of the meibomian lipid film giving it special properties that prevents the dewetting of the ocular surface. In these experiments, they measured at different surface pressures the viscoelasticity under shear stress of spread films of films of arachidyl alcohol, a phospholipid and meibomian lipids. Their equipment allowed them to form a lipid film at prescribed surface pressures (they used 15 mN/m and 25 mN/m) on a drop of water. They were able to slowly remove the water from beneath the lipid film to determine how thin the water drop could become before the film collapsed onto the silicon wafer surface. The characteristics of the film were studied by interference, and the thickness of the drops at collapse was measured. A characteristic of meibomian lipid films was a relatively large elastic modulus that increased with increasing compression of the film; for the alcohol and phospholipid, the elastic modulus was below detection, or low in value only at the highest compression of the film. Critical thickness before collapse of the alcohol, phospholipid and meibomian coated drops were respectively 16.4 μm, 13.6 μm and 9.8 μm at 15 mN/m and 14.9 μm, 12 μm and 7.6 μm at 25 mN/m. In addition, the dewetting associated with the meibomian lipid film was much less once dewetting had begun. The surface of the drop with the alcohol or phospholipid was smooth, whereas for meibum it was buckled and showed a distinct dip in the centre of the drop. However, there are two limitations of these experiments. The first was that they were carried out on a water sub-phase and hence possible effects of ions and proteins in the sub-phase were not examined. The second was that the temperature was room temperature, which is below the melt transition temperature of meibum. The latter points towards a critical consideration of meibomian lipids in this whole process: why is their transition temperature (Borchman et al., 2011) so close to the ocular surface temperature? Based on the experiments of Rosenfeld and Fuller (2012) an explanation could be that the meibomian lipid film needs to have properties of a “soft solid” in order to maintain its structure and resist the collapse of the tear film onto the ocular surface.

Deformation of the lipid layer during a blink cycle has been studied in situ by Forst (1985, 1988) using interference contrast microscopy, and is similar to the studies of Rosenfeld and Fuller (2012) who examined the deformation of the lipid layer in vitro on drops of water. Forst described creasing and rough graininess of the lipid layer. Over a contact lens, the observed substructure contained “micelle-like fields”. Similar work at about 10 times higher resolution has been reported by King-Smith et al. (2011) and this indicates that there are lipid lenses (the correct scientific term for the “micelles”) on a sub-phase that is possibly 20 nm thick. The increased resolution showed that many of these lenses were up to 20 μm in diameter as indicated by Forst (1988). In addition, King-Smith et al. (2011) observed bright objects of around 1 μm in diameter that they called spots. A similar arrangement was seen in vitro on a Langmuir trough and based on the appearance of the lipids and surface pressure measurements, Millar (2013), proposed a mechanism that would prevent the collapse of the tear film. This involved the formation of liquid crystals oriented at right angles to the surface and as such they would brace each other and resist collapse.

3.2. Role of ocular mucins and proteins in tear film stability

While there have been numerous studies linking the meibomian lipid layer with tear film stability, there has been less emphasis on a role for mucins and proteins. The distribution of ocular mucins in diseased states versus normal has been investigated due to the important role mucins play as wetting agents (Argüeso and Gipson, 2001; Gipson, 2004; Mantelli and Argüeso, 2008). The superficial corneal epithelial cells have a glycocalyx formed by membrane bound mucins, and this glycocalyx decreases with diseases linked to keratinization, or drying of the ocular surface. It has also been observed that there is a significant decrease in levels of MUC5AC, a main gel-forming mucin found in the aqueous, with patients with ocular disease (Argüeso and Gipson, 2001; Argüeso et al., 2002). While authors propose that the decrease of membrane bound mucins either alone or in combination with MUC5AC causes the formation of dry spots (this is likely due to the decrease in critical surface tension at the ocular surface—see Section 3.1), no direct evidence has been presented to confirm this.

Some insight can be gleaned from the work of Tiffany et al. (1989) as to the role the proteins and soluble mucins in the aqueous component have on tear film stability. Tiffany et al. (1989)
have shown that tears from patients suffering from dry eye have an average higher surface tension than those from normals (49.6 ± 2.2 mN/m versus 43.6 ± 2.7 mN/m for normals). This increased surface tension generally correlated with a lower NIBUT (all dry eye <20s, versus 53% of normals >30s) (Tiffany et al., 1989). On the premise that higher surface tension means a less stable tear film in vivo, Nagyova and Tiffany (1999) investigated the role of major tear proteins (β-lactoglobulin as a lipocalin, lactoferrin, lysozyme, IgA) to the surface tension of tears. Using a pooled sample of whole tears, they measured its surface tension (46 mN/m) and compared it with the surface tension of individual solutions of proteins (commercially available) and established that the surface tension closest to whole tears was attained from a combination of the four major proteins founds in tears, and free lipids. This study, as well as more recent studies using the Langmuir trough by Millar and co-workers (Miano et al., 2005; Tragoulias et al., 2005; Millar et al., 2006; Mudgil et al., 2006; Mudgil and Millar, 2008; Millar et al., 2009) suggests that proteins have a crucial role in lowering the surface tension of tears and therefore, in maintaining tear film stability.

4. Factors affecting tear film stability

4.1. Age, gender, race

Infants have highly stable tear films (Isenberg et al., 2003). As we age, the blink rate increases by up to 20 fold (Lawrenson et al., 2003) and tear film stability reduces (Patel and Farrell, 1989; Tonge et al., 1991; Cho and Yap, 1993; Ozdemir and Temizdemir, 2010; Sullivan et al., 2006).

Patel et al. (2000) used TTT to evaluate tear film stability with age in 55 males and females (110 subjects in total) ranging in age from 18 to 89 years. They reported tear film stability is lower in the aged eye regardless of gender. The major causes of the increased instability were hypothesised to be related to the quality of the tear binding surface and the efficacy of the eyelids during blinks. Borchman et al. (2012) measured changes in meibum composition with increasing age. The results suggested the tighter lipid-lipid interactions in infants and children compared with adolescents and adults contributed to the stability of the tear film by providing a better barrier to evaporation.

Maissa and Guillou (2010) evaluated the NIBUT using a Tearscope in 218 subjects, 160 less than 45 years of age and 58, 45 years and over with approximately 50% of each gender. They determined that whilst stability was influenced by age, with break-up being significantly shorter for the older group, changes were more marked in women than men. A statistically significantly longer median NIBUT was recorded for men than women in the under 45 years age group but no statistically significant difference in median NIBUT between male and female was observed in the older age group.

Studies on Caucasian subjects show NIBUT values generally far exceed those reported for Asian subjects. However, whether the results reflect a clear racial difference as opposed to effects of age, gender and technique differences remains unclear. Cho (1993) reported a NIBUT of 16s (9.4s) for Hong Kong Chinese subjects aged 25 to 32, with Mohdin et al. (2002) reporting 13s (6s) for a similar aged group of Malays. Patel et al. (1995) reported on different ethnic groups living in Scotland and showed significant differences with the Caucasians having the highest TIBUT values.

4.2. CLs wear

The stability of the tear film lipid layer is always disrupted to a greater or lesser extent by the presence on a contact lens (Cho and Yap, 1995; Panaser and Tighe, 2012) and is of major concern as it is associated with symptoms of dryness (Bruce et al., 1995; Fonn et al., 1999) as well as playing a role in maintenance of optical quality (Little and Bruce, 1994b).

In a study that involved 11 tolerant and 9 intolerant contact lens wearing subjects, six hours of HEMA based contact lens wear was reported to decrease NIBUT in both groups (Glasson et al., 2005). Long-term silicone hydrogel lens wear has also been reported to significantly lower TIBUT (Sengor et al., 2012).

4.3. Ocular surgery

Ocular surgery can also impact the stability of the tear film. Eyelid surgery, such as blepharoplasty that changes the anatomy of the lids and eyelid margin, can reduce tear film stability. Punctal cauterization as expected, increases BUT in dry eye patients (Hosaka et al., 2011).

In a hospital based prospective randomised trial of 48 eyes of 30 patients undergoing phacoemulsification, TIBUT was decreased significantly one day post operatively, but returned to close to preoperative levels one month after surgery (Oh et al., 2012). Liu et al. (2008) reported similar findings for diabetic patients undergoing phacoemulsification.

Refractive surgery particularly impacts the ocular surface and tear film dynamics (Chen and Wang, 1999; Albeitz et al., 2002; Goto et al., 2004b; Nejima et al., 2005; Huang et al., 2012). Irregularities in the corneal epithelium post-surgery have been suggested to influence tear film stability and that tear film break-up can be associated with local irregularities of the corneal topography resulting following corneal refractive procedures (Szczesna and Iskander, 2009).

4.4. Environmental stimuli

Tear film stability can be influenced by external environmental conditions, such as temperature, humidity, air conditioning, air pollution, including smoke and other atmospheric irritants, and even air currents (Purslow and Wolffsohn, 2007; Wolkoff, 2012). Stability can also be affected by daily activities and alcohol intake.

Wyon and Wyon (1987) investigated the effect of air velocity on TIBUT. They reported that after 30 min exposure to high air velocity (1.0 m/s) tear stability was significantly decreased in healthy eyes, but moderate air velocity (0.5 m/s) for the same time had no effect on TIBUT.

Korb et al. (1996) demonstrated that with increased humidity, the tear lipid layer thickness in dry eye patients increased significantly. Gonzalez-Garcia et al. (2007) showed that in mild dry eye patients, with a decrease in relative humidity from 34% to 19%, NIBUT decreased with and without contact lens wear (5.3—4.9 s and 12.4 to 8.3 s respectively).

A controlled adverse environment (CAE) has been developed that exacerbates the signs and symptoms of dry eye by controlling humidity, temperature, airflow and lighting conditions (Ousler et al., 2005b). Investigations using a controlled environmental chamber showed that a drop in RH from 40% to 5% produced an immediate reduction in NIBUT that plateaued after 40 min exposure (Abusharha and Pearce, 2013). Exposure to dry environments can reduce normal tear-film break-up to levels typical of dry eyes after 1 h exposure. Conversely, Abelson et al. (2012) did not show any changes in TIBUT in dry eye subjects in response to the ocular tear film analysis protocol (OP12.0) in the CAE. This was despite other metrics particularly, mean break-up area (MBA) decreasing significantly, and consequently being reported to be a superior metric to TIBUT.

Bron et al. (2004) postulated that higher temperatures resulted in a less stable lipid layer. TIBUT has been found to be lower in dry
and warm environments compared to that observed in cold and humid environments (Paschides et al., 1998). In a review, Wolkoff et al. (2005) reported TBUT decreased in response to environmental conditions associated with office environments, namely high room temperature, low relative humidity and irritants. Decreasing the ambient temperature and relative humidity environment of 11 soft lens wearers was found to produce thinning of the tear film and shorter NIBUTs, but not to affect NIBUT when contact lenses were not worn (Maruyama et al., 2004).

In a study of 500 people, those living and working in more polluted environments had significantly lower NIBUTs (12.97 (6.12) s vs 19.23 (5.70) s; p < 0.01) than people from less polluted areas (Saxena et al., 2003).

Dursu et al. (2005) studied 60 smokers and reported significantly lower BUT and failure of smooth lipid spread. Similarly, Altinors et al. (2006) reported smoking had deteriorating effects on the lipid layer of 60 smokers compared to a group of 34 healthy non-smokers. The mean break-up time was 5.3 s (range 1–10 s) for the smokers. They suggested that the oxidant species in cigarette smoke may damage the lipid layer via lipid peroxidation. The study of Thomas et al. (2012) emphasised the adverse effects of chronic smoking on the tear film, demonstrating that the reduction in TBUT increased with duration of smoking years (6.0 s for those who have smoked for 21 plus years vs 7.6 s for less than 20 years compared with 11.3 s for age and gender matched non-smokers). Increased tear film destabilisation has been reported with computer and visual display use (Himebaugh et al., 2009; Jansen et al., 2011) and this has been attributed to a reduced blink rate and frequent incomplete blinking pattern (Teoh et al., 2012). The combination of micro-environment glasses and artificial tears showed a marginally significant trend in increasing TBUT in a randomised clinical trial over 5 days of symptomatic and control patients using computers for three hours or more per day (Dawson et al., 2005). Topical lipid therapy has also been reported to significantly increase BUT (2.6–4.8 s) in 30 office workers with dry eyes that had not previously responded to conventional treatments (including punctual plugs, non-preserved artificial tears) (Goto et al., 2006).

Ingestion of ethanol has also been shown to adversely reduce TBUT (Kim et al., 2012). Orally ingested alcohol was detected in the tears and resulted in increased tear osmolality and a linked decrease TBUT. One postulated reason for the changes was that ethanol can act as an organic solvent for the lipid layer resulting in decreased tear evaporation which in turn leads to hyperosmolarity and subsequent tear film instability. It has also been hypothesised that hypohydration may cause changes in tear film stability by impairing lacrimal gland function as a consequence of decreased blood pressure and increase blood osmolality (Sollanek et al., 2012). However, further investigation is required as Kayikcioglu et al. (1998) reported marginal dehydration (~1.5% of body weight) had no effect on tear film stability: TBUT was measured at the beginning and at the end of fasting on 32 healthy male patients with a mean age of 22 years.

5. Conclusion and future directions

A number of procedures exist to measure tear film stability both in the clinical situation and for research purposes. Recent developments have focussed on technology that can examine the tear film dynamically and explain its kinetics. Further research directed to understanding what controls tear film stabilization is important. There are many individuals, some over the age of 50, with very stable tear films. These populations need to be identified and their tear film investigated along with those with dry eye symptoms to better inform our understanding of the mechanisms of tear film stability. If we can achieve an optimal level of tear film stabilization, it may be possible to provide therapy tailored to individual conditions and needs. This remains challenging due to the many aetiologies and the range of factors that impact the tear film.

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indicate that proteins are major contributors to the surface pressure. Cornea 24, 189–200.