Report of the Inaugural Meeting of the TFOS *i*^{2} = *i*nitiating *i*nnovation Series: Targeting the Unmet Need for Dry Eye Treatment (London, United Kingdom, March 21, 2015)

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ABSTRACT

In March 2015, a meeting was held in London, United Kingdom, to address the progress in targeting the unmet need for dry eye disease (DED) treatment. The meeting, which launched the ***i*²** = ***i***nitiating ***i***nnovation series, was sponsored by the Tear Film & Ocular Surface Society (TFOS; www.TearFilm.org) and supported by Dompé. The TFOS ***i*²** meeting was designed to review advances in the understanding of DED since publication of the 2007 TFOS International Dry Eye WorkShop (DEWS) report, and to help launch the highly anticipated sequel, DEWS II. The meeting was structured to discuss the scope of the DED problem, to review the clinical challenges of DED, and to consider the treatment challenges of DED. This article provides a synopsis of the presentations of this TFOS ***i*²** meeting.

KEYWORDS

Clinical trials, drug development, dry eye disease (DED), Dry Eye WorkShop II (DEWS II), ***i*²** = ***i***nitiating ***i***nnovation series, Tear Film & Ocular Surface Society

OUTLINE

I. Introduction
   A. TFOS Mission
   B. Treatment of DED, an Unmet Need
II. Scope of the DED Problem
   A. Epidemiology of DED
   B. Surgery-induced DED
   C. Contact Lens-associated DED
III. Clinical Challenges of DED
   A. Pathophysiology of DED
   B. Symptomology of DED: The Origin of Discomfort
   C. Diagnosis of DED
IV. Treatment Challenges of DED
   A. New Approaches for the Treatment of DED
   B. Regeneration of the Ocular Surface in Severe DED
   C. Regulatory Issues Associated with the Development of a DED Drug in Europe
V. Summary
VI. Appendix

I. INTRODUCTION

The Tear Film & Ocular Surface Society (TFOS; www.TearFilm.org) sponsored its inaugural meeting of the ***i*²** = ***i***nitiating ***i***nnovation series in March 2015 in London. The meeting was entitled "Targeting the Unmet Need for Dry Eye Treatment" and was designed to review progress in the understanding of dry eye disease (DED) since publication of the 2007 TFOS International Dry Eye WorkShop (DEWS) report.[1] The meeting was approved by the TFOS Board of Directors, organized by Dr. David A. Sullivan, coordinated by Ms. Amy Gallant Sullivan (TFOS Executive Director), and attended by a number of individuals from Europe. The speakers, panelists, and moderators, who were invited by TFOS to participate, are shown in Table 1. The specific topics to be addressed were three-fold: first, to discuss the scope of the DED problem, including its epidemiology, iatrogenic induction, and contact lens association; second, to review the clinical challenges of DED, including its pathophysiology, symptomatology, and diagnosis; and third, to consider the treatment challenges of DED, including new pharmaceutical, device and surgical approaches for its therapy, as well as the regulatory issues associated with the development of a DED drug in Europe. This article summarizes the presentations of this TFOS ***i*²** meeting.

Table 1. Meeting Presenters, Panelists and Moderators

Carlos Belmonte, MD, PhD (Speaker) Founder, Instituto de Neurociencias de Alicante, and Professor, Universidad Miguel Hernández-Consejo Superior de Investigaciones Científicas, and President, International Brain Research Organization, Spain
José Benitez del Castillo, MD (Speaker) Professor, Complutense University of Madrid and Hospital Clinico San Carlos, Madrid, Spain
Anthony Bron, FRCOphth, FMedSci (Speaker) Professor Emeritus, University of Oxford, Oxford, UK; Professor of Experimental Ophthalmology, Vision and Eye Research Unit (VERU), University of Anglia Ruskin, Cambridge, UK
Harminder Dua, MD, PhD (Speaker, Panelist) Professor and Chair, Department of Ophthalmology, University of Nottingham, UK
Eric Gabison, MD (Panelist) Deputy Head, Ophthalmology Department, Fondation Ophtalmologique Rothschild, and Professor, Fondation Ophtalmologique Rothschild & Hôpital Bichat Claude Bernard, Paris, France
Juha Holopainen, MD (Panelist) Professor, Department of Ophthalmology, Helsinki University Central Hospital, Finland
Florence Malet, MD (Panelist) Ophthalmology Service, Centre François-Xavier Michelet, Bordeaux and Past-President, European Contact Lens Society of Ophthalmologists, Bordeaux, France
Leonardo Mastropasqua, MD (Panelist) Professor, Faculty of Medicine and Surgery, University "G. d'Annunzio" of Chieti-Pescar, Italy
Elisabeth M. Messmer, MD (Panelist) Department of Ophthalmology, Ludwig Maximilian University, Munich, Germany
Kelly Nichols, OD, MPH, PhD, (Speaker, Moderator) Dean, School of Optometry, University of Alabama, Birmingham, AL, USA
Gary Novack, PhD (Speaker) President, PharmaLogic Development of San Rafael, CA, USA
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Deputy Executive Dean and Deputy Dean of Life and Health Sciences at Aston University, Birmingham, UK

I.A. TFOS Mission (David A Sullivan, MS, PhD, FARVO)

Dr. Sullivan opened the meeting by stating the mission of TFOS: To advance the research, literacy and educational aspects of the scientific field of the tear film and ocular surface. He thanked the invited speakers and panelists for their participation, Dompé for its generous financial support, and Ms. Sullivan for her excellent meeting coordination. Dr. Sullivan also noted that this ***i²*** meeting would help launch the much-anticipated sequel of the original TFOS DEWS.[1] This forthcoming Workshop, termed DEWS II, will soon begin, and will 1) update the definition, classification, and diagnosis of DED; 2) critically assess the etiology, mechanism, distribution, and global impact of this disorder; and 3) address its management and therapy.

I.B. Treatment of DED, an Unmet Need (Harminder Dua, MD, PhD)

Dr. Harminder Dua presented a clinical scenario to illustrate the causality dilemma posed by DED: a patient presents with complaints of dry eyes, and after a thorough evaluation (including detailed medical history, clinical examination, and diagnostic tests), the clinician concludes that the patient has dry eyes—a diagnosis that is circular in reasoning if it does not identify the underlying cause(s) of disease. Likewise, most available treatments target DED symptoms but do not address etiology. This problem is central to the unmet need in the treatment of DED, which Dr. Dua defined as five fundamental insufficiencies, summarized below.

I.B.1. A universally accepted set of criteria for diagnosis.

The first insufficiency is the uncertainty of whether "dry eye" represents a symptom or a diagnosis. From the patients' perspective, dry eye is a complaint (i.e., a symptom). The physicians use the term as a diagnosis. The connotation of the terms needs to be clarified. This is further complicated by the use of varying nomenclature to describe the condition (Table 2).

Table 2. Nomenclature and Symptoms of DED

Nomenclature	Symptoms
Dry eye disease	"Chilli powder in my eyes"*
Dry eye disorder	"Dust in my eyes"*
Dry eye syndrome	Burning*
Dry eyes	Discomfort*
Dysfunctional tear syndrome	Dryness*
Keratoconjunctivitis sicca (KCS)	Gritty/sandy eyes*
Non Sjögren's KCS	Itching
Sicca syndrome	Pain
Sjögren's KCS	Tearing or watery eyes
Xerophthalmia/xerosis	Visual aberrations

*May represent different symptoms or different expressions of the same symptom

Moreover, with the possible exception of tear osmolarity, tests used in the assessment of dry eye are notoriously nonpathognomic—lacking correlation between signs and symptoms—and have significant overlap between normal and abnormal values.[2] Foremost among the unmet needs in dry eye treatment is a uniform concept and minimum set of criteria for diagnosis. Attaining this goal will facilitate the design and interpretation of clinical studies, and make the results more meaningful and easier to compare.

I.B.2. Understanding the global magnitude of the problem

The 2007 TFOS DEWS Report thoroughly evaluated DED in its scope[3] and therapeutic management.[4] As one of the most frequent pathological conditions in ophthalmology, DED is a leading cause of patient visits to hospitals. Prevalence is as high as 50% (depending on criteria used), with more affected women than men. Prevalence is also higher in Asian and aging populations, though younger people are increasingly manifesting symptoms. There is a known disparity between patients requiring and receiving treatment, especially in Asian compared to Western countries, while figures for other regions are lacking. Thus, elucidating the true global magnitude of DED is another major unmet need in its management.

I.B.3. Understanding the underlying mechanisms of DED and translation to effective therapies.

According to the British National Formulary (BNF),[5] there are over 50 dry eye preparations in the UK alone, in addition to a variety of goggles, humidifiers, and other devices that deliver heat or massage to the lids. Despite the wide range of treatments available, an online survey conducted in March 2011 by Harris Interactive (commissioned by Allergan) found that of the respondents with symptoms of dry eye, 69% had not seen an eye care professional, and of those who did, 22% did not experience relief of symptoms with treatment.[6] As a potential contributing factor, many available treatments contain preservatives such as benzalkonium chloride (BAK or BAC, which has well-established toxic effects on the ocular surface) and disodium EDTA (a known irritant with potential epithelial toxicity).[4] Moreover, most available treatments overlook the underlying pathology involving corneal nerves, which are increasingly being recognized as key players in the pathology of postoperative dry eye and visual aberrations. As they are a major basis of symptoms (and DED is primarily a symptom-driven disease), corneal nerves need to be specifically studied. A greater understanding of nerve alterations in DED is an unmet need. There also is a great need for innovative therapeutic strategies that target the underlying biomechanics of DED; investigations of the ocular surface functions of lubricin,[7, 8] a proteoglycan and boundary lubricant, are exemplary in this regard, and represent a promising translational approach.

I.B.4. Integrated pathways of care and universal protocols for managing DED.

As the world population is expected to increase from 7.2 billion in 2012 to between 8.3–10.9 billion by 2050,[9] the concern of DED is expected to rise. According to The Lancet Series on Ageing, published in 2014, 2 billion people will be aged 60 years or older by the year 2050,[10] and assuming a prevalence of 25%, 500 million people will have the disease in this demographic alone. With approximately 19,000 ophthalmologists in the United States and just over 200,000 worldwide,[11] DED represents a growing burden on the ophthalmic care system, with a high ratio of DED patients to ophthalmologists and sight-threatening eye diseases competing for attention. Thus, there is a need to integrate the entire ophthalmic workforce: ophthalmologists, general practitioners, optometrists, opticians, nurses, orthoptists, staff, and patients. This includes universally accepted and regionally tailored protocols for treatment and referral, with increased education, heightened awareness, and adequate training.

I.B.5. Integrated prevention strategies in the management of DED.

The 2007 report of the TFOS DEWS Epidemiology Subcommittee identified both modifiable and nonmodifiable risk factors for DED.[3] Further study is needed to identify those at risk and influence modifiable risk factors. These advances will help integrate prevention and early intervention into the management of DED.

II. SCOPE OF THE DED PROBLEM

II.A. Epidemiology of DED (Kelly Nichols, OD, MPH, PhD)

Dr. Kelly Nichols stated that the 2007 report of the TFOS DEWS Epidemiology Subcommittee was a significant advance in a developing field with limited published research, and presented three epidemiology-related goals: 1) to assess and summarize current knowledge on the epidemiology of DED (prevalence, incidence, natural history); 2) to describe the risk factors for DED; and 3) to summarize existing questionnaires for DED and make recommendations for the use of such surveys in clinical trials.[3] In the current meeting, Dr. Nichols reviewed progress in each of these areas since the 2007 report, and undertook multiple approaches to estimate growth in the area of ocular surface epidemiology: reviewing the published literature, reviewing trials registered at clinical trials.gov, and reviewing surveys utilized in clinical trials.

II.A.1. Advances in DED Epidemiology

II.A.1.a. Prevalence of DED

The current global prevalence of dry eye can be assessed from available population-based studies, summarized in Table 3. At the time of 2007 report of the TFOS DEWS Epidemiology Subcommittee,[3] only eight population-based epidemiologic studies of DED had been published, with prevalence ranging from 5% to 30%.[12-19] Based on data from the largest U.S. studies of adults over 50 years of age, the Women's Health Study[17] and the Physicians' Health Study,[19, 20] 3.23 million women and 1.68 million men in the United States have mild-to-moderate or even severe symptoms of DED. The study in US women by Schaumberg and colleagues[17] was notable in that it used a short survey of just three questions.

By 2011, at least 7 additional population-based studies evaluating dry eye had been published,[21-27] generally using a symptom-based or a modified field-testing sequence. Prevalence ranged from approximately 5% to over 50%, which highlights the variation in how DED is defined in these studies.

Several more recent studies have provided estimates of the current global prevalence of dry eye, [28-38] with prevalence ranging from approximately 10% to 20% (again emphasizing the variability in defining DED). Prevalence tended to be higher in women compared to men. Studies conducted in Asian populations seem to indicate a higher prevalence of dry eye, and many of the studies have utilized similar symptom batteries as previously performed studies. The reasons for the higher prevalence values in Asia require further exploration.

It is now widely accepted that the prevalence of DED increases with age; however, depending on the ethnicity of the population in question, the universal value of 15% over the age of 65, based on a 1997 study by Schein et al.,[12] may be an underestimate of actual ocular surface disease prevalence, especially in Asian populations. There remains a discrepancy between those who have received diagnoses of "dry eye" and those with mild-to-moderate or even severe symptoms. Moreover, with our increased scrutiny on the prevalence of anterior blepharitis and MGD relative to evaporative dry eye, the lines become increasingly blurred, and it has been suggested that MGD should be included in the definition of DED.[39] Caution should be used in interpreting symptom-based diagnostic criteria, as multiple ocular surface and lid conditions can yield the same patient-reported symptoms.

Table 3. Global Prevalence of Dry Eye, 2015: Summary of population-based studies

Location, Study	Reference	Year	Prevalence (gender)

Africa, Nigeria	35	2014	19.2% (overall)
Asia, China (Beijing Eye Study)	24	2009	21.0% (overall)
Asia, China—Mainland	31	2014	17.0% (overall) 21.6% (females)
Asia, China—Southeast	38	2015	9.5% (overall)
Asia, Eastern India—West Bengal	30	2012	26% (overall)
Asia, Iran	34	2014	8.7% (overall)
Asia, Japan (Koumi Study)	28	2011	12.5% (males) 21.6% (females)
Asia, Japan (high school students)	22	2008	21.0% (males) 24.4% (females)
Asia, Japan (visual display terminal users)	23	2003	26.9% (males) 48.0% (females)
Asia, Korea (Korea National Health and Nutrition Examination Survey)	36	2014	8.0% (overall)
Asia, Mongolia (Henan Eye Study)	26	2010	50.1% (overall)
Asia, Singapore	37	2015	12.3% (overall)
Asia, Singapore (Singapore Malay Eye Study)	40	2010	6.5% (overall) 8.2% (males) 4.9% (females)
Asia, Indonesia—Sumatra*	15	2002	27.5% (overall)
Asia, Taiwan (Shihpai)*	16	2003	33.7% (overall)
Asia, Tibet	21	2008	52.4% (overall)
AUS (Extension Blue Mountains Eye Study)*	18	2003	16.6% (overall)
AUS, Melbourne*	13	1998	5.5% (overall)
EU, Spain (Saines Eye Study)	25	2009	11.0% (overall) 9.0% (males) 11.9% (females)
UK, Britain (TwinsUK)	32	2015	9.6% (females)
US, Wisconsin (Beaver Dam Eye Study)*	14	2000	14.4% (overall) 11.4% (males) 16.7% (females
US, Wisconsin (Beaver Dam Offspring Study)	33	2014	14.5% (overall) 10.5% (males) 17.9% (females)
US, Maryland—Salisbury*	12	1997	14.6% (overall)
US (Physicians' Health Study)*	19, 20	2009	4.34% (males)
US (Veterans Affairs Population)	29	2011	12% (males) 22% (females)
US (Women's Health Study)*	17	2003	7.8% (females)

*Included in the 2007 TFOS DEWS report
†Included in the 2007 TFOS DEWS report in abstract form

II.A.1.b. Incidence of DED

Regarding the incidence of dry eye (rate of new or newly diagnosed cases), 13.3% of subjects in the Beaver Dam Eye Study[41] developed dry eye over five years, and 21.6% developed dry eye over 10 years. The effect of age was relatively modest, and no difference between sexes was observed over a five-year period. However, over a 10-year period, the incidence was significantly greater in women (25.0%) than men (17.2%).

II.A.1.c. Natural History of DED

Since the 2007 TFOS DEWS report, there has been little progress in understanding the natural history and progression of dry eye. Progression between severity groups has been assessed in one small-scale study,[42] and is currently under investigation by Allergan. A study by Bron et al. considered the progression of DED, and suggested that over time aqueous-deficient dry eye (ADDE) may develop features of evaporative dry eye (EDE) and vice versa.[43] Overall, there remains an overall paucity of literature in this area.

II.A.1.d. Risk Factors for DED

The second major goal of the TFOS DEWS Epidemiology Subcommittee was to describe the risk factors for DED and grade them by level of evidence (mostly consistent, suggestive, or unclear).[3] The Subcommittee recommended that "future studies of risk factors for dry eye should concentrate on the examination of biologically compelling hypotheses in a detailed fashion, with appropriate attention to all aspects of good epidemiological study design," and that studies not designed to detect risk factors should be interpreted with caution.

Since the 2007 TFOS DEWS report, additional risk factors have been identified. In a study of the long-term incidence of DED, increased incidence was associated with age, female gender, poorer self-rated health, antidepressant or oral steroid use, and thyroid disease untreated with hormone; lower risk was associated with sedentary lifestyles or use of angiotensin-converting enzyme inhibitors.[41] In men (an under-evaluated population), data from the US Physicians' Health Studies suggested that risk factors for dry eye included benign prostatic hyperplasia (BPH), medications used to treat BPH, and antidepressant medications.[20] Patients with DED have also been demonstrated to have a significantly higher prevalence of medical comorbidities (including depression, ischemic heart disease, hyperlipidemia, peripheral vascular disorders, migraines, myasthenia gravis, rheumatoid arthritis, systemic lupus, pulmonary circulation disorders, diabetes with complications, hypothyroidism, liver diseases, peptic ulcers, hepatitis B, psychoses, and solid tumors without metastasis).[44] Taken together, these data suggest that medications used to treat various ocular and systemic disorders may affect ocular surface health.

II.A.2. Review of Clinical Trials of DED

II.A.2.a. Questionnaires

The third major goal of the TFOS DEWS Epidemiology Subcommittee was to review dry eye questionnaires.[3] To assess developments in this area, a literature review was conducted to identify questionnaires used in 1) epidemiologic studies to ascertain cases or study natural history, 2) in screening to identify individuals at risk, and 3) in clinical practice to assess treatment effects or disease

severity. Available data on validation, reproducibility, and responsiveness were assessed. Seven newer questionnaires were identified and summarized in Table 4.

Table 4. Dry Eye Questionnaires (published since 2007)

Description	Reference	Year
A questionnaire-based survey, Kanpur, India	45	2014
Development and validation of the Dry Eye-Related Quality-of-Life Score questionnaire	46	2013
Contact Lens Dry Eye Questionnaire-8 (CLDEQ-8)	47	2012
Comparison of ocular-surface disease index questionnaire, tear film break-up time, and Schirmer tests	48	2012
Development and validation of the impact of dry eye on everyday life (IDEEL) questionnaire	49	2011
Validation of the 5-Item Dry Eye Questionnaire (DEQ-5)	50	2010
McMonnies questionnaire: Rasch analysis	51	2009

The Ocular Surface Disease Index (OSDI)[52] featured prominently among these studies,[45, 50] and minimal clinically important difference (MCID) values have been assessed for various OSDI categories for DED.[53] A newer measure, the Ocular Comfort Index (OCI), uses Rasch analysis to produce estimates on a linear interval scale; it was shown to positively correlate with the OSDI score ($p < 0.0001$) and to negatively correlate with tear film break-up time ($p < 0.0001$), and was able to detect improvement in symptoms of dry eye in individuals before and after treatment ($p < 0.0001$).[54] Also of note was the 5-Item Dry Eye Questionnaire (DEQ-5),[50] in which frequency of watery eyes ($r=0.48$), discomfort ($r=0.41$), dryness ($r=0.35$), and late-day intensity of discomfort and dryness ($r=0.42$, 0.36) all significantly correlated to self-assessed severity. The nature of the items varied widely in terms of the parameter being assessed and response options—e.g., visual analogue scale (VAS) and categorical scales. In these recent reports, there was emphasis on the nature of dry eye symptoms, including irritation symptoms and describing how the eyes feel. Distinct (but equally important) was the domain of vision-targeted, health-related impact of DED, such as effects on visual function and quality of life (QoL). Further refinement is necessary to ensure that surveys are sufficiently sensitive to detect response to treatment.

II.A.2.b. Clinical Trials

To assess the progress in research and development of dry eye treatments since the 2007 TFOS DEWS report, a survey was performed on studies newly registered on ClinicalTrials.gov in 2010.[55] Of the 76 studies identified with the search words “dry” and “eye”, approximately 60 were related to DED (unrelated studies were on age-related macular degeneration). Of the DED studies, most were classified as interventional studies (Table 5), involving topical, oral, or surgical intervention for patient groups with varying degrees of dry eye. Interestingly, of the observational studies that were active in 2010, five were sponsored by companies,[56-60] and one of these was a natural history study of patients with DED.[59] These studies might be considered a direct result of the “call to action” of the 2007 TFOS DEWS report. It is also interesting to note that there were five registered studies of oral omega fatty acid supplementation for dry eye,[61-65] multiple tear lubrication (artificial tear) studies, and several pharmaceutical studies in phase II and phase III. Collectively, this indicates positive growth in the field and active research and development programs in the pharmaceutical companies.

Table 5. Dry Eye Trials Registered on ClinicalTrials.gov (2010)

Primary Outcome(s)	**Number of Trials**
OSDI (n=13) or other Sx	19
Staining	13
Schirmer	6
TBUT	4
Design	
Interventional	48
Observational	9
Phase	
I	3
II	12
III	6
IV	16
Not listed	20
Interventions (n=48)	
Oral	5
Topical	39
Surgical or other	4
Sponsor	
Industry	39
University/Government	18

II.A.3. Summary and Conclusions

Since 2007, significant advances have been made in the global prevalence assessment of DED. Prevalence is approximately 9% based on tests and 22% based on symptoms. Moreover, prevalence is upwards of 30% in Asian populations (over 50% in certain demographics). Recent ten-year incidence data (21%) and risk factor assessments (e.g., antidepressants, other medications, and systemic comorbidities) have refined our existing knowledge base. There has been an overwhelming increase in studies on DED, including clinical trials, since 2007—a testament to the impact of TFOS and DEWS. However, further research is needed on the natural history of DED. Newer surveys need to be validated to ensure that they are able to monitor therapeutic response, and the impact of MGD on survey reporting requires further assessment.

II.B. Surgery-induced DED (José Benitez del Castillo, MD)

Dry eye is a common complication of ocular surgery, and involves a complex interplay between altered corneal innervation and ocular surface tear dynamics. Not only can dry eye affect patient satisfaction, but it also may affect surgical outcome. Dr. Benitez del Castillo reviewed the pathophysiology of surgery-induced dry eye and discussed strategies for prevention and treatment.

II.B.1. Corneal Refractive Surgery

The cornea is the most densely innervated tissue in the body, and refractive procedures are known to cause corneal sensitivity impairment. This may lessen reflex-induced lacrimal secretion and reduce blink rate—thus promoting evaporative loss and inducing or worsening dry eye.[66] Dry eye is a common patient complaint after refractive surgery, as well as a common reason why patients initially seek refractive surgery. Refractive surgery may cause various problems on the ocular surface; conversely, certain ocular surface diseases (including dry eye) may affect refractive surgery results.

II.B.1.a. Pathophysiology of LASIK-induced dry eye

Dry eye is the major cause of patient dissatisfaction following laser-assisted in situ keratomileusis (LASIK), causing frustration for both patients and surgeons.[67, 68] LASIK causes a temporary decrease in ocular surface health, and both tear secretion and corneal sensitivity are reduced three months after surgery, with recovery to normal values at six months postsurgically.[69] Detailed analysis of mechanical, chemical, and thermal sensation using the Belmonte noncontact esthesiometer showed that corneal sensitivity did not recover in many cases until 54 months after surgery, and recovery was slower after hypermetropic LASIK.[70]

Nerve bundles enter the cornea (middle third of the stroma) at the periphery in a radial fashion parallel to the corneal surface, and lose their myelin sheaths within 1 mm of the limbus.[70] Of the corneal nerves, 20% are mechanonociceptors that respond to only mechanical forces; 70% are polymodal nociceptors that respond to chemicals, heat, and inflammatory mediators in addition to mechanical forces; and 10% are cold thermoreceptors that respond to evaporative cooling.[71] It was previously believed that most nerves enter the stroma nasally and temporally,[72] but subsequent studies have suggested that equal numbers of nerves penetrate in all quadrants.[73] Indeed, corneal sensation and dry eye following LASIK are not affected by position or angle of the corneal flap hinge, which disrupts afferent sensory nerve fibers.[74] In patients without previous dry eye, rose bengal staining confined to the flap is likely due to LASIK-induced neurotrophic epitheliopathy (LINE), as Schirmer's test is normal; LINE resolves 6–8 months after surgery.[75]

Corneal nerve alterations have been observed in dry eye of various origins, including Sjögren's syndrome and non-Sjögren's etiologies.[70, 76] The high prevalence of dry eye symptoms after refractive surgery, without dramatic changes in tear secretion, has been suggested to represent neuropathic pain or neurotrophic keratopathy (LINE) rather than actual tear film deficiency.[77] In neuropathic pain, central sensitization becomes an autonomous activity that is independent of the stimulus, and the transition from chronic to neuropathic pain does not have a discrete boundary. Evidence of neuropathic pain after LASIK includes disparity between signs and symptoms; depressed corneal sensitivity to touch; decreased and abnormal corneal nerves on confocal microscopy; unexplained photophobia and blepharospasm; exaggerated pain in response to instilled drops, Schirmer´s testing and airflow incident on the eye; and persistent burning after pharmacologically induced corneal anesthesia.[78] After corneal nerves are cut in refractive procedures, the pathophysiology of neuropathy may be due to invading nerve fibers into the denervated areas, as well as regenerating axons and developing microneuromas, leading to hyperalgesia.

In addition to reduced corneal sensation and tear secretion,[69] LASIK has been shown to reduce tear clearance, with concomitant increases in proinflammatory interleukin-1 concentrations and activity of matrix-degrading enzymes MMP-3 and MMP-9 in tear fluid.[79] Reductions in goblet cell density may also contribute to the pathology of postoperative dry eye. In LASIK, application of the microkeratome suction ring has been correlated with reduced goblet cell density and development of dry eye.[80, 81]

In summary, in LASIK-induced ocular surface disease, corneal nerve alterations are central to a vicious circle of hypo- and hyperstimulation, reduced blink rate, reduced goblet cell density, and tear film instability (comprising reduced tear secretion/clearance and meibomian lipids). The resulting hyperosmolarity may be compounded by cytokines that may further distort sensory information and inhibit mucin production.

II.B.1.b. Dry eye in other corneal refractive procedures

In superficial corneal refractive procedures such as photorefractive keratectomy (PRK) and laser-assisted subepithelial keratomileusis (LASEK), neuronal injury is restricted to the basal epithelial-subepithelial (sub-Bowman's) nerve plexus, and transient sensory loss correlates with ablation depth.[82] It has been suggested that higher postoperative levels of nerve growth factor (NGF) may explain the milder dry eye symptoms and corneal sensory loss observed after PRK compared to LASIK.[83] Small incision lenticule extraction (SMILE) also causes fewer alterations to the ocular surface and corneal innervation than LASIK; accordingly, preliminary results suggest a decreased DED incidence with this procedure.[84] Controlled studies, with large samples and objective methods for measuring and quantifying subbasal nerve density, are necessary to establish the safety of mitomycin C for preventing postoperative corneal haze.

II.B.1.c. Risks and risk factors

The risk factors for developing LASIK-induced dry eye have been examined,[85-87] and reported to be increased in women and Asians, and associated with include high refractive defects, ablation depth, low preoperative Schirmer's test, preoperative dry eye, and long duration of contact lens wear.

Contact lens intolerance due to dry eye is a common reason why patients seek refractive surgery. It has been estimated that more than 75% of patients seeking LASIK have symptoms of dry eye, and while preexisting dry eye does not appear to affect LASIK efficacy, it does predispose patients to severe postoperative dry eye.[88]

Post-LASIK dry eye may affect refractive results. Increased rates of refractive regression have been observed in patients who developed dry eye after LASIK for hyperopia[89] and myopia.[90] Although it is not clear whether there is a causal association between dry eye and refractive regression, proposed pathological mechanisms include epithelial hyperplasia, altered growth factors, and abnormal remodeling.[90]

II.B.1.d. Prevention and treatment

In order to optimize results and minimize dry eye following refractive procedures, several tests and precautions should be taken preceding and following surgery.

A pre-LASIK ocular surface study is recommended, which includes asking the patient about existing dry eye symptoms (including contact lens intolerance). Preferred screening and diagnostic tests for dry eye have been previously reviewed by the 2007 TFOS DEWS Diagnostic Methodology Subcommittee,[2] and

include tear film break-up time (TFBUT), Schirmer's test, slit lamp examination (particularly of Meibomian gland orifices), fluorescein and lissamine green staining, and osmolarity.

Preoperative treatment should aim to achieve the best ocular surface for as long as possible before surgery. Patients should avoid contact lens wear and maintain good lid hygiene. Recommendations for dry eye management, based on disease severity and patient profiles, have been summarized by the 2007 TFOS DEWS Management and Therapy Subcommittee,[4] and preoperative treatment may include artificial tears, steroids, punctal/canalicular plugs, oral tetracycline, 0.05% cyclosporine, and omega 3 fatty acid supplementation. Physicians may also wait six months before excluding patients as LASIK candidates, and if there are no symptoms and examination is normal, offer surgery and continue treatment for at least six months after surgery.

Intraoperative treatment should minimize the use of topical anesthetic. Lubrication should be achieved with substances of low coefficient of friction (e.g., hyaluronate, carboxymethylcellulose 1%, carbomers) rather than high coefficient of friction (balanced salt solution, polyvinylalcohol, carboxymethylcellulose 0.5%, and hydroxypropylmethylcellulose).

Postoperatively, the patient should keep the eyes closed as long as possible during the early hours. Postoperative treatment may be similar to preoperative treatment, and include artificial tears, steroids, punctal/canalicular plugs, oral tetracycline, 0.05% cyclosporine, and omega 3 fatty acid supplementation. Other treatments may include autologous serum, and platelet-rich plasma.

Neuropathic pain after refractive surgery may be treated with autologous serum (with or without lidocaine drops, in doses ranging from 0.01% to 0.1%); pregabalin (Lyrica®), a common treatment for peripheral neuropathic pain, in doses starting at 150 mg/day and increasing to a maximum of 600 mg/day; duloxetine (Cymbalta®), a common treatment for diabetic neuropathy, starting at 30 mg/day and increasing to 120 mg/day, with evaluation at 2 months; and therapeutic contact lenses such as the BostonSight® PROSE (prosthetic replacement of the ocular surface ecosystem).

II.B.2. Cataract Surgery

Cataract surgery may also alter corneal sensitivity and ocular surface dynamics. Deteriorated corneal sensitivity and tear physiology have been observed immediately following phacoemulsification, with recovery times of approximately one month for tear function and three months for corneal sensitivity.[91] The incidence of dry eye after phacoemulsification has been reported to approach 10%.[92]

II.B.2.a. Pathophysiology

The pathophysiology of dry eye induced by cataract surgery involves altered tear turnover. A standardized protocol has been developed for measuring basal tear turnover based on the decay of fluorescein concentration in tears,[93] and in normal subjects, mean tear turnover rate is 17.5%/min +/- 3.4, and declined approximately 0.15%/min for each additional year of age. In patients experiencing dry eye after cataract surgery, investigations have found a reduction in lacrimal flow, decreased TFBUT and Schirmer values, and squamous metaplasia in the lower lid-covered conjunctiva.[94] Alterations in eyelid anatomy and function have also been reported after phacoemulsification. Ptosis has also been reported in patients following phacoemulsification (<1mm ptosis in 30% of patients and 1-2.5mmin 8%), along with altered margin reflex distances, decreasing tear clearance.[95]

II.B.2.c. Risks and risk factors

The risk factors for dry eye induced by cataract surgery are unclear, but may involve speculum time and type (e.g., aspirating vs. nonaspirating speculum).[96] Retrobulbar anesthesia may also be associated with an increased frequency of ptosis. Other risk factors of dry eye following cataract surgery include contact lens intolerance.

Dry eye has also been associated with increased risk of visual complications following cataract surgery. Retinal image quality is reported to be decreased in patients with dry eye following phacoemulsification.[92] Following multifocal intraocular lens implantation, dry eye has been reported to cause 15% of patient complaints of blurred vision and 5% of photic phenomena.[97]

II.B.2.d. Prevention and treatment

Preoperative treatment should aim to protect and improve the ocular surface. Antibiotics and toxic preservatives should be avoided. Additionally, preoperative surveys and tests will aid in selecting good surgical candidates in order to reduce the risk of dry eye following cataract surgery. Risk factors include contact lens intolerance. Good surgical candidates have high TFBUT (>8 seconds), whereas poor candidates have low values (<4 seconds). High tear volume (Schirmer test >10 mm) is also favorable, because if there is no reflex tear production, the patient is not a good candidate. Ocular surface stress tests may uncover underlying pathologies that may inform treatment; if significant keratitis is observed after the application of diagnostic drops in the clinic, this indicates that special therapies may be necessary in the perioperative period. Finally, patients should be alerted to the risks in the informed consent.

Intraoperative treatment should similarly aim to preserve the ocular surface. Anesthetics and other substances used on the ocular surface should be low toxicity, and speculum time should be minimized to reduce associated trauma. Intrasurgical desiccation should be avoided, and incisions should be as small as possible.

Postoperative treatment should aim to avoid further damage to an already-unstable ocular surface. Antibiotic use should be minimized. After cataract surgery, the addition of preservative-free artificial tears containing hydroxypropyl (HP)-Guar to standard treatment has been found to reduce ocular surface inflammation and dry eye compared to standard treatment alone (tobramycin and dexamethasone eye drops);[98] thus, preservative-free artificial tears may be an important postsurgical consideration.

II.B.3. Glaucoma Surgery

Glaucoma surgery represents a different scenario than refractive or cataract surgery. Patients typically have lower expectations and are more accepting of ocular surface discomfort, since eye irritation may arise in response to glaucoma medical treatment. Some glaucoma medications have an adverse effect on the ocular surface, so DED epitheliopathy, subconjunctival fibrosis, and dry eye-like symptoms may be present perioperatively in some patients.

A study comparing the long-term results of filtering surgery using either a limbal-based or fornix-based flap found differences in relation to dry eye symptoms (sicca score).[99] However, if mitomycin C is used, dendritiform inflammatory cells increase in blebs, and the transcellular pathway of the aqueous humor, which has been proposed to involve ocular surface goblet cells, is decreased.[100]

An injured ocular surface appears to decrease success of glaucoma surgery. Lower expression of human leucocyte antigen-DR (a marker of conjunctival inflammation) and higher expression of trefoil factor family 1 and MUC5AC are observed in successful glaucoma surgeries, suggesting less injury and improved healing of the ocular surface in successful surgeries compared to failures.[101] It is likely that long-term use

of topical glaucoma medications may have adverse effects on the ocular surface (and thus on surgical outcomes); however, pressure control is understandably a high priority for glaucoma patients and surgeons.

II.B.4. Summary and Conclusions

Many patients seeking ocular surgery have preexisting dry eye, and surgery may worsen or induce dry eye and alter the ocular surface, thus affecting the patient´s satisfaction. Moreover, ocular surface injury may affect surgical results. Therefore, the ocular surface should be examined preoperatively, because damage is higher if the ocular surface is already abnormal. Careful patient selection and education, combined with preoperative, intraoperative, and postoperative treatment, will improve refractive results and patient satisfaction. The routine addition of tear substitutes to postoperative treatment may improve results and patient satisfaction.

II.C. Contact Lens-associated DED (Mark Willcox, PhD)

The 2013 report of the TFOS International Workshop on Contact Lens Discomfort (TFOS CLDW) provided a critical assessment of the field of contact lens discomfort, and laid the groundwork for further investigation and clinical development.[102] In the current meeting, Dr. Willcox summarized the previous findings of the TFOS International Workshop on Contact Lens Discomfort and reviewed recent advancements.

II.C.1. Epidemiology of Contact Lens Discomfort

The Subcommittee on Epidemiology of the TFOS CLDW found the frequency of contact lens discomfort to be approximately 50% (reported range of ~30% to ~80%), with variations attributed to the populations evaluated, questionnaires used, symptoms evaluated, contact lens types, and care solutions.[103]

Recent studies have identified significant associations between contact lens wear and dry eye. In Singapore, the prevalence of symptomatic DED (12.3%) had a significant association with contact lens wear (OR 2.96, 95% CI: 1.81-4.83).[37] In the Beaver Dam Offspring Study (BOSS), dry eye prevalence (14.5%) was also significantly associated with contact lens wear (2.01; 95% CI, 1.53-2.64).[33]

II.C.2. Definition and Classification of Contact Lens Discomfort

The TFOS CLDW report of the Definition and Classification Subcommittee defined contact lens discomfort as "a condition characterized by episodic or persistent adverse ocular sensations related to lens wear, either with or without visual disturbance, resulting from reduced compatibility between the contact lens and the ocular environment, which can lead to decreased wearing time and discontinuation of contact lens wear."[104] Indeed, discomfort is the leading reason for contact lens dropout, accounting for approximately 50% of discontinuations in an international web-based survey.[105]

The Definition and Classification Subcommittee categorized contact lens discomfort into two major subclasses, which were further subdivided based on potential contributing factors (Figure 1). This classification scheme provides a framework for assessing the etiology and pathophysiology of contact lens discomfort, discussed below.

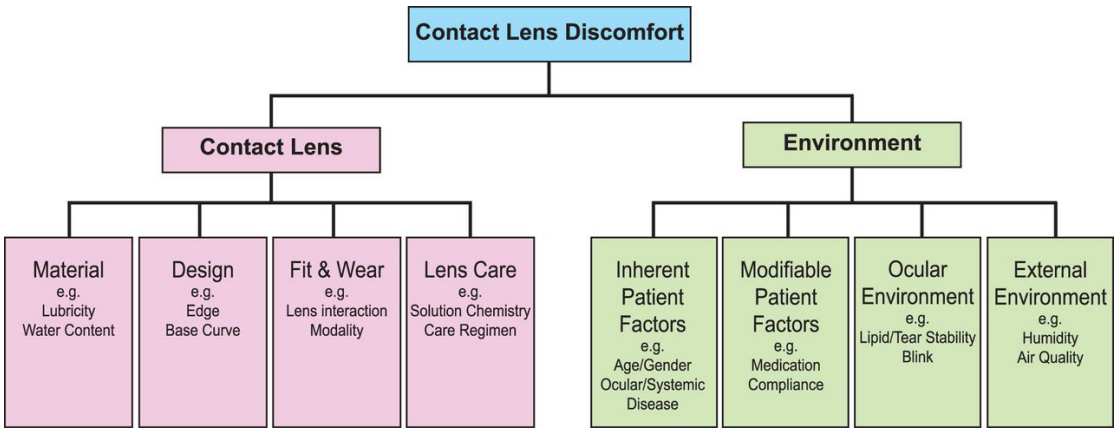


Figure 1. Classification of contact lens discomfort. Reprinted from Nichols KK, Redfern RL, Jacob JT, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the definition and classification subcommittee. *Invest Ophthalmol Vis Sci* 2013;54:TFOS14-19. Copyright: Association for Research in Vision and Ophthalmology.

II.C.3 Etiology and pathophysiology

Based on the previously described classification scheme (Figure 1), the etiology and pathophysiology of contact lens discomfort are interrelated with the characteristics of contact lenses (including materials, design, fit and wear, and lens care systems) or the contact lens environment. The latter includes the ocular environment (structures of the eye and adnexa, tear film, and associated glands), patient factors (inherent or modifiable), and the external environment.

II.C.3.a. Contact lenses and lens care products

Contact lens characteristics that may contribute to discomfort have been reviewed in detail in the TFOS CLDW report of the Contact Lens Materials, Design, and Care Subcommittee.[106] These included water content, surface friction, lens fit, tear film deposits, time of day, and lens care products. Among the attributes of contact lens materials, friction was the sole factor that appeared to be associated with comfort. It was also unclear whether care solutions were associated with comfort levels, although wearing lenses on a daily disposable basis has been reported to increase the comfort of lenses when compared to using them on a daily wear basis with care solutions. Other factors affecting contact lens comfort were associated with design (e.g., size, shape, and contour of lens edges).

A recent study found almost no correlation between contact lens-associated dry eye with compliance factors related to aspects of lens care (replacement of lenses, lens cases, and solutions) or wear (e.g., sleeping with lenses), even despite overall low compliance.[107] Another recent study found no relation between contact lens replacement during the day and discomfort.[108] These findings highlight the need for further research on etiological factors other than compliance or lens replacement, including stimulation of ocular surface tissues or nociceptors by the contact lens.

II.C.3.b. Contact lens interactions with the ocular surface & adnexa

The TFOS CLDW report of the Contact Lens Interactions with the Ocular Surface & Adnexa Subcommittee examined the impact of contact lenses on the ocular surface, with an emphasis on contact lens discomfort.[109] There was insufficient evidence to support a relation between contact lens discomfort with limbal vessel engorgement (redness), limbal stem cell deficiency, and corneal staining. However, there was some evidence of more frequent contact lens discomfort in patients with bulbar conjunctival staining, and lid wiper epitheliopathy (LWE) appeared to be the most likely cause of discomfort. Lid parallel conjunctival folds also had a positive correlation with dry eye in contact lens wearers, with possible similar mechanical and friction-related etiologies as LWE. Palpebral roughness and lid sensitivity were also found to have a possible correlations discomfort. CL wear may have a causative association with meibomian gland dysfunction (MGD).

Recent studies have examined a possible link between *Demodex* infestation (eyelash mites) and dry eye, but while contact lens wearers were found to harbor higher numbers of *Demodex* than non-wearers, there was no association with either dry eye or contact lens discomfort.[110, 111]

II.C.3.c. Contact lens interactions with the tear film

The TFOS CLDW report of the Contact Lens Interactions with the Tear Film Subcommittee determined that contact lens discomfort was associated with several tear variables, including lipid layer integrity and spread, tear film stability, evaporation, and tear volume.[112]

A major hurdle identified by the Subcommittee, with regard to drawing conclusions about biochemical changes in the tear film, has been the inconsistency in tear collection methods used, which has resulted in differences in the tear types collected.[112] For example, more invasive techniques induce more reflex tearing than less invasive methods. Nonetheless, the Subcommittee found that the most likely lipidomic tear film changes are to the polar lipid component, where reduced phospholipid levels possibly due to increased secretory phospholipase 2 (sPLA2) activity likely contributes to contact lens discomfort, possibly through an effect on tear film lipid layer integrity. The available literature failed to demonstrate consistent changes in the tear proteome with contact lens wear, with the possible exception of increased lipocalin-1 in contact lens intolerance. Cytokine levels may also be increased with contact lens wear, but variations in collection method have resulted in a wide range of cytokine levels being reported in different studies, confounding the results, and it was not known whether cytokine levels were associated with contact lens discomfort. There also appeared to be an inconclusive relationship between contact lens intolerance and mucins, though there was a possible relation with the pattern of mucin degradation.

A recent study examined cytokine changes in tears and their relationship to ocular discomfort with or without contact lens wear.[113] Ninety participants were divided into two groups (with or without contact lenses) and asked to rate ocular comfort and collect their tears (morning and night) for 10 days. Of the cytokines examined, vascular endothelial growth factor (VEGF), best known for its role in angiogenesis, was the only factor that positively associated with greater ocular discomfort; however, this was not affected by contact lens wear.

II.C.4. Management and Therapy of Contact Lens Discomfort

The aim of the Management and Therapy Subcommittee of the TFOS CLDW was to develop an evidence-based regime to alleviate contact lens discomfort.[114] As data were often equivocal, evidence was categorized based on objective criteria (Table 6) adapted from the American Academy of Ophthalmology Practice Guidelines and were used in previous TFOS reports.[1] The approaches that were assessed (with corresponding level of available evidence) are summarized in Table 7.

Table 6. Grading Level of Evidence of Clinical and Basic Research Studies Clinical studies

	Clinical Studies	Basic Science
Level I	Evidence obtained from at least 1 properly conducted, well- designed randomized controlled trial or evidence from studies applying rigorous statistical approaches	Well-performed studies confirming a hypothesis with adequate controls published in a peer-reviewed journal
Level II	Evidence obtained from 1 of the following: • Well-designed controlled trial without randomization • Well-designed cohort or case-control analytic study from 1 (preferably more) center(s) • Well-designed study accessible to more rigorous statistical analysis	Preliminary or limited published study
Level III	Evidence obtained from 1 of the following: • Descriptive studies • Case reports • Reports of expert committees • Expert opinion • Meeting abstracts, unpublished proceedings	Meeting abstracts or unpublished presentations

Adapted from: Papas EB, Ciolino JB, Jacobs D, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the management and therapy subcommittee. *Invest Ophthalmol Vis Sci* 2013;54:TFOS183-203.

Table 7. Summary of Evidence Supporting Various Potential Management Strategies for CLD

Treatment Strategy	Level of Supporting Evidence		
	I	II	III
Adjust replacement frequency		†	
Change material		*	
Add internal wetting agents		*	
Add external wetting agents		†	†
Eliminate the care system		†	
Alter lens design factors	†	‡	†
Change the care system		†	
Use tear supplements, wetting agents, lacrimal inserts		†	
Dietary supplementation (omega-6 FAs/evening primrose oil)	†		
Punctal occlusion		†	†
Topical medication (azithromycin)		†	
Improve environment			†
Alter blink behavior		*	
Switch soft to RGP lens		*	
Switch RGP to soft lens		†	
Orthokeratology		*	
Refractive surgery		*	
Spectacles			†, ‡

* Insufficient or contradictory evidence
† Soft contact lenses
‡ Rigid contact lenses
Blank cells indicate no data available.
Adapted from: Jones L, Brennan NA, Gonzalez-Meijome J, et al. The TFOS International Workshop on Contact Lens Discomfort: report of the contact lens materials, design, and care subcommittee. *Invest Ophthalmol Vis Sci* 2013;54:TFOS37-70. See source for details.

II.C.4.a. Management strategies related to contact lenses and care systems

According to the TFOS CLDW report of the Subcommittee on Management and Therapy,[114] evidence supports adjusting contact lens replacement frequency by using daily disposables, and the mechanism may be elimination of the care system. Changing the care system—namely, removing multi-purpose

disinfecting solution (MPDS), appears to be supported by evidence. Wetting agents (packaging solution additives or pre-conditioning treatments) have short-term benefits evident early in the wearing cycle.

Mildly favorable evidence supports changing lens material, though additional well-designed studies were called for. Altering lens design was supported by varying levels of evidence (depending on the particular attribute); however, a practical issue with this approach is the difficulty in manipulating individual parameters due to design component interdependence, and unless lenses are custom made, control may lie with manufacturer.

II.C.4.b. Management strategies related to contact lens environment

Tear supplementation (eye drops and wetting agents) were widely regarded as mainstay of treatment, and clinical benefit is generally evident in trials (with more-recent studies tending to favor complex solutions over saline alone).[114] Data have since emerged that degraded lipids and a wax esters in the tear film may be associated with a lower non-invasive surface drying time (NISDT), suggesting that lipid supplements may alleviate contact lens discomfort by increasing NISDT.[115]

In the TFOS CLDW report,[114] there was generally little evidence supporting topical medications, with only one relevant study supporting the use of azithromycin.[116] Two studies on cyclosporine produced conflicting results,[117, 118] and potential risks do not justify the use of steroids or anesthetics (especially long-term). Data existed on non-steroidal 0.1% diclofenac for reducing post-fitting adaptation time for rigid gas-permeable lenses.[119]

The TFOS CLDW report of the Subcommittee on Management and Therapy found support for dietary supplementation with omega-6 fatty acids (evening primrose oil), but evidence was lacking for hydration and omega-3 fatty acid supplementation.[114] However, recent data on omega-3 fatty acid supplementation showed improved symptom scores and increased tear break-up time compared to placebo (corn oil).[120]

Concerning punctal occlusion, the balance of evidence suggested that increased tear volume is beneficial, and that silicone plugs are superior to dissolvable collagen, and that both upper and lower occlusion was better than lower lid alone.[114]

II.C.4.c. Summary of management strategies for contact lens related discomfort

The management strategies for contact lens related discomfort may be summarized as follows: 1) determine the most likely cause, 2) identify corresponding treatment strategy, and 3) stepwise (additive) application of treatments to achieve maximum effect.

II.C.5. Summary and Conclusions

The 2013 report of the TFOS CLDW formed the basis for further investigation and clinical development.[102] Significant momentum is evident in several studies that have since been published, which will inform further investigations on contact lens-associated discomfort and DED.

III. CLINICAL CHALLENGES OF DED

III.A. Pathophysiology of DED (Anthony Bron, FRCOphth, FMedSci)

The 2007 TFOS DEWS Report comprehensively assessed the available literature on the pathogenesis and natural history of DED, and provided a framework for further investigation.[1] Dr. Bron summarized significant multidisciplinary developments since the publication of the DEWS Report and synthesized the current understanding of the pathophysiology of DED.

III.A.1. Tear Physiology

III.A.1.a. Tear film composition

The human tear film has been described in detail in the 2011 report of the TFOS International Workshop on Meibomian Gland Dysfunction (MGDW),[121] and comprises multiple layers that overlie the ocular surface, as well as many tear components that maintain ocular surface health (Figure 2).

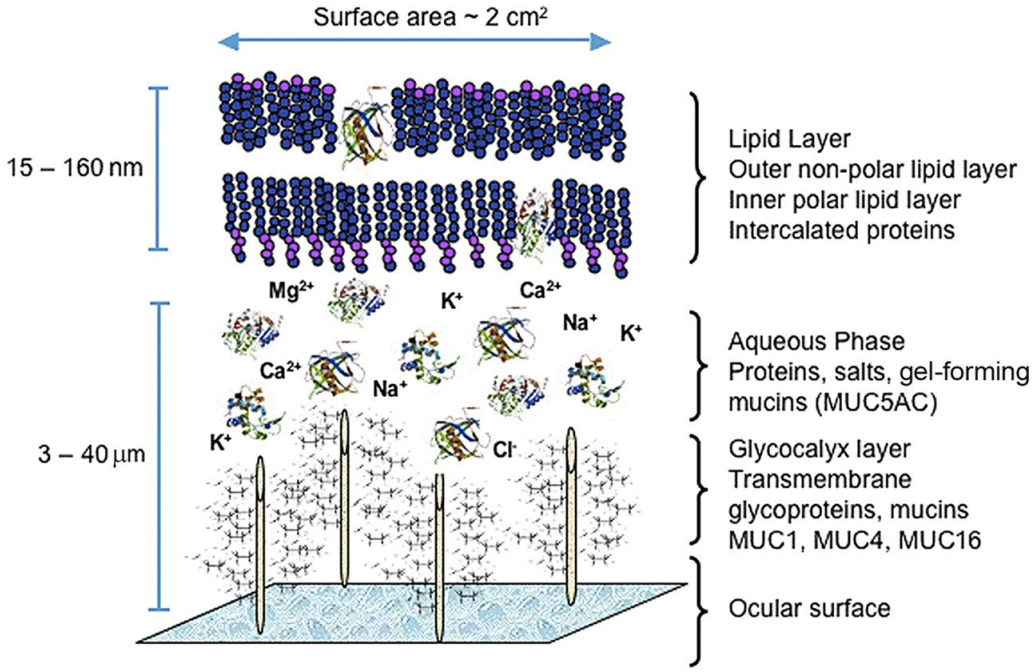


Figure 2. The proposed composition of the human tear film. Reprinted from The International Workshop on Meibomian Gland Dysfunction. *Invest Ophthalmol Vis Sci* 2011;52:1917-2085. Copyright: Association for Research in Vision and Ophthalmology.

The lacrimal gland produces the aqueous layer (the largest proportion of the tear film) and various growth factors therein, including epidermal growth factor (EGF), hepatocyte growth factor (HGF), nerve growth factor (NGF), and retinol.[122-124] The lacrimal gland also releases the defense proteins, lysozyme, lactoferrin and lung surfactant protein D.[124] Plasma cells within the gland produce IgA, which is delivered into the tears as secretory IgA (sIgA).

The ocular surface epithelia express glycocalyx mucins, which ensure the wettability of the epithelium.[125, 126] Transmembrane mucins, expressed by the corneal epithelium (MUC1, MUC4, and MUC16) or the conjunctival epithelium (MUC1, MUC2, and MUC 4), are major components of the glycocalyx, reinforced by the presence of galectin-3. Conjunctival goblet cells secrete MUC5AC into the tears,[126] and the functions of this gel-forming mucin include lubrication, surface tension lowering, antimicrobial actions, and scavenging of reactive oxygen species (ROS). Lubricin (proteoglycan-4), a boundary surface lubricant

that is also produced by ocular surface epithelia, serves to decrease shear stress generated during blinking and eye movements.[7]

The meibomian glands are the primary source of tear film lipids, which retard evaporation by 8–10%.[127, 128] The tear film lipid layer (TFLL), described in the 2011 report of the TFOS MGDW Subcommittee on Tear Film Lipids and Lipid-Protein Interactions in Health and Disease,[129] is a highly stable film consisting of 3–8 monolayers (Figure 2). The outer non-polar layer is composed of wax and sterol esters, and triglycerides,[130] whereas the deep polar layer is mainly (O-acyl)-omega-hydroxy fatty acids (OAHFAs) and phospholipids, though the exact composition is controversial.[131] These polar lipids, interfacing with the aqueous phase of the film, facilitate the rapid spread of the lipid film over the ocular surface with each blink. The viscoelastic tear film stabilizes within 1-2 seconds of the blink, ensuring the low turnover of the TFLL (approximately 10% of the turnover rate of the aqueous layer).[132] Lipocalin, a lacrimal lipid-binding protein, likely stabilizes this interface. Alterations in the physical properties of meibomian lipids may disrupt the structure of the TFLL, as seen in MGD,[133] discussed later.

III.A.1.b. The lacrimal functional unit

The ocular surface, lacrimal system, meibomian glands, and neural interconnections are components of the lacrimal functional unit.[134] This integrated unit, which maintains ocular surface homeostasis and is central to the pathophysiology of aqueous-deficient dry eye,[135] was discussed in detail the 2007 report of the TFOS DEWS Definition and Classification Subcommittee[66] and reviewed by the TFOS CLDW Report.[109] Sensory impulses from the ocular surface regulate secretions (lacrimal, conjunctival, and possibly Meibomian). Blink action is supported by central nervous, sympathetic, endocrine, and immunological influences. This unit promotes tear film stability, corneal transparency, and accurate retinal image formation.

III.A.1.c. The tear meniscus

In the interblink, the tears reside in three compartments: the fornical sac, the tear menisci and the tear film. The tear menisci form as a result of surface tension and separate from the tear film immediately after the blink. In the presence of fluorescein, this separation is seen as a black line (Figure 3).[136] Fornical tears drain into the menisci and then into the nasolacrimal duct; importantly, tears are also lost by evaporation. Because meniscus volume reflects both total tear volume and lacrimal secretion rate, meniscus parameters (such as radius and curvature) are used in the diagnosis of DED.

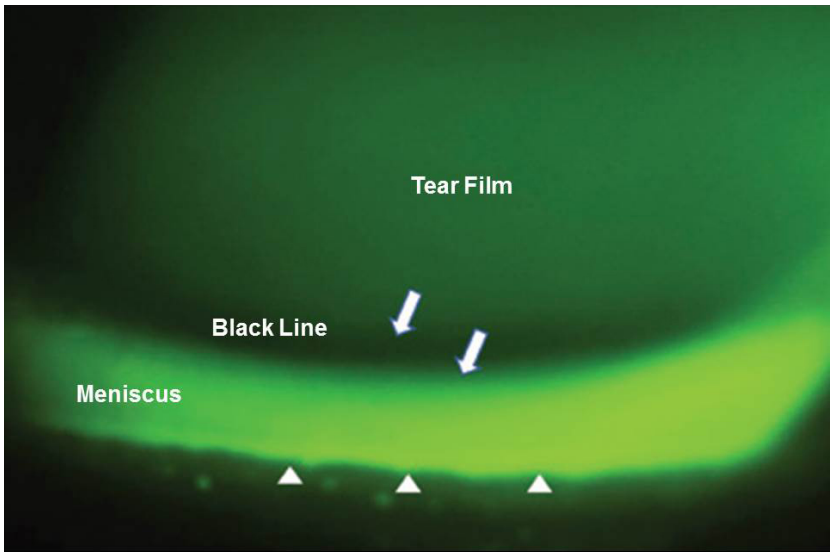


Figure 3. Fluorescein-stained, lower tear meniscus with arrowheads indicating the mucocutaneous junction at the peripheral apex of the meniscus, and arrows indicating peripheral and central boundaries of the "black line" above the central apex of the meniscus. Adapted from Bron AJ, Yokoi N, Gaffney EA, Tiffany JM. A solute gradient in the tear meniscus. I. A hypothesis to explain Marx's line. Ocul Surf. 2011 Apr;9(2):70-91.

III.A.2. Central Mechanisms of DED

The pathological mechanisms of DED may have different etiologies, depending on the disease subtype,[124] but their central mechanisms converge and are interrelated. This is reflected in the definition of DED and its classification. The key features of dry eye were outlined in the 2007 report of the TFOS DEWS Definition and Classification Subcommittee as hyperosmolarity and inflammation at the ocular surface.[66] Other mechanisms, occurring in parallel, may contribute to dry eye and complicate the disease state.

III.A.2.a. Hyperosmolarity

Tear osmolarity relates to plasma osmolarity and is modulated by evaporation and tightly regulated by blink rate and tear secretion.[124] Thus, the lacrimal functional unit is central to the homeostasis of tear osmolarity. Tear hyperosmolarity may result from reduced aqueous tear flow (a consequence of lacrimal failure) and/or increased evaporation from the tear film. Environmental conditions that favor evaporative loss include low humidity and high airflow; physiological conditions include MGD and resulting instability of the TFLL.

As illustrated in Figure 4, tear film instability and tear hyperosmolarity drive the core mechanisms of dry eye.[137] Importantly, this implies hyperosmolarity of the exposed ocular surface cells, with aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE) as the major disease forms. This definition is a practical convenience; since evaporation is the basis of hyperosmolarity in both forms; lid closure or prevention of evaporation in theory should reverse all those features of DED due to tear hyperosmolarity. However, while it is a core mechanism, hyperosmolarity does not preclude etiological factors such as preservative toxicity, or direct inflammatory attack of the ocular surface in Sjögren's syndrome, from inducing ocular surface damage, leading to tear film breakup and exacerbating tear hyperosmolarity. Baudouin and colleagues have stressed that tear hyperosmolarity leads to a "vicious circle" of inflammatory events so that the disease becomes self-perpetuating.[138] The resulting autonomous state might not respond to efforts to prevent evaporation, and might require other measures to reverse.

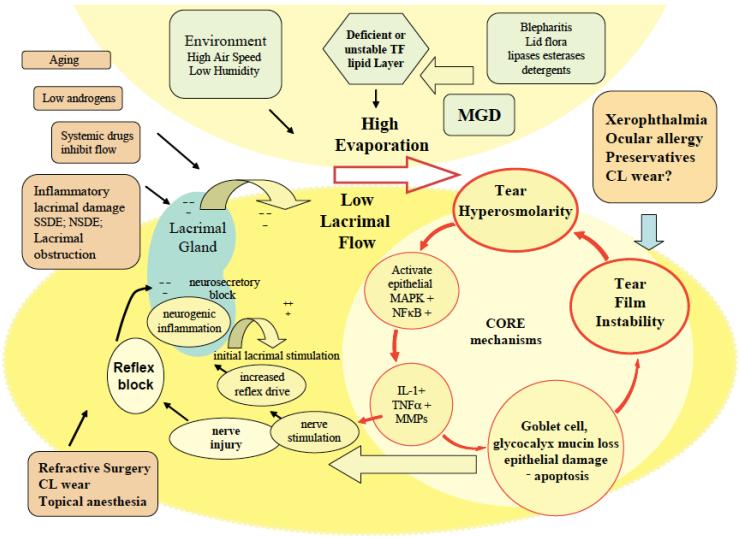


Figure 4. Mechanisms of dry eye. Reprinted with permission from DEWS Definition and Classification Subcommittee. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf* 2007;5:75-92.

Recent evidence suggests that spatial and temporal variations in tear osmolarity may affect tear film stability, and the magnitude of these variations may in turn vary between normal and symptomatic eyes. Local increases in evaporation, resulting from prolonged interblinks or environmental factors (such as relative humidity and wind speed), drive tear film breakup, and massive increases in osmolarity are predicted at the center of tear film ruptures.[133] Spots of ocular surface cooling, assessed by infrared thermography, colocalize with tear film breakup assessed by fluorescein instillation, and reflect local increases in evaporation.[139] Mathematical modeling of the mass and solute balance of the tears, suggests that, in healthy eyes, osmolarity is higher in the tear film than in the menisci, but that the differential is much greater in DED.[140] Higher tear osmolarity and greater variation over time are also observed in patients with dry eye symptoms compared to normal subjects.[141] Inter-eye variation in tear osmolarity, which was demonstrated earlier to be greater in DED,[142] has recently been found to correlate significantly with increasing disease severity.[143]

III.A.2.b. Inflammation

As outlined in the 2007 report of the TFOS DEWS Definition and Classification Subcommittee,[66] damage to the ocular surface epithelium, driven by tear hyperosmolarity and other etiological factors, activates an inflammatory cascade at the ocular surface, which in turn releases inflammatory mediators into the tears. Epithelial damage can also affect the goblet cells and the epithelial cells that produce glycocalyx mucin, causing tear film instability and further exacerbating hyperosmolarity. Various etiologies, including xerophthalmia, ocular allergy, topical preservatives, and contact lens wear, may also trigger tear film instability independently of tear hyperosmolarity.

In recent years, there has increasing evidence that ocular surface stress leads to infiltration by autoreactive T-cells. Experimental models of desiccating stress have demonstrated that infiltrating autoreactive T-cells are involved in the immunopathogenesis of murine DED.[144-148] In brief, desiccating stress alters epithelial cytokine expression (e.g., inducing IL-1, TNFα and IL-6) to favor maturation of antigen-presenting cells. These migrate to the regional lymph nodes, where they engage with naïve T lymphocytes to stimulate a T helper (Th) cell response. Meanwhile, at the ocular surface, IFNγ not only induces apoptosis and squamous metaplasia of ocular surface epithelia, but also upregulates chemokines and adhesion molecules (CAMs) such as ICAM-1. IFNγ and ICAM-1 may also underlie the induction of the cornified envelope proteins responsible for squamous metaplasia.[149]

Recent studies have examined the correlation between inflammatory mediators and parameters of the tear film and ocular surface, yielding insight into the diagnostic potential of cytokine and chemokine profiles. Yoon et al.[150] observed increased expression of IFNγ-inducible chemokines CXCL9, 10, 11, and CXCR3 in the tear film and ocular surface of patients with DED, particularly in patients with Sjögren's syndrome vs. non-Sjögren's dry eye. CXCL11 levels, in particular, correlated significantly with parameters such as low basal Schirmer values, low tear clearance, kerato-epitheliopathy, and reduced goblet cell density. Lam et al. examined cytokine profiles in dysfunctional tear syndrome using a Luminex bead assay,[151] and found that many of the cytokines that are elevated in dry eye are also markers for ocular surface inflammation in seasonal allergic conjunctivitis (SAC), vernal keratoconjunctivitis (VKC), and atopic keratoconjunctivitis (AKC). Increases in interleukins (IL 1α, 1β, 6, 8), IFNα, and MIP-1α correlated with DEWS severity grade, and EGF levels correlated positively with Schirmer values and inversely with corneal staining. IL-6 showed the greatest correlation with clinical severity (signs and symptoms). Moreover, the ratio between IFNγ (marker for Th-1 inflammation) and IL-13 (marker for Th-2

inflammation) was increased in dry eye, correlating with goblet cell loss and metaplasia in a dry eye model.

III.A.2.c. Symptoms

The central mechanisms of DED are important causative factors in symptomology. Tear hyperosmolarity, which may be diffuse,[152] or focal when accompanying tear break up,[133] is a potent source of irritation and pain. Reduced lubrication,[153] due to loss of mucin gel and glycocalyx (and fluid in ADDE), is another. This may be compounded by lid wiper epitheliopathy,[154] or by parallel conjunctival folds (LIPCOF).[155] Inflammatory mediators may stimulate nociceptors directly or lower the threshold of pain-sensitive neurons, resulting in hyperesthesia.[156] Sensory nerve damage also may lead to chronic, neuropathic firing.[157] While the highest density of sensory innervation is at the center of the cornea (over 10 times that of dental nerve pulp), the posterior lid margin mucosa has similar sensitivity, and could be an important source of symptoms.[158, 159] However, it is unclear what happens to lid margin sensitivity through the course of any form of DED.

Dry eye symptoms may not only derive from multiple sources, but may also change during the evolution of the disease. For example, it is possible that inflammatory events release algesic mediators to cause early symptoms. Corneal hypersensitivity may occur both in early and advanced disease. Alterations in the surface glycocalyx, a loss of surface wettability, and loss of goblet cell mucin may result from inflammation; combined with insufficient aqueous volume, these events are potential contributors to shear-related symptoms. Current tests of pathology may be insufficient to delineate the spatio-temporal complexity of these events, which may explain why correlations between signs and symptoms are so poor or variable. In the clinical management of DED, an unmet need is the development of biomarkers that correlate with symptoms, which may provide more successful correlations of signs and symptoms in the future. On the other hand, biomarkers that do not correlate may instead characterize other features of the disease, such as inflammation and may confirm intrinsic mechanisms.

III.A.3. Some Etiologies and Mechanisms of DED

III.A.3.a. MGD

The anatomy, physiology, and pathophysiology of the meibomian gland were discussed in detail in the 2011 TFOS MGDW report.[160] Briefly, secretion from meibomian gland occurs under the constant force of multiple holocrine secretory acini, and contraction (during lid movement) of Riolan's muscle drives the delivery of meibomian oil onto the lid margin and tear film. In MGD, quantitative reduction of, and chemical changes in, meibum lipids and proteins occur; moreover, there is a loss of the polar lipid, OAHFA, which is critical for the interaction of the TFLL with the aqueous subphase.[129] Thus, the TFLL loses its structure, becoming more heterogeneous, and thinner. Spreading of the TFLL and subsequent stabilization are both impaired. This is accompanied by increased permeability, and the aforementioned hotspots of evaporation and hyperosmolarity.[133] Noninvasive meibography has shown that meibomian gland loss increases with age.[161] Tear film interferometry has demonstrated vertical patterns in the TFLL in MGD, in contrast with horizontal in normal patients.[162]

According to the TFOS MGDW Definition and Classification Subcommittee, MGD is classified into two major categories based secretion: low-delivery and high-delivery states, the latter being less well characterized. Low-delivery MGD is further classified as hyposecretory, or obstructive. In the non-cicatricial form of obstructive MGD (see below), the key pathological event is occlusion of terminal duct due to hyperkeratinization, leading to progressive ductal dilatation and acinar atrophy. Whether inflammatory events lead to hyperkeratinization is speculative at the moment. We have proposed that Marx's line may provide a route by which inflammatory mediators gain access to the terminal duct and

stimulate the production of cornified envelope proteins that are a part of the keratinization process.[136] A similar mechanism may explain the forward movement of Marx's line and the mucocutaneous junction with age and its relation to MGD.[163]

Obstructive MGD is further subcategorized as cicatricial or noncicatricial.[164] In noncicatricial MGD, meibomian orifices are in their normal anatomic positions, anterior to the mucocutaneous junction (MCJ) and Marx's line, whereas in cicatricial MGD the ducts and orifices are dragged posteriorly across the MCJ into the mucosa of the lid margin and tarsal plate. Occlusion occurs by a cicatricial process. This form may occur in conjunction with the non-cicatricial form but is particularly associated with scarring conjunctival disease, such as mucous membrane pemphigoid, Stevens-Johnson syndrome, and trachoma.

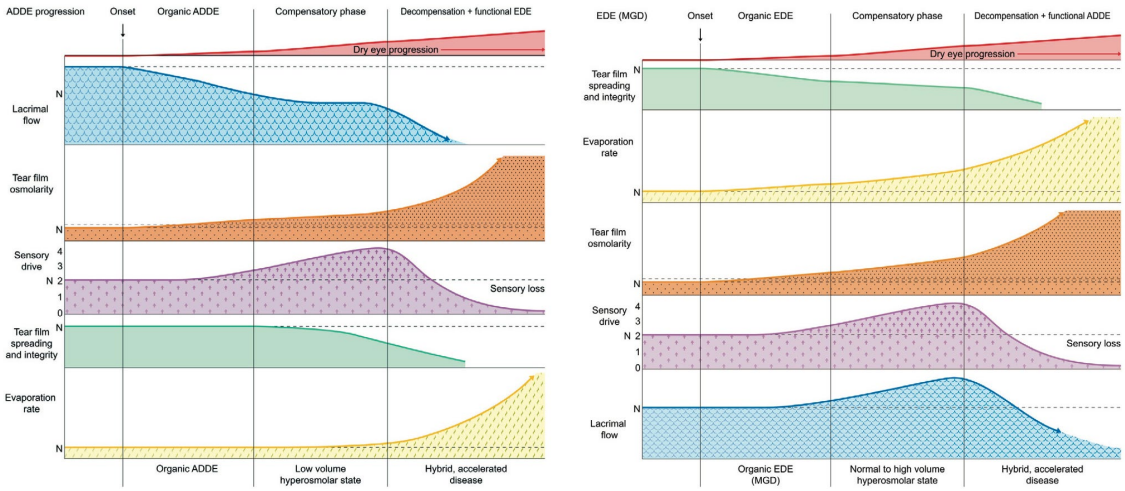
While it remains unclear whether inflammation in non-cicatricial MGD is a cause or a result of meibomian gland obstruction, IFNγ, as mentioned earlier, may underlie the induction of the cornified envelope proteins responsible for squamous metaplasia, contributes to terminal duct obstruction in MGD.[149] The cornified proteins calgranulin A and B (also termed S100A8 and S100A9, and together calprotectin), which have roles in innate immunity, barrier functions, and stress signaling, have been shown in proteomic studies to correlate with MGD severity signs or redness, and visual blur.[165] These tear film proteins may originate, at least in part, from the meibomian gland, given that their gene expression is significantly increased in this tissue in human MGD.[166]

III.A.3.b. Reflex block

As discussed by the 2007 report of the TFOS DEWS Definition and Classification Subcommittee,[66] epithelial injury in DED stimulates corneal nerve endings, leading to discomfort, increased blinking and compensatory reflex lacrimal tearing. It has been suggested that a high reflex input underlies neurogenic inflammation within the gland, which not only causes tissue damage but also a reflex secretory block. A receptor block may be also caused by circulating antibodies to the M3 receptor. Chronic surface damage results in reductions in corneal sensitivity and reflex tear secretion. The loss of sensory reflex drive from the ocular surface to the lacrimal gland may reduce tear delivery. Reflex secretory block may also underlie the pathology of eye disease caused by ocular surgery, contact lens wear and certainly occurs with the chronic use of topical anesthetics. Indeed, Jordan and Baum showed in 1980 that bilateral topical anesthesia (i.e., total loss of ocular sensory drive) reduces tear secretion.[167] However, bilateral topical anesthesia causes a reduction in blink rate,[168] similar to the significant fall in blink rate following refractive surgery, which may persist for several months after surgery.[88] It is likely that trigeminal inputs feed centrally to reflex centers that regulate lacrimal secretion and blinking, and to cognitive centers that mediate pain and other sensory modalities.

III.A.3.c. Lacrimal inflammation

DED, from various etiologies, is conveniently classified according to whether there is insufficient lacrimal secretion (ADDE) or excessive evaporative loss (EDE). However, despite this established nomenclature, both forms of DED have a component of evaporative loss; i.e., almost all dry eye is evaporative, with MGD as the archetypal cause. DED also may involve a combination of ADDE and EDE,[43] as illustrated in Figure 5. Indeed, in a 2011 multicenter study of 299 subjects in USA and Europe, [143] 224 were designated as having DED; and using a cut-off of < 7 mm Schirmer for ADDE and an MGD score cut-off of >5, 79 subjects were considered pure MGD-EDE, 23 pure ADDE, and 57 to have a combination of MGD with ADDE. The ratio of pure EDE to pure ADDE was found to be 3:1, and could have been as high as 7:1 if the Schirmer cut-off was reduced to 5 mm or lower.



A) B)

Figure 5. Predicted natural history of A) aqueous-deficient dry eye (ADDE) for a typical etiology such as non-Sjögren's syndrome dry eye, and B) (EDE) for a typical etiology, such as meibomian gland dysfunction (MGD). Clinical events are represented on the left, and time progresses toward the right (top row). Reprinted with permission from Bron AJ, Yokoi N, Gafney E, Tiffany JM. Predicted phenotypes of dry eye: proposed consequences of its natural history. Ocul Surf. 2009;7:78-92.

Feedback events via the lacrimal functional unit raise the possibility that in advanced DED, organic dry eye states may be modified by an added functional component (Figure 5). Thus it is suggested that in aqueous-deficient dry eye, defective spreading of the TFLL adds an evaporative (EDE) component to the ADDE. In EDE on the other hand, it is proposed that ocular surface damage caused by tear hyperosmolarity blocks the compensatory reflex sensory drive to the lacrimal gland, thereby adding an aqueous-deficient component. In this way, hybrid dry eye states could occur.[43]

Lacrimal gland inflammation, involving infiltration of T-cells followed by B-cells and plasma cells,[169] could compound meibomian terminal duct obstruction. Inflammatory products of the lacrimal gland or lid margin could modify the surface phenotype and induce cornified envelope proteins responsible for squamous metaplasia, contributing to terminal duct obstruction.[149] This is supported by the proteomic correlation of calgranulin A and B with MGD severity.[165] Thus, it is possible that inflammation is a key mechanism of keratinization process, via production of cornified envelope proteins.[136]

Inflammatory lacrimal damage is a key pathological mechanism in systemic autoimmune disorders such as Sjögren syndrome, as well as in non-Sjögren syndrome dry eye (NSSDE). In Sjögren syndrome, described by the TFOS DEWS Definition and Classification Subcommittee,[66] the immune system targets the lacrimal and salivary glands, giving rise to dry eye and dry mouth and affecting several other organ systems. In Sjögren syndrome (and to a lesser extent NSSDE), the lacrimal gland is infiltrated by T-cells, predominantly CD4+ but also cytotoxic CD8+ T-cells; these cluster around acinar epithelial cells and induce apoptosis.[169] Although primary Sjögren syndrome has been considered to be a T-cell-driven autoimmune disorder, B-cells also play a key role. IFNα, overexpressed in the salivary gland, may induce the production of B-cell activating factor (BAFF) by infiltrating monocytes, dendritic cells and resident epithelial cells.[170] Immune responses to viral triggers, as well as genetic and hormonal influences, may also contribute to inflammatory lacrimal damage.

III.A.4. Summary and Conclusions

Hyperosmolarity is a measure of DED severity and distinguishes it from other ocular surface disorders. A combination of hyperosmolarity and inflammation leads to keratitis, loss of goblet cells and epithelial glycocalyx, with loss of lubrication. Initiating events in DED include ocular surface alterations, meibomian dysfunction, lacrimal dysfunction, or a combination of all three; whatever the cause, hyperosmolarity is amplified by tear film breakup within the blink interval and further exacerbated later, by loss of compensatory feedback. There is an urgent need to improve the understanding of TFLL properties and its role in stabilizing the tear film and preventing excessive evaporation. Additionally, newer concepts in TFLL barrier properties may lead us to reevaluate our understanding of ADDE and EDE. Also needed is a full description of the natural history of dry eye, from hyperosmolarity to the initiation of inflammation and its self-perpetuation. Finally, as correlations continue to be poor between signs and symptoms, reliable biomarkers are urgently needed, to improve the monitoring and management of DED.

III.B. Symptomatology of DED: The origin of discomfort (Carlos Belmonte, MD, PhD)

Symptoms of discomfort are integral to the definition of DED.[66] However, according to the 2007 TFOS DEWS report, "the basis for symptoms in dry eye is not truly known but may be surmised from a consideration of the etiologies, mechanisms, and responses of dry eye to therapy." Activation of nociceptors, which form the largest class of corneal sensory neurons, was believed to underlie the symptoms of DED. Since the 2007 report, a distinct class of cold thermoreceptors has emerged as an additional key modulator of ocular surface sensations and tearing. Dr. Belmonte discussed the functional roles of corneal sensory nerves, their altered responses in ocular surface pathology, and their relation to the symptoms of dryness, discomfort, and pain in DED and other ocular surface pathologies.

III.B.1. Functional Roles of Corneal Innervation

In the human body, the cornea contains the highest density of sensory nerve endings of any surface tissue. In normal conditions, a variety of sensations (including pain and discomfort) serve physiological roles in maintaining ocular health. In addition to sensory functions, corneal nerves serve important protective roles by detecting potentially injurious stimuli and eliciting defensive reflexes (such as blinking and lacrimation); moreover, corneal nerves have important trophic functions, including the modulation of immune responses and wound healing processes.[71]

Impulses at the ocular surface are transmitted to the central nervous system (CNS) via the sensory terminals of trigeminal ganglion neurons, which innervate the cornea and conjunctiva. Nerve bundles enter the stroma at the corneoscleral limbus, and through repetitive branching give rise to the corneal epithelial nerves and the subbasal nerve plexus.[171] Functional recordings of corneal sensory activity may be performed at various levels, including the CNS (central neurons and analyzing sensations), sensory nerves (primarily the peripheral neurons and branches of the trigeminal tract), and at single nerve terminals.

III.B.1.a. Functional characteristics of ocular sensory receptors

Ocular sensory receptors fall into functionally distinct types, grouped by the nature of their specific stimuli and expression of transducing channels.[157] Most are nociceptor sensory neurons, which are activated by injurious stimuli that evoke corneal pain sensations. Mechanonociceptors and polymodal nociceptors respond to mechanical forces, with Piezo2 and TRPA1 as major ion channels.[78] Polymodal nociceptors also respond to a wide array of additional stimuli, including heat, chemicals, and endogenous inflammatory mediators;[71, 172-174] major transducing channels include TRPV1-4, TRPA1, and ASICs.[78]

The cornea is also innervated by cold thermoreceptors (a class of sensory neurons distinct from nociceptors), which confer unique and specific sensory properties to the cornea and conjunctiva. Cold thermoreceptors are extremely sensitive to small temperature changes; spontaneously and continuously firing at normal background temperatures, they are able to rapidly discern transient temperature variations of 0.5°C or less.[173, 175] Corneal cold receptor activity depends on TRPM8, two-pore domain K channels, and Kv1 channels, which exert opposite influences on thermal threshold, allowing fine-tuning of the excitability of cold thermoreceptors.[176, 177] There is also evidence suggesting the existence of high-threshold cold receptors, which detect large temperature variations.[178]

III.B.1.b. Evoked sensations and reflex effects

The cornea is able to distinguish information coming from distinct nerve populations, thus discerning different sensations (Figure 6) and evoking various reflexes through specific sensory receptors and their respective transducing channels.[71]

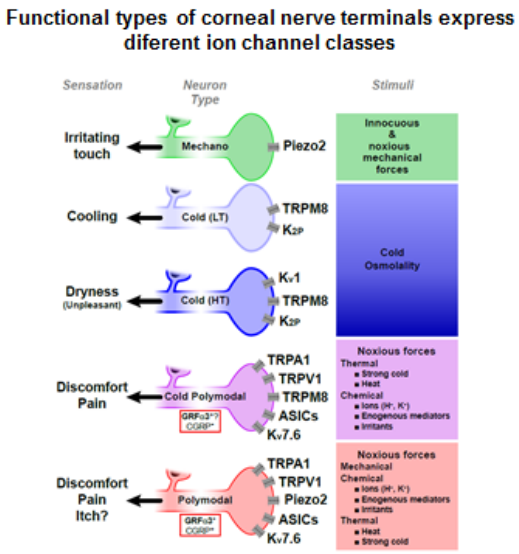


Figure 6. Graphical summary of the qualitative sensations of the ocular surface (left), the classes of ocular surface sensory neurons and their major transducing channels (center), and the specific stimuli (right). Modified from Belmonte C, Acosta MC, Merayo-Lloves J, Gallar J. What Causes Eye Pain? Curr Ophthalmol Rep. 2015;3:111-21.

In polymodal and mechanoreceptive neurons, stimulation results in reflex tearing, blinking, and sensations of discomfort (e.g., pain, irritation). Conversely, basal tearing is maintained by cold thermoreceptors via TRPM8 channels.[177] Basal blinking is likely mediated by cold thermoreceptors as well.[179] During blinking, tear film dynamics have different effects on ocular surface sensory receptors, and cold thermoreceptors seem to convey sensory information regarding ocular surface dryness. [175] Thermoreceptors expressing TRPM8 channels are also activated by increased osmolality and inhibited by reduced osmolality; furthermore, *Trpm8*-/- mice display reduced blinking compared with wild-type mice, suggesting that TRPM8 has osmosensory functions in the regulation of normal blinking.[179] As discussed in the previous section, during the interblink periods, spots of ocular surface cooling coincide with tear film breakup, and reflect local increases in evaporation.[139] Prolonged eye opening or enhanced corneal evaporation may result in further temperature decreases and thus more cold fiber activity.[157] Experimentally, cold thermoreceptors have been shown to be activated by ocular surface drying and hyperosmolarity.[180] It is possible that ocular surface sensory receptors are stimulated by tear film breakup, with stimulation of cold thermoreceptor activity with decreases in ocular surface temperature and increases in osmolarity, and nociceptor activation with increased mechanical stress and injury of epithelium cells.

III.B.2. Sensory Receptor Responses in Ocular Surface Pathologies

In contrast to the aforementioned sensory receptor responses that are part of normal physiological processes, altered activation occurs in inflammation and peripheral nerve injury. These two pathological processes are often inextricably linked, and depending on the level (and duration) of inflammation and/or injury, symptoms may differ in terms of evoked sensations.

In polymodal nociceptors, inflammatory mediators can cause excitatory increases and sensitization.[172] This occurs through the opening of TRPV1 and TRPA1 ion channels, resulting in membrane depolarization, sensitization of nociceptor endings, and hyperesthesia—and thus an augmented response to virtually any stimulus. Chronic inflammation can further cause long-term changes in ion channel expression, perpetuating this situation and evoking chronic inflammatory pain.[78] Nerve injury from a variety of causes (including physical damage, toxicity, and infection) may lead to inflammation; however,

in many cases (such as in ocular surgery), ocular nerves are directly severed or destroyed, and the effects of nerve damage can persist beyond transient inflammation. After surgery-induced nerve damage, an immediate reduction in corneal sensitivity is followed by transient hyperesthesia to mechanical stimuli; 3–5 months later, there is hyposensitivity to both mechanical and chemical stimuli,[181] and further central neural aberrations may result in neuropathic pain.[78]

Whereas polymodal nociceptors are sensitized by inflammatory mediators released during inflammation, an "inflammatory soup" (including mediators such as bradykinin, prostaglandins, and histamine) inhibits TRPM8 in cold thermoreceptors, thus reducing cold-evoked activation.[182] Nerve injury to cold thermoreceptors markedly increases their ongoing activity at 34°C. Like polymodal nociceptors, changes have been surmised to involve alterations in the expression of ion channels.[78]

In pathological conditions of the ocular surface, varying levels of inflammation and nerve injury result in various symptoms of discomfort (Figure 7). Symptomologies will arise from the specific aberrations in the impulse activity of sensory nerve pathways, resulting in typical sets of symptoms for different ocular surface pathologies.

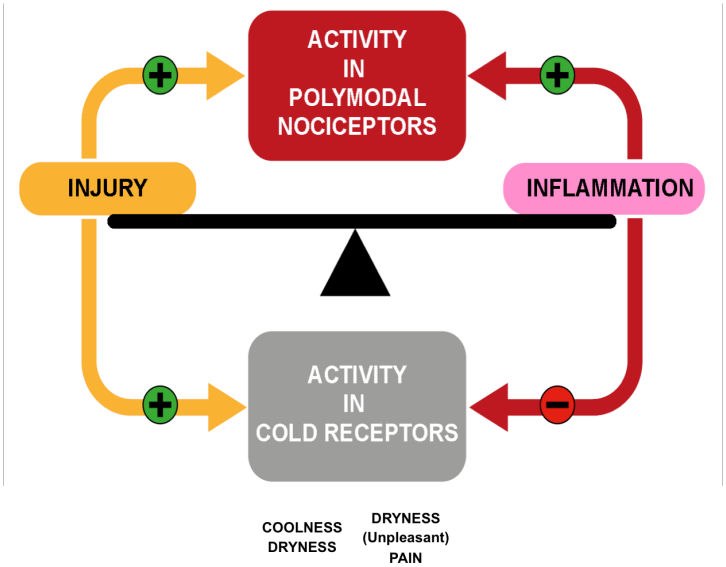


Figure 7. Graphical summary of the potential effects of pathological processes on ocular surface sensory nerve terminals and evoked sensations. Right: Inflammation activates polymodal nociceptor fibers, causing sensitization and inflammatory pain; in contrast, inflammation tends to inhibit TRPM8-dependent impulse activity in cold thermoreceptors. Left: injury of polymodal nociceptors can cause temporal aberrations in activity and neuropathic pain, whereas injury in cold thermoreceptors can result in sensations of cooling, dryness, discomfort, and pain. Reprinted from Belmonte C, Acosta MC, Merayo-Lloves J, Gallar J. What Causes Eye Pain? Curr Ophthalmol Rep. 2015;3:111-21.

III.B.2.a. DED

Uncomfortable dryness is a defining feature of DED.[66] Ocular surface inflammation and damage likely evoke sensations of discomfort and pain through polymodal nociceptors; additionally, lid action over a dry ocular surface is likely to result in activation of mechano- and polymodal nociceptors.[71] Cold thermoreceptors may play an even greater role in evoking unpleasant dryness sensations, as they are activated by evaporation-induced cooling[177] and, importantly, by hyperosmolarity,[183] a core mechanism of DED. These notions are supported by recent evidence from a DED model, in which lacrimal gland

removal resulted in transient inflammation and polymodal nociceptor sensitization, yet progressive and ongoing activation of cold thermoreceptors after inflammation subsided.[184]

III.B.2.b. Refractive surgery

Refractive surgery causes injury to corneal sensory nerve endings,[185] and dry eye is a common complication of refractive procedures despite the lack of dramatic changes in tear dynamics.[88] The effects of inflammation are minimal compared to the nerve damage, which alters the transducing capacity of mechano- and polymodal nociceptors, resulting in pain immediately after photorefractive surgery[186] and loss of mechanical and chemical sensitivity.[181] In contrast, cold thermoreceptors display abnormally high background firing and warmer thresholds for cooling following refractive surgery.[78] Thus, postsurgical sensations of dryness may be more neuropathic in origin, and less related to tear film deficiency.[77, 88]

III.B.2.c. Allergic conjunctivitis and UV-induced keratitis

Allergic conjunctivitis comprises several forms, and generally involves allergen-triggered release of inflammatory cytokines and associated symptoms of swelling and irritation.[66] In a model of experimental allergic conjunctivitis, mechano- and polymodal nociceptors were sensitized,[187] while low-threshold cold thermoreceptors displayed reduced activity, presumably due to the inhibition of TRPM8 by inflammatory mediators.[182] An experimental model of UV-induced keratitis resulted in a similar profile of nociceptor sensitization and suppression of cold thermoreceptor activity (likely due to the inhibitory effects of inflammatory mediators on TRPM8 channels).[188] Taken together, these response profiles are likely to produce the symptoms of discomfort in conjunctivitis and UV-induced keratitis.

III.B.3. Summary and Conclusions

Although ocular surface pathologies have diverse etiologies, their symptoms often converge on altered responses of corneal sensory nerves. Distinct symptomologies arise from the varying involvement of mechanonociceptors, polymodal nociceptors, and cold thermoreceptors. In recent years, there have been significant advances in the understanding of cold thermoreceptors and their roles in ocular surface disorders. Furthermore, several lines of investigation have yielded great insight into the molecular physiology of nociceptors and thermoreceptors, their alterations in pathological processes, and the specific sensations evoked in ocular surface disorders.

III.C. Diagnosis of DED (James Wolffsohn, OD, PhD)

The 2007 report of the TFOS DEWS Diagnostic Methodology Subcommittee provided a thorough assessment of DED parameters, and compiled and validated a comprehensive database of assessment techniques for disease diagnosis and monitoring.[2] Prof. Wolffsohn reviewed the 2007 report and the current state of DED diagnosis, including remaining unmet needs, recent advances, and novel testing approaches.

III.C.1. Current State of DED Diagnosis

DED is a multifactorial disease with many subtypes;[66] as such, accurate diagnosis is heavily dependent on robust criteria for defining and classifying the disease. However, the definition of DED has evolved since the1995 Report of the National Eye Institute (NEI)/Industry Dry Eye Workshop,[189] which defined DED as follows:

> "*Dry eye a disorder of the tear film due to tear deficiency or excessive evaporation, which causes damage to the interpalpebral ocular surface and is associated with symptoms of ocular discomfort.*"

Subsequently, the 2007 TFOS DEWS report updated the definition and classifications of DED based on etiology, mechanisms, and severity:[66]

> "*Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.*"

The TFOS DEWS Diagnostic Methodology Subcommittee (representing a global collaboration of scientists, clinicians, and industry representatives) reevaluated the available diagnostic tests in the context of the contemporary understanding of DED, including the updated definition. These efforts resulted in a searchable database of tests for diagnosing and monitoring DED, with individual tests compiled and assessed by experts in the field and presented within standard templates (available at www.tearfilm.org/dewsreport).[2] The DEWS report highlighted the importance of differential diagnosis of dry eye vs. non-DED, as well as their subtypes, which are not mutually exclusive and may be interrelated (Figure 8).

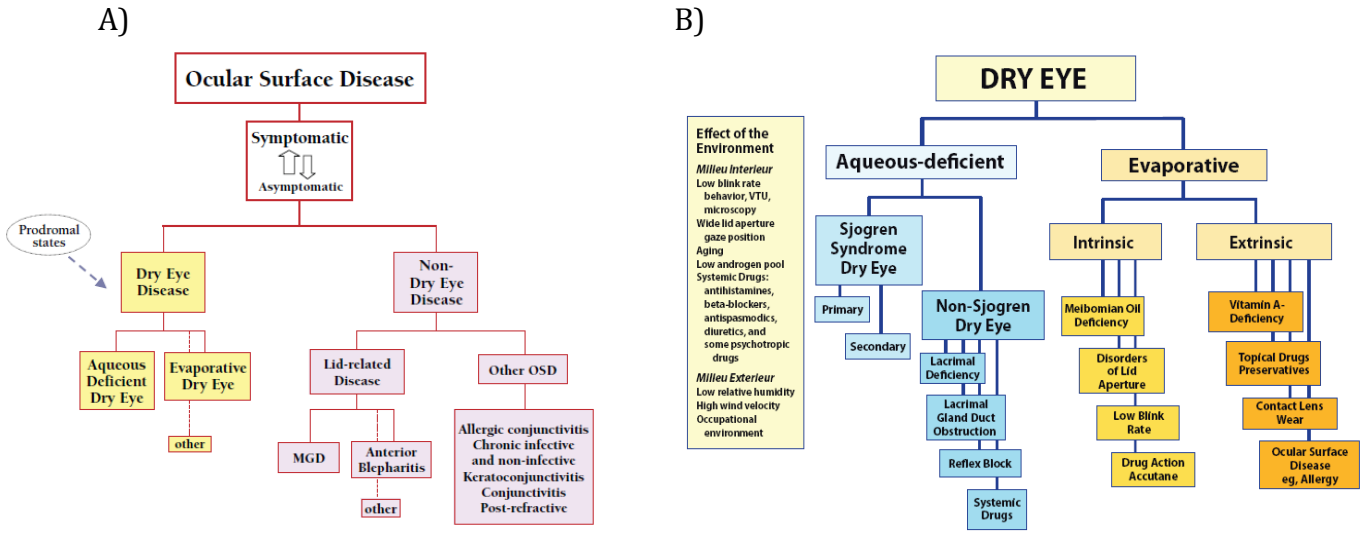


Figure 8. A) Schematic illustration of the relationship between DED and other ocular surface disorders. B) Subtypes of DED (right) and potential environmental factors (left). It is important to

note that the various subtypes are not mutually exclusive and may coexist. From: 2007 Report of the International Dry Eye WorkShop (DEWS). Ocul Surf. 2007;5:65-204.

In light of the updated definition and classifications of DED, the DEWS Diagnostic Methodology Subcommittee determined that among the numerous tests that are currently used for diagnosing and monitoring DED, non-invasive tear film break-up time (NITFBUT) likely represented the best means of evaluating tear film stability for office-based physicians, with moderately high sensitivity and good overall accuracy.[2] However, an objective measure of dry eye hyperosmolarity was still desired. Furthermore, available tests were hindered by shortcomings such as selection bias (introduced by the method used for selecting subjects in an experiment) and spectrum bias (due to differences in the features of different populations, which influence the sensitivity and/or specificity of a test).

Although there have been recent advances for eye care practitioners regarding differential diagnosis, particularly for MGD/blepharitis (Figure 9),[190, 191] Sjögren's syndrome,[192] and contact lens-associated DED,[193] it remains difficult to tease out the different subtypes of DED in the clinic. In pharmacy settings, "mystery shopper" studies have shown that differential diagnosis of DED and other ocular surface disorders is particularly poor.[194, 195] Finally, it is not entirely clear whether performing multiple tests will have additive diagnostic value. For example, in a recent evaluation of multiple dry eye tests, we found that various tear stability tests and likewise tear volume tests and ocular surface damage tests produced largely comparable results (suggesting that a single test in each 'category' may suffice), and the predictive values of different treatments may vary depending on the outcomes evaluated.[196]

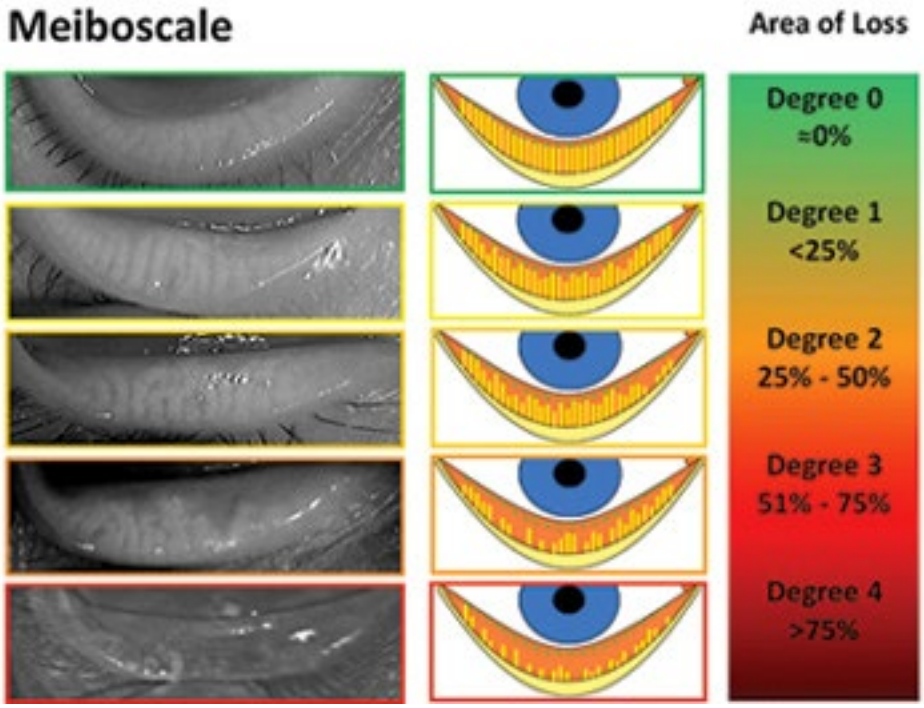


Figure 9. The five-grade pictorial "Meiboscale" for grading and classifying meibomian gland loss. Image courtesy of Heiko Pult, MSc, PhD, and available at www.heiko-pult.de.

For a multifactorial disease with variable phenotypes such as dry eye, a strong definition and classification scheme is necessary to drive diagnosis, epidemiology, clinical trial design, and basic scientific research. Additionally, for a diagnosis to be useful, it would need to be able to inform management of different forms of DED. However, this continues to be challenged by the persistent lack of uniform concepts and minimum sets of diagnostic criteria for DED and its subtypes. This is illustrated by the variable methods used in epidemiological studies reviewed in the 2007 report of the TFOS DEWS Epidemiology Subcommittee,[3] as well as in Section II of this report. A strong definition of DED would

form a solid foundation on which to base diagnostic methodologies of DED, which would in turn facilitate management, research, and clinical development of effective therapeutic strategies.

III.C.2. Assessments Based on the Current Definition of DED

Several diagnostic test categories for DED, discussed briefly here, are based on five factors included in the current definition of the disease (emphases added): 1) symptoms of discomfort, 2) visual disturbance, 3) tear film instability with *potential damage to the ocular surface*, 4) increased osmolarity of the tear film, and 5) inflammation of the ocular surface. The current definition requires each of these factors to be present for dry eye to occur, but this was not articulated in the subsequent diagnosis report or in subsequent research studies, limiting the ability to better understand and develop treatments for dry eyes and its sub-classification conditions. Damage to the ocular surface (qualified by the operative word "potential"), and other factors not included in the current definition of DED, are discussed in the following section. Most of the assessments for these diagnostic test categories were described in detail in the 2007 report of the TFOS DEWS Diagnostic Methodology Subcommittee.[2]

III.C.2.a. Symptoms

Symptoms of discomfort are the foremost defining factors of the current definition of DED.[66] Symptomology also forms the basis of validated dry eye questionnaires, which are noninvasive, highly accessible, and widely used in the clinic and in population-based studies. Surveys used for diagnosing DED (and recommendations for their use in clinical trials) were detailed in the 2007 report of the TFOS DEWS Epidemiology Subcommittee.[3] Questions include supplementary history (e.g., age and gender, systemic conditions/atopy, medication, environment, and smoking), which often relates to risk factors for DED. Many dry eye questionnaires, such as the McMonnies questionnaire,[197] utilize a systematic approach for diagnosing DED. Others, such as the Ocular Surface Disease Index© (OSDI©),[198] have been adapted to smart phones and tablets, which appeals to patients because they facilitate self-monitoring and regular feedback. Other notable questionnaires include the Dry Eye Questionnaire (DEQ)[50, 199, 200] and the survey of 39,876 women participating in the U.S. Women's Health Study, which involved only three questions.[17]

III.C.2.b. Visual disturbance

Although visual disturbance is included in the 2007 DEWS definition of DED, it is often dismissed by patients as a normal course (e.g., aging), and usually this symptom is only revealed through certain questionnaires.[2] There is also a paucity of research on objective measures of visual disturbance in DED. The 2007 report of the TFOS DEWS Diagnostic Methodology Subcommittee mentioned one functional measurement system for assessing dynamic visual acuity changes,[201] and a recent study comparing two ocular lubricants (or a combination of the two) found no significant differences in visual quality or higher order aberrations.[202] Better objective measures of visual disturbance are still needed in the evaluation of DED.

III.C.2.c. Tear film instability

Tear film stability has been a major cornerstone of clinical tests for dry eye, with the main metric being tear film break-up time (TFBUT). Fluorescein is often instilled to enhance visibility of the tear film, and this mildly invasive tear stability measurement is often referred to as the fluorescein break-up time (FBUT). There is significant variability among reference values for dry eye diagnosis, ranging from the longstanding cut-off time of <10 seconds,[203] to the more-recently proposed cut-off time of <5 seconds for smaller volumes of fluorescein.[204] Non-invasive TFBUT was determined by the DEWS Diagnostic Methodology Subcommittee as likely the best assessment of tear film stability for eye care practitioners,

with moderately high sensitivity (83%) with good overall accuracy (85%),[2] and may be measured noninvasively with instruments such as the Tearscope. We have found highly correlated results from non-invasive keratograph break-up time (NIKBUT – although tear instability is generally detected more quickly with objective methodology), Tearscope NIBUT, and FBUT tear stability tests, suggesting that the predictive value of FBUT may be redundant with less invasive tests.[196]

III.C.2.d. Osmolarity

Tear osmolarity, which has long been considered to be a possible "gold standard" of dry eye diagnosis, was introduced as a defining measure of DED in the 2007 TFOS DEWS report.[66] However, an objective measure of dry eye hyperosmolarity was still lacking at the time.[2] For diagnosis of DED, a reference limit of 316 MOsm/L is generally accepted,[205] yet the widespread utility of osmolarity testing has been limited by the lack of reliable measurement techniques. The development and validation (Tomlinson et al., 2010; Eperjesi et al., 2012; Yoon et al., 2014; Bunya et al., 2015) of a commercial osmometer, the TearLab Osmolarity System, has significantly advance the diagnosis and management of DED based on osmolarity. A tear osmolarity >308 mOsm/L is considered as the appropriate cut-off of dry eye disease, with the maximum of two eyes used in the diagnosis, acknowledging heteroscedasticity as a an indicator of tear film instability (Sullivan et al., 2014). Since the initial DEWS report osmolarity has been shown not be strongly associated with other tear film metrics such as TFBUT (Schmidl et al., 2015; Yeh et al., 2015), but has been shown to be more sensitive to diagnosing dry eyes in general (Khanal et al., 2018; Sullivan et al., 2010; Lemp et al., 2011) and dry eyes induced by other conditions such as LASIK (Chao et al., 2015), type 2 diabetes (Sagdik et al., 2013; Sullivan et al., 2014; Alves et al., 2014; Najafi et al., 2015), rheumatoid arthtitis (Turkyilmaz et al., 2013) and identify patients who are more likely to have unexpected refractive errors following cataract surgery due to disruption of the ocular surface when performing pre-operative biometry (Epitropoulos et al., 2015). It has also been shown sensitive to detecting and monitoring graft versus host disease (Schargus et al., 2015; Na et al., 2015). With effective treatment of most dry eye related conditions, tear film osmolarity returns to normal and its variability reduces (Bron et al., 2014). It has recently been recommended as a test to inform the clinical management of Sjogren Disease (Foulks et al., 2015)

Structural and functional changes in corneal innervation after laser in situ keratomileusis and their relationship with dry eye.
By: Chao, Cecilia; Stapleton, Fiona; Zhou, Xiangtian; et al.
Graefe's archive for clinical and experimental ophthalmology = Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie Volume: 253 Issue: 11 Pages: 2029-39 Published: 2015-Nov (Epub 2015 Aug 11)

Effect of tear osmolarity on repeatability ofkeratometry for cataract surgery planning.
By: Epitropoulos, Alice T; Matossian, Cynthia; Berdy, Gregg J; et al.
Journal of cataract and refractive surgery Volume: 41 Issue: 8 Pages: 1672-7 Published: 2015-Aug

Relationships among Tear Film Stability, Osmolarity, and Dryness Symptoms
By: Yeh, Thao N.; Graham, Andrew D.; Lin, Meng C.
OPTOMETRY AND VISION SCIENCE Volume: 92 Issue: 9 Pages: E264-E272 Published: SEP 2015

Correlation Between Tear Film Osmolarity and the Disease Score of the International Chronic Ocular Graft-Versus- Host-Disease Consensus Group in Hematopoietic Stem Cell Transplantation Patients
By: Schargus, Marc; Meyer-ter-Vehn, Tobias; Menrath, Julia; et al.
CORNEA Volume: 34 Issue: 8 Pages: 911-916 Published: AUG 2015

Tear Osmolarity and Ocular Surface Parameters as Diagnostic Markers of Ocular Graft-Versus-Host Disease
By: Na, Kyung-Sun; Yoo, Young-Sik; Hwang, Kyu-Yeon; et al.
AMERICAN JOURNAL OF OPHTHALMOLOGY Volume: 160 Issue: 1 Pages: 143-149 Published: JUL 2015

Variability of Tear Osmolarity in Patients With Dry Eye
By: Bunya, Vatinee Y.; Fuerst, Nicole M.; Pistilli, Maxwell; et al.
JAMA OPHTHALMOLOGY Volume: 133 Issue: 6 Pages: 662-667 Published: JUN 2015

Correlation of Tear Film Osmolarity and 2 Different MMP-9 Tests With Common Dry Eye Tests in a Cohort of Non-Dry Eye Patients
By: Schargus, Marc; Ivanova, Svetlana; Kakkassery, Vinodh; et al.
CORNEA Volume: 34 Issue: 7 Pages: 739-744 Published: JUL 2015

Clinical Guidelines for Management of Dry Eye Associated with Sjogren Disease
By: Foulks, Gary N.; Forstot, S. Lance; Donshik, Peter C.; et al.
OCULAR SURFACE Volume: 13 Issue: 2 Pages: 118-132 Published: APR 2015

The Association Between Subjective and Objective Parameters for the Assessment of Dry-Eye Syndrome
By: Schmidl, Doreen; Witkowska, Katarzyna Jadwiga; Kaya, Semira; et al.
INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE Volume: 56 Issue: 3 Pages: 1467-1472 Published: MAR 2015

Dry eye disease in type 2 diabetes mellitus; comparison of the tear osmolarity test with other common diagnostic tests: a diagnostic accuracy study using STARD standard.
By: Najafi, Laily; Malek, Mojtaba; Valojerdi, Ameneh Ebrahim; et al.
Journal of diabetes and metabolic disorders Volume: 14 Pages: 39 Published: 2015

Precision and Accuracy of TearLab Osmometer in Measuring Osmolarity of Salt Solutions
By: Yoon, Dan; Gadaria-Rathod, Neha; Oh, Cheongeun; et al.
CURRENT EYE RESEARCH Volume: 39 Issue: 12 Pages: 1247-1250 Published: DEC 2014

Comparison of Diagnostic Tests in Distinct Well-Defined Conditions Related to Dry Eye Disease
By: Alves, Monica; Reinach, Peter Sol; Paula, Jayter Silva; et al.
PLOS ONE Volume: 9 Issue: 5 Article Number: e97921 Published: MAY 21 2014

Rethinking Dry Eye Disease: A Perspective on Clinical Implications
By: Bron, Anthony J.; Tomlinson, Alan; Foulks, Gary N.; et al.
OCULAR SURFACE Volume: 12 Supplement: 1 Pages: S1-S31 Published: APR 2014

Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: clinical implications
By: Sullivan, Benjamin D.; Crews, Leslie A.; Messmer, Elisabeth M.; et al.
ACTA OPHTHALMOLOGICA Volume: 92 Issue: 2 Pages: 161-166 Published: MAR 2014

Investigation of tear osmolarity in early rheumatoid arthritis: relation to disease activity
By: Turkyilmaz, Kemal; Turkyilmaz, Aysegul Kucukali; Kurt, Ali; et al.
CANADIAN JOURNAL OF OPHTHALMOLOGY-JOURNAL CANADIEN D OPHTALMOLOGIE Volume: 48 Issue: 4 Pages: 235-239 Published: AUG 2013

Tear Film Osmolarity in Patients with Diabetes Mellitus
By: Sagdik, H. Murat; Tetikoglu, Mehmet; Ucar, Fatma; et al.
OPHTHALMIC RESEARCH Volume: 50 Issue: 1 Pages: 1-5 Published: 2013

Reproducibility and repeatability of the OcuSense TearLab (TM) osmometer
By: Eperjesi, Frank; Aujla, Maana; Bartlett, Hannah
GRAEFES ARCHIVE FOR CLINICAL AND EXPERIMENTAL OPHTHALMOLOGY Volume: 250 Issue: 8 Pages: 1201-1205 Published: AUG 2012

Tear Osmolarity in the Diagnosis and Management of Dry Eye Disease
By: Lemp, Michael A.; Bron, Anthony J.; Baudouin, Christophe; et al.
AMERICAN JOURNAL OF OPHTHALMOLOGY Volume: 151 Issue: 5 Pages: 792-798 Published: MAY 2011

An Objective Approach to Dry Eye Disease Severity
By: Sullivan, Benjamin D.; Whitmer, Diane; Nichols, Kelly K.; et al.
INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE Volume: 51 Issue: 12 Pages: 6125-6130 Published: DEC 2010

Comparison of Human Tear Film Osmolarity Measured by Electrical Impedance and Freezing Point Depression Techniques
By: Tomlinson, Alan; McCann, Louise C.; Pearce, Edward I.
CORNEA Volume: 29 Issue: 9 Pages: 1036-1041 Published: SEP 2010

Dry eye diagnosis
By: Khanal, Santosh; Tomlinson, Alan; McFadyen, Angus; et al.
INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE Volume: 49 Issue: 4 Pages: 1407-1414 Published: APR 2008

Sullivan B. Challenges in Using Signs and Symptoms to Evaluate New Biomarkers of Dry Eye Disease. Ocular Surface 2014;12:2-9.
III.C.2.e. Inflammation

DEWS diagnostic templates have been developed for bulbar hyperemia scales, which may be used to assess the degree of redness (due to vessel dilation) of the bulbar conjunctiva. Although such scales have helped with quantifying ocular surface inflammation, it remains a “noisy” subjective measure, and the natural phenomenon of whole number bias may further limit the accuracy and sensitivity of these subjective scales. Prior to the 2007 DEWS report, we developed methods for monitoring ocular physiology, including hyperemia, using objective image analysis,[206-208] and recent studies have demonstrated greater sensitivity and reliability compared to subjective grading methods.[209] Further development may result in the adoption of these objective measures and integration with current techniques for assessing ocular surface inflammation.[210] Other new approaches for evaluation of ocular surface inflammation include point-of-care testing for inflammatory biomarkers. One such test by Rapid Pathogen Screening, Inc. (RPS) detects tear levels of both active and latent MMP-9 (test cutoff value of 40 pg/mL), an inflammatory marker that has been shown to be elevated in DED.[211]+ Schargus et al., 2015. However, initial research suggests MMP-9 is only detected in late stage dry eye (Schargus et al., 2015), in a similar manner to fluorescein staining (Bron et al., 2015).

III.C.3. Other Assessments of DED

A number of tests for DED are based on factors that under the current definition are not absolute characteristics of the disease. Nonetheless, because there is no single definitive test or diagnostic battery for DED, these tests may be complementary and/or aid in evaluating disease severity. Moreover, some measures obtained in certain tests may affect the interpretation of other test outcomes.

III.C.3.a. Damage to the ocular surface

Whereas "damage to the interpalpebral ocular surface" was a defining feature of DED in the1995 Report of the NEI/Industry Workshop,[189] one notable distinction of the 2007 TFOS DEWS report was the qualification of "potential damage to the ocular surface" in DED.[66] Nonetheless, it is common practice to assess ocular surface damage by grading staining by fluorescein dye, lissamine green or (to a lesser extent) rose bengal.

Fluorescein was described in 1882 by Pflüger for evaluating the corneal & conjunctival abrasions in rabbits,[212] and its first clinical use is attributed to Fromm & Groenouw in 1891.[213] Today, fluorescein is the most widely used diagnostic dye by eye care practitioners, and the longstanding belief is that fluorescein highlights defects in the corneal and conjunctival epithelium by specifically entering damaged cells. However, in a recent critical review of the literature,[214] alternative cellular mechanisms that may cause corneal fluorescence were examined, beyond the previously suggested surface pooling, such as cellular uptake, and ingress around cells due to disruption of cell-cell junctions.[215] Thus, a better understanding of the mechanisms of fluorescein staining may alter the interpretation of this diagnostic method. In a recent review, Bron and colleagues (Bron et al., 2015) noted a proportion of normal corneas show sparse, scattered time-dependent, punctate fluorescein uptake, which, they hypothesise is due to a graded loss of the glycocalyx barrier, permitting transcellular entry into pre-shed cells. The minimal or absent epithelial damage noted in short break-up time dry eye in symptomatic office workers who use visual display units suggests that corneal staining may onset relatively late in the development of dry eye disease (Yokoi et al., 2015).

Clinical staining of the ocular surface: Mechanisms and interpretations
By: Bron, A. J.; Argueeso, P.; Irkec, M.; et al.
PROGRESS IN RETINAL AND EYE RESEARCH Volume: 44 Pages: 36-61 Published: JAN 2015

Importance of Tear Film Instability in Dry Eye Disease in Office Workers Using Visual Display Terminals: The Osaka Study
By: Yokoi, Norihiko; Uchino, Miki; Uchino, Yuichi; et al.
AMERICAN JOURNAL OF OPHTHALMOLOGY Volume: 159 Issue: 4 Pages: 748-754 Published: APR 2015

Lissamine green was introduced in 1973 by Norn,[216] and has been demonstrated to stain membrane-degenerate cells and dead cells; furthermore, lissamine staining is not blocked by mucin.[217] Lissamine green stains the ocular surface in a manner similar to rose bengal (although rose bengal is blocked by mucin; Argueso et al., 2006), but with less irritation and toxicity to eyes.[218] Because it fades within four minutes, repeat instillation of 10-20µl of lissamine are required.[219] In a recent evaluation of the efficacy of fluorescein, rose bengal, lissamine green for ocular surface staining, a mixture of 2% fluorescein and 1% lissamine green was found to be superior to individual dyes.[220]

Mucin characteristics of human corneal-limbal epithelial cells that exclude the rose bengal anionic dye
By: Argueso, P; Tisdale, A; Spur-Michaud, S; et al.
INVESTIGATIVE OPHTHALMOLOGY & VISUAL SCIENCE Volume: 47 Issue: 1 Pages: 113-119 Published: JAN 2006

III.C.3.b. Tear volume

The phenol red thread test provides an index of tear volume, and likewise Schirmer testing (with or without anesthesia or nasal stimulation) provides an assessment of tear secretion. Newer techniques, such as the use of smart phones or tablets for assessing tear meniscus curvature and its relationship to tear volume,[221] may provide reliable and sensitive (yet noninvasive) estimates of tear volume.

III.C.3.c. Lid parallel conjunctival folds

Lid parallel conjunctival folds (LPCOFs or LIPCOFs) are subclinical folds in the lateral, lower quadrant of the bulbar conjunctiva, parallel to the lower lid margin, and may be predictive of dry eye.[222, 223] They are evaluated by slit lamp (typically using a 2-3 mm vertical slit, 18-24x magnification, and angle 20°-30°). Of note, LIPCOFs may cause tear meniscus height measurements to be underestimated,[155] which is an important consideration when interpreting estimates of tear volume.

III.C.3.d. Lid wiper epitheliopathy

The lid wiper is the edge (0.4-0.6mm wide) of the marginal conjunctiva of the upper eyelid, which slides over ocular surface, during blinking, approximately 8,000 times per day. Noting that the epithelium of the lid wiper is squamous (not columnar), Parsons suggested that the lid wiper is the only part of the conjunctiva that closely contacts the ocular surface.[224] Sequential instillations with dyes (e.g., fluorescein, lissamine green, or rose bengal) stains the lid wipers of the majority of patients with symptomatic DED, and grading is typically based on horizontal length of staining or a combination of length and width.[154, 225] Lid wiper epitheliopathy is commonly found in patients experiencing symptoms of DED, but displaying normal findings with other tests (e.g., FBUT, Schirmer test).[154] It has been reported that 80% of symptomatic contact lens wearers (vs. 13% of asymptomatic lens wearers) have staining indicative of lid wiper epitheliopathy,[154] and that damage increases after 6 months of lens wear, although other tests such as tear volume assessed by the phenol red test remains constant.[196] Of note, assessment of LWE requires understanding of Kessing's space, which separates the ocular surface from tarsal palpebral conjunctiva of the upper eyelid.[223]

III.C.4. Summary and Conclusions

Achieving a robust, comprehensive diagnostic battery remains an important clinical challenge for DED. Due to the inherent heterogeneity of the disease itself and the populations affected, this requires clear definitions of disease subtypes and unbiased physiological ranges of what is 'normal' adjusted for demographics. Other significant considerations include invasiveness and cost effectiveness (both in terms of consumable costs and requirements for time and skills). For a comprehensive diagnostic battery, it is also paramount to evaluate the usefulness of each component in order to optimize diagnostic yield and to better inform treatment.

IV. TREATMENT CHALLENGES OF DED

IV.A. New Approaches for the Treatment of DED (David A. Sullivan, MS, PhD, FARVO)

Despite the fact that hundreds of millions of people throughout the world suffer from DED, there is no approved global cure for this condition. Dr. Sullivan noted that, whatever the cause, the final common denominators of DED are tear film hyperosmolarity and instability (Figure 4).[1] Hyperosmolarity induces the symptoms of discomfort by acting on the cold thermoreceptors of the cornea (See Section III.B. Symptomatology of DED). Hyperosmolarity also causes loss of epithelial cell surface microplicae, which were first described in 1976 and postulated to hold a protective layer of mucin.[226] Loss of the microplicae results in loss of the negative charge of the glycocalyx,[227] detrimental effects on tear film stability, and further increases in evaporation.[228] Ultimately, tear hyperosmolarity leads to a "vicious circle" of increased ocular surface stress, friction, inflammation and damage, and visual impairment.[138]

Dr. Sullivan highlighted a number of new therapeutic approaches for DED, which target either the underlying disease mechanisms or their sequelae. Dr. Sullivan also reviewed shared attributes of failed interventional trials for DED, underscoring the unmet needs in appropriate disease indicators and preclinical models.

IV.A.1. Approved Treatments for DED

Very few treatments for DED, described briefly below, have achieved approval by regulatory agencies in certain countries.

IV.A.1.a. Cyclosporine A (Restasis®, Ikervis®)

Cyclosporine A ophthalmic emulsion 0.05%, manufactured by Allergan, Inc. and marketed as Restasis®, is the only drug approved by the U.S. Food and Drug Administration (FDA) for the treatment of DED,[229] with estimated net sales of $1 billion in 2014.[230] According to the U.S. package insert,[231] "Restasis is indicated to increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca" and has demonstrated significant increases (~10 mm) in Schirmer wetting in 15% of treated patients vs. 5% of vehicle treated patients.[232] Restasis® was approved in December 2002 by the FDA after post-hoc data analyses. A topical formulation of cyclosporine A, marketed as Ikervis® by Santen (formerly known as Novagali Pharma), was recently made available in Europe (discussed in Section IV.C).

IV.A.1.b. Diquafosol (Diquas™, Prolacria™)

Diquafosol is a P2Y2 purinergic receptor agonist that stimulates conjunctival fluid flow and mucin secretion.[233] In 2010, diquafosol tetrasodium ophthalmic solution 3% (Santen Pharmaceutical Co., Ltd.) was approved for dry eye treatment in Japan, where it is marketed as Diquas™.[234] However, in the United States, diquafosol tetrasodium 2% (Prolacria™, Inspire Pharmaceuticals, Inc.) did not achieve primary and secondary endpoints in Phase III clinical trials,[235] and it did not achieve FDA approval.

IV.A.1.c. Rebamipide (Mucosta™)

Rebamipide is a quinolinone derivative that induces mucus secretion by activating cyclooxygenase 2, and has been shown to decrease lissamine green conjunctival staining and foreign body sensation, as compared to 0.1% sodium hyaluronate.[236, 237] In Japan, rebamipide is approved and marketed for the treatment of dry eye syndrome as Mucosta® Ophthalmic Suspension UD2%. However, Phase III trials by

Novartis[238, 239] and Acucela, Inc.[240] did not appear to provide at least two well-controlled, replicative studies showing efficacy in the United States.

IV.A.1.d. Hyaluronate (Hyalein®)

Topical sodium hyaluronate 0.3%, marketed by Santen Pharmaceutical Co., Ltd. as Hyalein®, was introduced in 1995 as Japan's first treatment for disorders of the cornea and conjunctival epithelium. River Plate pursued an NDA for sodium hyaluronate in the treatment of keratoconjunctivis sicca. An FDA advisory committee meeting recommended against approval in July 2009. While sodium hyaluronate for dry eye treatment is regulated as a drug in the United States, some manufacturers have included it as an excipient in over-the-counter (OTC) eye drops. OTC preparations are also widely available in numerous countries in Asia and Europe.[241]

IV.A.2. New Treatment Targets

The underlying pathophysiology of DED, described in Section III.A and illustrated in Figure 4, provides several potential treatment targets for DED. Indeed, more than 200 interventional clinical trials for the treatment of DED have been registered since 2010 (see Section II.A: Epidemiology of DED). New therapeutic strategies (with varying levels of supportive evidence) may be classified based on these targets, and are summarized below.

IV.A.2.a Symptoms of discomfort

Symptoms of discomfort are fundamental to the definition of DED,[66] and arise from various pathological processes (see Figure 4) and converge on altered responses of corneal sensory nerves. Acupuncture and traditional Chinese medicine have been suggested to relieve dry eye symptoms by stimulating tear secretion and/or decreasing pain, while more-recently developed treatments aim to decrease pain by targeting major transducing channels of ocular sensory receptors (Table 8).

Table 8. Treatments Targeting Symptoms of Discomfort

Treatment	Potential Effects
Prosthetic Replacement of the Ocular Surface Ecosystem (PROSE)	Scleral lens device to maintain a reservoir of tears to wet and lubricate the ocular surface[242, 243]
Acupuncture	Possible effects on dry eye signs and symptoms, as compared to sham-treated controls or artificial tears[244-246]
ShengJinRun ZaoYangXue granules	Improve tear flow and reduce dry eye symptoms[247]
Korean red ginseng	Improve tear film stability and reduce dry eye symptoms[248]
Suppressing transient receptor potential vanilloid 1 (TRPV1) receptor	Decrease pain associated with DED[249]

IV.A.2.b. Mucin deficiency

Hydrophilic mucins are critical for holding tears onto the ocular surface, and alterations in mucin levels have been demonstrated in DED.[250] Various therapeutic approaches have aimed to stimulate, stabilize, or mimic mucins on the ocular surface (Table 9).

Table 9. Treatments Targeting Mucin Deficiency

Treatment	Potential Effects
Galectin 3	Stabilize ocular surface mucins; promote corneal wound healing[251]
Mycophenolate mofetil	Increase MUC5AC[252]
DA-6034 (Eupatilin)	Stimulate MUCs 1, 2, 4, 5AC, 5B & 16[253]
Trefoil factor family peptide 3 (TFF3)	Stabilize mucous layer [254, 255]
Nerve growth factor & mimetics	Increase goblet cell number and MUC5AC production; increase corneal sensitivity; promote corneal epithelial wound healing[256, 257]
Tamarind seed polysaccharide	Mimic the mucoadhesive mucin properties[258-261]
Nanoparticles	Deliver plasmids coding for MUC5AC protein to the ocular surface[262, 263]

IV.A.2.c. Aqueous tear deficiency

Multiple pathological processes promote aqueous tear deficiency (see Figure 4), and artificial tears form the largest treatment class for this condition, with a market that is expected to exceed $2 billion USD by 2018.[264] Other treatment strategies that target tear deficiency include tear supplementation, hydration, tear film conservation, and aqueous tear stimulation (Table 10). Of note, vitamin A metabolites have been proposed to increase the Schirmer wetting score,[265] but are also known to cause MGD and evaporative DED.[266]

Table 10. Treatments Targeting Aqueous Tear Deficiency

Treatment	Potential Effects
Tear film supplements (e.g., albumin, alginic acid in contact lenses, autologous serum, cationic oil emulsion, chitosan, chondroitin sulfate/xantham gum, hyaluronate, hydroxypropyl-methylcellulose, hydroxypropyl-guar, n-acetyl-aspartyl-glutamic acid, phospholipids, propylene glycol, selenoproteins)	Improve physical properties of the ocular surface (e.g., wetting, lubrication; many clinical studies and trials have been completed, or are underway, to evaluate these supplements)[267]
Oral sea buckthorn oil	Reduce tear osmolarity[268]
7eye (Panoptyx)	Provide hydration via moisture chamber glasses
Botulinum toxin injection near lower puncta	Prevent tear drainage and alleviate the clinical manifestations of severe DED[269, 270]
Salivary gland transplantation	Replace dysfunctional lacrimal gland [271-273]
Generation of two- and three-dimensional lacrimal gland constructs for lacrimal gland regeneration	Serve as bioengineered lacrimal gland replacement[274]
Oculeve electrode device	Stimulate the lacrimal nerve to induce aqueous tear production[275-277]

Oral anethole triothine	Increase Schirmer wetting score[278]
Oral uridine	Stimulate tear and mucin secretion after metabolism into P2Y2 agonist[279]
Oral hydroxychloroquine	Increase Schirmer wetting score in in Sjögren's syndrome patients with anti-fodrin antibodies[280]
Oral pilocarpine and cevimeline	Reduce subjective eye symptoms in Sjögren's syndrome patients, with no effect on tear volume[281-283]
Lacritin and synthetic derivatives	Promote basal tearing[284, 285]
Abdominal (diaphragmatic) breathing	Increase tear meniscus volume[286]

IV.A.2.d. Epithelial cell desiccation

Several osmoprotectants have been proposed to improve cell water management, avoid cell membrane lipid oxidation and protein denaturation, and preserve epithelial cell life in hyperosmotic conditions. (Table 11). Combination formulas containing osmoprotectants (along with other agents) have also been reported to alleviate ocular symptoms and staining in patients with DED.[287]

Table 11. Treatments Targeting Epithelial Cell Desiccation

Treatment	**Potential Effects**
Betaine	Reduce apoptosis, hyperosmotic stress, and expression of proinflammatory cytokines[288-292]
Ectoine	Fluidize lipids and reduce evaporation[293]
Erythritol	Reduce hyperosmotic stress and expression of proinflammatory cytokines[288, 289, 291, 292]
L-carnitine	Reduce hyperosmotic stress and expression of proinflammatory cytokines[288, 289, 291, 292]
Taurine	Reduce inflammation-related proteins[294]
Trehalose	Prevent desiccation-induced corneal epithelial cell death[295, 296]

IV.A.2.e. Mitochondrial oxidation

A small molecule SkQ1 (also called Visomitin or plastoquinonyl decyltriphenyl phosphonium) has been engineered to reduce oxidative stress inside mitochondria (Table 12), with the goal of decreasing the apoptosis of ocular surface epithelial cells during aging.[297] This topical drug is currently in DED clinical trials in the United States.[298] An ophthalmic formulation of Visomitin is already marketed in Russia.[299]

Table 12. Treatments Targeting Mitochondrial Oxidation

Treatment	**Potential Effects**
plastoquinonyl decyltriphenyl phosphonium (SkQ1; visomitin)	Decrease mitochondria-generated ROS during aging and reduce apoptosis of ocular surface epithelial cells[297, 298]

IV.A.2.f. Ocular surface inflammation

As defined in the TFOS Dry Eye WorkShop (DEWS) report,[66] "Dry eye is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface." This DED association with inflammation, especially given the approval of Restasis by the USA FDA, has led to the development of numerous anti-inflammatory compounds for possible use in DED treatment (Table 13). However, despite this effort, interventional clinical trials of anti-inflammatory and immunomodulatory agents have invariably failed to meet their primary endpoints (e.g. typically corneal fluorescein staining; discussed later).

Table 13. Treatments Targeting Inflammation

Treatment category	Potential Effects
Omega 3 fatty acid supplementation	Alter tear metabolic profile;[300] decrease tear evaporation rate;[301] improve TBUT and Schirmer scores[302]
Resolvins	Metabolite of omega-3 fatty acids; reduce corneal epithelial barrier disruption and goblet cell loss
Glucocorticoids	
Dexamethasone & transscleral iontophoresis	
Mapracorat	
Loteprednol	
Fluorometholone	
Difluprednate	
Non-steroidal anti-inflammatory drugs (e.g., bromfenac, diclofenac and pranoprofen)	Inhibit cyclooxygenases 1 and 2 and promote mucous production
Calcineurin inhibitors	Immunosuppression via inhibition of calcineurin
Tacrolimus (Prograf)	
Cyclokat® (0.1% cyclosporine)	
Cationic emulsions	
Pimecrolimus (Elidel)	
Antibodies	
Secukinumab	Neutralize IL-17α
ESBA105	Inhibit TNF-α
Belimuma	Inhibit B cell activation factor (BAFF)
VAY736	Inhibit BAFF-receptor
Canakinumab	anti-IL-1β
Tocilizumab	anti-IL-6 receptor
Baminercept	Inhibit lymphotoxin-β and LIGHT pathway
Abatacept	Inhibit T cell activation
Other anti-inflammatory drugs	

Lifitegrast (SAR 1118)	Bind to LFA-1 and prevent ICAM-1 interaction, inhibiting adhesion, migration, proliferation, and cytokine release
CAN-FITE-101	Oral adenosine3 receptor agonist; induce inflammatory cell apoptosis
Regenerx RGN259 ()	Topical thymosin β4
Perceiva	Sirolimus, subconjunctival injection, inhibit response to IL-2
Tofacitinib	Inhibit Janus kinase 3
EBI-005	Antagonize IL-1 receptor signaling
Cis-urocanic acid	Reduce inflammation and free radicals
DNase	Remove neutrophil extracellular traps
XG-104	Inhibit c-Jun N-terminal kinase, T cell differentiation and cytokine production
R932348	Inhibit Janus kinase 3 and Syk kinase
Mesenchymal stem/stromal cells	Suppress inflammation
FK506 binding proteins	Inhibit cytokine production
SB203580	Decrease lacrimal gland inflammation
Rivoglitazone & WY14643	Bind to PPARs
Cilomilast	Phosphodiesterase 4 inhibitor
DA-6034	Inhibit NF-κB activation
Chitosan-N-acetylcysteine conjugate	Thiolated polymer; suppress inflammation, no effect on corneal staining
Tranilast	Inhibit lipid mediator and cytokine release
N-acetyl aspartyl glutamic acid	Neuropeptide, antiinflammatory
Astaxanthin	Oral carotenoid, antiinflammatory
Curcumin	Natural polyphenol extracted from turmeric, antiinflammatory
Catechins	From green tea, antiinflammatory
KLS-0611 & KCT-0809	Treat surface damage, antiinflammatory
Mimetogen MIM-D3	Tyrosine kinase TrkA receptor agonist[303]

IV.A.2.g. MGD

Meibomian glands play an extremely important role in the health and well-being of the ocular surface. The acinar epithelial cells of these glands secrete a proteinaceous lipid mixture (i.e., meibum) that promotes the stability and prevents the evaporation of the tear film.[121] Conversely, MGD, and the resulting meibum insufficiency, destabilizes the tear film, increases its evaporation and osmolarity, and is the most common cause of DED (Figure 10).[121]

The 2011 TFOS MGDW Subcommittee on Management and Treatment recommended treatment options for various stages of MGD.[304] In the United States, azithromycin (used off label) is the most common treatment for MGD,[305] and was thought to decrease inflammation and bacterial growth. Recent studies

have shown, though, that azithromycin's efficacy may also be due to stimulating the function of meibomian gland epithelial cells.[306, 307] Tetracycline-based antibiotics do not duplicate these azithromycin actions on human meibomian gland epithelial cells.[308]

Another MGD treatment approach is topical testosterone, given that androgen deficiency is a significant risk factor for the development of MGD.[160, 309, 310] Topical testosterone drops were tested in a Phase 2 clinical trial by Allergan and reportedly led to a significant decrease in the signs and symptoms of MGD.[311] Allergan has recently continued these trials in the United States.

Figure 10 illustrates the events that cause progression of MGD to a self-propagating, chronic condition, and represents multiple new therapeutic approaches, summarized in Table 14.

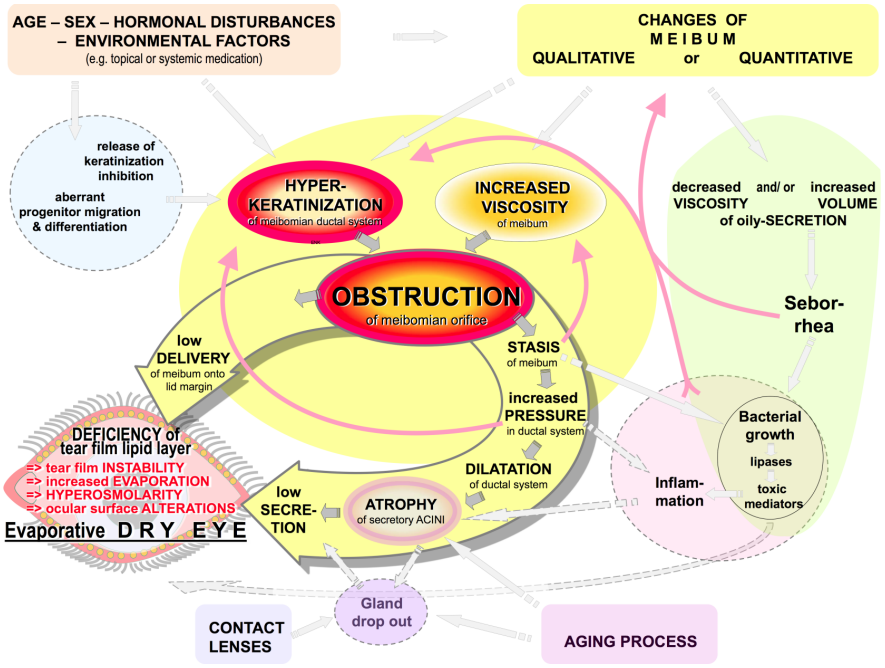


Figure 10. The interacting pathways in meibomian gland dysfunction (MGD). The core mechanisms of obstruction (via epithelial hyperkeratinization) with increased viscosity (of meibum) result in two effector limbs (arrows) that lead to low delivery of oil onto the lid margin and tear film, or via the atrophic pathway to a secondary low secretion of oil. Both effector arms result in evaporative dry eye. This process is based on underlying factors (shown in square colored boxes in the periphery) and influenced by associated functional complexes (shown on rounded colored areas). Vicious circles (indicated by red arrows) act back and can reinforce the pathological process into a self-propagating disease, if not limited by timely diagnosis and therapy. Reprinted from: Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. Invest Ophthalmol Vis Sci. 2011;52:1938-78. Copyright: Association for Research in Vision and Ophthalmology.

Table 14. Treatments Targeting MGD

Treatment	Potential Effects
Lipid-based tear substitutes	Promote lipid spreading, lubrication and tear stability, and reduce tear evaporation
Intraductal probe	Remove the terminal duct obstruction

Eyelid warming devices (e.g., LipiFlow®, Blephasteam®, Eyegiene®, MGDRx EyeBag®, MediBeads®, warm compresses)	Alleviate meibomian duct obstruction by improving eyelid hygiene (warming and humidifying the eyelid to liquefy meibum)[312-321]
Intense pulsed light (IPL)	Warm meibomian secretions; decrease inflammatory cytokines and microbial growth[322-324]
Topical testosterone	Decrease hyperkeratinization of the terminal duct, stimulate meibomian gland function and increase the quantity and quality of meibum[160, 311]

IV.A.2.h. Ocular surface stress

DED hyperosmolarity leads to increased ocular surface friction and stress. Recent studies have suggested that lubricin, an anti-friction and anti-adhesion protein, may serve as an important barrier to the generation of such friction and stress, and especially the corneal and conjunctival epitheliopathies that occur in DED [7, 8] If so, then topical lubricin could prevent the DED-associated downstream development of ocular surface inflammation and damage (Table 15). A clinical trial is currently underway in Europe to evaluate the therapeutic potential of lubricin in alleviating DED.

Table 15. Treatments Targeting Ocular Surface Stress

Treatment	Potential Effects
Lubricin	Decrease ocular surface friction and stress in DED[7, 8]

IV.A.3. Reasons for Failure in Clinical Trials for DED

Despite supportive preclinical evidence, the vast majority of therapeutic candidates for DED have failed in clinical trials. The low success rate may be due to the multitude of potential confounders, including clinical trial length (in relation to time taken to reverse end points), numerous environmental factors, heterogeneity in study populations (e.g., age, gender, comorbidities), and the heterogeneity of DED itself (in terms of causes, symptoms, and clinical manifestations). While some trials of potential therapies (such as rebamipide[325] and pimecrolimus[326]) have ended quietly with little explanation, the results of other failed studies have highlighted overarching issues that plague clinical trials for DED.

IV.A.3.a. Dependence on corneal staining

Although some clinical trials for DED have met certain co-primary or secondary endpoints, the vast majority of studies have failed to meet endpoints related to corneal staining (Table 16). Corneal staining is generally accepted as a sign of ocular surface damage (and therefore indicative of DED); however, it is important to note that corneal staining does not occur in all cases of dry eye, nor is it sufficient to diagnose DED under the current definition,[66] as discussed in Section III.C of this report. Finally, corneal staining may not be the most robust measure of dry eye severity.[143, 327] Therefore, the dependence on corneal staining to assess treatment response may confound the interpretation of clinical trial results.

IV.A.3.b. Lack of correlation between clinical signs and symptoms.

Although symptoms of discomfort are the foremost feature in the current definition of DED,[66] symptoms alone are insufficient for the diagnosis or management of DED, and their subjective nature limits the sensitivity of symptom scores for assessing treatment response. Furthermore, for DED, the correlation between signs and symptoms is notably poor,[328] as is the correlation among individual dry eye tests.[329] This lack of correlation between the signs and symptoms of DED complicates the clinical evaluation of candidate therapies. It may be that a consensus of clinical signs may better reflect all aspects of the disease.[329]

Table 16. Summary of Recent Dry Eye Trials that Failed to Meet Corneal Staining Endpoints

Compound	Mechanism of Action	Company	Result
Lifitegrast	Small-molecule integrin inhibitor; ICAM-1 decoy	SARcode Bioscience, Shire	Results of the OPUS-1 trial (presented in 2012) met primary endpoint of change from baseline in inferior corneal staining score vs. placebo (p=0.0007), and showed significantly reduced corneal fluorescein staining (superior and total) and conjunctival lissamine staining vs. placebo;[330] however, top-line results of the OPUS-2 trial did not meet co-primary endpoint of change from baseline in inferior corneal staining score (p=0.6186 vs. placebo)[331]
CF101	A3 adenosine receptor agonist	OphthaliX Inc. (subsidiary of Can-Fite BioPharma, Ltd.)	Did not meet primary efficacy endpoint of complete clearing of corneal staining[332]
Diquafosol tetrasodium 2% (Prolacria)	Purinoreceptor P2Y(2) receptor agonist; promotes tear and mucin secretion by increasing intracellular Ca^{2+} concentrations	Inspire Pharmaceuticals	Did not achieve significance of the % of patients with complete corneal staining clearance (primary p = 0.526) or its secondary endpoint of a two-unit reduction in staining (p = 0.368)[235]
RGN-259 [Thymosin beta 4 (Tβ4)]	Promotes cell migration; increases cell adhesion via increased laminin-5 production; anti-apoptotic and anti-inflammatory	RegeneRx Biopharmaceuticals	Did not meet all pre-specified primary outcome measures including inferior corneal and conjunctival staining[333-335]
Bromfenac (REMURA™)	Cyclooxygenase 1 and 2 inhibitor	ISTA Pharmaceuticals, Inc.	Staining was not statistically better than placebo in the entire patient cohort[336]
EGP-437	Novel formulation of dexamethasone phosphate delivered iontophoretically	Eyegate Pharmaceuticals, Inc.	Primary efficacy endpoints of corneal staining and ocular discomfort were not achieved[337]

RX-10045	Synthetic analog of RvE1, a resolvin with anti-inflammatory and cell-sparing activities	Resolvyx Pharmaceuticals, Inc.	There was a significant reduction in CAE-induced staining from baseline, but the change was not significantly different than placebo (p=0.11)[338]
MIM-D3	TrkA receptor agonist (NGF mimetic)	Mimetogen Pharmaceuticals Inc.	Pre-specified primary outcome measures of fluorescein corneal staining and worst symptom scores over 28 days were not met[303]
EBI-005	Interleukin-1 (IL-1) receptor blocker	Eleven Biotherapeutics, Inc.	Differences from baseline in change in total corneal fluorescein staining and patient-reported ocular pain and discomfort based on the ocular surface disease index (OSDI), were not statistically significant when compared to patients who received vehicle control[339]
R348	Ophthalmic JAK/SYK inhibitor	Rigel Pharmaceuticals, Inc.	Failed to meet changes in corneal fluorescein staining, conjunctival staining, tear production, and dry eye symptom scores from baseline over 12 weeks of treatment vs. placebo[340]

IV.A.3.c. Dependence on murine preclinical models

For many human conditions, murine models have been invaluable for elucidating the underlying physiological and pathological processes. However, therapeutic candidates that have been identified and preclinically validated in mouse models do not always translate successfully to humans. This is may be particularly true for therapies targeting inflammatory pathways, as genomic responses to inflammatory challenges have shown poor correlation between different mouse models in comparisons with human responses.[341] Although some studies show that mouse results can be predictive of therapeutic success in humans,[342, 343] the fact remains that dozens of clinical trials involving investigational anti-inflammatory therapies based on mouse data have failed, including several potential treatments for DED (Table 16). These findings underscore the need to demonstrate whether a mouse model mimics, or fails to mimic, a relevant human disease.[341, 344, 345]

IV.A.4. Summary and Conclusions

A major unmet need in the treatment of DED is the lack of effective global treatments. Recent advances in the understanding of underlying pathological processes have uncovered novel disease targets, which may lead to innovative therapeutic strategies for this complex disease. Clinical trials of candidate therapies, though largely unsuccessful to date, have provided insight for future lines of investigation and therapeutic development—emphasizing the need for caution when extrapolating basic scientific findings from animal models to human therapies.

IV.B. Regeneration of the Ocular Surface in Severe DED (Stefan Schrader, MD, PhD)

Dry eye is a disease with many different causes, as described in the 2007 report of the TFOS DEWS Definition and Classification Subcommittee[66] and elsewhere in this report (see Section III.C. and Figure 8). As such, multiple pathologic mechanisms can interact and synergize to damage the ocular surface (Figure 4).

For DED, most available therapies either aim to relieve symptoms or to curb inflammation of the ocular surface (see Section IV.A, New Approaches for the Treatment of DED). Although these treatment strategies are targeting fundamental attributes of DED, they do not fully address the unmet needs in disease management—especially in severe cases. By definition, symptomatic treatments do not address the underlying cause(s) of disease; furthermore, immunomodulatory treatments are largely targeting the final, self-propagating pathways of DED, and might only serve to prevent or slow the progression of damage. A more meaningful approach would target the specific causes of DED and regenerate damaged structures—thus reversing the pathological processes. To this end, three approaches are presented for regenerating the ocular surface and adnexa in severe DED: 1) support of regeneration by a "tear-like" substitution, 2) regeneration/reconstruction of secretory tissue, and 3) regeneration/reconstruction of the ocular surface itself.

IV.B.1. Support of Regeneration by "Tear-Like" Substitution

The ocular surface environment contains an array of growth factors, which suggests that they support tissue maintenance and wound healing. Artificial tears, which form the largest treatment class for DED, improve lubrication and hydration; however, they lack the biological properties of natural tears.[346] Thus, a more tear-like substitution may be necessary to support regeneration of the ocular surface.

IV.B.1.a. Autologous serum

Like natural tears, the serum contains numerous growth factors, including epidermal growth factor (EGF), transforming growth factor beta (TGF-β), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF); thus, autologous serum has been considered for potential therapeutic use in ocular surface disorders.[347] The clinical use of autologous serum to support corneal regeneration can be traced to the mid-1970s, when Ralph, Doane, and Dohlman perfused various fluids (including autologous and homologous sera) to the ocular surfaces of patients following keratoplasty, severe chemical burns, or lid/fornix reconstruction for severe DED.[348] About a decade later, artificial tears containing autologous serum were reported to have beneficial effects for keratoconjunctivitis sicca.[349] In 1999, autologous serum was reported to improve ocular surface staining (rose bengal and fluorescein) in Sjögren's syndrome[350] and to successfully treat persistent epithelial defects.[351] This triggered more widespread use of serum eye drops as topical epitheliotrophic treatments, and optimized protocols were developed for the production of autologous serum for this purpose.[352] Serum has also been successfully used in combination with other therapies. For example, combined with hydrogel bandage contact lenses, autologous serum eye drops demonstrated a significant improvement of healing of persistent epithelial defects.[353]

However, in the growing body of literature evaluating autologous serum for DED, there has been mixed evidence of success in terms of clinical utility. The efficacy of autologous serum was evaluated in the 2007 report of the TFOS DEWS Management and Therapy Subcommittee,[4] which found that the efficacy of serum eye drops for DED varied substantially between studies.[350, 354-357] In addition to significant variations in patient populations, there have been large disparities in production, storage, and treatment protocols.[352] The most convincing evidence of success has been for the treatment of persistent epithelial defects, in which efficacy is more evident in "healing" of the defects.[358] For objective measures of DED, the

benefits are less clear. A recent randomized, double-blind, crossover clinical trial demonstrated improvement in symptoms after short-term autologous serum compared to artificial tears (Systane®), but no change in objective parameters such as tear break up time (TBUT) and Oxford score for corneal and conjunctival fluorescein staining.[359] A recent Cochrane systematic review and meta-analysis, published in 2013, found inconsistency in the potential benefits of autologous serum on TBUT and subjective symptoms, and overall lack of effect based on objective clinical measures.[360] To properly evaluate the benefits of autologous serum for DED, well-designed studies are warranted, using standardized questionnaires, reliable objective assessments, and objective biomarkers. To further complicate the evaluation of autologous serum, it is not only difficult to compare different trials but also individual patients within trials, as serum epitheliotrophic/anti-inflammatory factors will vary between patients. Moreover, it is still unclear which factors are the key components of autologous serum. Nonetheless, in the absence of other tear substitutes containing epitheliotrophic factors, autologous serum can be considered as the current gold standard in this treatment approach, and a valuable therapeutic option for severe DED.

IV.B.1.b. Nerve growth factor (NGF)

Regeneration of innervation is an important consideration for restoring ocular surface homeostasis, which is regulated coordinately by neural inputs and other components of the integrated lacrimal functional unit. As detailed elsewhere in this report, the pathologic processes of DED (e.g., inflammation, hyperosmolarity) can alter corneal sensitivity and disrupt the lacrimal functional unit, thus reducing tear secretion and tear film stability (see Section III.A, Pathophysiology of DED). Significantly lower corneal subbasal nerve density has been observed in non-Sjögren DED compared with age-matched controls.[361] Direct injury to the corneal nerves, which can occur in various ocular procedures, also can disrupt tear dynamics and/or cause neurotrophic keratopathy (see Section II.B, Surgery-induced DED). Indeed, almost half the patients who undergo laser in situ keratomileusis (LASIK) experience neuropathic symptoms of dry eye.[362]

Specific and defined treatments must be developed in order to improve corneal nerve function and support regeneration of innervation. In this context, tear-like substitutions containing nerve growth factor (NGF) hold much potential for modulating corneal innervation and improving epithelial cell healing. In patients with neurotrophic keratopathy treated with autologous serum, the restoration of ocular surface epithelial integrity has been attributed to the NGF content of the serum.[363, 364] In rats with capsaicin-induced corneal damage, NGF-containing eye drops improved Schirmer values, promoted corneal healing, and reversed corneal sensory denervation.[365] This is further corroborated by a canine model of dry eye disease experimentally induced by bilateral lacrimal gland removal, in which topical application of NGF significantly improved all evaluated parameters compared to baseline values and control contralateral eyes, including keratopathy, corneal haze, conjunctival goblet cell density and impression cytology, Schirmer values, and corneal staining.[366]

There is evidence that NGF also is involved in the modulation of tear function. In patients following refractive surgery, differences in six-month postoperative corneal sensitivity and sensations of ocular surface dryness were correlated with tear levels of NGF in the early postoperative period.[83] In preclinical investigations, NGF has recently been shown to regulate conjunctival goblet cell secretion in rats.[367] Upregulation of NGF has also been correlated with reduced corneal epithelial cell apoptosis after hyperosmolar stress in vitro, suggesting that NGF may promote recovery from hyperosmolarity-induced corneal damage.[368]

Recombinant human NGF (rhNGF) has now been investigated clinically in patients with various ocular surface conditions. In patients with dry eye disease, rhNGF eye drops (4 or 20 μg/mL) have been tested in an open-label, monocentric Phase II study, to be completed in 2015.[369] NGF is also being investigated in

the REPARO Study, a Phase I/II randomized, double-blinded, multicenter trial evaluating the safety and efficacy rhNGF eye drops in patients with neurotrophic keratitis stage 2 (persistent epithelial defect) and stage 3 (corneal ulcer).[370] Preliminary results from the Phase I segment demonstrated that the treatment was safe and well tolerated, and suggested favorable effects on healing of corneal lesions in the majority of patients with neurotrophic keratitis caused by diabetes, surgery, and viral infection.[371] Promising preliminary results have also been obtained in patients with neurotrophic keratitis caused by chemical burns.

IV.B.2. Reconstruction/Regeneration of Secretory Tissue

Aqueous-deficient dry eye (ADDE) is a major class of DED, and mainly signifies lacrimal gland deficiency (Figure 8), although insufficient aqueous secretion by the conjunctiva may also underlie ADDE.[66] However, most efforts to restore secretory tissue in severe DED have focused on lacrimal gland reconstruction or regeneration, and have involved several approaches.

IV.B.2.a. Salivary gland transplantation

One approach of lacrimal gland replacement involves heterotopic salivary gland transplantation. Various glands have been used for this purpose, such as the major salivary glands (e.g., the submandibular gland)[272, 372] and minor salivary glands.[273] This method is capable of restoring seromucinous secretions (with submandibular gland transplantation) as well as the aqueous tear film layer, and significant improvements are seen in Schirmer values, FBUT, corneal staining, and symptoms of discomfort. However, vision is not affected; moreover, it is important to note that the osmolarity of saliva is very different than tears. In fact, the hypoosmolar salivary secretions can induce a microcystic corneal edema, which can in turn lead to epithelial defects. Thus, lacrimal gland replacement is only for selected patients with end-stage DED with absolute aqueous tear deficiency.

IV.B.2.b. Lacrimal gland reconstruction

Tissue engineering for lacrimal gland reconstruction is still very much experimental, as the reconstruction of a complex functional secretory tissue (such as the lacrimal gland) poses several challenges. As with virtually all bioengineered tissues, cells first need to be isolated, then expanded in sufficient clinical-grade quantities, and characterized to ensure function. Finally, cells need to be grown in functional quantities on biocompatible, three-dimensional matrices that ensure mechanical stability and bioavailability. Excellent reviews on strategies and tools for tissue engineering have been published,[373, 374] and techniques have been described for isolating and culturing lacrimal gland epithelial cells.[375, 376] Secretory function can be tested using the ß-hexosaminidase assay.[377] Briefly, ß-hexosaminidase activity is a biomarker for lysosomes and is an established test for acinar cell secretion. Enzymatic activity of ß-hexosaminidase is measured in the cell culture supernatant at baseline and after parasympathetic stimulation (usually with carbachol or phorbol esters). Work by our group[378] and others have focused on developing model constructs for lacrimal gland reconstruction. A simulated microgravity environment[379] as well as a lacrimal gland in vitro model based on decellularized lacrimal gland tissue[380] have been developed, and facilitate the development of three-dimensional constructs containing functional acinar cells with secretory responses to carbachol. Hirayama et al. recently bioengineered fully functional lacrimal gland replacements by transplantation of a bioengineered organ germs.[274] These recent advances represent great strides toward the goal of lacrimal gland replacement for DED.

IV.B.3. Reconstruction/Regeneration of the Ocular Surface

In severe cases of ocular surface disease or injury, reconstruction of the ocular surface is often necessary to prevent corneal opacity and recurrence of the disease. Approaches for reconstructing and regenerating

the ocular surface range from established grafting techniques to promising possibilities from advances in tissue engineering.

IV.B.3.a. Amniotic membrane transplantation

For reconstructing the ocular surface, the amniotic membrane is the most commonly used matrix; this approach is simple and most reliable, and can promote healing of the ocular surface in spite of severe tear film deficiencies.[381] The amniotic membrane possesses many characteristics that directly address the pathophysiology of DED: it can act as a supportive substrate for epithelial cells; it has anti-inflammatory properties; and the amnion harbors various growth factors (e.g., NGF, TGF-ß, KGF, EGF) that are beneficial for ocular surface repair and regeneration.

The amniotic membrane can be used in various ways for corneal surface reconstruction. As an inlay graft (typically for sterile ulcers), one or more layers of amniotic membrane can integrate into the cornea—filling stromal defects and acting as a basement membrane for the epithelium.[382] As a protective onlay (typically for epithelial defects), the amniotic membrane acts as a natural bandage contact lens to support epithelial closure, and usually falls off after 1–2 weeks. A sandwich technique, combining both onlay and inlay grafts, can be used for severe ocular surface defects.

The amnion also may be used for conjunctival reconstruction, however with some limitations. Efficacy of conjunctival reconstruction is dependent on whether the bulbar conjunctiva or the fornix has to be restored: for the bulbar conjunctiva, results are typically favorable, whereas in fornix reconstruction, fornix depth is often lost.[383] Oral mucosa transplantation can be combined with amniotic membrane for reconstruction of the conjunctiva, with the amnion mostly used for the bulbar conjunctiva and the more bulky and stable oral mucosa for tarsal reconstruction.

While amniotic membrane has many advantages for ocular surface reconstruction (mostly relating to its elastic, basement membrane, anti-inflammatory, and epitheliotrophic properties), there are some drawbacks. Transparency can be variable, often limiting best-corrected visual acuity to 6/60. There also may be variability in tissue properties and epitheliotrophic factors, depending on donors and storage methods. Furthermore, highly inflamed ocular surfaces may result in quick enzymatic digestion and loss of membranes. Finally, disease transmission is an inherent concern with allogeneic transplants. Thus, there is a need for the development of new matrices to standardize mechanical properties and growth factor content.

IV.B.3.b. Bioengineered ocular surface substitutes

Many of the potential limitations of both autologous and allogeneic grafts may be circumvented with bioengineered tissue grafts. These can be produced in a highly controlled, standardized, and scalable manner, with high potential for growth factor integration.

Equine type I collagen membranes showed satisfactory re-epithelialization after ocular surface reconstruction in rabbits, and thus may be suitable for corneal surface reconstruction in patients with persistent, nonhealing ulcerations.[384] As transparency is an important concern in corneal surface reconstruction, keratin matrices made of human hair may serve as suitable matrices for corneal epithelial progenitor cells. Chemically processed and gamma sterilized, the keratin matrix is displays excellent transparency in vitro, suitable biomechanical properties, and good surgical applicability in suturing to the ocular surface.[385, 386] In biocompatibility experiments in a rabbit model, keratin films exhibited excellent transparency and served as a good surface for limbal epithelial cell growth.[387] These bioengineered matrices may have clinical utility for reconstructing the corneal surface.

While there are few reports in the scientific literature on bioengineered conjunctival substitutes, modified poly(lactide-co-glycolide) (PLGA) scaffolds have been investigated in vivo for reconstruction of conjunctival wounds.[388] PLGA/collagen scaffolds were grafted for four weeks, and exhibited complete epithelialization and reduced scarring/contraction. However, this type of scaffold lacks elasticity, which poses a problem for reconstructing the fornix. Conjunctival tissue substitutes based on compressed collagen gels may be useful for this purpose; these serve as good substrates for conjunctival cells and display favorable biomechanics due the compression process, resulting in very good surgical handling.[389] While much of the work on ocular surface bioengineering is still in the preclinical stage (particularly for conjunctival reconstruction), there are many exciting developments that may lead to future clinical trials and integration into the management for severe DED.

IV.B.3. Summary and Conclusions

For the treatment of severe DED, there have been dramatic developments in the areas of tear-like substitution, regeneration of secretory tissues, and ocular surface reconstruction. The key factors of existing treatments have to be identified in order to develop new more effective therapies—particularly in addressing the specific causes of DED and mechanisms of damage. Ongoing research on NGF and other neurotrophic and epitheliotrophic factors may yield new possibilities for targeted treatment of DED subtypes. The growing fields of regenerative medicine and tissue engineering offer promising new approaches, particularly for lacrimal gland regeneration and reconstruction, as well as reconstruction of the ocular surface.

IV.C. Regulatory Issues Associated with the Development of a DED Drug in Europe (Gary D. Novack, PhD)

Although DED is a significant global problem and the focus of ample research and development, very few pharmacologic treatments have achieved regulatory approval in limited markets. In the 2010 TFOS/ARVO Symposium on Global Treatments for DED, barriers were identified in the development of pharmacotherapies for DED. As noted in the published report from that symposium in 2012, there were no pharmacological approvals in Europe.[390] In a 2014 article, Dr. Novack noted that Restasis® was approved in some Eastern European countries.[391] In the present meeting (2015), Dr. Novack reviewed regulatory issues in gaining regulatory approval in Europe for pharmacological treatment of DED.

IV.C.1. European Medicines Agency Workshop on Medicines for Eye Disorders

In October 2011, the European Medicines Agency (EMA) hosted the inaugural Workshop on Clinical Development and Scientific Advice in Ophthalmology.[392] This workshop gathered experts from academia, industry, and regulatory agencies to review the regulatory and scientific challenges in developing medicines for eye disorders. One session was dedicated to DED, and featured a review of new therapeutic approaches and challenges for the treatment of DED by Dr. David Sullivan,[393] an update of the TFOS MGDW by Dr. Kelly Nichols,[394] and an overview of ocular surface biomarkers and inflammation by Prof. Anthony Bron.[395] Additionally, industry perspectives were provided by Dr. Auli Ropo of Santen,[396] and regulatory perspectives by Dr. Kerstin Wickström of the Swedish Medical Products Agency (MPA).[397]

In her presentation, Dr. Wickström noted that there were no centrally approved pharmacological therapies for DED in the EU, although oral pilocarpine has been approved for symptomatic treatment of dry eyes in Sjögren's syndrome via the mutual recognition procedure (MRP). Also used are oral bromhexine and evening primrose (*Oenothera glazioviana*).

European submissions also require a pediatric investigation plan to be submitted relatively early in the development process. This is a non-trivial plan, as well as typically a non-trivial clinical trial to conduct.

IV.C.2. Recent European approval

On January 23, 2015, the EMA's Committee for Medicinal Products for Human Use (CHMP) adopted a positive opinion recommending marketing authorization for Santen's Ikervis® (cyclosporine 1% emulsion), and marketing authorization (valid throughout the European Union) was issued March 19, 2015.[398] The indication is "treatment of severe keratitis in adult patients with DED, which has not improved despite treatment with tear substitutes." At the time of the meeting (March 21, 2015), efficacy results underlying the approval were not available. Subsequently, that data became available on the EMA's website (July 2015). The Sponsor conducted two randomized, vehicle-controlled double-masked studies, with the acronyms of SANSIKA (12 months) and SICCANOVE (6 months).

In SANSIKA, the primary outcome was a co-primary evaluation of signs and symptoms. A small but statistically significant difference in CFS improvement was observed between the treatment groups at Month 6 in favor of the active (mean change from baseline in CFS -1.05 with IKERVIS and -0.82 with vehicle, p=0.009). The mean change from baseline in ocular discomfort score (assessed using a Visual Analogic Scale) was -12.82 with IKERVIS and -11.21 with vehicle (p=0.808).

IV.C.3. Regulatory advances in other major markets

As of the time of the meeting, there had been no regulatory approvals for pharmacological treatment of DED since 2012 in either the U.S. or Japan. In 2012, lifitegrast ophthalmic solution demonstrated efficacy in the OPUS-1 Phase III clinical trial.[330] A new drug application (NDA) for lifitegrast was submitted by Shire in 2015 to the U.S. Food and Drug Administration (FDA).

IV.C.4. Current status and Perspective

The regulatory status of pharmacological therapies for DED is major markets as of March 2015 is shown in Table 17.

Table 17. Regulatory Status of Therapies for DED

	Country/Region (Year Approved)			
Product	**USA**	**Canada**	**Japan**	**Europe**
Cyclosporine	Restasis® (2002)	Restasis® (2010)		**Ikervis® (2015)**
Hyaluronic Acid*	---	---	Hyalein® (1995)	**---**
Diquafasol	---	---	Diquas® (2010)	**---**
Rebamapide	---	---	Mucosta® (2011)	**---**

Modified from Sullivan DA, Hammitt KM, Schaumberg DA, et al. Report of the TFOS/ARVO Symposium on global treatments for DED: an unmet need. Ocul Surf. 2012;10:108-16.

By way of perspective in the risks of pharmaceutical development, Dr. Novack cited a 2010 report of all investigational compounds that entered clinical testing between 1999 and 2004. In this analysis based upon confidential data provided to the authors, only 16% eventually gained marketing approval.[399] A second report assessed the reasons for U.S. FDA rejection of initial NDAs. The major reasons were deficiencies in efficacy, safety, or chemistry.[400]

In a recent interview, Dr. Timothy J. Garnett, Chief Medical Officer and Senior Vice President of Eli Lilly & Company, maintained that inadequate attention to dosing during Phase 2 testing may result in costly dose finding (and increased risk of failure) in Phase 3 trials.[401] Studies on pharmacokinetics and pharmacodynamics are relatively uncommon in ophthalmology compared to other fields of medicine, and pharmacogenomics is virtually nonexistent for DED.[391]

IV.C.5. Summary and Conclusions

With the 2015 approval of Ikervis®, there is now a regulatory precedent in Europe. As noted previously by Novack (2014),[391] development of a pharmacological treatment for dry eye is risky, and dependent upon having the right drug, the right dose, the right disease (i.e. patient selection) and the right endpoints. Fortunately, ophthalmology continues to be an area of investment,[402] and thus it is hopeful that new pharmacotherapies will have the opportunity to be developed.

V. SUMMARY (*Kelly Nichols, OD, MPH, PhD*)

In the closing session, Dr. Nichols expressed gratitude to TFOS—particularly Amy Sullivan, Executive Director—for coordinating and raising funds for the meeting; and Dompé for generous financial support. The speakers, moderators, panelists, and attendees were also thanked for sharing their insight and fostering collective intellectual curiosity. Dr. Nichols highlighted key statements from the presentations and panel discussions to provide a global summary of the meeting; identify recurring themes among the diverse topics; and emphasize important areas of investigation towards meeting the unmet needs in dry eye treatment.

V.A. Scope of the DED Problem

Over 20 large-scale, population-based studies have demonstrated clearly that DED is a global issue with prevalence ranging from approximately 8–50%, depending on the population(s) studied and the diagnostic criteria and questionnaires used. However, there is a striking lack of consensus on a minimum diagnostic battery, which becomes an even greater concern as it carries over from the clinic into clinical trials.

Treatment options remain limited for patients, and even newly approved therapies merely represent expansion of markets for existing treatments. More therapeutic options are urgently needed; additionally, management protocols must integrate education and prevention in order to deliver treatment to a broader clinical audience.

Incidence and natural history data are still lacking for DED, and ongoing research may improve the identification and classification of the disease state. Existing surveys and questionnaires have many limitations in terms of monitoring changes over time or with intervention. A persistent problem is the lack of correlation between signs and symptoms of DED.

Various ophthalmic procedures (e.g., refractive surgery, trabeculectomy, and cataract surgery) are known to induce DED; conversely, DED can affect surgical outcome. Thus, greater consideration of ocular surface health is needed in the arena of ocular surgery. Nonetheless, surgically induced dry eye is a promising model in which to study causative etiologies, diagnosis, and disease management. Contact lens discomfort may also be a promising model for studying dry eye pathogenesis, particularly as it affects 50% of contact lens wearers and represents a two- to three-fold higher odds ratio for DED.

V.B. Clinical Challenges of DED

The clinical challenges of DED include its pathophysiology and symptomology, which collectively drive diagnosis. While it is clear that modifiable environmental factors are important in dry eye pathophysiology, there is still a lack of consistent evidence to convince clinicians and patients alike to address these factors.

Nonetheless, advances in the understanding of core pathological mechanisms may lead to improved diagnosis and management. Tear osmolarity is emerging as a meaningful diagnostic measure, and the forthcoming DEWS II presents an opportunity to systematically assess the existing osmolarity data in order to make reliable recommendations. Other emerging concepts also promise to drive the reciprocal translation between clinical and scientific investigation. These include Marx's line position in the diagnosis of MGD; compositional changes in the tear lipid layer; and the improved understanding of ocular surface innervation and nerve responses associated with dry eye symptomology, osmolarity, blinking, and tearing.

The definition and classification of DED is a continuing clinical challenge for diagnosis and management. Of note, the overlap between ADDE and EDE warrants re-exploration of the impact of evaporation on DED subtypes and their diagnosis. It is abundantly clear that DED is not a single disease, but rather multiple interrelated and overlapping conditions with distinct etiologies. Therefore, specific targeted treatments are needed for the various subtypes of DED. Accordingly, these targeted treatments require specific test batteries and clinical trial designs—reemphasizing the need for a minimum set of diagnostic criteria for DED, and perhaps distinct tests for screening and diagnosis.

V.C. Treatment Challenges of DED

Despite dozens of clinical trials and therapeutic candidates at some level of development, there are only three pharmacologics approved for the treatment of dry eye in limited markets. It continues to be difficult to demonstrate efficacy for dry eye therapeutics in clinical trials. In this regard, considerations include the precision of the diagnostic batteries, the suitability of the study designs (i.e., reflecting the mechanism of action of the candidate therapeutics), and the relevance of the outcome measures.

Nonetheless, there is encouraging research on novel approaches for treating DED, particularly for patients with severe and end-stage disease. Significant advances have been made in the study of autologous serum and specific neurotrophic factors with the aim of supporting ocular surface healing and regeneration. There are also innovative surgical approaches for reconstructing and regenerating the ocular surface, reconstruction of secretory tissue, and transplantation of amniotic membranes or bioengineered tissues. Continued investigation and investment in DED, combined with a newly established therapeutic precedent in Europe, suggests that the global market is verging on new pharmacologic treatments.

VI. APPENDIX

DISCLOSURES OF FINANCIAL RELATIONSHIPS OF AUTHORS WITH COMPANIES WITH PRODUCTS OR INTERESTS RELATED TO DED

Carlos Belmonte has a personal financial interest in Avizorex Pharma and is a named inventor on patents licensed to Avizorex Pharma; and has served as a consultant to Vistakon.

José Benitez del Castillo has provided consulting for Alcon, Laboratoires Thea, and Allergan, and has received research support from Alcon, Laboratoires Thea, and Allergan.

Anthony Bron has a personal financial interest in TearLab, and has been a consultant to Diagnostear, Pharmaglobal, Redwood Pharma, Santen, SARcode and Semba.

Wendy Chao has no proprietary interest in any company with products or interests related to dry eye.

Harminder Dua has received travel support and honoraria from Thea, Nicox, Croma, and Allergan.

Kelly Nichols has been a consultant to Allergan, Alcon, Bausch & Lomb, Eleven Biotherapeutics, InSite, Kala, Nicox, Shire, Science Based Health, and Tear Innovations. She has had research support from Allergan, Alcon, SARcode, NIH, Vistakon, Tear Science, Shire, Kala, and Eleven Biotherapeutics.

Gary Novack is a consultant to numerous pharmaceutical and medical device firms.

Stefan Schrader has no proprietary interest in any company with products or interests related to dry eye.

David A. Sullivan has a personal financial interest in Lμbris, serves on an Advisory Board for TearLab, and has received research support from NIH, GlaxoSmithKline, and the Margaret C. Sinon Scholar in Ocular Surface Research Fund.

Mark Willcox has a personal financial interest in the Brien Holden Vision Institute and has provided consulting for CooperVision, Johnson & Johnson, and Vistakon. He received financial support from Allergan and Bausch & Lomb, and research support from Allergan.

James Wolffsohn has a personal financial interest in Aston EyeTech and has received research support from Alcon, AMO, Bausch & Lomb, Bespak Europe, CooperVision, EMPharma, European Union, Innovate UK, Johnson & Johnson, Lenstec, Optimec, Rayner, TearLab, Thea, and Visioncare Research. He has provided consulting for Alcon and Johnson & Johnson.

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