Published in final edited form as:
Graefes Arch Clin Exp Ophthalmol. 2014 June ; 252(6): 857–872. doi:10.1007/s00417-014-2618-2.

IMAGE-GUIDED EVALUATION AND MONITORING OF TREATMENT RESPONSE IN PATIENTS WITH DRY EYE DISEASE

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Abstract

Dry eye disease (DED) is one of the most common ocular disorders worldwide. The pathophysiological mechanisms involved in the development of DED are not well understood and thus treating DED has been a significant challenge for ophthalmologists. Most of the currently available diagnostic tests demonstrate low correlation to patient symptoms and have low reproducibility. Recently, sophisticated *in vivo* imaging modalities have become available for patient care, namely, *in vivo* confocal microscopy (IVCM) and optical coherence tomography (OCT). These emerging modalities are powerful and non-invasive, allowing real-time visualization of cellular and anatomical structures of the cornea and ocular surface. Here we discuss how, by providing both qualitative and quantitative assessment, these techniques can be used to demonstrate early subclinical disease, grade layer-by-layer severity, and allow monitoring of disease severity by cellular alterations. Imaging-guided stratification of patients may also be possible in conjunction with clinical examination methods. Visualization of subclinical changes and stratification of patients *in vivo*, allows objective image-guided evaluation of tailored treatment response based on cellular morphological alterations specific to each patient. This image-guided approach to DED may ultimately improve patient outcomes and allow studying the efficacy of novel therapies in clinical trials.

Keywords

IVCM; OCT; Dry Eye Disease; meibomian gland dysfunction; confocal microscopy

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Conflict of interest: none for all authors.

INTRODUCTION

The Dry Eye Workshop (DEWS) conducted in 2007 defined dry eye disease (DED) as, "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."[1] DED is a global health problem with several large population-based studies estimating the prevalence ranging from approximately 30% to 50% of the population at various ages.[2-8] In the United States alone, DED is estimated to affect around 5 million people, aged 50 years or more, with the disease being more prevalent in the elderly and female population.[9-14] However, with increasing contact lens wear and use of electronic digital devices, an increased prevalence of DED is now seen among the younger population as well. Recent epidemiological studies report dry eye symptoms in about 25% of high school students and 30 – 65% office workers.[15-21]

The quality of life (QOL) in patients with DED is significantly affected due to their altered visual acuity and discomfort, resulting in the inability to carry out activities of daily living and hampering social functioning.[22-28] Several utility assessment models have shown that the impact of moderate to severe DED is comparable to moderate to severe angina.[29,30] Moreover, the economic impact of DED is also significant, due to both direct medical and healthcare related costs, as well as due to indirect productivity losses.[31-34] A recent study, for example, estimated a US prevalence-adjusted average annual cost for managing DED patients at $3.84 billion, underscoring the accompanying economic burden of disease.[35]

The reported therapeutic success of current regimens in DED is variable.[36-38] In part, this has been attributed to the heterogeneity within a patient cohort in terms of severity, multitude of confounding factors and underlying etiology in DED, as well as unreliable evaluation of inflammation pre- and post-treatment, and the overall variability in measurements of clinical signs. Thus, it has been very difficult for clinicians to assess objective improvement in disease and the efficacy of a particular treatment regimen. Similarly, the pharmaceutical industry has faced significant challenges in obtaining approval of new therapeutic drugs over the past decade, as the improvement of clinical signs has not been possible with the current clinical endpoints available.[39-41] Thus there is an urgent need for the development of new biomarkers for DED that are reliable, reproducible, consistent with symptoms, and reflect the underlying pathogenesis and severity of disease.[42] Herein we discuss a novel, non-invasive *in vivo* imaging approach that can potentially emerge as a tool for quantitative evaluation of disease and monitoring of treatment efficacy in DED, supplementing the current standard diagnostic tests. Through quantitative impressions of *in vivo* confocal micrographs based on densities of the subepithelial corneal immune cells, palpebral conjunctival immune cells and sub-basal nerves, when taken together with clinical diagnostic tests, *in vivo* imaging may enhance the clinical management of dry eye disease.

METHODS

A PubMed literature search was conducted for papers, using the keywords: in vivo confocal microscopy, dry eye, in vivo confocal microscopy in cornea, meibomian gland dysfunction, optical coherence tomography, and ocular imaging. Bibliography of selected manuscripts were also reviewed and referenced in our manuscript. Limits for our literature search filters included papers in English from 1980 till 2013, including both human and animal studies published as case reports, review papers or original research. We also referenced product information brochures and manufacturers' webpages for technical information regarding medical devices discussed in this review paper.

BACKGROUND, CLASSIFICATION AND GRADING OF DRY EYE

Background

The tear film is composed of three layers, the innermost mucous, middle aqueous, and outermost lipid layers. The mucin layer, composed of gel-forming mucins, soluble mucins, electrolytes and water, is largely secreted by conjunctival goblet cells with some contribution of soluble mucins form the lacrimal gland, corneal and conjunctival cells.[43-45] This hydrophilic mucin layer of the tear film rests upon the mucin of the glycocalyx, which is formed by the corneal and conjunctival epithelial membrane-spanning mucins.[43,46] While both the mucin of the glycocalyx and the mucin layer of the tear film serve to provide hydration and lubrication to the ocular surface, they also help retard colonization of pathogens through restricting cell adhesion (mucin of the glycocalyx) and acting as a lavage to wash out pathogens, debris and inflammatory molecules in the tear film (mucin layer).[47] Both the mucin of the glycocalyx and mucin layer of the tear film can be affected in DED.[48,49] Lacrimal glands are the main contributors to the aqueous layer of the tear film that provides hydration and lubrication of the ocular surface,[50] while meibomian glands maintain the outermost lipid layer to retard tear evaporation.[51]

Classification

Based on abnormalities of the tear film composition, DED has been broadly classified into two categories, aqueous-deficient dry eye (ADDE) and evaporative dry eye (EDE), corresponding to disorders of the lacrimal and meibomian glands, respectively. ADDE can further be classified into Sjögren's syndrome (SS) and non-Sjögren's syndrome (NSS) dry eye. EDE can be classified into intrinsic and extrinsic causes, with meibomian gland dysfunction (MGD) being the most common cause of EDE. As ADDE and EDE are not mutually exclusive and may act together to produce signs and symptoms, [52] it may be challenging to identify pure forms of each disease due to mixed forms of DED using the current system of classification. In addition, the initial underlying dry eye mechanism may lead to involvement of additional mechanisms, thus resulting in misclassification at different stages of disease. Therefore, with time and progression of disease severity, the clinical phenotype of DED may be altered as well, often leading mixed clinical phenotypes.[53]

Grading

Currently, dry eye disease severity is graded into four categories based on a combination of patient symptoms and clinical signs.[1] However, several studies have demonstrated the lack of correlation between the severity of symptoms and signs.[54-59] For example, patients in early stages of DED may demonstrate symptoms, without clear clinical signs.[60-62] Conversely, patients may show mild or severe objective clinical signs of DED, but may be symptom free.[6,11,63] The disparity between signs and symptoms is multifactorial, but may in part be explained by the reduction of corneal nerve density with dry eye disease; reduced or loss of corneal sensation secondary to reduced corneal sub-basal nerve density from nerve damage in DED, may explain the presence of clinical signs of ocular surface damage in the absence of concordant symptoms of ocular discomfort.[64]

METHODS AND LIMITATIONS IN THE DIAGNOSIS AND TREATMENT OF AQUEOUS DEFICIENT DRY EYE DISEASE

The current diagnostic protocol for DED is based on the recommendations of the 2007 DEWS.[65] This includes symptomatology questionnaires, such as the OSDI, in combination with the various clinical diagnostic tests. The traditional clinical diagnostic tests include corneal fluorescein staining, conjunctival Lissamine Green or Rose Bengal staining, tear film break-up time (TBUT), Schirmer's tests, and more recently tear osmolarity. Each of these tests address a specific and necessary component of the complex nature of DED. However, given their varied reproducibility, they have limitations in meeting the prerequisites of ideal diagnostic tests, which include high specificity, sensitivity, reliability and validity.[66-68] Of the existing clinical examinations, studies have shown better reproducibility with tear hyperosmolarity testing,[69-72] even though significant overlap exists between the scores of normal subjects and patients with DED. This overlap of scores between normal subjects and dry eye patients is seen with other dry eye tests as well. Moreover, while tear osmolarity may has been shown to be reproducible as a diagnostic test, the utility of this test for following dry eye patients may be more limited.[73] A recent multicenter study evaluated the most commonly used clinical tools to grade severity of DED and concluded that there is a significant overlap in symptom severity scores amongst the prospectively defined normal subjects and patients with DED[70]. In addition, technical variations in measurement, variable conditions while conducting the tests,[74] and potential diurnal variation of tear parameters,[75-77] may all lead to increased variability of these tests.

The current approach to treatment of DED is based on the recommendations of the Management and Therapy subcommittee of the International Dry Eye Workshop 2007. Based on review of literature, preferred practice patterns by the American Academy of Ophthalmology and the International Task Force (ITF) Delphi Panel on DED, the subcommittee proposed treatment guidelines based on the severity of disease. [78]

METHODS AND LIMITATIONS IN THE DIAGNOSIS AND TREATMENT OF MEIBOMIAN GLAND DYSFUCTION

MGD lends a significant burden of disease given its high prevalence, making for up to half or more of all patients that present with symptoms of dry eye.[63,79-82] Population-based studies indicate that Asians have higher prevalence of MGD, ranging from 60.8% to 69.3% in those aged over 40 years.[83] However, two large population-based studies on Caucasians aged 40 to 97 years, revealed prevalence rates from 3.5% to 19.9%.[83] Unfortunately, despite its widespread global prevalence, treatment of MGD is complicated by issues ranging from lack of sensitive diagnostic tests, a poorly understood natural history of disease to paucity of quantifiable methods for assessment of therapeutic efficacy, thereby stalling the development of effective and sustainable therapeutic strategies.

The recent 2011 International Workshop on Meibomian Gland Dysfunction (IWMGD 2011) defined MGD as “a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. It may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease”.[61,83] This definition illustrates the complexity of mechanisms underlying MGD, much of which are still poorly understood. The precise pathogenesis of MGD is yet to be determined, however, there are several hypotheses that implicate the role of gonadal steroids,[84,85] bacteria,[86-88] and androgens[89-92]. These etiological factors lead to hyperkeratinization of the terminal ducts and an increased viscosity of expressed meibum. Hyperkeratinization may then lead to glandular obstruction, and these changes may result in subsequent alterations in tear film stability resulting in increased tear evaporation. Obstructive MGD is by far the most common presentation of MGD.[60,93] Moreover, while previously controversial, the importance of inflammation in the pathogenesis of MGD has now been recognized by the 2011 IWMGD.

There remain two key issues when tackling MGD: (a) presence of an early, more prevalent, asymptomatic stage of disease, making early diagnosis toward effective treatment difficult using the existing standard clinical examination methods;[61,63] (b) a disconnect between symptoms and signs, making both diagnosis and evaluation of therapy challenging for the physician given the reliance on solely traditional clinical methods of examination.[62,63,94,95] Traditional methods of diagnosis and assessment of MGD involve a clinical slit-lamp examination with a focus on the health and appearance of the lid margin (telangiectasia, orifice plugging or obstruction, lid swelling, lid tenderness), lissamine green staining of the conjunctiva and eyelid margin indicative of compromised cells, meibum expressibility and quality to assess gland obstruction and inflammation respectively, meibography to assess glandular dropout and appearance of the acini, and assessment of tear film stability and production through TBUT and Schirmer's test to help categorize disease. Most of these clinical methods are prone to subjectivity and lack of reliability due to inter-user variation in grading and assessment.[96] Powell and colleagues determined kappa values ranging from 0.23 to 0.5 at best for inter-user reliability using meibography and slit-lamp bio-microscopy in the assessment of MGD.[96] Nichols and group also found similarly

low kappa values for inter-observer reliability using meibography (simple κ= 0.38, weighted κ= 0.57).[97] With the advances in meibography employing computerized grading (100-grade scale), there has been some improvement in inter-observer reliability as compared to subjective methods, but the inter-observer variability still remains prominent (mean difference in score of 19.3 between observers).[98] Koh and colleagues developed a semi-automated method to digitally quantify infra-red meibographs that can objectively measure meibomian gland length and width. [99] This software advancement is exciting in that it has high sensitivity and specificity, but data regarding inter-observer reliability are not available. [99] Perhaps with the recent development of 3D reconstruction using FD-OCT meibography, further quantifiable parameters are likely to be developed toward improved assessment and grading systems.[100] Despite advances made to date, as a function of the limited magnification offered by these devices, current methods fail to detect cellular detail and consequently identification of patients with subclinical inflammation, which may account for symptoms with undetectable signs using traditional examination methods. [101,102]

Developing sensitive parameters using cutting-edge tools to detect subclinical inflammation in a clinical setting is critical for the early diagnosis, tailored management and prevention of irreversible damage to the ocular surface in patients with MGD. The role of inflammation in the pathogenesis of MGD may be intertwined with glandular obstruction and bacterial colonization of the eyelid margin. While the evolution of MGD remains elusive, it has been postulated that obstructive MGD increases intraglandular pressure, resulting in cell stress of ductal and acinar epithelia, which can subsequently trigger activity of cell proteins, leading to the release of inflammatory mediators, and subsequent local inflammation. Moreover, it possible that hyperkeratinization of terminal ducts could be triggered by inflammatory events. Likewise, increased bacterial growth is linked with subclinical inflammatory events through the release of free fatty acids that may irritate the tissue.[86,87,93,103,104] Inflammatory cytokines such as interleukin (IL)-1α may then activate epithelial cells,[105] which in turn produce further inflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1α and –β, thereby maintaining a chronic subclinical inflammatory milieu, resulting in obstructive glandular disease.[106,107] Currently, slit-lamp bio-microscopy, which provides a maximal magnification of x32 is the gold standard tool in the assessment of the ocular surface. However, subclinical inflammation, which may be present at the initial stages of disease cannot be detected using this method, potentially leading to disparity between patient's symptoms and clinical signs.[62,95] Given these techniques and the associated subjectivity in diagnostic assessment, there is lack of a consensus about the definition of MGD, and consequently its diagnosis.[93,108-116] Therefore, there is an unmet need to develop alternative sensitive, more reproducible and reliable clinical parameters for the diagnosis and evaluation of disease severity in MGD. In addition, the time lag between the detection of early signs and onset of symptomatic disease is currently unknown, as are the usefulness of particular early signs as biomarkers of established symptomatic disease.

Treatment of MGD varies greatly among eye care professionals, ranging from conservative management to medical therapy or a combination of both. Conservative measures have a proven role in the management of MGD: such as lubrication for symptomatic relief from mechanical friction,[117,118] hot compresses with lid massage for gentle but firm

expression of inspissated meibum from the ducts,[119,120] which can be performed at home, or in clinic using recent devices that work on the same principle of heating followed by compression.[121-127] While lid hygiene is indicated primarily for anterior blepharitis, it does serve a role in the management of MGD (posterior blepharitis), possibly by exfoliation of lid debris and reducing lid margin bacterial load.[117,128,129]. Anti-microbials such as systemic tetracyclines (doxycyline, minocycline),[130-134] and topical macrolides (1% azithromycin), that also have anti-inflammatory properties are the most commonly prescribed treatments of MGD.[132,135,136] Sytemic tetracyclines work by inhibiting microbial lipase production thereby reducing release of proinflammatory free fatty acids and diglycerides onto the lid margin.[87,137-140] They also inhibit the activity of tissue matrix metalloproteinases (MMP) such as MMP-2 and MMP-9.[141] Macrolides serve as immunomodulatory and anti-inflamamtory drugs through a myriad of mechanisms including increase in phagocytosis, downregulation of adhesion molecules,[142-144] and reduction of inflammatory cytokines such as IL-6,[145]. Current topical treatments include off-label azithromycin,[136,146,147] or an antibiotic–steroid combination[148] for acute exacerbations.[149] Thus, inflammation has emerged as a target in the management of MGD. Despite the evolving role of inflammation in MGD, only 12 randomized controlled trials have been published studying interventions in this disease.[150] The results are inconclusive with regards to anti-inflammatory therapy (topical corticosteroid, oral antibiotics) in MGD,[150] explaining the paucity of evidence-based treatment guidelines and continued controversy regarding the role of topical corticosteroids in the treatment of MGD, with or without adjuvant antibiotic therapy. This controversy arises from the lack of clinically apparent inflammation in the early stages of disease,[60-62] necessitating the development of tools that can detect and reliably quantify subclinical inflammation or activated immune response in the face of an unremarkable clinical examination, yet a symptomatic patient.

The 2011 MGD workshop concluded that “new methods to assess MGD, both clinically and biologically, are needed to further the field, alone and in conjunction with dry eye disease”, pointing out the lack of objective evaluation parameters.[83,94] Towards that goal, with the advent of a high-magnification (x800), non-invasive, fast, laser-scanning *in vivo* confocal microscope, (IVCM), detection of subclinical inflammation in MGD has become possible.[151] Given the speed with which it can be performed in an out-patient setting, and the near histological details acquired in a non-invasive manner, IVCM has very quickly begun to provide new, quantifiable insights into the evaluation of MGD.[115,152-157]

DIAGNOSTIC IMAGING IN DRY EYE DISEASE

Sophisticated *in vivo* imaging techniques such as *in vivo* confocal microscopy and anterior segment optical coherence tomography (OCT) have recently made headway in the evaluation of dry eye disease. Their diagnostic utility is discussed in the following sections in a tissue-specific manner, with a focus on the cornea, palpebral conjunctiva and meibomian glands. Figures 1, 2 and 3 illustrate the superior resolution, high magnification and detailed structural information gathered using these *in vivo* imaging techniques in DED and MGD.

Principles of Laser-Scanning *In Vivo* Confocal Microscopy (IVCM)

The principle of confocal microscopy is its conjugate alignment of light rays focused on the tissue with those reflected by the tissue and transmitted to the observer, therefore the term "confocal[158] The Heidelberg Retina Tomograph with the Rostock Cornea Module (HRT/RCM, Heidelberg Engineering, Dossenheim, Germany), is a laser-scanning IVCM, using a 670nm diode laser.[159] It allows quasi-histological, real-time imaging of the cornea, conjunctiva and meibomian glands, generating a 400 × 400μm images (384 pixels × 384 pixels) with a magnification of close to 800-fold and a lateral resolution of 1μm/pixel. [115,157,160,161] The high-resolution HRT/RCM has become a powerful tool in understanding the microstructural anatomy in both ocular health and disease.

In Vivo Confocal Microscopy-Guided Diagnosis of Dry Eye

Cornea—*In vivo* confocal microscopy is emerging as a promising tool in the study of corneal ultrastructure, both in health and disease. The ability of IVCM to examine each layer of the cornea in detail and to identify pathological changes at the cellular level is particularly useful in DED where the changes are often subclinical and are not picked up by the standard slit lamp bio-microscopy. IVCM provides high resolution images of the individual layers of the cornea i.e. the epithelium, Bowman's layer, stroma, Descemet's membrane and the endothelium. Further, the subbasal corneal nerve plexus, stromal nerves, [162-165] and dendritiform and non-dendritic immune cells can be clearly identified.

Several studies using IVCM in DED have described the changes induced in the corneal layers and provide an insight to the underlying pathophysiological mechanisms.

The outermost corneal layer, the epithelium consists of the superficial epithelial layer, an intermediate and the innermost basal cell layer. Being an ocular surface disease, DED affects the superficial epithelial cells. IVCM reveals reduced density and morphological alterations of superficial epithelial cells. [166-171]. These corneal superficial epithelial cells increase in size and cell borders are hyperreflective and irregular. Further, the nuclei become prominent, hyperreflective and increase in size.[166] These changes are attributed to desquamation and squamous metaplasia. Erdelyi et al. also reported a decrease in the intermediate cell layer.[168] Moreover, increased epithelial basal cell density has been reported in a number of studies,[167-169] which has been postulated to be due to the fact that there is an increased turnover of epithelial cells as a result of the epitheliopathy.

Epithelial dendritic cells are the major type of immune antigen presenting cells in the cornea and are responsible for generating an immune response or maintaining tolerance.[172,173] These dendritic cells are primarily located in the subbasal layer in close proximity of the subbasal nerve plexus, with their density declining from the periphery towards to center. [173-175] Using confocal microscopy, Lin et al. have shown an increased number of dendritic cells in DED, both in the center and the periphery [176]. They also suggest that that the numbers of dendrites represent an active stage of these cells. Similarly, Tuisko et al. showed an increased infiltration of the subbasal nerve plexus with purportedly mature antigen presenting cells.[174] A similar increase in central corneal dendritic cells has also been reported in conditions such as infectious keratitis, with the area covered by these cells

(cell field) and their number of dendrites demonstrating the mature and active stage of these cells.[177] Finally, an increase in the number of non-dendritic leukocytes has also been noted in the cornea in DED.[176,177] Both quantitative and qualitative changes in the nerve plexus have been reported in DED. Examination of the corneal nerve morphology using IVCM has shown various abnormalities such as increased tortuosity, reflectivity and beading.[167,169,178,179] These morphological changes are believed to be due to the damage and subsequent attempted regeneration of subbasal nerves. Different studies have demonstrated somewhat conflicting results regarding nerve density. Benitez et al. and Villani et al in 2007 have reported a decrease in the density of the nerves [167,178,180] and this reduction in density is in concordance with decreased clinical corneal sensitivity. [167,174,178,180-182]. However, some studies have shown no change or even an increase in the nerve density [166,179,183] and hypersensitivity of the cornea has been reported clinically.[174,184] These changes may be explained by the fact that different stages and severity of DED induce different degeneration/regeneration patterns of nerves.

The basic underlying pathophysiological mechanism responsible for the above noted changes in DED is likely increased inflammation.[185] Several pro-inflammatory cytokines are increased; the type and concentration of which vary with the underlying etiology of DED.[186-191] These cytokines could lead to damage of the epithelial cells and corneal nerves, which in turn can increase pro-inflammatory mediators. The release of pro-inflammatory mediators can subsequently activate or enhance an immune or inflammatory response.[192] The observed decrease in corneal nerves can induce further damage to the epithelium, due to the diminishment of neurotrophic mediators.[193,194] In addition, neuronal damage may serves as a stimulus for inflammatory cells, called neurogenic inflammation, leading to a vicious cycle of increased inflammation with simultaneous nerve and epithelial cell damage.[195,196]

IVCM has been reported to correlate well with patient reported symptoms as well as other diagnostic tests.[197,198] Thus, by being able to demonstrate alterations in epithelial layers, corneal nerves and immune cell changes at a cellular level that correlate with clinical signs and symptoms of DED, IVCM may serve as a useful assessment tool supplementary toclinical diagnostic modalities. The density and morphology of dendritic immune cells and superficial epithelial cells, as well as the status of subbasal corneal nerves have the potential to serve as imaging biomarkers for inflammation in DED. However, additional studies are required to validate these imaging markers and their utility in clinical practice, which could be used for treatment stratification and measurement of therapeutic efficacy when taken together with clinical tests.

Conjunctiva and Meibomian Glands—In 2005, Kobayashi and colleagues pioneered the application of laser-scanning IVCM to study normal human bulbar and palpebral conjunctiva in a set of four healthy volunteers.[157] For the first time, a layer-by-layer description of the conjunctival epithelium was provided, and glandular structures beneath the palpebral conjunctiva were postulated to be meibomian glands.[157] Since then, the application of IVCM to conjunctival and meibomian gland imaging has started to expand, generating insightful and novel data from conditions such as Sjögren's syndrome,[153] contact lens wear,[152] and aging,[156]. Later, Matsumoto et al. defined the first set of

diagnostic quantitative imaging parameters in MGD, namely, acinar unit density and diameter, and tested them against normal controls.[115] They demonstrated that both acinar unit density and diameter were significantly altered in MGD.[115]

Ibrahim et al. tested additional quantifiable acinar parameters in the evaluation of MGD, and logically progressed work in the field by determining the sensitivity and specificity of glandular IVCM parameters in the diagnosis of MGD.[199] They demonstrated that meibomian gland acinar imaging parameters not only correlated strongly with clinical tests for tear film stability and ocular surface integrity, but that when taken together, they yielded high specificity and sensitivity in diagnosing MGD.[199] The first, and to date, the only qualitative diagnostic IVCM grading system of meibomian glands was devised and described by Villani et al in 2011.[153] They described a 4-point scoring system based on a subjective assessment of the hyperreflectivity of meibum, and hyperreflective speckling of the acinar epithelium and interstitial tissue; the higher the score, the more likely the presence of MGD.[153] The observed hyperreflectivity and speckling of the meibum, acinar epithelial and interstitium may be explained by the recent detection of subclinical inflammation in MGD.[151,200,201] Qazi and colleagues identified and described a unique layer by layer distribution of palpebral conjunctival inflammatory cells in MGD; dense populations of inflammatory cells were seen in the palpebral conjunctival epithelium, as well as the conjunctival substantia propria. Occasionally inflammatory cells can be seen encircling meibomian gland ducts, and lying embedded in the acinar epithelium. However, most remarkably, inflammatory cells can be seen residing within meibomian glands and are associated with ductal dilatation and in some cases, frank glandular obstruction.[200] Following these glands post-treatment can be made possible by following a systematic imaging protocol, scanning multiple serial regions and maintaining consistency of method and regions imaged. The sequences of images acquired can then be compared between visits. Given the high magnification of IVCM (x800), which allows imaging to cellular and microstructural detail, it becomes pivotal to scan all meridians (central, nasal, temporal, superior, inferior) of the palpebral conjunctiva in order to reach a clinical impression based on mean values of multiple representative images. These findings bear imminence in potentially being able to guide physicians towards tailored therapy. Observing concurrent epithelial and stromal inflammation may provide for evidence-based decisions regarding topical versus systemic therapy for MGD, where adjuvant systemic therapy would be critical to eradicating the deeper seated inflammation and bringing symptomatic relief. Treating patients topically without an assessment of the subclinical severity of disease, often results in poor patient satisfaction and refractory symptoms, suggestive of continued stromal or intraductal inflammation.[151] Similarly, visualization of intraductal inflammatory cells could prove to be an indicator of the need for intraductal meibomian gland probing to relieve symptoms attributable to glandular obstruction. IVCM is thus emerging as an informative and objective supplementary tool in the evaluation of MGD and associated dry eye disease. Nevertheless, additional studies are required to validate these parameters and to demonstrate their clinical utility.

IVCM-Aided Assessment of Therapeutic Efficacy

Cornea—Anti-inflammatory therapy is now a major component of treatment of DED. However, inflammation and/or changes in immune cells may not be appreciated in the initial stages or in mild disease using slit-lamp examination. Confocal microscopy can assess subclinical immune cell changes and hence may be used as a tool to determine the need for anti-inflammatory therapy. Moreover, serial imaging may be used for objective assessment of therapeutic success of anti-inflammatory therapy. Based on the dysplastic changes of the superficial epithelial cells in DED,[166] improvement in density and morphology can be used to follow the epithelial healing with treatment. Further, as dry eye disease has been associated with decreased corneal nerve density, [167,178] nerve regeneration can be monitored by IVCM during the course of treatment and if required can be accelerated using therapies that focus on providing neurotrophic support. Finally, improvement in superficial epithelial health, nerve parameters and reduction in inflammation by IVCM may be used as objective end points in clinical trials and to validate and standardize treatment protocols for DED.

Conjunctiva and meibomian glands—Translation of therapeutic success of medical and conservative therapy in MGD into subjective improvement by patients is not immediate and typically a lag of symptoms and signs by slit-lamp bio-microscopy are observed. [200,201] This lag of clinical improvement, while on treatment, can be distressing for the patient. Patient dissatisfaction with the inability to feel any change in symptoms can result in poor compliance with the therapeutic regimen. In our experience, medical therapy requires approximately two to five months of stringent compliance before therapeutic effects may be appreciated by the patient.[200] IVCM allows sensitive detection of changes in subclinical immune cell changes in all layers of the palpebral conjunctiva and within meibomian glands as early as four to six weeks post-therapy.[200] These findings of reduction in epithelial inflammation are consistent with those determined by Matsumoto and colleagues, [149] and aid in the determination of success of therapy. To ensure patient compliance in the early stages of therapy when symptomatic improvement lags clinical response,[201] and patients may therefore be prone to discontinuing medical treatment, our anecdotal experience suggests that using visual aids of the patient's response to treatment through *in vivo* confocal micrographs of conjunctival immune cells, allows continued compliance with therapy until symptomatic relief is also achieved.

One of the more invasive therapeutic strategies that is gaining popularity, is intraductal probing of meibomian glands using the Maskin intraductal probe.[202] In a pilot study of 25 patients, Maskin demonstrated that intraductal probing has provided immediate symptomatic relief in 96% of patients, 80% of whom only required 1 treatment in a follow-up period of 11.5 months.[202] Similar results were seen by an independent investigator who followed ten patients for a period of 6 months post-probing.[203] These patients have thus far only been assessed using symptom questionnaires, making IVCM-aided quantitative evaluation meibomian gland morphology a necessary next step in validating this promising therapeutic intervention.

Principles of Anterior Segment-Optical Coherence Tomography (AS-OCT)

Optical coherent tomography (OCT) is a non-contact *in vivo* imaging method that incorporates the principle of low-coherence interferometry to generate cross-sectional images of ocular tissues and its microstructures.[204]. AS-OCT allows near-histological visualization and biometry of the anatomical structures starting from the tear film, corneal epithelium, stroma, endothelium, corneo-scleral junction, sclera, anterior chamber, trabecular meshwork, irido-corneal angle, anterior pigment of the iris and anterior capsule of the lens, making it an indispensable tool for clinical and surgical management of anterior segment pathology.[205-214]

Methods of signal acquisition and processing determine features of the OCT such as speed of imaging, and resolution of the images. Based on these technical differences in design, the two most popular types of commercially available OCT for anterior segment imaging are time-domain OCT (TD-OCT), such as the Visante-omni and Visante OCT (Carl Zeiss, Meditec Inc.),[215,216] and spectral-domain OCT (SD-OCT), available as slit-lamp OCT (SL-OCT, Heidelberg Engineering, Vista, CA), and more recently, the Envisu C-class (Bioptigen, Durham, NC, USA).[217-219] Moreover, the RTVue OCT (Optovue) with the anterior segment cornea module provides high resolution anterior segment imaging. Technical features of these two techniques with respect to AS-OCT are listed in Table 1.

The ultra-high resolution OCT (UHROCT), which has a lateral resolution of 10μm is also used extensively for *in vivo* corneal and anterior chamber imaging in ophthalmic practice. [220-222] It provides good tissue penetration, resolution for diagnostic assessment and the ability to use images for 3D reconstruction.[223]. Quantitative parametric and volumetric analysis of the cornea and lacrimal functional unit becomes possible with the UHROCT.

AS-OCT in the Diagnosis and Assessment of Therapy in Dry Eye

It is only in the past two years that AS-OCT has emerged as a quick, non-invasive and quantitative diagnostic tool in the assessment dry eye disease, particularly ADDE.[215] In the assessment of DED, of particular interest is tear film biometry. The measurements taken include the tear meniscus height (TMH), a tear meniscus depth (TMD) and tear meniscus area (TMA).[215,224] These parameters are significantly decreased in patients with dry eye disease, especially secondary to Sjögren's syndrome, as compared to controls (Fig. 2), thereby serving as reliable, accurate, non-invasive and quick diagnostic markers of Sjögren's disease in the evaluation DED.[215,225] Based on stronger correlation with other ocular surface tests, the lower tear menisci measurements seem to be more clinically relevant than upper menisci in the diagnosis of dry eye.[226] Ibrahim et al. showed that TMH, with a sensitivity and specificity of 67% and 81% respectively, is reduced in dry eye disease along with other tests measuring the tear film such as TMH on slit-lamp examination, tear meniscometry, tear break-up time and Schirmer's test.[215] Furthermore, they proceeded to test the role of OCT-derived TMH measurements in assessing response to punctal plug therapy for dry eye, which confirmed TMH as a quick and sensitive parameter in the diagnosis and therapeutic assessment of dry eye.[227] Other groups have confirmed the correlation of AS-OCT tear film biometry with Schirmer's test,[228] while retaining high reliability of central tear film measurements (intraclass correlation coefficient = 0.97),

making AS-OCT a reliable, precise and accurate tool in tear film biometry.[229] However, it is worthwhile to note that tear film biometry may not be useful in the assessment of MGD-dependent EDE.

Further, Chen et al. found that tear meniscus volume measurements using AS-OCT are reduced in Sjögren's patients, correlating strongly with corneal staining and TBUT.[230] It has been determined that OCT-derived tear menisci measurements are most useful in diagnosing and following patients with Sjögren's syndrome-related dry eye than patients with non-Sjögren's dry eye or evaporative dry eye.[224] Dry eye symptoms may also be associated with conjunctivochalasis. Several groups have successfully adapted OCT-derived tear menisci measurements to evaluate and classify these lid-parallel conjunctival folds toward developing further objective parameters in the assessment of DED.[214,231,232]

CONCLUSIONS

Diagnostic assessment methods for dry eye disease have burgeoned towards objective and quantitative measures that reduce inter-user variability and encourage a consensus among physicians in the assessment and management of dry eye disorders. Early detection of ongoing subclinical immune cells changes and inflammation may explain the lack of clinical signs in the presence of symptoms, making it imperative to treat the underlying pathology aggressively, with the goal of preventing tissue damage and retarding subclinical inflammation. IVCM may allow clinicians to make rapid assessments of the patient's tissue immune response in out-patient settings and prescribe tailored management early in the disease in an image-guided fashion, gearing towards potentially better clinical outcomes. IVCM also provides objective parameters for evaluating and monitoring disease severity. Prospective randomized clinical trials studies using *in vivo* imaging tools such as IVCM and AS-OCT are needed, to not only determine their absolute utility, but also to test their true worth as objective *in vivo* tissue biomarkers of disease. While AS-OCT provides high-resolution, magnified imaging of the anterior segment with fast acquisition times, this technology is limited by the structural details provided. AS-OCT is unable to provide visualization of the microstructural anatomy and cellular detail unlike IVCM. Furthermore, depth of imaging is contingent upon the wavelength of the light source, with reduced tissue penetration for shorter wavelengths. Some of the issues currently facing IVCM are: (a) the level of expertise and training required to efficiently and adequately acquire high-quality images of the region of interest; (b) the user's ability to re-register at exactly the same location each time, especially with regards to the lid margin and palpebral conjunctiva, which would be important in monitoring response to therapy for specific procedures such as meibomian duct probing; (c) identification of the phenotype of cells visualized using IVCM. While the former two issues can be addressed through rigorous training, experience and following a systematic protocol, unequivocal identification of cell phenotype makes for an inviting avenue to explore by interested groups. While the phenotype of immune cells is largely consistent and distinctive on IVCM of the cornea, given the cellular architecture of the palpebral conjunctiva with hyper-reflective goblet and epithelial cells, it becomes more challenging to identify immune cells that may not conform to commonly accepted morphology. Therefore, this makes adherence to a strict and meticulous image analysis protocol even more important. Nevertheless, in order to derive definitive conclusions about

the immune response from IVCM studies, exploratory immunohistochemistry studies are needed toward establishing corneal and conjunctival cell phenotypes in correlation to IVCM

Acknowledgments

We would like to thank our sponsors for their generous support through grants from the National Institute of Health (NIH K08-EY020575 to PH), Research to Prevent Blindness Career Development Award (PH) and Falk Medical Research Foundation (PH).

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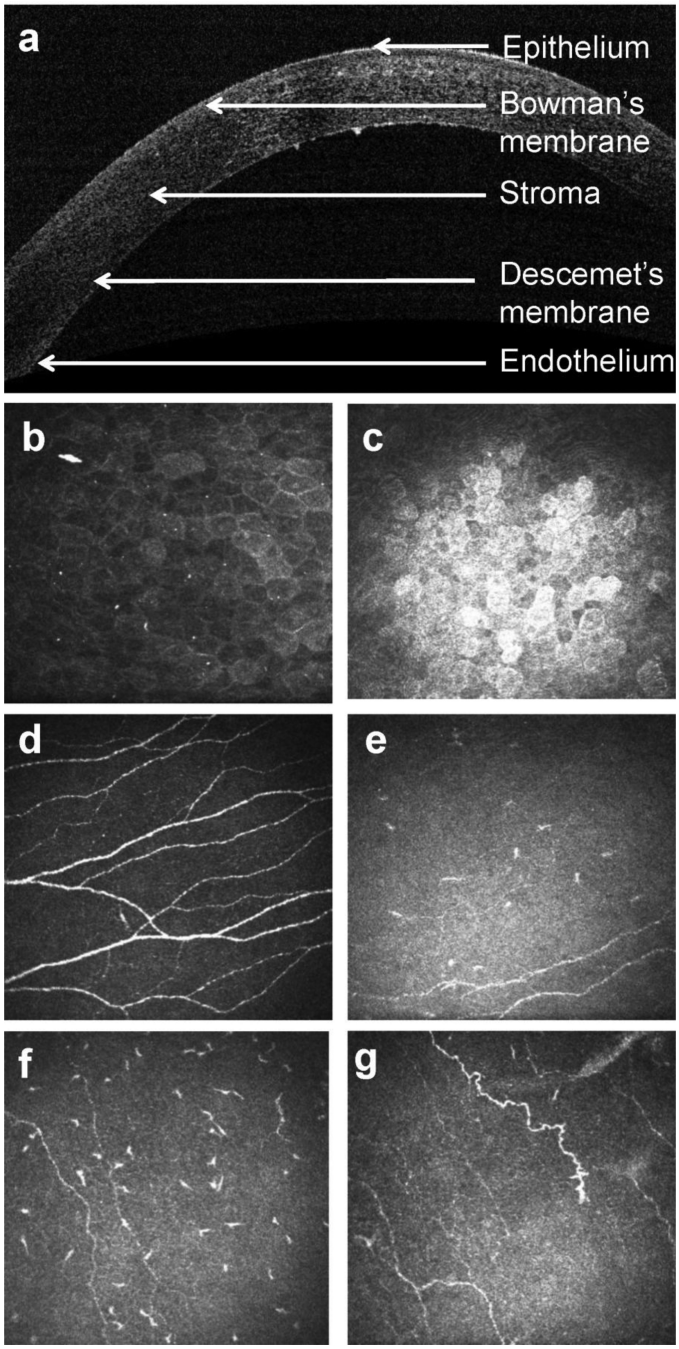

Fig. 1.

Corneal imaging in health and dry eye disease. (a) Horizontal OCT section of the normal human cornea showing the epithelium, bowman's membrane, stroma, descemet's membrane and endothelium. *In vivo* confocal micrographs (IVCM) of the cornea showing in normal and dry eye subjects (b-g). (b) Normal superficial epithelium showing regularly arranged cells with dark nuclei, (c) superficial epithelium in dry eye disease showing squamous metaplasia, hyperreflectivity and lower cell density as compared to normal,(d) subbasal layer showing normal corneal nerve plexus and dendritic cells, (e) subbasal layer in dry eye

with increased density of dendritic cells, (f) reduced density of nerves tortuous, and (g) hyperreflective nerves. IVCM image: magnification: x800.

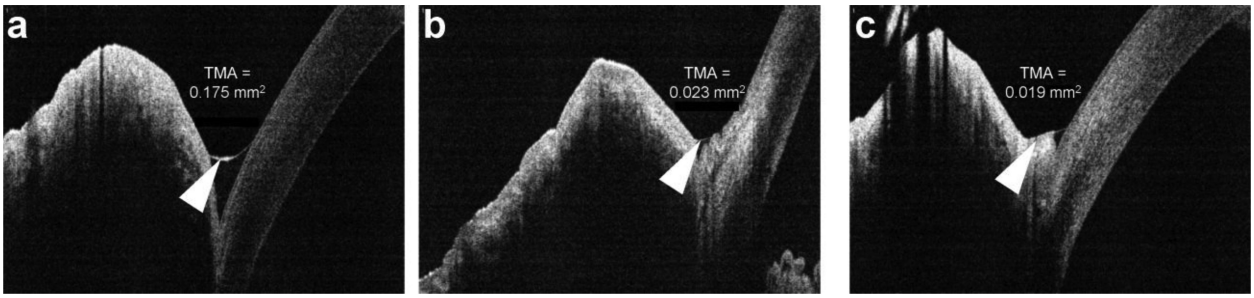

Fig. 2.

RTVue optical coherence tomography (OCT) images showing tear meniscus area (TMA) in health and dry eye disease. (a) Normal, TMA = 0.175mm^2, (b) dry eye disease, TMA = 0.023 mm^2, (c) conjuctival chalasis, TMA = 0.019 mm^2. Arrowheads (a, b) indicate the tear meniscus, and (c) conjunctival chalasis.

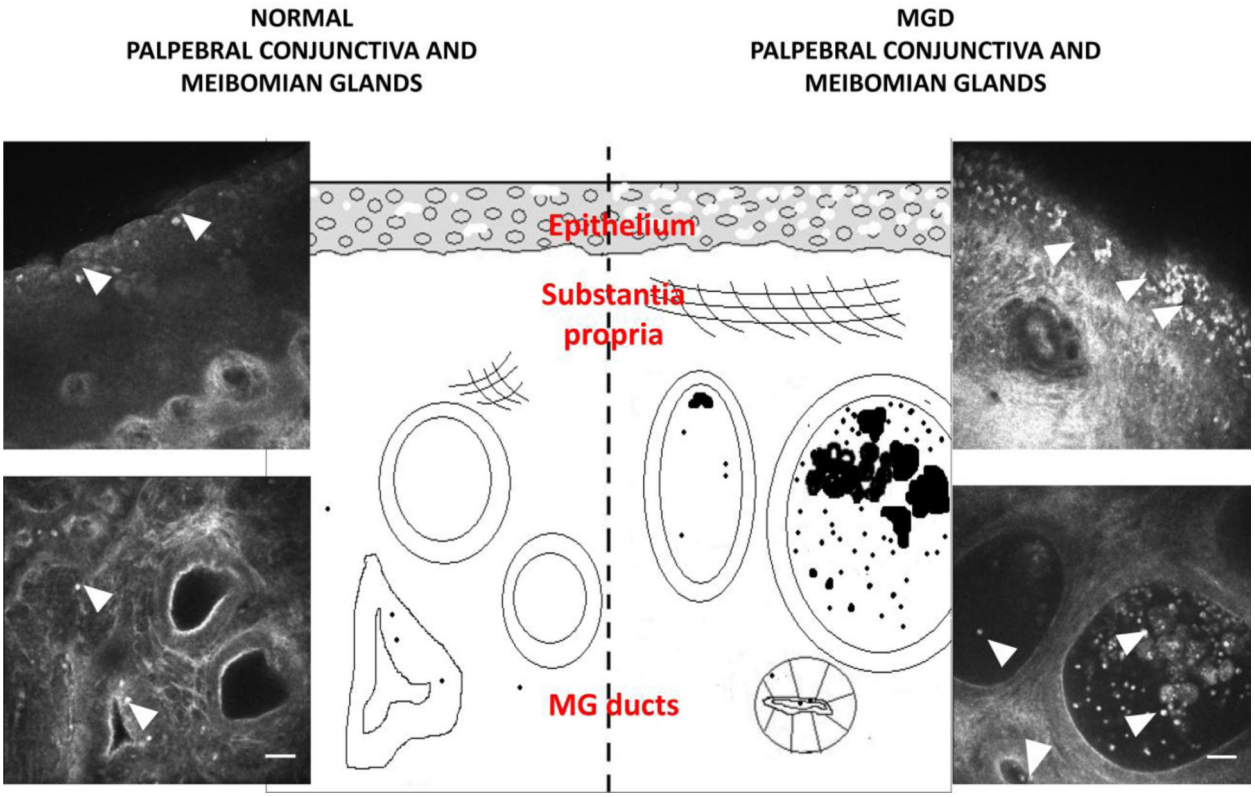

Fig. 3.

Schematic representation of the palpebral conjunctiva and meibomian glands on *in vivo* confocal microscopy (IVCM) in normals and meibomian gland dysfunction (MGD). Bright, hyperreflective, structures (arrowheads) represent immune cells. Scale bar, 40μm

Table 1

A comparison of commercially available time- and spectral-domain optical coherence tomography (OCT) in anterior segment imaging

	Visante OCT (Carl Zeiss)[233,234] (time-domain OCT)	Envisu-C class (Bioptigen)[235] (spectral-domain OCT)	SL-OCT (Heidelberg) [234, 236] (spectral-domain OCT)	RTVue100 (Optovue Inc.)[237] (spectral-domain OCT)
Illumination wavelength (nm)	1310	1310	1310	840
Imaging depth (tissue; mm)	6	1.7 - 2.5	7	2.0- 2.3
Imaging speed (A-scans/second)	2000	32000	200	26000
Speed of image capture	Fast	Fastest	Least fast	Faster
Optical axial resolution (tissue; μm)	18	1.6 - 2.4	25	5
Area of image	16 mm × 6 mm	20 mm × 2.5mm	15 mm × 7 mm	13 mm × 9 mm
Cellular observations	None	None	None	None