The International Workshop on Meibomian Gland Dysfunction: Introduction

Kelly K. Nichols

There are rare occasions in a field of science when significant advances occur in leaps and bounds, rather than in small, deliberate steps. This moment is imminent in the field of meibomian gland dysfunction (MGD)—and therefore in dry eye disease. The goals of the International Workshop on Meibomian Gland Dysfunction were twofold: first, to develop a consensus understanding of the meibomian gland in health and disease; second, to disseminate the knowledge broadly to further the field.

Over the past several years, although the body of knowledge about dry eye has been expanding, it has become clear that significant detail and direction relative to the impact of the meibomian gland in dry eye have been lacking. The Tear Film and Ocular Surface Society (TFOS; http://www.tearfilm.org), a nonprofit organization, launched the International Workshop on Meibomian Gland Dysfunction (www.tearfilm.org/mgdworkshop/index.html) in conjunction with generous industry sponsors that supported the workshop process through unrestricted grants, allowing volunteers to come together to plan, execute, translate, and present the findings of the workshop at a variety of meetings worldwide.

OBJECTIVES

International workshops, such as the Dry Eye Workshop (DEWS) and this workshop on MGD, provide a consensus overview of the field as a snapshot in time. In addition to an exhaustive international literature–based review of the salient clinical, translational, and basic research, new concepts—often assimilated through the process of refining the reports—are also included here. Thus, this report is the most current, definitive summary of the meibomian gland in health and disease. As such, the objectives defined by the Steering Committee were as follows:

- to develop a contemporary understanding of the definition and classification of MGD;
- to conduct an evidence-based evaluation of meibomian gland structure and function in health and disease;
- to critically assess the structure of meibomian lipid and the interaction of the secreted lipid with additional components of the tear film;
- to evaluate the prevalence and associated risk factors for MGD;
- to assess methods of diagnosis, evaluation, and grading of severity of MGD;
- to evaluate existing recommendations and provide a diagnostic/therapeutic algorithm for the management and therapy of MGD;
- to evaluate existing clinical trials of pharmaceutical interventions for the treatment of MGD and provide recommendations for future clinical trial design; and
- to create an executive summary of recommendations for future research in MGD.

PROCESS

More than 50 international experts participated in the workshop, which occurred over a 2-year period. The initial steering committee meeting was held in November 2008, at which time subcommittee chairs and committees were selected on the basis of expertise within the field. After the appointments, the steering committee and the subcommittees met via conference call, Skype, and in person to create draft outlines, assign writing topics, and create draft subcommittee reports. The draft outlines were reviewed by the membership at an MGD workshop meeting after the Association for Ophthalmology and Visual Science (ARVO) annual meeting in May 2009. After that meeting, draft reports were written and circulated for review by the membership at large, including members of the industry liaison committee. Each subcommittee reviewed comments, and suggestions were incorporated into the reports. The “final” draft reports were reviewed by the writing committee at a meeting in April 2010. The committee used this meeting to identify areas in the reports that required harmonization, when overlap classification was needed. After this process and revision by the subcommittees with writing committee guidance, the finalized reports were submitted to the subcommittees for final approval. The steering committee members, writing committee members, subcommittee chairs, and subcommittee members are listed in Tables 1 and 2.

OVERARCHING ISSUES AND FORWARD-LOOKING STATEMENTS

Assembling a group of experts in any field provides the opportunity for discussion, agreement, and disagreement, all of which tend to move a field forward. During the MGD Workshop process, each subcommittee grappled with the controversies within each topical area. Several of the key issues are identified in the following sections. In addition, several appear in more than one report, indicating that there are overarching topics related to MGD that we have yet to fully understand.

Relation of MGD and Dry Eye Disease

It is believed that MGD may be the most common cause of evaporative dry eye and may also have some association with
aqueous-deficient dry eye. Overview reports on dry eye have suggested “meibomian oil deficiency” as an intrinsic factor associated with the disease. The field now understands that the meibomian gland is a key component in the etiology of dry eye and contributes to the evaporative status of the tear film. Most clinicians now assess the lid/meibomian glands in a severity-grading scheme for dry eye, which includes terminology such as “MGD variably present” to “frequent” and “trichiasis, keratinization, symblepharon” at the severe end of the scale. The inclusion of meibomian gland pathophysiology indicates a consensus that the meibomian gland plays a role in dry eye disease.

What is perhaps less clear is the causative relation involved, as well as the binomial classification of dry eye (aqueous-deficient versus evaporative). From a clinical perspective, patients can present with various degrees of MGD and aqueous deficiency and of the two currently accepted forms of dry eye, evaporative dry eye is thought to be significantly more common than aqueous-deficient dry eye. One could hypothesize that abnormalities in meibomian gland structure or function (e.g., lipid quality and/or quantity) are the leading contributors to dry eye disease. Several key questions should be answered, including but not limited to the following:

1. Can MGD be considered a leading cause of dry eye?
2. Can aqueous-deficient dry eye and evaporative dry eye co-exist? Further, can aqueous-deficient dry eye lead to evaporative dry eye, and vice versa?
3. Should MGD be diagnosed and managed within the dry eye paradigm or as an independent condition?
4. Should MGD be considered to be a separate entity or within the dry eye context when evaluating the prevalence of dry eye?
5. Can symptom-based definitions of dry eye discriminate between aqueous-deficient dry eye and evaporative dry eye?
eye that may be related to the pathophysiology of the meibomian glands?

**Terminology and Definitions**

It became clear very early in the MGD workshop process that previously reported terminology had been used interchangeably, with lack of agreement regarding preferred terminology. The term *meibomian gland dysfunction* and its description first came to our attention in the mid-1980s. Since that time, terms such as posterior blepharitis, meibomian gland disease, meibomitis, meibomianitis, meibomian gland dysfunction, MGD (with no reference to disease or dysfunction), and meibomian keratoconjunctivitis have been used by clinicians and researchers to describe clinical conditions involving meibomian gland and/or lid disease. This report provides a new definition for MGD and clearly defines additional terminology, to allow the field to move forward.

**Clinical Outcomes and Design of Clinical Trials**

Central to both issues (relation to dry eye and terminology) is an appropriate clinical diagnosis and diagnostic technology. Definitions are only as good as the ability to appropriately classify disease, and like dry eye, there is no agreed upon gold standard diagnostic test for MGD. Emerging technology, biochemical (lipidomic and proteomic) analyses, and improved clinical grading schemes for individual lid and meibomian gland parameters, as well as for the co-morbid dry eye/MGD clinical condition, should be further explored and validated. The key questions include:

1. Can a gold standard diagnostic test for MGD be developed? Could a single clinical subjective parameter (e.g., meibomian gland expressibility or meibum quality) or objective parameter (e.g., tear osmolarity) differentiate subcategories of ocular surface disease?
2. Can any eyelid or meibomian gland parameter demonstrate appropriate change over time or with treatment? Can a biochemical or physical measure (biomarker) demonstrate change?
3. Will a battery of tests be required to adequately diagnose MGD for clinical trials? What tests should be included to determine entry/exclusion criteria as well as clinical outcomes?
4. Can standardized testing protocols be developed, validated, and adopted for individual tests or batteries of tests in MGD?

Furthermore, without a known natural history of MGD, including progression, it remains a challenge to determine which clinical findings constitute the natural aging process and which findings indicate disease. Importantly, natural history studies were recommended by nearly every subcommittee to better elucidate methods to define, detect, manage, and monitor MGD, including the design of clinical trials for MGD. Standardizing of clinical outcomes for MGD, dry eye, and other ocular surface conditions has been determined to be a major need within the community.

**Specific Subcommittee Controversies**

Within each subcommittee, debate about controversial issues, or lack of group consensus, often was a subtle indicator of areas in which further research or knowledge was needed to bring about agreement or resolution. External (to the committee) review of the reports by workshop participants also provided an indication of discord, and while agreement, for the most part, is indicated in the reports, it is important to recognize areas in which the committee struggled. Several of these issues are highlighted by subcommittee in the following section.

**Definition and Classification.** As mentioned, the lack of consensus regarding terminology and the need for a working definition and classification scheme provided the backdrop for this committee. The committee acknowledged the significant contributions in the past while creating a reference point with a new definition and classification scheme for future clinical and basic studies in the field of MGD.

**Anatomy and Pathophysiology.** Although MGD has been described as a condition for more than 100 years, its etiology remains in dispute. Significant evidence regarding the etiology of MGD is reported by this committee, yet systemic and ocular contributions have yet to be fully understood. In addition, it is unclear whether changes in meibomian gland structure result in alterations to the meibomian lipid and whether this process can be halted or reversed. The presence or absence of inflammation and infection in the meibomian gland was considerably debated relative to the etiology and pathogenesis of MGD and requires further exploration.

**Lipids.** Lipid production and subsequent delivery onto the lid margin as meibum, as well as the interaction of meibum with the tear film, are generally understood on a clinical level; however, specific chemical and biochemical interactions in the lipid production process, as well as in the tear film, are poorly understood. Newer techniques allow for determination of the molecular and physical structure of lipids, and the most controversial issue related to lipid measurement is the phospholipid content, once thought to be critical to the maintenance of tear film stability. Given the holocrine nature of lipid production, phospholipid detection in meibum is expected, although the mass spectrometry techniques currently used to assess meibum have demonstrated relatively low levels of phospholipids in the meibum. In addition, methods of comparing lipid profiles statistically are needed to determine the differences between health and disease.

**Epidemiology.** The natural history of MGD and of dry eye disease in general has not been established; therefore, a fundamental understanding of disease etiology, clinical presentation across severities, and disease progression have yet to be determined. Population-based studies are needed, to better assess prevalence and determine incidence, as well as to assess differences in subtypes of dry eye disease. The impact of potential causative factors, including contact lens wear, medication use, and hormone status should be explored further. In addition, survey instruments specific to MGD should be developed, as well as methods of classifying and analyzing clinical data for which the validity and repeatability are unknown.

**Diagnosis and Management.** Historically, MGD has been evaluated primarily in clinical and basic research settings, although it has often been overlooked or underdiagnosed in clinical care. In writing this report, the diagnosis and management committees struggled to create algorithms for both research and clinical applications. Each committee approached the task from opposing points of view, and their work, while harmonized as much as possible, reveals some of the controversy within the profession regarding the grading of clinical findings and the appropriate paired clinical management approaches. In both scenarios, the evidence supporting therapies across severity levels must be studied to achieve a consensus.

**Clinical Trials.** The clinical trials report highlights 26 clinical trials in MGD, most of which were small and were not randomized, controlled, and/or masked. Additional studies are needed in which diagnostic criteria are established for MGD, with terminology that is widely accepted, such that new or existing treatments can be assessed and compared across studies. New methods to assess MGD, both clinically and biologically, are needed to further the field, alone and in conjunction with dry eye disease.

This process has been an incredible experience for everyone involved. The countless hours spent by committee members reading the literature, writing, and reviewing the reports could easily go unnoticed. Therefore, it is with gratitude that I would...
like to thank everyone who played a role in the creation of this report, for giving encouragement, knowledge, and insight into the process. It is my hope that this report will provide the framework needed to move to the next level of achievement in this field and will inspire research that will ultimately benefit clinical care of patients with MGD across the world for years to come.

Acknowledgments

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Dedication

This workshop report is dedicated to our colleague, Jeffrey P. Gilbard, MD, and all past, current, and future researchers in the field of meibomian gland dysfunction.

APPENDIX

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The International Workshop on Meibomian Gland Dysfunction: Executive Summary

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Meibomian gland dysfunction (MGD) may well be the leading cause of dry eye disease throughout the world. Although this condition affects the health and well-being of millions of people, there is no global consensus on the definition, classification, diagnosis, or therapy for MGD. To achieve such a consensus, the Tear Film and Ocular Surface Society (TFOS; http://www.tearfilm.org), a nonprofit organization, launched the International Workshop on Meibomian Gland Dysfunction (www.tearfilm.org/mgdworkshop/index.html). The objectives of the workshop were to:

- conduct an evidence-based evaluation of meibomian gland structure and function in health and disease;
- develop a contemporary understanding of the definition and classification of MGD;
- assess methods of diagnosis, evaluation, and grading of the severity of MGD;
- develop recommendations for the management and therapy of MGD;
- develop appropriate norms of clinical trial design to evaluate pharmaceutical interventions for the treatment of MGD; and
- create a summary of recommendations for future research in MGD.

The report of the Workshop on MGD, which required more than 2 years to complete, was finalized in 2010. This effort involved more than 50 leading clinical and basic research experts from around the world. These participants, who were assigned to subcommittees, reviewed published data and examined the levels of supporting evidence. Subcommittee reports were circulated among all workshop participants, presented in open forum, and discussed in an interactive manner.

From the 1College of Optometry, Ohio State University, Columbus, Ohio; the 2Department of Ophthalmology and Visual Sciences, Kentucky Lions Eye Center, Louisville, Kentucky; the 3Nuffield Laboratory of Ophthalmology, Oxford University, Oxford, United Kingdom; the departments of 4Ophthalmology and 5Pathology and Laboratory Science, Jules Stein Eye Institute, University of California Los Angeles, Los Angeles, California; the 6Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan; 7Georgetown University, Washington, DC; 8Schepps Eye Research Institute and the 9Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts.

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The entire workshop report is published in English in this issue of IOVS. The report has also been translated, at least in part, into Chinese, Dutch, French, German, Greek, Italian, Japanese, Polish, Portuguese, Spanish, Russian and Turkish; these translations are available on the TFOS website.

An executive summary of the conclusions and recommendations of the TFOS Workshop on MGD is presented in this article. The material is abstracted from the full report, and thus, additional details and references can be obtained in the open-access, online version.

DEFINITION AND CLASSIFICATION OF MGD

Meibomian gland dysfunction (MGD) is 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.

There are several evidence-based explanations for the terminology used in this definition. The term dysfunction is used because the function of the meibomian glands is disturbed. The term diffuse is used because the disorder involves most of the meibomian glands. Localized involvement of meibomian glands, such as in chalazion, tends not to cause abnormalities in the tear film or ocular surface epithelia and therefore is not considered to belong within the context of MGD. Obstruction of the meibomian gland orifices and terminal ducts and qualitative and/or quantitative changes in meibomian gland secretions are identified as the most prominent aspects of MGD. In addition, subjective symptoms of eye irritation are included in the definition, as it is the symptoms that are of greatest concern to the patient and often to the clinician. Improvement in the patient’s symptoms is the major goal in the treatment of MGD. The role of inflammation in the etiology of MGD is controversial and uncertain.

Recent literature has used the terms posterior blepharitis and MGD as if they were synonymous, but these terms are not interchangeable. Posterior blepharitis describes inflammatory conditions of the posterior lid margin, of which MGD is only one possible cause. In its earliest stages, MGD may not be associated with clinical signs characteristic of posterior blepharitis. At this stage, affected individuals may be symptomatic, but alternatively, they may be asymptomatic and the condition regarded as subclinical. As MGD progresses, symptoms develop and lid margin signs, such as changes in meibum expressibility and quality and lid margin redness, may become more visible. At this point, an MGD-related posterior blepharitis is said to be present.

The term MGD is regarded as appropriate for describing the functional abnormalities of the meibomian glands. Meibomian gland disease is used to describe a broader range of meibomian gland disorders, including neoplasia and congenital dis-
ease. Other terms such as meibomitis or meibomianitis describe a subset of disorders of MGD associated with inflammation of the meibomian glands. Although inflammation may be important in the classification and in the therapy of MGD, these terms are not sufficiently general, as inflammation is not always present.

MGD may be classified according to anatomic changes, pathophysiological changes, or the severity of disease. Any classification system must meet the needs of the clinician and researcher alike. A classification based on pathophysiology is deemed to best meet these needs.

A classification of MGD into two major categories based on meibomian gland secretion is proposed: low-delivery states and high-delivery states (Fig. 1). Low-delivery states are further classified as hyposecretory or obstructive, with cicatricial and noncicatricial subcategories. Hyposecretory MGD describes the condition of decreased meibum delivery due to abnormalities in meibomian glands without remarkable obstruction. Obstructive MGD is due to terminal duct obstruction. In the cicatricial form, the duct orifices are dragged posteriorly into the mucosa, whereas these orifices remain in their normal positions in noncicatricial MGD. High-delivery, hypersecretory MGD is characterized by the release of a large volume of lipid at the lid margin that becomes visible on application of pressure onto the tarsus during examination. Each MGD category also has primary causes, referring to conditions for which there are no discernible underlying causes or etiology.

Overall, MGD can lead to alterations of the tear film, symptoms of eye irritation, inflammation, and dry eye.

**ANATOMY, PHYSIOLOGY, AND PATHOPHYSIOLOGY OF MGD**

The meibomian glands are large sebaceous glands located in the tarsal plates of the eyelids. These glands actively synthesize and secrete lipids and proteins that are delivered at the upper and lower eyelid margins just anterior to the mucocutaneous junctions. The glandular lipids spread onto the tear film, promote its stability, and prevent its evaporation.

Meibomian glands, unlike other sebaceous glands, do not have direct contact with hair follicles. Each meibomian gland consists of multiple secretory acini-containing meibocytes, lateral ductules, a central duct, and a terminal excretory duct that opens at the posterior lid margin. The number and volume of meibomian glands is greater in the upper than in the lower lid, but the relative functional contribution of the upper and lower lid glands to the tear film remains to be determined. Also unknown is the source or sources of stem cells for this gland.

Meibomian glands are densely innervated, and their function is regulated by androgens, estrogens, progestins, retinoic acid, and growth factors, and possibly by neurotransmitters. The glands produce polar and nonpolar lipids through a complex and incompletely understood process. These lipids are secreted into the ducts through a holocrine process. Meibum delivery onto the lid margin occurs with muscular contraction during lid movement.

Meibomian gland dysfunction is caused primarily by terminal duct obstruction with thickened opaque meibum containing keratinized cell material. The obstruction, in turn, is due to hyperkeratinization of the ductal epithelium and increased meibum viscosity (Fig. 2). The obstructive process is influenced by endogenous factors, such as age, sex, and hormonal disturbances, as well as by exogenous factors such as topical medication. The obstruction may lead to intraglandular cystic dilatation, meibocyte atrophy, gland dropout, and low secretion, effects that do not typically involve inflammatory cells. The outcome of MGD is a reduced availability of meibum to the lid margin and tear film. The consequence of insufficient lipids may be increased evaporation, hyperosmolarity and instability of the tear film, increased bacterial growth on the lid margin,
evaporative dry eye, and ocular surface inflammation and damage.

Overall, MGD is an extremely important condition, conceivably underestimated, and very likely the most frequent cause of dry eye disease.

**TEAR FILM LIPIDS AND LIPID–PROTEIN INTERACTIONS IN HEALTH AND DISEASE**

The meibomian glands are the main source of lipids for the human tear film. The meibomian gland secretions consist of a complex mixture of various polar and nonpolar lipids containing cholesterol and wax esters, diesters, triacylglycerol, free cholesterol, free fatty acids, and phospholipids. The meibum spreads onto the tear film and functions to slow evaporation of the aqueous component, preserve a clear optical surface, and form a barrier to protect the eye from microbial agents and organic matter such as dust and pollen.

A proposed model of the human tear film is shown in Figure 3. This model incorporates proteins (e.g., lipocalin, lysozyme, and the surfactant proteins B and C) intercalated in and/or adsorbed to the outer lipid layer. These protein interactions appear to influence the physical properties and surface tension of the tear film lipid layer. The proposed model also features very long chain (O-acyl)-ω-hydroxy fatty acids, which may act in the formation of an intermediate surfactant lipid sublayer.
between the outermost nonpolar lipids and the aqueous layer of the tear film.

The lipid patterns of human meibum show many similarities among normal individuals, but may differ from those in persons with MGD. Some of these differences may be due to an increased presence of certain types of commensal lid bacteria that can hydrolyze lipids. Indeed, the ability of antibiotics to inhibit bacterial lipolytic enzymes may in part explain the effectiveness of such pharmaceuticals in the treatment of MGD.

Lipid profiles in human meibum differ from those in the tear film. Of particular interest, the absolute and relative amounts of polar lipids in both the meibum and the tear film have yet to be resolved.

Another attribute of tear film lipids is that they appear to be essential for ease and comfort in contact lens wear, but also form deposits on these lenses. It is possible that contact lens wear itself disrupts the meibomian glands and/or lipid layer and leads to tear film evaporation and ocular surface discomfort.

### Table 1. Population-Based Studies Providing Estimates of the Prevalence of MGD

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Ethnicity</th>
<th>Parameter</th>
<th>Prevalence (%)</th>
<th>Age (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beijing Eye Study</td>
<td>1957</td>
<td>Mainland Chinese</td>
<td>Telangiectasia (asymptomatic)</td>
<td>68</td>
<td>&gt;40</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Telangiectasia (symptomatic of dry eye)</td>
<td>69.3</td>
<td></td>
</tr>
<tr>
<td>Japanese study</td>
<td>113 pensioners</td>
<td>Japanese</td>
<td>Gland dropout, expressibility and nature of meibum secretion</td>
<td>61.9</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Shihpai Eye Study</td>
<td>1361</td>
<td>Taiwanese Chinese</td>
<td>Telangiectasia or meibomian gland orifice plugging</td>
<td>60.8</td>
<td>&gt;65</td>
</tr>
<tr>
<td>Melbourne Visual Impairment Project</td>
<td>926</td>
<td>Caucasian</td>
<td>Tear break up time &lt; 1 SD (10 s)</td>
<td>19.9</td>
<td>40–97</td>
</tr>
<tr>
<td>Salisbury Eye Evaluation</td>
<td>2482</td>
<td>Caucasian</td>
<td>Tear break up time &lt; 1.5 SD (8 s)</td>
<td>8.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Meibomian gland plugging or collarettes (grades 2 and 3)</td>
<td>3.5</td>
<td>&gt;65</td>
</tr>
</tbody>
</table>

### Table 2. Specialized and Nonspecialized Tests for MGD and MGD-Related Disease

<table>
<thead>
<tr>
<th>Testing Category</th>
<th>Specific Test (s)</th>
<th>Tests for a General Clinic</th>
<th>Tests for a Specialized Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptoms</strong></td>
<td>Questionnaires</td>
<td>McMonnies; Schein; OSDI; DEQ; OCI; SPEED; and others</td>
<td>McMonnies; Schein; OSDI; DEQ; OCI; SPEED; and others</td>
</tr>
<tr>
<td><strong>Signs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meibomian function</td>
<td>Lid morphology</td>
<td>Slit lamp microscopy</td>
<td>Slit lamp microscopy; confocal microscopy</td>
</tr>
<tr>
<td></td>
<td>Meibomian gland mass</td>
<td>Slit lamp microscopy</td>
<td>Meibography</td>
</tr>
<tr>
<td></td>
<td>Gland expressibility; expressed oil quality and volume</td>
<td></td>
<td>Slit lamp microscopy</td>
</tr>
<tr>
<td></td>
<td>Lid margin reservoir</td>
<td>Interferometry, slit lamp</td>
<td>Meibometry</td>
</tr>
<tr>
<td></td>
<td>Tear film lipid layer; thickness, spread time, spread rate</td>
<td></td>
<td>Interferometry; slit lamp; video interferometry</td>
</tr>
<tr>
<td><strong>Evaporation Tears</strong></td>
<td>Evaporimetry</td>
<td></td>
<td>Evaporimetry</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>Osmolarity</td>
<td>TearLab device, other</td>
<td>TearLab device, other</td>
</tr>
<tr>
<td>Stability</td>
<td>Tear film</td>
<td>TFBUT; Ocular protection index</td>
<td>TFBUT; Ocular protection index</td>
</tr>
<tr>
<td></td>
<td>Tear film lipid layer</td>
<td>Spread time</td>
<td>Interferometry; spread rate; pattern</td>
</tr>
<tr>
<td>Indices of volume and secretion</td>
<td>Tear secretion</td>
<td>Not available</td>
<td>Fluorophotometry/fluorescein clearance rate</td>
</tr>
<tr>
<td></td>
<td>Tear volume</td>
<td>Schirmer 1</td>
<td>Volume by fluorophotometry</td>
</tr>
<tr>
<td></td>
<td>Tear volume</td>
<td>Meniscus height</td>
<td>Meniscus radius of curvature; meniscometry</td>
</tr>
<tr>
<td></td>
<td>Tear clearance</td>
<td>Tear film index</td>
<td>Tear film index</td>
</tr>
<tr>
<td>Ocular surface</td>
<td>Ocular surface staining</td>
<td>Oxford scheme; NEI/industry scheme</td>
<td>Oxford scheme; NEI/industry scheme</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Biomarkers</td>
<td></td>
<td>Flow cytometry; bead arrays; microarrays; mass spectrometry; cytokines and other mediators; interleukins; matrix metalloproteinases</td>
</tr>
</tbody>
</table>

Tests of glandular function are presented first followed by those for related disorders such as dry eye. OSDI, Ocular Surface Disease Index; DEQ, Dry Eye Questionnaire; OCI, Ocular Comfort Index; SPEED, Standard Patient Evaluation of Eye Dryness.
mian gland damage and altered meibum delivery or instead arise from subsequent damage to other ocular surface tissues.

The reported prevalence of MGD varies widely. A striking observation is that the prevalence of MGD appears to be much higher in Asian populations (Table 1), often reported as greater than 60% in different Asian population-based studies. In contrast, the prevalence in Caucasians spans from 3.5% to 19.9%. Many people with the clinical signs of MGD also have overlapping symptoms of dry eye disease.

Several ophthalmic, systemic, and medication-related factors may coexist with, or plausibly contribute to, the pathogenesis of MGD. Ophthalmic factors include anterior blepharitis, contact lens wear, Demodex folliculorum, and dry eye disease. Systemic factors that may promote MGD include, among others, androgen deficiency, menopause, aging, Sjögren's syndrome, cholesterol levels, psoriasis, atopy, rosacea, hypertension, and benign prostatic hyperplasia (BPH). Medications associated with the pathogenesis of MGD include antiandrogens, medications used to treat BPH, postmenopausal hormone therapy (e.g., estrogens and progestins), antihistamines, antidepressants, and retinoids. The ω-3 fatty acids may be protective.

In summary, MGD appears to be a prevalent problem, with detriments that are potentially damaging to well-being. Nonetheless, even basic information regarding its prevalence, demographic and geographic distributions, risk factors, and impact on ocular health and quality of life are only beginning to emerge. The same was said of dry eye disease more than a decade ago, and since that time, research efforts have grown exponentially. We are confident that the time has now come to embark on the systematic study of MGD as well. It is through such efforts that a better understanding of the disease will be gained, and strategies for prevention and treatment will begin to be developed.

**Diagnosis of MGD**

The diagnosis of MGD, whether in isolation or associated with ocular surface damage or dry eye, should be viewed in the context of diagnosing any ocular surface disease. Tests should be performed in an order that minimizes the extent to which one test influences the results of the tests that follow. A series of tests that are recommended for use in the diagnosis of MGD and in MGD-related disorders, including evaporative dry eye, is presented in Table 2.

**Tests for MGD**

In asymptomatic adults, it is appropriate to include gland expression (e.g., by the application of moderate digital pressure to the central lower lid) to the routine workup of the patient, to detect asymptomatic, nonobvious MGD. A diagnosis of MGD may require that the patient be further assessed for ocular surface damage and dry eye, using appropriate diagnostic techniques.

In patients with ocular surface symptoms or morphologic lid signs of MGD (e.g., orifice plugging and other orifice or lid margin signs), meibomian gland functionality should be assessed by digital pressure over the central (± nasal) third of the lower and upper lids, to determine the extent and severity of the MGD (expressiveibility and secretion quality). The examination should be performed with moderate digital pressure or by a standardized technique. The patient should be further assessed for evidence of ocular surface damage and dry eye.

**Tests for MGD-Related Dry Eye**

A two-tiered approach to the diagnosis of MGD-related dry eye is recommended. In the first step, normal subjects are distinguished from patients with dry eye of any type (generic dry eye). The second step involves the differential diagnosis of MGD-related evaporative dry eye and aqueous-deficient dry eye.

Two approaches are proposed: one suitable for practitioners working in a general clinic and the other for investigators working in specialized units. The evidence base of the tests proposed varies according to the clinical setting.

A suitable sequence of tests to perform in a general clinic for the diagnosis of MGD-related disease in patients presenting with symptoms of ocular surface disease is as follows:

1. Administration of a symptom questionnaire;
2. Measurement of the blink rate and calculation the blink interval;
3. Measurement of lower tear meniscus height;
4. Measurement of tear osmolarity (if available);
5. Installation of fluorescein and measurement of the tear film breakup time (TFBUT) and Ocular Protection Index (OPI);
6. Grading of corneal and conjunctival fluorescein staining;
7. Schirmer test or alternate (phenol red thread test).

Positive (abnormal) results in tests 1, 4, 5, and 6 provide partial evidence of the presence of a generic dry eye, without specifying whether it is aqueous-deficient or evaporative. Evidence of aqueous-deficient dry eye may be obtained by measuring tear flow or an assessment of aqueous volume on the basis of tear meniscus height or Schirmer test.

8. If MGD has not been characterized (symptomatic/asymptomatic) at a previous visit, then it can be assessed at the end of this sequence as follows:
   a. Quantification of morphologic lid features
   b. Expression: quantification of meibum expressibility and quality
   c. Meibography: quantification of dropout.

If testing suggests the diagnosis of a generic dry eye and tests of tear flow and volume are normal, then an evaporative dry eye is implied and quantification of MGD will indicate the meibomian gland contribution. This test sequence also permits a diagnosis of symptomatic MGD to be made, with or without ocular surface staining and with or without dry eye. The graded scores for each test can be used to monitor the disease during treatment.

An "ideal" or comprehensive test series for corneal specialists or for investigators engaged in clinical trials is also proposed for clinics that have access to a wider range of diagnostic equipment. Some of the tests listed are alternatives and are more research based. It is suggested again that the diagnosis be

<table>
<thead>
<tr>
<th>Stage</th>
<th>MGD Grade</th>
<th>Symptoms</th>
<th>Corneal Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+ (minimally altered expressibility and secretion quality)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>++ (mildly altered expressibility and secretion quality)</td>
<td>Minimal to mild</td>
<td>None to limited</td>
</tr>
<tr>
<td>3</td>
<td>+++ (moderately altered expressibility and secretion quality)</td>
<td>Moderate</td>
<td>Mild to moderate; mainly peripheral</td>
</tr>
<tr>
<td>4</td>
<td>++++ (severely altered expressibility and secretion quality)</td>
<td>Marked</td>
<td>Marked; central in addition</td>
</tr>
</tbody>
</table>

"Plus" disease Co-existing or accompanying disorders of the ocular surface and/or eyelids
### TABLE 4. Treatment Algorithm for MGD

<table>
<thead>
<tr>
<th>Stage</th>
<th>Clinical Description</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No symptoms of ocular discomfort, itching, or photophobia</td>
<td>Inform patient about MGD, the potential impact of diet, and the effect of work/home environments on tear evaporation, and the possible drying effect of certain systemic medications</td>
</tr>
</tbody>
</table>
|       | **Clinical signs** of MGD based on gland expression  
Minimally altered secretions: grade ≥2–4  
Expressibility: 1 | Consider eyelid hygiene including warming/expressing as described below (±) |
|       | No ocular surface staining |           |
| 2     | Minimal to mild symptoms of ocular discomfort, itching, or photophobia  
Minimal to mild MGD clinical signs  
Scattered lid margin features  
Mildly altered secretions: grade ≥4–<8  
Expressibility: 1 | Advise patient on improving ambient humidity; optimizing workstations and increasing dietary omega-3 fatty acid intake (±)  
Institute eyelid hygiene with eyelid warming (a minimum of four minutes, once or twice daily) followed by moderate to firm massage and expression of MG secretions (+)  
**All the above, plus** (±)  
Artificial lubricants (for frequent use, non-preserved preferred)  
Topical azithromycin  
Topical emollient lubricant or liposomal spray  
Consider oral tetracycline derivatives |
|       | None to limited ocular surface staining: DEWS grade 0–7; Oxford grade 0–5 |           |
| 3     | Moderate symptoms of ocular discomfort, itching, or photophobia  
with limitations of activities  
Moderate MGD clinical signs  
↑ lid margin features: plugging, vascularity  
Moderately altered secretions: grade ≥8 to <13  
Expressibility: 2 | **All the above, plus**  
Oral tetracycline derivatives (+)  
Lubricant ointment at bedtime (±)  
Anti-inflammatory therapy for dry eye as indicated (±) |
|       | Mild to moderate conjunctival and peripheral corneal staining, often inferior: DEWS grade 8–23; Oxford grade 4–10 |           |
| 4     | Marked symptoms of ocular discomfort, itching or photophobia  
with definite limitation of activities  
Severe MGD clinical signs  
↑ lid margin features: dropout, displacement  
Severely altered secretions: grade ≥13  
Expressibility: 3 | **All the above, plus**  
Anti-inflammatory therapy for dry eye (+) |
|       | Increased conjunctival and corneal staining, including central staining: DEWS grade 24–33; Oxford grade 11–15 |           |
|       | ↑ signs of inflammation: ≥moderate conjunctival hyperemia, phlyctenules |           |

**“Plus” disease** Specific conditions occurring at any stage and requiring treatment. May be causal of, or secondary to, MGD or may occur incidentally:

1. Exacerbated inflammatory ocular surface disease
2. Mucosal keratinization
3. Phlyctenular keratitis
4. Trichiasis (e.g. in cicatrical conjunctivitis, ocular cicatricial pemphigoid)
5. Chalazion
6. Anterior blepharitis
7. Demodex-related anterior blepharitis, with cylindrical dandruff

1. Pulsed soft steroid as indicated
2. Bandage contact lens/scleral contact lens
3. Steroid therapy
4. Epilation, cryotherapy
5. IntraleSIONAL steroid or excision
6. Topical antibiotic or antibiotic/steroid
7. Tea tree oil scrubs

*Meibum quality* is assessed in each of eight glands of the central third of the lower lid on a scale of 0 to 3 for each gland: 0, clear; 1, cloudy; 2, cloudy with debris (granular); and 3, thick, like toothpaste (total score range, 0–24). *Expressibility* is assessed on a scale of 0 to 3 in five glands in the lower or upper lid, according to the number of glands expressible: 0, all glands; 1, three to four glands; 2, one to two glands; and 3, no glands. *Staining scores* are obtained by summing the scores of the exposed cornea and conjunctiva. Oxford staining score range, 1–15; DEWS staining score range, 0–33.
performed in two steps: first to diagnose generic dry eye, and then to subtype with the grade of MGD.

This test series consists of a symptom assessment (e.g., the Ocular Surface Disease Index [OSDI] and the Dry Eye Questionnaire [DEQ]) and measurements of the osmolarity, secretion, volume, stability, and evaporation of tears. Tests of ocular surface damage, such as corneal and conjunctival staining, are also included in the diagnostic series. The results of tests of inflammatory mediators, the presence of inflammatory cell markers, and other proteomic and lipidomic mass spectrometry analyses can also be assessed to provide information regarding overall ocular surface inflammatory status, although the link to MGD specifically is not known at this time. Specific measures of tear production for the diagnosis of aqueous-deficient dry eye are also recommended.

**MANAGEMENT AND THERAPY OF MGD**

Treatment of MGD varies greatly among eye care providers on different continents. Underreporting makes it difficult to assess practice patterns accurately, but most practitioners agree that underdiagnosis is common and clinical follow-up irregular.

Without generally accepted definitions for a staging system of clinical severity of MGD, it is problematic to propose a treatment plan based on disease stage. Nonetheless, in the hope of assisting eye care providers attempting to fashion a logical, evidence-based treatment approach, a disease-staging summary (Table 3) and staged treatment algorithm (Table 4) are proposed.

In the staging of disease, it is recognized that it is difficult clinically to separate the effects of MGD and the effects of aqueous deficiency on the ocular surface. In addition, co-morbid diseases are often present. Thus, Table 3 represents a clinical picture of staged disease. Co-morbid conditions, defined as plus disease may require concurrent management according to standard-of-care protocols.

Table 4 reflects an evidence-based approach to the management of MGD. At each treatment level, lack of response to therapy moves treatment to the next level. A ± sign means that the evidence to support the use of the treatment at that level is limited or emerging, thus its use should be based on clinical judgment. A + sign indicates that the treatment is supported by the evidence at that stage of disease. The quality of expressed meibum and meibum expressibility are key features in the clinical assessment of MGD.

As outlined in Table 4, meibum quality is assessed in each of eight glands of the central third of the lower lid, and meibum expressibility is assessed in the five glands in the lower or upper lid. The numerical staining scores refer to a summed score of staining of the exposed cornea and conjunctiva. Note that corneal staining with topically instilled fluorescein can occur in normal subjects on a sporadic basis, therefore pathologic staining should be identified as repeatedly observed staining of the same or adjacent portions of the cornea.

With every systemic medication, systemic side effects have to be considered. With the treatment algorithm in Table 4 in mind, the phototoxicity caused by systemic tetracycline derivatives and the anticoagulant effects of essential fatty acids (EFAs) are of specific concern. EFAs are nutritional supplements that have received much attention, but with only one published clinical study so far supporting their efficacy in MGD. This is also true of the use of sex hormones, for which there is no published clinical trial regarding efficacy, and there is no licensed product available. Hence, the panel agreed not to assign this potential treatment modality to a grade of disease. The risks of prolonged topical corticosteroid therapy (e.g., induction of cataract and elevated intraocular pressure) are

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**Table 5.** Key Issues and Subcommittee Findings in the MGD Clinical Trials Review

<table>
<thead>
<tr>
<th>Key Issues</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial objective(s)</td>
<td>Most of the studies evaluated were interventional treatment trials. Approximately one third were comparative (e.g. warm compresses or artificial tears).</td>
</tr>
<tr>
<td>Trial design/methodology</td>
<td>Studies were primarily small trials (&lt;40 subjects) of short (&lt;3 months) duration. Most were prospective, three utilized a randomized controlled design, and two were double masked.</td>
</tr>
<tr>
<td>Study sample</td>
<td>In general, patients with chronic disease were recruited, but selection criteria were not uniformly defined.</td>
</tr>
<tr>
<td>Inclusion criteria</td>
<td>Lid changes and symptoms were the most common clinical characteristics utilized in recruitment and selection.</td>
</tr>
<tr>
<td>Exclusion criteria</td>
<td>Classification of exclusion criteria fall into three different categories: 1. Ocular disease-related or contact lens wear (most common) 2. Iatrogenic (e.g., surgery, one third of studies) 3. Systemic disease-related or pregnancy (15%)</td>
</tr>
<tr>
<td>Outcome measures</td>
<td>No specific and consistent outcomes were reported. The most common outcomes included symptoms (typically of dry eye), lid margin signs, and dry eye clinical findings (Schirmer, TIBUT): 1. Symptoms 2. TBUT 3. MG secretion/expression 4. Schirmer 5. Corneal staining 6. MG obstruction 7. Eyelids 8. Lipid layer assessment (e.g., interferometry)</td>
</tr>
<tr>
<td>Treatment</td>
<td>Most studies lacked a washout period and did not check for relapse. Approximately one half allowed concurrent use of other treatment and one third had a treatment in the control group. Large variability was seen in treatment duration, but pharmacologic trials tended to be longer with more follow-up.</td>
</tr>
<tr>
<td>Statistics</td>
<td>There are a limited number of randomized, controlled, clinical trials available for comparison. With nonuniform outcome variables and small samples, it is difficult to calculate effect size, power, or required sample size. There is limited information on how missing data (e.g. loss to follow-up, exclusion due to noncompliance) were handled.</td>
</tr>
</tbody>
</table>
well known. Consequently, the use of such medications should be reserved for the treatment of acute exacerbations in MGD and are not recommended for long-term therapy. Regular monitoring of intraocular pressure is mandatory with the use of topical corticosteroids.

Management of plus disease conditions should follow the standard of care and is not limited to the treatments listed in Table 4.

**CLINICAL TRIALS**

There are significant limitations in assessing the available literature regarding clinical trial methodology in MGD. The lack of consensus in terminology and the broad array of clinical tests performed in clinical trials involving the meibomian gland and eyelid create a discordance in comparing results across studies.

Table 5 provides an overview of the topical areas in clinical trials (objectives, design, sample, inclusion, exclusion, outcomes, treatments, and statistical design) that were reviewed in the 26 papers identified as clinical trials involving MGD.

A recommendation for the design of clinical trials specific to MGD is to include well-defined objectives. These objectives should be clearly stated and allow for concise and specific questions to be answered. There are important and basic questions and considerations to address in MGD clinical trial design:

- Studies must be designed to distinguish between MGD and dry eye disease. A review of past clinical trials of MGD suggested that there is no clear consensus on what such studies should entail. Some include subjects with dry eye, others exclude them, and still others fail to consider dry eye status altogether. Studies that evaluate the possible role of MGD in aqueous-deficient dry eye and the overlap of the two would also be welcome.
- Given that there is considerable uncertainty between MGD and dry eye disease, trials that evaluate the association between the two would be beneficial, as would observational trials that assess the natural history of MGD. Of special value would be a standardized symptom questionnaire that could distinguish MGD lid disease from dry eye disease.
  - Developing alternative or indirect ways of assessing and testing MGD would also be desirable. Accurate, repeatable measures of symptoms are of obvious value as outcome measures and are directly relevant to the patient’s health. Quantitative measures of disease may also be useful, especially if it can be shown that reversal improves long-term health. Examples include osmolarity, interferometry, high resolution OCT, tests that can measure visual function and interblink visual acuity decay, and techniques that discriminate differences in the meibum. It is important that clinical studies that demonstrate the correlation between the results of these tests and clinical findings, such as symptoms and signs, be conducted first.

Overall, the most desirable clinical trials for the evaluation of MGD treatments are prospective, randomized, controlled, and double-masked. To date, very few trials have met these criteria, and it is unknown when, if ever, results from those ongoing trials will be published.

We suggest the following main priorities in future clinical trials in MGD:

- Determine the natural history of MGD;
- Clarify the association between MGD and dry eye disease;
- Develop a specific and validated questionnaire for symptoms of MGD;
- Create a standardized grading for lid and other signs in MGD;
- Evaluate the feasibility and clinical value of lipid and protein biomarkers;
- Validate surrogate clinical outcomes related to MGD.

**Acknowledgments**

The authors thank Michelle Dalton (www.dalton-and-associates.com) for her professional assistance with this Workshop highlight report.
The International Workshop on Meibomian Gland Dysfunction: Report of the Definition and Classification Subcommittee

J. Daniel Nelson,1 Jun Shimazaki,2 Jose M. Benitez-del-Castillo,3 Jennifer P. Craig,4 James P. McCulley,5 Seika Den,2 and Gary N. Foulkes6

Recommended definition of MGD: Meibomian gland dysfunction (MGD) is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. This may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease.

Previous definitions and criteria of MGD: There is no firmly established definition of MGD published in the literature. Most researchers have used a criterion-based approach to describe the condition, with combinations of objective findings and measurements. Anatomic changes of the lid margin, expressibility of meibomian lipids, gland dropout by meibography, evaporimetry, and meibometry are most commonly used (Table 1).

Terminology

The terminology associated with MGD is described in Table 2. The term blepharitis is a general one, describing inflammation of the lid as a whole. Marginal blepharitis is applied to inflammation of the lid margin and includes both anterior and posterior blepharitis.

Anterior blepharitis describes inflammation of the lid margin anterior to the gray line and centered around the lashes.16 The gray line represents the location of the marginal region of the orbicularis muscle (the muscle of Riolan) seen through the lid skin.12 It divides the lid into an anterior lamella (skin and muscle) and a posterior lamella (tarsus and conjunctiva).13 Anterior blepharitis may be accompanied by squamous debris or collarettes around the base of the lashes and vascular changes of the lid skin.

Posterior blepharitis is used to describe inflammatory conditions of the posterior lid margin,7 including MGD. Indeed, recent literature has used the terms posterior blepharitis and meibomian gland dysfunction or MGD as if they were synonymous,14-18 but these terms are not interchangeable.19 Distinct from the portion of lid margin anterior to the gray line, which includes the skin and eyelashes, the posterior lid margin contains the marginal mucosa, the mucocutaneous junction, the meibomian gland orifices and associated terminal ductules, and the neighboring keratinized skin. Posterior blepharitis is a term used to describe inflammatory conditions of the posterior lid margin,20 of which MGD7 is only one cause. Other causes include infectious20 or allergic20 conjunctivitis and systemic conditions such as acne rosacea.15,20

Clinically, a diagnosis of posterior blepharitis implies the presence of inflammation affecting the posterior lid margin. However, in its earliest stages, MGD may not be associated with the biomicroscopic lid margin signs characteristic of posterior blepharitis.21,22 At this stage, affected individuals may be asymptomatic, but alternatively they may be asymptomatic, and the condition may be regarded as subclinical.22,23 In either case, MGD may be diagnosed by meibomian gland expression alone, with demonstration of an altered quality of expressed secretions, and/or by a loss of gland functionality (decreased or absent expressibility). As it progresses, symptoms develop and lid margin signs may become visible with biomicroscopy. At this point, an MGD-related posterior blepharitis is said to be present.

The term meibomian gland dysfunction (MGD), first used in the early 1980s by Korb and Henriquez22 and then by others,14,25,24 is considered to be appropriate for describing the functional abnormalities of the meibomian glands. The general term meibomian gland disease is used to describe a broader range of meibomian gland disorders, including neoplasia and congenital disease.7,25 The more specific term MGD also emphasizes the important role that the meibomian glands play in the tear film and ocular surface.

Other terms such as meibomitis or meibomianitis describe a subset of disorders of MGD associated with inflammation of the meibomian glands. Although inflammation may be important in the classification and in the treatment of MGD, these terms are not sufficiently general, as inflammation is not always present in MGD.

When MGD occurs with increased secretion of meibomian lipids, the terms hypersecretory MGD and seborrheic MGD have been used. Confusion arises with the term seborrheic dermatitis, which is a chronic, relapsing inflammatory skin condition occurring in areas rich in sebaceous glands.26 This condition is not regularly associated with excessive secretion of sebum, nor are the sebaceous glands primarily involved. The actual underlying etiology may be related to fungal infection (genus Malassezia).26 Therefore the more appropriate and

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1. Health Partners Medical Group, Minneapolis, Minnesota; the 2Tokyo Dental College, Chiba, Japan; the 3Unidad Superficie Ocular, Hospital Clinico San Carlos, Madrid, Spain; the 4Department of Ophthalmology, University of Auckland, Auckland, New Zealand; the 5Department of Ophthalmology, The University of Texas Southwestern Medical Center, Dallas, Texas; and the 6Department of Ophthalmology and Visual Science, The Kentucky Lions Eye Center, University of Louisville, Louisville, Kentucky.

2. Supported by the Tear Film and Ocular Surface Society (TFOS; http://www.tearfilm.org); individual author support is listed in the Appendix of the Introduction.

3. Submitted for publication December 6, 2010; accepted March 23, 2011.

4. Disclosure: Each Workshop Participant’s disclosure data can be found in the Appendix of the Introduction.

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6. DOI:10.1167/iovs.10-6997b

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Table 1. Criteria of Meibomian Gland Dysfunction Used in Previous Works

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mathers et al.</td>
<td>Meibomian gland dropout and normal tear secretion. Thickened secretions following meibomian gland expression in most cases.</td>
</tr>
<tr>
<td>Shimazaki et al.</td>
<td>Obstructive MGD characterized by: The presence of gland dropout at the central two thirds of the lower tarsus. A lack of meibum secretion after application of moderate digital pressure.</td>
</tr>
<tr>
<td>Lee and Tseng</td>
<td>Abnormal meibomian gland function characterized by: Lack of active inflammation. Poor meibum expression. Orifice squamous metaplasia or acinar atrophy.</td>
</tr>
<tr>
<td>Yokoi et al.</td>
<td>Abnormal findings on the lid margin (three positive findings) Reduced oil expression (a negative score). An Oxford staining grade of ≥2 but no abnormalities in the Schirmer and cotton-thread test results.</td>
</tr>
<tr>
<td>Goto et al.</td>
<td>Noninflamed, obstructive MGD characterized by: Presence of meibomian gland dropout by meibography. No or poor meibum expression by digital compression. No or negligible inflammation in the lid margin.</td>
</tr>
<tr>
<td>Foulks and Bron</td>
<td>MGD defined as a symptomatic or asymptomatic condition with typical morphologic lid features and the following additional features: Tear flow: normal or increased. Ocular surface damage: present. Dropout: &gt;1 (central two thirds of the lid). Oil volume: &lt;0.5 mm. Oil viscosity: criterion not stated. Meibometry reading: normal (≤800 meibometer units). Evaporation rate: increased. Seborrheic MGD can be defined as a symptomatic or asymptomatic condition with typical morphological lid features and the following additional features: Tear flow: normal. Ocular surface damage: variable. Dropout: nil. Oil volume: &gt;0.8 mm. Oil viscosity: normal. Meibometry reading. Evaporation rate: normal.</td>
</tr>
<tr>
<td>Matsumoto et al.</td>
<td>Occluded MG orifices. Cloudy or inspissated glandular secretion with lack of clear meibum secretion after the application of moderate digital pressure on the tarsus of the upper and lower eye lid. Presence of keratinization or displacement of the mucocutaneous junction. Absence of inflammatory lid disease such as blepharitis as well as inflammatory skin disorders such as atopic dermatitis, seborrhea sicca, and acne rosacea. Absence of a history of cicatricial eyelid and conjunctival diseases, such as trachoma, erythema multiforme, ocular cicatricial pemphigoid, and chemical, thermal, or radiation injury. Absence of excessive meibomian lipid secretion (seborrheic MGD).</td>
</tr>
<tr>
<td>Arita et al.</td>
<td>Obstructive MGD should be suspected when any two of the three scores are abnormal. 1. Symptom score ≥5†. 2. Lid margin abnormality score ≥2‡. 3. Meibo-score ≥5§.</td>
</tr>
<tr>
<td>Amano et al.</td>
<td>Obstructive MGD is considered to be present when all of the following three signs/findings are present. 1. Chronic ocular discomfort. 2. Anatomical abnormalities around the meibomian gland orifices (present of one or more of the following is considered positive): a. Vascular engorgement. b. Anterior or posterior displacement of mucocutaneous junction. c. Irregularity of the lid margin. 3. Obstruction of the meibomian glands (presence of both is considered positive): a. Obstructive findings of the gland orifices by slit lamp biomicroscopy (pouting, plugging, and ridge). b. Decreased meibum expression by digital pressure with moderate pressure.</td>
</tr>
</tbody>
</table>

* A combination of the classifications published by Mathers and Lane and Yokoi et al.† Symptom score: based on the questionnaire about 14 ocular symptoms: ocular fatigue, discharge, foreign body sensation, dryness, uncomfortable sensation, sticky sensation, pain, epiphora, itching, redness, heavy sensation, glare, excessive blinking, and a history of chalazion or hordeolum. Symptoms were scored from 0 through 14, according to the number of symptoms present.‡ Lid margin abnormality score: irregular lid margin, vascular engorgement, plugged meibomian gland orifices, and anterior or posterior replacement of the mucocutaneous junction scored from 0 through 4 according to the number of these abnormalities present in each eye.§ Meibo-score is a semiquantitative score of the results of noncontact meibography: Grade 0, no loss of meibomian glands; Grade 1, area loss less than one third of the total meibomian gland area; Grade 2, area loss between one third and two thirds of the total meibomian gland area; Grade 3, area loss more than two thirds of the total meibomian gland area; Meibo-scores summed for the upper and lower eyelids to obtain a score of 0–6 for each eye.
clinically understandable term is hypersecretory MGD. Similarly, the term hyposecretory MGD is used instead of obstructive MGD. Obliteration of meibomian gland ducts and orifice obstruction due to hyperkeratinization is an important finding in hyposecretory MGD. However, decreased lipid secretion can occur due to abnormalities in meibomian glands without concurrent remarkable obstruction. Therefore, hyposecretory MGD is used, as it covers a wider range of manifestations of the disorder.

### MGD Definition Background

The term dysfunction is used because the function of meibomian glands is disturbed (Table 3). Alteration of these functions leads to decreased tear film stability (evidenced by increased evaporation, increased surface tension, contamination with sebum, unsealed lid during sleep) and/or symptoms. The dysfunction is a result of anatomic abnormalities or abnormalities in meibomian gland secretion.

The term diffuse is used in the definition of MGD, because the disorder involves most of the meibomian glands. Localized involvement of meibomian glands, such as in chalazia, does not tend to cause abnormalities in the tear film or ocular surface epithelia, and therefore is not considered to be within the context of MGD. Obstruction of the meibomian gland orifice and terminal duct is identified as the most prominent aspect of MGD (see the Report on Anatomy, Physiology, and Pathophysiology).

Subjective symptoms of eye irritation are included in the definition of MGD, as symptoms are of greatest concern to the patient, and often to the clinician. Improving patient symptoms is the major goal in the treatment of MGD. The enigma still exists, as in dry eye, that signs and symptoms frequently show disparity.

The role of inflammation in the etiology of MGD is controversial and uncertain. Although the cause-and-effect relationship is unclear, associations between meibomian gland dropout and ocular surface inflammatory diseases, such as chronic blepharitis, giant papillary conjunctivitis, and Sjögren syndrome, have been reported. Histopathologic observation in meibomian glands obtained at autopsy have revealed lipogranulomatous inflammation around the gland lobules in 18.6% of cases.

Clinically, increased vascularization of the posterior lid margin is one of the major signs of inflammation and of MGD, and its importance in diagnosis and treatment is widely accepted. It should be noted, however, that the finding has been shown to be related to aging.

McCulley and Sciallis described a condition called meibomian keratoconjunctivitis (MKC), often associated with anterior blepharitis, with the most prominent changes centered on the meibomian glands. It is usually associated with some form of skin disease and is characterized by tear film instability, ocular surface inflammation, and ocular surface damage. MKC is an important cause of symptoms in severe chronic blepharitis.

### Classification of MGD

#### Overall Consideration

MGD may be classified according to anatomic changes, pathophysiological changes, or the severity of disease. Any classification system must meet the needs of the clinician and researcher alike; therefore, a classification based on pathophysiology is deemed to best meet these needs and is presented here.

#### Previous Works on Classification of MGD

At least five different classifications have been published previously (Table 4). The first was proposed in 1921 by Gifford, who classified meibomian gland changes in chronic blepharoconjunctivitis. His classification emphasized the involvement of adjacent tissues (e.g., conjunctival and tarsal concretions).

Second, in the 1980s McCulley et al. classified chronic blepharitis into four primary categories, including both anterior and posterior blepharitis: (1) staphylococcal, (2) seborrheic, (3) primary meibomitis, and (4) other (including atopy, psoriatic, and fungal). The seborrheic category was further divided into four subcategories: (2a) seborrheic alone, (2b) mixed seborrheic/staphylococcal, (2c) seborrheic with meibomian gland seborrhrea, and (2d) seborrheic with secondary meibomitis. Categories 2c, 2d, and 3 were associated with the posterior lid margin and the meibomian glands. The classification was observational and based on appearance, including meibomian gland orifice obstruction and inflammation around the glands.

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**Table 3. The Functions of Healthy Meibomian Lipids**

<table>
<thead>
<tr>
<th>Function</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Provide a smooth optical surface for the cornea at the air-lipid interface</td>
</tr>
<tr>
<td>2.</td>
<td>Reduce evaporation of the tear film</td>
</tr>
<tr>
<td>3.</td>
<td>Enhance the stability of the tear film</td>
</tr>
<tr>
<td>4.</td>
<td>Enhance spreading of the tear film</td>
</tr>
<tr>
<td>5.</td>
<td>Prevent spillover of tears from the lid margin</td>
</tr>
<tr>
<td>6.</td>
<td>Prevent contamination of the tear film by sebum</td>
</tr>
<tr>
<td>7.</td>
<td>Seal the apposing lid margins during sleep</td>
</tr>
</tbody>
</table>

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**Table 2. Terminology of Blepharitis**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blepharitis</td>
<td><em>Blepharitis</em> is a general term describing inflammation of the lid as a whole; <em>marginal blepharitis</em> is inflammation of the lid margin and includes both anterior and posterior blepharitis.</td>
</tr>
<tr>
<td>Anterior blepharitis</td>
<td><em>Anterior blepharitis</em> describes an inflammation of the lid margin anterior to the gray line and concentrated around the lashes. It may be accompanied by squamous debris or collarettes around the lashes, and inflammation may spill onto the posterior lid margin.</td>
</tr>
<tr>
<td>Posterior blepharitis</td>
<td><em>Posterior blepharitis</em> describes an inflammation of the posterior lid margin, which may have different causes, including MGD, conjunctival inflammation (allergic or infective), and/or other conditions, such as acne rosacea.</td>
</tr>
<tr>
<td>MGD</td>
<td><em>MGD</em> describes 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.</td>
</tr>
</tbody>
</table>
**Table 4. Classification Systems for Meibomian Gland Dysfunction**

**Gifford**

Meibomian Gland Dysfunction
- Simple hypersecretion
- Simple chronic meibomitis (simple inflammation)
- Chronic meibomitis with hypertrophy
- Chronic meibomitis with chalazia
- Chronic meibomitis secondary to chronic conjunctivitis
- Chronic meibomitis associated with tarsal concretions

**McCulley et al.**

Chronic Blepharitis
1. Staphylococcal: anterior lid inflammation with collarettes and madarosis
2. Seborrheic
   a. Seborrheic alone: Less inflammation with greasy scales on the anterior lid margin
   b. Mixed seborrheic and staphylococcal: a combination of the seborrheic and staphylococcal features described above
   c. Seborrheic with meibomian seborrhea: patients with meibomian gland hypersecretion but without obstruction
   d. Seborrheic with secondary meibomitis: patients with occluded and inflamed meibomian glands in a spotty distribution
3. Primary meibomitis (also known as meibomian keratoconjunctivitis): patients with obstruction and inflammation of all the meibomian glands in association with seborrheic dermatitis or acne rosacea
4. Other, including atopy, psoriatic, and fungal

**Mathers et al.**

Chronic blepharitis
- Seborrheic MGD: patients with hypersecretion, normal gland morphology, and low or normal tear osmolarity
- Obstructive MGD: patients with low excretion or high gland dropout on meibography and increased tear osmolarity but normal Schirmer test results
- Obstructive with sicca: patients with the same findings as for obstructive but with a low Schirmer test result
- Sicca: normal gland morphology, high tear osmolarity, and low Schirmer test result

**Foulks and Bron**

Meibomian Gland Dysfunction
- Hypersecretory
  - Meibomian seborrhea
- Hypos secretory
- Obstructive
  - Simple (SMGD) or cicatricial (CMGD)
    - Focal or diffuse; obstructive/cicatrical
    - Primary
    - Secondary to:
      - Local disease: Anterior blepharitis,
        Conjunctionitis (e.g., trachoma; pemphigoid; atopy)
        Chemical burns
      - Systemic disease: Seborrheic dermatitis
        Acne rosacea
        Atopy
        Ichthyosis
        Psoriasis
        Anhydrotic ectodermal dysplasia
        Ectodactyly syndrome
        Turner syndrome
        Fungal infection,
        Toxic (e.g., 13-cis retinoic acid,
        polychlorinated biphenols
        epinephrine (in the rabbit))
      - Other disease: Internal hordeolum, chalazion, concretions

**Amano et al.**

Meibomian Gland Dysfunction
- Obstructive
  - Primary
  - Congenital
  - Atrophic
  - Secondary
  - Atopy
  - Stevens Johnson Syndrome
  - Infection
  - Graft vs. host disease, etc.
- Seborrhoeic
  - Primary
  - Secondary
  - Allergy
  - Infection, etc.
Mathers et al.\textsuperscript{29} classified chronic blepharitis into four groups in 1991: (1) seborrheic MGD, (2) obstructive MGD, (3) obstructive MGD with sicca, and (4) sicca. Three parameters were used to classify MGD: (1) meibomian gland morphology using meibography, (2) tear osmolarity, and (3) Schirmer’s test. This classification system was oriented more toward tear film changes rather than the changes in function or anatomy of the meibomian glands.

The 1991 classification by Bron et al.\textsuperscript{25} was based on detailed observation of the lid margins. This observational classification described the lid changes observed on slit lamp biomicroscopy and classified meibomian gland diseases into five main subcategories: (1) absence/deficiency, (2) replacement, (3) meibomian seborrhea, (4) meibomitis, and (5) meibomian neoplasia. Changes in the meibomian glands were described in terms of mucocutaneous changes, ducts, acini, and secretory performance of the gland. Each factor was graded in a semi-quantitative fashion.

A more recent classification of MGD was published by Foulks and Bron\textsuperscript{7} in 2003. This system integrated the observation of anatomic changes and gland expressibility with biochemical alteration of meibomian gland lipids and the underlying etiology (Fig. 1). Bron and Tiffany\textsuperscript{27} presented a unique circular diagram breaking down the etiologies of meibomian gland disease into primary cicatricial and non-cicatricial, secondary, and hypersecretory causes (Fig. 2).

**Recommended Classification of MGD by the Subcommittee**

Under the broad category term, meibomian gland dysfunction, MGD is further classified into two major categories based on meibomian gland secretion (Fig. 3): low-delivery and high-delivery states. Low-delivery states are further classified as hyposecretory (meibomian sicca) and obstructive, with cicatricial and noncicatricial subcategories. Primary causes are listed under each category and refer to conditions for which there is no discernible underlying cause or etiology.

**Low-Delivery States.** Low delivery of meibomian gland secretions is further classified into two major categories: hyposecretion and obstructive conditions. *Meibomian gland hyposecretion* is characterized by decreased meibomian lipid secretion without gland obstruction. Although there is no published and verified evidence of primary hyposecretion, this disorder is associated clinically with gland atrophy. A decrease in the number of functional meibomian glands is associated with contact lens wear, and this decrease appears to be proportional to the duration of contact lens wear.\textsuperscript{35}

The other category under low-delivery states is *meibomian gland obstruction*. This is probably the most common form of MGD.\textsuperscript{3,7,32} Histopathologic changes include hypertrophy of the duct epithelium and keratinization of the orifice epithelium. Low delivery is caused by glandular obstruction due to either terminal duct obstruction or altered secretion. The disorder is seen in older subjects or after the use of retinoids for acne treatment.\textsuperscript{36} Androgen insufficiency or lack of androgen receptors is also associated with keratinization, obstruction, and alteration of meibomian gland secretions (see the Anatomy, Physiology, and Pathophysiology report).\textsuperscript{37} Obstructive causes may be further classified as cicatricial and noncicatricial. In *noncicatricial*
obstructive MGD the ducts and orifices remain in their normal anatomic position; in cicatricial obstructive MGD the ducts and orifices are dragged posteriorly into the mucosa. Causes of cicatricial obstructive MGD include trachoma, ocular cicatricial pemphigoid, erythema multiforme, and atopic eye disease. Noncicatricial obstructive MGD may be seen in Sjögren’s syndrome, seborrheic dermatitis, acne rosacea, atopy, and psoriasis. Inflammation in adjacent tissues is commonly seen in conjunctivitis and anterior blepharitis, for example. Although inflammation is frequently associated with meibomian gland obstruction (the term meibomitis has been used as a synonym), whether the inflammation is a cause or a result of meibomian gland obstruction remains unclear.

High-Delivery States. Hypersecretory MGD is characterized by the release of a large volume of meibomian lipid at the lid margin in response to pressure on the tarsus. Although it has been reported that hypersecretory MGD is associated with seborrheic dermatitis in 100% of cases,16 this eyelid disorder is believed to occur in other diseases as well, including atopic disease and acne rosacea (secondary hypersecretory MGD). There also have been cases without the association of other diseases (primary/idiopathic hypersecretory MGD). It is not certain whether increased lipid is a result of true hypersecretion of the meibomian glands, or a result of damming back of secretions in the presence of mild obstruction. The disorder is not associated with active inflammation, and no remarkable changes in gland structure are noted by meibography. There is a recognized association between hypersecretory MGD and acne, and the evidence of the potential for increased lipid secretion by meibomian glands comes from the finding of increased sebum excretion as a major factor in the pathophysiology of acne.38 An end-organ hyperresponse of the glands to androgens is the most likely explanation for the seborrhea.39 In women with acne, the total sebum excretion rate is higher than normal. Although sebum production is influenced both by the number of active follicles and their individual capacity to excrete sebum, the severity of seborrhea most probably depends on an increased excretion of sebum by a few glands rather than on an increased number of active sebaceous follicles.40

Relationship to Ocular Surface Disease and the Tear Film

MGD can lead to alterations in the normal lipid composition in meibomian gland secretions.41–44 Lipid abnormalities can lead to abnormalities of tear film composition and function resulting in evaporative dry eye.45
References


The tarsal glands of Meibom (glandulae tarsales) are large sebaceous glands located in the eyelids and, unlike those of the skin, are unassociated with hairs. According to Duke-Elder and Wyller,1 they were first mentioned by Galenus in 200 AD and later, in 1666, they were described in more detail by the German physician and anatomist Heinrich Meibom, after whom they are named.

Lipids produced by the meibomian glands are the main component of the superficial lipid layer of the tear film that protects it against evaporation of the aqueous phase and is believed also to stabilize the tear film by lowering surface tension.2 Hence, meibomian lipids are essential for the maintenance of ocular surface health and integrity. Although they share certain principal characteristics with ordinary sebaceous glands, they have several distinct differences in anatomy, location, secretory regulation, composition of their secretory product, and function.

Functional disorders of the meibomian glands, referred to today as meibomian gland dysfunction (MGD),3 are increasingly recognized as a discrete disease entity.4–8 In patients with dry eye disease, alterations in the lipid phase that point to MGD are reportedly more frequent than isolated alterations in the aqueous phase. In a study by Heiligenhaus et al.,9 a lipid deficiency occurred in 76.6% of dry eye patients compared with only 11.1% of those with isolated alterations of the aqueous phase. This result is in line with the observations by Shimazaki et al.10 of a prevalence of MGD in the absolute majority of eyes with ocular discomfort defined as dry eye symptoms. These observations noted that 64.6% of all such eyes and 74.5% of those excluding a deficiency of aqueous tear secretion were found to have obstructive MGD, or a loss of glandular tissue, or both.10 Horwath-Winter et al.11 reported MGD in 78% of dry eye patients or, if only non-Sjögren patients are considered, in 87% compared with 13% with isolated aqueous tear deficiency. It may thus be accepted that MGD is important, conceivably underestimated, and possibly the most frequent cause of dry eye disease due to increased evaporation of the aqueous tears.5,9–12

After some excellent reviews of MGD4,7,8,13,14 in the past, many new findings have been reported in recent years, and other questions remain to be identified and resolved. A sound understanding of meibomian gland structure and function and its role in the functional anatomy of the ocular surface15 is needed, to understand the contribution of the meibomian glands to dysfunction and disease. Herein, we seek to provide a comprehensive review of physiological and pathophysiological aspects of the meibomian glands.

HEINRICH MEIBOM

Heinrich Meibom the younger (1638–1700; Fig. 1)16 came from a scholarly family. He was the son of the physician Johann Heinrich Meibom and the grandson of the German historian and poet Heinrich Meibom the elder (1555–1625),17 who was professor of history and poetry at the University of Helmstedt in Germany. Heinrich Meibom the younger was born on June 29, 1638, in Lübeck, Germany, and later traveled around in Europe and received a cosmopolitan education. In a short article18 that commemorated the 300th anniversary of his birth in 1938, the British Medical Journal characterized him as follows: “Like so many of his contemporaries, he was indeed a child of Apollo, god of culture, poetry, rhetoric, and healing. While still a medical student (he became MD at Angers in 1663) he was appointed to, and in 1664 took up, the professorship of medicine in the University of Helmstadt. Fourteen years later he accepted the additional chairs of history and of poetry. He further showed his versatility by straying into the pleasant fields of archaeology, philology, and philosophy, and all his life he was an insatiate traveller.” Apparently a man of many talents, in 1666, shortly after receiving the chair of medicine, Heinrich Meibom published the first detailed description, including a drawing, of the oil glands inside the tarsus of the eyelid, that later were named the meibomian glands. His description appeared as a book with the title De Vasis Palpebrarum Noris Episeta.19 (This early drawing showed basic characteristics of the glands, such as multiple single gland streaks along the extension of the tarsus with openings onto the lid margin, similar to but not as detailed as another much later and more well-known drawing (Fig. 2).)

ANATOMY, EMBRYOLOGY, AND PHYSIOLOGY

Anatomy of the Meibomian Glands

Arrangement. A single meibomian gland is composed of clusters of secretory acini that are arranged circularly around a
long central duct and connected to it by short ductules. This arrangement has been compared with a chain of onions.\textsuperscript{20} One end of the central duct is blind, and the other end opens close to the posterior lid border, just anterior to the mucocutaneous junction, at the lid margin, where the oily secretion is delivered onto the tear meniscus.\textsuperscript{21}

These separate glands are arranged in parallel in a single row throughout the length of the tarsal plates in the upper and lower lids,\textsuperscript{1,2,22,23} and they presumably act in a coordinated fashion that is influenced by hormonal and neural regulation and by the mechanical forces of muscle contraction during the eye blink.\textsuperscript{24}

The extent of the meibomian glands roughly corresponds to the dimensions of the tarsal plates in the upper and lower eyelids and hence differs between them (Fig. 2). In the upper lid the tarsal plate has the shape of a half circle that extends upward centrally for approximately 1 cm and narrows on the temporal and nasal sides, whereas the tarsal plate in the lower lids is smaller and forms a strip of rather equal length (~0.5 cm) from the nasal to the temporal side.\textsuperscript{24}

**Dimensions and Number of Glands.** The reported dimensions of the meibomian glands differ to a certain extent in different studies. The number of separate glands in the upper lid is given in one study\textsuperscript{20} as 25 and in another\textsuperscript{1} as 40, with a median number of approximately 31.\textsuperscript{25} The number of glands in the lower lid is given in the former study\textsuperscript{20} as 20 and in the latter\textsuperscript{1} as 30, with a median of approximately 26 glands.\textsuperscript{25} The length of the individual glands is reported as approximately 5.5 mm in the middle of the upper lid and approximately 2 mm in the lower lid, and hence their calculated total volume is also higher: approximately double in the upper lid (26 μL) versus the lower lid (13 μL).\textsuperscript{25} The meibomian glands in the lower lids tend to be wider than those in the upper lids. The number of secretory acini along a single meibomian gland is reported\textsuperscript{20} to be approximately 10 to 15 and is also higher in the upper than in the lower lid.

The secretory capacity of the meibomian glands in the upper lids should therefore be roughly double of that in the lower lids, but most investigations focus on the lower lid because of its greater accessibility. The differential secretory capacity in the upper versus the lower lid has not been investigated.

**Embryologic Development of the Meibomian Gland**

The embryologic growth of the meibomian glands occurs from the third to the seventh month of gestation, during the sealed-lid phase of eyelid development.\textsuperscript{20–28} During this time, the loose connective tissue of the mesoderm in the lid folds differentiates into the tarsal plate and muscles (orbicularis and Riolan's muscle), the blood vessels, and the loose connective tissue underlying the outer lid skin and the conjunctiva. The development of the meibomian glands from the anlage (the initial clustering of embryonic cells that serves as a foundation from which the organ develops) of the meibomian glands shows considerable similarities to that of the hair follicles, the hair anlage. Both of them grow from the ectodermal sheet, which seals the fused lid folds down into the mesoderm, although the meibomian anlage is larger, grows deeper, and takes longer for complete development as investigated in detail by Ehler's group.\textsuperscript{28}

Similar to the hair anlage of the eyelashes, which develops associated glands (holocrine sebaceous glands of Zeis and modified sweat glands of Moll), the epithelial cord of the meibomian anlage develops lateral outgrowths that later differentiate into the connecting ductules and secretory holocrine sebaceous acini. Inside the epithelial cylinder of the meibomian anlage, similar to the hair anlage, the production of lipids leads to the formation of a central canal that later develops into the central duct. The production of lipids is followed by the occurrence of keratohyalin granules in the luminal epithelial
cells, and therefore the lipid synthesis and keratinization events were once assumed to be somehow related.\textsuperscript{28} Lipid production by the more mature meibomian anlage and by the ciliary glands of Zeis has also been found to be related to the formation of a canal between the two sealed lid folds, which leads to the separation of the then fully differentiated upper and lower lids in the seventh month of gestation.\textsuperscript{28} In the mouse, it is thought that an increasing amount of keratinization, rather than lipid secretion, contributes to the separation of the upper and lower eyelids.\textsuperscript{29}

Hence, the central meibomian duct can be compared to the hair follicles of the eyelashes in embryology and also shows distinct similarities in structure and epithelial differentiation, including the keratinization status, in the adult. The meibomian gland can hence be regarded as a ‘hair follicle without a hair shaft.’\textsuperscript{30} This observation may offer the conclusion that hyperkeratinization is a typical disease of the meibomian gland.

**Histologic Appearance of the Meibomian Gland**

The meibomian glands are composed of secretory acini that are connected via smaller ductules to the larger, long, straight central duct (Fig. 3) that extends throughout the length of the tarsal plate and opens onto the free lid margin close to the posterior lid border. The whole internal ductal system is lined by a stratified squamous epithelium with signs of incipient keratinization. Full keratinization (cornification), as indicated by the presence of luminal keratin lamellae, is physiologically only present in the terminal part of the central duct that is lined by an ingrowth of the cornified epidermis from the surface of the free lid margin.\textsuperscript{1,23,24,31,32}

**Acinus.** As a special type of sebaceous gland, the secretory acini of the meibomian glands follow a holocrine secretion mode that is reflected by their structure (Fig. 4A). The numerous secretory acini have an elongated or spherical shape of approximately 150 to 200 \(\mu\)m diameter. They are completely filled with secretory cells, termed meibocytes.\textsuperscript{33} The basal cells are smaller and darker. The meibocytes, located more toward the center of the acinus, show a progressive accumulation of lipids in the cytoplasm and hence appear increasingly foamy and pale in conventional histology of paraffin-embedded sections because of the extraction of the lipids during processing. During their maturation, the most central cells undergo shrinkage, compaction, and disintegration of the nucleus (pyknosis).

Eventually, disintegration of the cell membrane occurs at the transition from the acinus to the ductule. Hence, the whole cell contents form the oily secretory product termed meibum.\textsuperscript{33} A gradient in maturation with more undifferentiated, immature cells in the basal layer is also supported by transmission electron microscopy in mouse\textsuperscript{34} and human\textsuperscript{35} meibomian glands. The basal acinar cells contain a medium dense nucleus that is rich in heterochromatin and has a prominent nucleolus. The basal acinar cells have sparse cytoplasm that contains a large number of keratin filament bundles together with numerous mitochondria and many free ribosomes, as is characteristic for synthesis of internal cell proteins, whereas the rough endoplasmic reticulum and Golgi apparatus, for export of secretory products, are scarce. The basal layer of meibocytes in the periphery of the acinus serves as a proliferating progenitor cell population that constantly gives rise to new meibocytes.\textsuperscript{36}

**Connecting Ductule.** Typically one, or sometimes more, acini are connected to a ductule that is approximately 150 \(\mu\)m long and has a luminal diameter of approximately 30 to 50 \(\mu\)m. The ductules are lined by a stratified (four layers) squamous epithelium. At the junction from the acinus to the ductule, a sharp transition from the peripheral layer of basal meibocytes to the ductal epithelium has been reported in electron microscopy of the monkey and rabbit.\textsuperscript{32} This conclusion was reached from the observation that the epithelial cells of the ductule did not contain lipid droplets, as found in neighboring meibocytes, but instead contained keratohyalin granules. Histology in the human meibomian gland does not necessarily confirm a sharp transition based on the shape and arrangement of the epithelial cells in this region, because the spherical brighter epithelial cells of the basal meibocytes are seen to transform gradually into the cells of the multilayered ductal epithelium that have a slightly denser cytoplasm and a more elongated shape (Fig. 4B). Keratohyalin granules are also observed in ductal epithelial cells of the normal human meibomian gland (Knop E, et al. IOVS 2009;50:ARVO E-Abstract 4833).

**Central Duct.** The connecting ductules enter the long central duct, typically in an oblique direction that leads to the formation of a sharp tissue spur that is mainly composed of epithelial cells and contains a narrow internal core of connective tissue at the entrance into the central duct (Fig. 4C).

The central duct is also lined by a four-layered, stratified squamous epithelium but has a wider lumen approximately 100 to 150 \(\mu\)m in diameter. The central duct extends through-
out the total length of the gland, which corresponds roughly to the extension of the tarsus.\textsuperscript{1,20,25,37}

Around the terminal part of the central duct and among the terminal acini close to the free lid margin, there are various amounts of striated fibers of Riolan’s muscle (Fig. 5, also noted in Fig. 18A) that are split from the orbicularis muscle by the down-growth of the hair anlage of the cilia, deep into the tarsal fold during embryologic development. These muscle fibers appear to encircle the terminal part of the meibomian gland.\textsuperscript{20,38} The terminal part of the central duct preceding the excretory duct is often slightly dilated, thus forming a kind of ampulla, conceivably due to its physiological content of secreted meibum.

**Excretory Duct.** The cornified epithelium of the free lid margin (epidermis) extends into the terminal part of the mei-
borian gland for about 0.5 mm. It has a keratinizing layer that contains numerous dense keratinyl granules (granular layer) and a superficial layer of fully keratinized (cornified) keratin lamellae. At about 0.5 mm internal to the orifice, the epithelium gradually transforms into the ordinary ductal epithelium of the meibomian gland by losing the keratin lamellae and the granular layer and by reducing the stratification from about six to eight layers to four layers. Since the epithelium in this terminal part has a different structure compared with the rest of the central duct, it appears justified to term it as an excretory duct (Knop E, et al. IOVS 2009;50:ARVO E-Abstract 4833).

**Physiology of the Meibomian Glands**

**Secretion Mode.** Basal meibocytes move during their maturation, which includes the production and accumulation of lipids, from the basal compartment of the acinus toward its orifice, which includes the production and accumulation of lipids, proteins, and nucleic acids contribute to the oily secretion, which is also integrated into the lipid droplets and hence contribute to the oily secretion (Fig. 4, also indicated in Fig. 3). The biochemical characteristics of the secretion process and its products are considered in the section on lipid synthesis.

During this process, the meibocytes go through several stages that can be differentiated morphologically (basal, differentiating, mature, and hypermature), depending on several structural characteristics as described by Gorgas. Cell organelles that are necessary for lipid production inside the cells increase in number and size during this process—in particular, the smooth-surfaced endoplasmic reticulum (sER) and peroxisomes. The lipid droplets are encircled by multilamellar membrane structures that are assumed to originate from the membrane of the sER. Similar multilamellar structures are also integrated into the lipid droplets and hence contribute to the meibum. All the components of the whole cell including lipids, proteins, and nucleic acids contribute to the oily secretary product, which is also called meibum.

The ductal system may contribute to the final secretory product that is released onto the posterior lid margin in an active or passive way, since (1) nerve fibers are observed not only around the acini but also around the ductal system, and (2) the originally secreted meibomian lipids are conceivably, at least in part, modified by hydrolyzing enzymes from commensal bacteria inside the ductal system that lead to a breakdown of triglycerides into free fatty acids and small portions of monoo- and diglycerides and other modifications in patients with blepharitis. and even in the normal condition. Commensal bacterial species have been cultured in most expressed meibum samples from blepharitis patients, but similarly also from normal subjects.

**Mechanisms of Secretion and Delivery.** Because of the length of the meibomian glands, there is frequently a long distance between the cell biological process of secretion of the meibum in the secretory acini and its actual delivery onto the lid margin where it exerts its functions. Therefore, it appears advisable to follow the nomenclature suggested by Bron and Tiffany and to separate secretion of the meibum from its delivery. The constant production of new meibocytes in the secretory acini and their disintegration into the final secretory product generates a continuous secretory force that drives the meibomian oils within the ductal system of the gland toward the orifice at the free lid margin (Fig. 5). The hypothesis of a continuous production is not only supported by the observed generation time of new meibocytes but also by the finding that in the morning after sleep, during which the lids are closed, an increased amount of lipid that has apparently accumulated within the ductal system is then delivered in increased amounts onto the lid margin. The constant secretion of meibum further represents a basis for the generation of increased pressure within an obstructed gland.

The same observation further supports the conclusion that the mechanical action of the lid muscles contributes to delivery (Fig. 5), as suggested by Linton et al. During the movement of the eyelids during a blink, the orbicularis muscle, located on the external side of the tarsal plate, generates a compression of the tarsal plate and the enclosed tarsal glands of Meibom. It was concluded that this “would promote the flow of secretion by a milking action.” It has been suggested that the embedding of the glands inside the tarsal tissue provides for a homogeneous effect on compression of the individual glands along the lid margin. The contraction of Riolan’s muscle conceivably exerts compression of the terminal part of the ductal system and acini and contributes to the delivery of the oily meibum onto the surface of the lid margin. This notion may be supported by the incidental observation that meibum is released onto the lid margin in the form of jets of liquid. It has been assumed that the constrictive forces of Riolan’s muscle may also close the terminal part of the meibomian gland and hence prevent the outflow (i.e., act in a somewhat antagonistic way on the flow of meibum compared to the pretarsal orbicularis muscle). It has been further speculated that, in the act of a blink, the pretarsal orbicularis performs a “milking action” while Riolan’s muscle is relaxed and, conversely, between blinks, while the orbicularis is relaxed, Riolan’s muscle contracts to prevent the outflow of meibum. Contraction of Riolan’s muscle may also aid in limiting unwanted outflow of meibum (e.g., at night), although then the orbicularis muscle is relaxed, according to Linton et al., and does not perform a propulsive action on the meibum. Although these speculations may offer attractive explanations for the delivery of meibum, there is no evidence of such an antagonistic action of the marginal muscle of Riolan compared with the pretarsal orbicularis muscle. There is also no evidence of a potential influence of the smooth fibers of the superior and inferior tarsal muscles or of an influence of age-related or pathologic changes in the composition and shape of the tarsal plate and lids that could influence meibomian gland function and meibum delivery. These questions may need further investigation.

After an absence of blinking and muscular action overnight, the accumulated meibum from within the ductal system is delivered in increased amounts. This action has been observed by meibometry in the morning during the first hour or so after awaking and has been clinically observed after a prolonged time of concentrated work associated with reduced blinking frequency. Consequently, muscular action during repeated enforced blinks significantly increases the lipid layer thickness and is also an appropriate therapeutic approach to overcoming a certain minor degree of obstruction in patients with incipient obstructive meibomian gland disease. The differential contribution of the meibomian glands in the upper versus lower eyelids has been insufficiently investigated to date. Because of the calculated higher volume of the meibomian glands in the upper lids, it can be assumed that they also have a higher secretary capacity and contribute more to the lipid pool at the lid margin and subsequently to the tear film lipid layer. However, because of the better accessibility of the margin of the lower lids, most investigations of the morphology and secretory capacity of the meibomian glands have focused on the lower lids.

This focus applies in particular to meibometry, introduced by Bron and Tiffany and Chew and colleagues, which is able to measure effective amounts of lipid on the lid margin. Research has shown the amount of meibomian lipids on the lid margin, and the rate of delivery is dependent on age, sex, and diurnal conditions. In these investigations it has been as-
sumed that mixing of the meibum of the upper and lower lids during blinking means that the lower reservoir is likely to be similar to the value obtained from the upper lid. The amount of lipid in the marginal reservoir is lower in children younger than 14 years compared with adults, higher in adult males than in adult females, equal in both sexes after the age of 50 years, and stable or even slightly increased up to the eighth decade of age. The raising amount of lipids in the marginal reservoir with age is in some contrast to the described decrease of active meibomian glands with age and can probably be explained by a decreased removal of lipids from the lipid layer and reservoir.

The secretory activity of glands, as analyzed by Norn by the staining of delivered lipid at the meibomian orifice, found active delivery in only 45% of gland openings at one time point and a decrease of active glands by 50% from the age of 20 years to the age of 80 years. The morphologic equivalent of such a decrease in function may be represented by an age-dependent disappearance of gland tissue (gland dropout) as observed more recently by meibography. The secretory activity of glands was analyzed in more detail by Korb and Blackie by their ability to deliver a liquid secretory product on diagnostic expression involving application of mild external pressure in the physiological range of 1.25 g/mm², performed to reveal delivery without overcoming a potential obstruction of the orifice. These studies supported that not all glands deliver oil at the same time. In addition, it was observed for the first time, that the number of active glands in lower lids depends on their location along the lid margin and is highest in the nasal third, lower in the middle of the lid, and lower still in the temporal third meibomian glands. It was also observed that there is a correlation between the number of actively delivering meibomian glands in the lower eyelid and dry eye symptoms. The time necessary for full expression of a gland, at the specified mild pressure until delivery stopped was approximately 12 seconds, on average, and the recovery time until new oil could again be expressed from the same gland was approximately 2 hours. When individual glands were repeatedly expressed, with intervals of 3 hours between expressions over a daytime period of 9 hours (i.e., four times), it was observed that a single gland is capable of secreting oil on demand over the course of a working day. This continuous activity of the meibomian glands also showed a similar dependence on the position along the lid margin, as observed before.

Investigations on the secretory activity and capacity of the meibomian glands in the upper lid are desirable, to better analyze the physiological functions of the glands and their alterations in different types of disease. In contrast to the sebaceous glands of the skin elsewhere in the body that are mainly regulated via hormones and other factors, the meibomian glands also have a distinct neural innervation.

The meibomian glands of the human have a dense meshwork of unmyelinated nerve fibers (nerve plexus) around the acini that are described by electron microscopy and also by histochemistry. The network consists of nerve fibers that have terminal buttons containing small vesicles filled by granules that contain neurotransmitters. The nerve endings are located closely around the acini but remain outside the basement membrane and constitute so-called synapses en passant that lack a direct postsynaptic structure in the target cell, as is characteristic of the autonomic nervous system. Such nerve fibers are also observed around the ductal system, which may indicate that the ductal epithelium contributes to the composition of the finally delivered meibum. Many nerve fibers occur around and within the wall of the small vessels that build a dense meshwork around the acini.

The nerve fibers of the human meibomian gland are mainly positive for acetylcholinesterase and are hence supposed to represent a part of the cholinergic parasympathetic nervous system. In addition they contain the neuropeptides calcitonin gene-related peptide (CGRP) and substance P, which are markers for the sensory nervous system but also occur in the parasympathetic system, and, in addition, the parasympathetic vasoactive intestinal polypeptide. These results substantiate a prevailing parasympathetic innervation of the meibomian gland.

In summary, the innervation of the meibomian glands is maintained via a dense meshwork of nerve fibers that contain different neurotransmitters and originate from different sources. They include, besides the mainly parasympathetic nerves from the pterygopalatine ganglion, sympathetic nerves from the superior cervical ganglion and sensory fibers from the trigeminal ganglion. In the rat it has been shown, that the parasympathetic fibers via the pterygopalatine ganglion originate from the superior salivatory nucleus that is also responsible for the innervation of the lacrimal gland. This innervation pattern offers the possibility of a common regulation of the ocular surface glands that contribute the different components of the tear film (meibomian glands for the lipids and lacrimal gland and accessory glands for the aqueous phase) to achieve an optimal composition of the tear film. The goblet cells that produce the secreted mucins which represent the main component of the mucous phase of the precorneal tear film appear to be regulated in the same way. Whether and how the meibomian glands are actually integrated into the neural feedback loop, similar to the lacrimal gland, is yet to be learned.

Less information is available at present on the release of the transmitters observed in the nerve fibers, on respective receptors on the target tissue, and on the mode of action that is transmitted by their interaction.

Keratinization

Process of Epithelial Keratinization. Meibomian glands share principal features of the embryologic developmental course and of the structural organization with the hair follicles of the eyelashes. These features include a general commitment of the epithelium to keratinization. Keratohyalin granules represent incipient stages of keratinization and contain proteins such as filaggrin that are later released into the cytoplasm and serve the function of interconnecting the intermediate keratin filaments leading to the formation of a densely packed meshwork. The keratin meshwork increasingly occupies the cytoplasm of the keratinizing epidermal cells, termed keratinocytes. The cross-linking of keratin bundles goes along with an enforcement of the cell membrane that is transformed into the cornified envelope. After degeneration and loss of their nuclei, these cells form the superficial keratin lamellae that indicate full keratinization (cornification) and serve the purpose of protecting against physical and chemical stress factors.

Incipient Keratinization

Electron microscopy has shown that the ductal epithelium of normal meibomian glands contains keratohyalin granules in the apical cell layer of the rabbit and monkey. Although no obvious keratinization occurs in the normal human meibomian gland, recent histologic investigations (Knop E, et al. IOVS 2009;50:ARVO E-Abstract 4833) could verify that the whole epithelium of the central duct and ductules of the human meibomian gland also contains keratohyalin granules and hence preserves a commitment to keratinization. Thus, the meibomian gland can in principle be regarded as a “hair follicle without a hair shaft.”
MEIBOMIAN GLAND STEM CELLS AND CELL DYNAMICS

As a special type of large sebaceous gland, the meibomian glands, unassociated with hairs, share certain principle rules of biology and cell dynamics with the more conventional hair-associated sebaceous glands of the skin. However, compared with the latter, the knowledge of basic information on stem cells and cell dynamics is very limited for the meibomian gland in general and in particular for the human. The same applies to another sebaceous gland without association to hairs, the preputial gland. Therefore, it is at present frequently necessary to consider more general phenomena of sebaceous gland biology in the skin, the validity of which is awaiting experimental proof for the meibomian gland.

Meibocyte Generation and Migration

As a sebaceous gland, the meibomian gland produces its secretum (meibum) by the holocrine secretion mechanism. This means that the contents of the whole glandular cells form the meibum, as shown in Figure 4A. After a process of maturation including lipid synthesis and accumulation, centripetal cell movement, and eventual cell degeneration and membrane disintegration, the lipids and other cell components are shed into the lumen of the ductal system. This holocrine secretion process hence results in the structural consequence that the whole secretory acinus is filled by secretory cells and in the dynamic consequence that secretory cells are continuously lost and replaced. This process is in distinct contrast to the merocrine secretion mode of the aqueous lacrimal gland, where only the secretory products are released from intact secretory cells.\(^{65}\) The continuous loss of acinar cells requires a consequent continuous production of new cells and therefore a continuous cell turnover and differentiation within the acinus. Even though it has long been assumed that the regeneration of the meibocytes arises from the peripheral layer of basal cells located on the basement membrane,\(^{66}\) in a way that is equivalent to the regeneration of other epithelial tissues from their basal layer,\(^{67}\) this was only proven in 2001 by Olami et al.\(^{68}\) A gradient in maturation, with more undifferentiated immature cells in the basal layer, has been observed by transmission electron microscopy in the mouse\(^{34}\) and the human\(^{75}\) meibomian gland and has also been indicated by histology.

Olami et al.\(^{69}\) labeled dividing cells in the mouse meibomian gland with the radioactive nucleotide [\(^{3}\)H]-thymidine, which is integrated as a marker into the nuclei during mitosis. They were able to show, after observation at different time points, that the labeled dividing cells were initially only found in the basal cell layer. At later time points, the number of labeled cells gradually increased, indicating basal cell mitosis and multiplication. Later, labeled cells were observed in locations closer to the center of the acinus, thus verifying the assumed centripetal movement of meibocytes. It has been calculated that in the mouse meibomian gland, the meibocytes have a generation time of 4.1 days between each division. Newly formed cells move from the basal layer at a velocity of 0.62 µm per day toward the center of the acinus and need approximately 9 days from their formation in the basal layer for their movement and eventual shedding in the center. These results\(^{66}\) showed for the first time the location of meibocyte progenitor cells in the basal cell layer, together with the constant and synchronous centripetal movement of their progeny. This movement explains the constant secretion of meibum, as observed by meibometry,\(^{44}\) and provides the basis for a previously assumed constant secretory force\(^{45}\) that, together with the muscular action\(^{46}\) of the orbicularis muscle on the outside of the tarsal plate and of Riolan’s muscle around the terminal parts of the meibomian glands, leads to the delivery of the oil onto the posterior lid margin. It can be assumed that basic similar characteristics also apply to the human meibomian gland, but, since the human acinus is larger in diameter and is filled by more cells, the exact numerical values may be slightly different.

Meibomian Gland Stem Cells

Olami et al. concluded from their observations that the stem cells of the meibomian glands lie at the circumference of each acinus. There have been almost no investigations of stem cells in the meibomian gland to date, with the exception of Olami’s work and one abstract (Lavker RM, et al. IOVS 2003;44:ARVO E-Abstract 3781). The latter group was concerned with meibomian gland stem cells in general and also reported that, after labeling with bromodeoxyuridine (BrDU) or [\(^{3}\)H]-thymidine, most of the rapidly cycling cells were seen in the “basal sebocytes,” which refers to the basal acinar meibocytes. However, these cells are not regarded as real stem cells which are defined as a slow-cycling cell population,\(^{66}\) similar to those in the corneal limbus\(^{66–71}\) and skin,\(^{72}\) analogous to those defined in the hematopoietic system.\(^{73}\) Rather they are regarded as progenitor cells (transient amplifying [TA] cells). TA cells are daughters of the stem cells, with more rapid division but a limited further number of divisions and a restricted differentiation program,\(^{68}\) that eventually give rise to terminally differentiated cells. There were only a few if any slow-cycling stem cells observed in the acini (Lavker RM, et al. IOVS 2003;44: ARVO E-Abstract 3781), but most of the fast-cycling cells of the meibomian glands were located there.

The presence of fast-cycling TA cells in the basal acinar epithelium appears sufficient to explain the continuous generation of meibocytes, if it is additionally assumed that these basal cells are continuously replenished by the migration of young TA cells from a stem cell source outside the acinus. Similarly, the corneal epithelium is apparently maintained by the migration and division of TA cells that originate from the stem cell source at the limbus and can also remain intact after a prolonged time of limbal stem cell insufficiency.\(^{69–71}\) The same applies to the skin epithelium, which is also regenerated by several generations of basal TA cells that originate from the stem cells located in the hair follicles\(^{68}\) or in interfollicular epidermal rete pegs.\(^{74}\)

Slow-cycling, and hence label retaining, putative stem cells have been found concentrated in the ductal epithelium of the meibomian gland (Lavker RM, et al. IOVS 2003;44:ARVO E-Abstract 3781). In addition, many of the cells “in the uppermost portion of the meibomian gland ductal epithelium” have show label dilution indicative of rapid dividing TA cells (Lavker RM, et al. IOVS 2003;44: ARVO E-Abstract 3781). Such highly proliferative cells also occurred farther out in the zone of the mucocutaneous junction on the inner lid margin.

Similarities to Hair Follicles. This situation in the meibomian glands shows immediate similarity with the arrangement of stem cells and TA cells in the hair follicle—consistently downward with the lower hair root epithelium and upward with the skin epithelium. Cotsarelis et al.\(^{75}\) observed that, in the hair–skin unit, the slow-cycling putative stem cells are almost exclusively localized in a specific zone (the hair bulge) at about the middle of the hair follicles where the permanent upper part of the hair follicle ends and the arrector pili muscle inserts into the follicle. Later investigations by Taylor et al.\(^{68}\) confirmed the initial hypothesis that TA cells originate from slow-cycling cells in the hair-bulge zone and populate two different tissue compartments. From the differential location and cell-cycling characteristics it was concluded that they form (1) the lower hair follicle.
differentiation status maintained by differential regulation of
structural and functional domains (Fig. 7) that require a unique
The meibomian glands have conceivably at least three struc-
tural and functional compartments. These are (1) the ho-
locrine acinus with its basal cycling and luminal differenti-
ating, lipid-producing meibocytes that produce the meibomian
oil, (2) the four-layered stratified squamous epithelium of the
ductal system (connecting ductules and long central duct),
which has physiological incipient keratinization; and (3) the
epidermis of the excretory duct, which represents an
ingrowth of the stratified squamous, fully cornified epidermis
of the skin from the free lid margin. It can be assumed from
studies of stem cells of the epidermis and of hair-associated
sebaceous glands that each of these compartments is provided
with lineage-committed progenitor cells. The basement mem-
brane that separates the epithelial tissues from the underly-
ing connective tissue is indicated by a dotted line. Schematic
drawing of a section through a meibomian gland and its
components is presented in Fig. 7. Modified from Knop N,
Knop E. [Meibomian glands. Part I: anatomy, embryology and
histology of the meibomian glands]. Mei-
bom-Drüsen, Teil I: Anatomie, Embryologie und Histologie der Mei-
bom-Drüsen. Ophthalmo- loge. 2009;106:872–883 with the kind per-
m ission of Springer Science and Business Medi a.
Location and Lineage Commitment of the Sebaceous Gland Stem Cells. Even though there is a relatively large body of literature on sebaceous glands, the formation of the sebaceous gland tissue from stem cells is still insufficiently understood. From Taylor et al., it can be assumed that the subpopulation of hair-bulge stem cells that migrate upward to populate the epidermis also represent the stem cells for the hair-associated sebaceous glands that are located on the way from the bulge toward the epidermal surface. This notion is supported by studies in which it was found that isolated and transplanted hair-bulge stem cells can form all skin cell lineages and constitute the respective tissues (i.e., hair follicles, sebaceous glands, and epidermis). More recent results indicate that epidermal stem cells from regions between hair follicles (interfollicular epidermis) are also bipotent, similar to the hair-bulge stem cells and form two lineages. One of them differentiates into epidermal cells that express keratinization markers, and the other differentiates into sebocytes under the influence of the transcription factor c-myc. Another study indicated multiple classes of stem cells are present in cutaneous epithelium, independent of the hair-bulge cells, and can contribute to the development of epidermal structures including hairs and sebaceous glands. This group also showed, by retroviral lineage tracing in the mouse, that in approximately one third of the labeled hair units, only the sebaceous gland was selectively labeled, which indicates the presence of a pool of long-lived, slow-cycling cells that conceivably represents a pool of lineage-specific glandular stem cells (Fig. 8). These presumed gland progenitors are located at the transition zone from the acinus to the hair follicle, and, in the same position, presumed mouse sebaceous gland-specific progenitors were later characterized by a transcription factor. This finding is in some contrast to the location of slow-cycling progenitors in the ductal epithelium of the mouse meibomian gland (Lavker RM, et al. IOVS 2003;44:E-Abstract 3781). Differences in the location of holocrine acinar stem cells may be related to the fact that the mouse hair-associated skin sebaceous glands apparently do not have a distinct connecting ductule, in contrast to the human hair sebaceous glands and the meibomian glands.

At present, it is thought that there are at least three independent stem cell populations in the skin: the multipotent hair-bulge stem cells, for the cyclic reformation of the hair, and lineage-committed stem cells for the sebaceous glands and the interfollicular epidermis. In addition, there are isthmus-resident cells in the upper hair follicle close to the sebaceous gland. Under pathologic conditions such as wounding, the bulge stem cells become activated and can in fact replenish the sebaceous glands and the epidermis.

Development of Sebaceous Glands. The transcription factor c-myc governs the expression of a large number of genes, including some that are essential for skin development. In skin, c-myc represents a kind of switch that determines the development of stem cells into keratinizing epidermal cells versus sebaceous gland cells. Increased expression of c-myc favors their differentiation into sebocytes. The differentiation of sebocytes can also be induced by c-myc activation in stem cells of the interfollicular epidermis between hair follicles.

The further differentiation and proliferation of stem cells committed to sebaceous gland development is governed by the transcription factor B lymphocyte-induced maturation protein 1 (BLIMP1), originally discovered as a factor that inhibits the further proliferation but promotes the differentiation of B-lymphocytes into antibody-secreting plasma cells. Experiments in the mouse showed that a loss of the Blimp1-positive gland progenitor cells at the transition from the acinus to the hair follicle sebaceous gland (Fig. 8) resulted in increased cell activity in the hair-bulge stem cell compartment and led to reformation of gland tissue, although in a hyperplastic state. From these observations it was concluded that the BLIMP1-positive cells represented the unipotent, lineage-committed progenitor cells that control the development and homeostasis of the sebaceous gland. A later study in the mouse and human, however, did not show that BLIMP1 distinguishes stem cells undergoing differentiation into sebaceous gland versus hair follicle versus interfollicular epidermal cells. Recently, it was observed that the BLIMP1 protein does in fact occur in all appendages of the skin including hair follicles, nail organs, sebaceous glands, and the epidermal granular layer, at least in the human. In addition, BLIMP1 protein was mainly found in the most mature cells; therefore, the authors concluded that BLIMP1 has a major role in terminal differentiation, including a central function in skin barrier homeostasis, as indicated by its presence in the epidermal granular layer. However, the function of BLIMP1 may be more complex, since in BLIMP1 knockouts, an increased cell division activity has been observed in the multipotent stem cells of the hair bulge, conceivably serving to replace the dysfunctional sebaceous gland cells. This occurrence results in a disturbance of sebaceous gland homeostasis with formation of enlarged, hyperplastic glands and an

Figure 8. Location of sebaceous gland progenitors in the mouse skin. The expression of sebaceous gland-committed progenitor cells was found to be restricted to the transition zone between the acinus and the hair follicle in skin sebaceous glands. Such progenitor cells, labeled by retroviral transfer, are seen in a hair met in longitudinal section (A) as well as in cross-sections (B, C). Another marker (BLIMP1) that is assumed to characterize lineage-committed sebaceous gland progenitors indicates respective cells at the same position in a schematic drawing (D). (A–C) Reprinted by permission from Macmillan Publishers Ltd: EMBO J. Ghazizadeh S, Taichman LB. Multiple classes of stem cells in cutaneous epithelium: a lineage analysis of adult mouse skin. 2001;20:1215–22, © 2001 Knop et al. IOVS, Special Issue 2011, Vol. 52, No. 4.

2001. (D) Reprinted from Cell, 126, Horsley V, O’Carroll D, Tooze R et al., Blimp1 defines a progenitor population that governs cellular input to the sebaceous gland, 597–609, © 2006 with, permission from Cell Press.
oily fur. Other regulatory factors also contribute to sebocyte differentiation and proliferation via the Hedgehog, Wnt, Notch, and other signaling pathways. Hedgehog signaling stimulates sebocyte proliferation, whereas their inhibition results in suppression of gland development.

**Terminal Maturation of Sebocytes.** The maturation of sebocytes within the sebaceous gland involves signaling by peroxisome proliferator activated receptor-γ (PPAR-γ), a member of the PPAR subfamily of nuclear hormone receptors. It is a ligand-activated transcription factor that plays an important role in the control of gene expression in a large number of tissues with lipid-producing cell types, even including keratinocytes, and is activated by fatty acids. PPARs regulate multiple lipid metabolic genes via PPAR response elements located in cell organelles, such as peroxisomes, microsomes, and mitochondria, which are involved in lipid metabolism. PPAR-γ is necessary for the differentiation of adipose tissue in vivo and in vitro. Signaling to PPAR-γ (e.g., by long-chain fatty acids such as linoleic acid) initiates lipogenesis and the accumulation of large intracellular lipid droplets. PPAR-γ is also involved in the terminal differentiation of sebocytes in the non-hair-associated rat preputial gland. The growth and development of sebaceous glands is dependent on androgens, but they are not sufficient for the full maturation of the sebocytes, as dissected in cell culture experiments. Androgens appear to influence early steps of sebocyte differentiation, probably including the upregulation of PPAR-γ, whereas full differentiation and maturation of sebocytes, including the formation of the typical large lipid droplets, requires the action of PPAR-γ. Therefore, the action of androgens is related to but distinct from that of PPAR-γ. Still, in cell culture, the effects of dihydrotestosterone and the specific PPAR-γ ligand thiazolidinedione BRL-49653 were additive in increasing the lipogenesis of rat preputial sebocytes, which points to the importance of sex hormones—in particular, androgens—in the regulation of sebaceous gland function.

**Cell Differentiation and Dynamics in the Meibomian Gland**

Molecules that are involved in the differentiation and function of sebaceous glands are also present in the meibomian glands, as observed by Nien et al. BLIMP1 was described as a differentiation marker for the sebaceous lineage stem cells, but it was observed later in the epidermis and basically in all skin appendages and was also found in the meibomian gland ductal system and in the luminal layers of the eye lid epidermis. The sebocyte differentiation marker PPAR-γ was also observed in mouse meibocytes. It showed an age dependence and changed from a cytoplasmatic staining of basal cells of young animals, to cytoplasmatic staining in most meibocytes of young adults, to a nuclear staining of all meibocytes in old animals. This rearrangement was paralleled by a decrease in acinar size and in lipid production, as verified by oil red O staining. Furthermore, in young and young adult animals, many of the basal acinar cells were recognized as proliferative by Ki67 staining, whereas the proliferation rate decreased in older animals. The noted absence of BLIMP1 from basal acinar cells may be explained by the recent finding that it does not represent a lineage marker for sebaceous stem cells, as originally assumed and does not discriminate between stem cells of different lineages in the mouse, but in fact occurs everywhere in the human epidermis.

The terminal differentiation of meibocytes shares similarities with that of the secretory cells (sebocytes) of sebaceous glands, as can be assumed from their structure and function for lipid production. Intracellular lipids, after their synthesis via a PPAR-γ-dependent pathway, are maintained inside specialized compartments (lipid droplets) that contain the associated molecule adipose differentiation-related protein (ADRP, or adipophilin) in their periphery. ADRP is an intrinsic lipid storage protein found in lipid droplets of different cell types and in all cells that produce lipids to any degree, from muscle cells to adipocytes. ADRP stimulates the uptake of long-chain fatty acids and its own expression is also upregulated by the presence of these fatty acids that represent a prominent component in the meibomian oil (Green-Church KB, et al. JOVS 2009;50:ARVO E-Abstract 533). ADPR has been described in rat meibomian gland tissue by Northern and Western blot analysis and by immunohistochemistry. The latter showed ADRP localization at the margins of the lipid droplets with a generally higher level of expression in the more mature meibocytes located toward the center of the acinus in situ and in nonmature cells in the cell culture of isolated meibocytes. ADPR was hence suggested to be a differentiation marker for mature meibocytes.

**Relation of Stem Cells to Meibomian Gland Disease**

Defects in stem cell formation and their migration may contribute to MGD and disease. This assumption is mainly based on observations in cutaneous hair-associated sebaceous glands, but also on findings in the meibomian glands itself.

In the skin, these defects are relevant (e.g., in the onset of acne and sebaceous cancer). Related diseases also occur in the meibomian gland in the form of hyperkeratinizing seborrheic conditions and cancers of the meibomian gland tissue.

Alterations of sebaceous glands can occur on all levels of development, from commitment to stem cell lineage, to gland tissue formation, to terminal sebocyte maturation inside the acini, as discussed by Horsley et al. Overexpression of the basal transcription factor c-myc, which acts as a kind of switch between the developmental directions into epidermis versus sebaceous gland formation, favors sebaceous gland hyperplasia, which conceivably results in primary seborrhea. BLIMP1, a more downstream transcription factor that represses c-myc and inhibits further proliferation but stimulates the differentiation of progenitor cells, is essential for lineage determination of sebaceous gland stem cells. Disturbance of sebaceous gland homeostasis due to loss of BLIMP1-positive, gland-committed progenitors can result in excessive repair processes with formation of enlarged, hyperplastic glands and seborrhea. The terminal maturation of sebocytes inside the acini is maintained by PPAR-γ, which is a key factor for all lipid-producing cells. Cells with deficiency of PPAR, such as knockout cells, can only poorly contribute to the formation of secretory acini. In line with this observation in sebaceous glands may be the finding that the expression pattern of PPAR-γ gradually redistributes from a cytoplasmatic location in young and young adult mice to a nuclear location in old mice. This change in location is paralleled by development of acinar atrophy with a decrease in the size of the acini in general and of the lipid droplets within individual meibocytes in particular. A lack of PPAR-γ could therefore contribute to potential age-related atrophic processes of the meibomian gland. Agonists and antagonists of PPAR-γ can modulate the function of sebaceous glands and may also provide potential therapeutic approaches.

In wounded conditions, stem cell differentiation tends to develop a higher degree of plasticity and the lineage commitment can switch, leading to a replacement of altered or lost progenitor cells. This process may also apply to events observed in MGD that may exert stress on the cells, such as that due to stasis with downstream mechanical pressure stress.
in obstruction or due to increased bacterial growth and downstream release of bacterial lipases, toxic mediators, or inflammatory mediators. Stem cell-based repair mechanisms, however, do not always result in intact tissue reconstruction but can lead to alterations in structure and function. This alteration can result in hyperplastic acini with increased lipid production that could contribute to the pathogenesis of MGD, as similarly described in the pathogenesis of acne. \(^{107}\) but it may also be involved in the development of meibomian gland cancer. \(^{111}\)

**LIPID SYNTHESIS IN MEIBOMIAN GLANDS**

**Overview**

There are few studies on lipid synthesis or uptake in the meibomian gland. A primary reason for the scant number of studies is that information about which lipids are synthesized has been lacking. Meibomian lipid characterization has reached a point where it may now be possible to identify those lipid synthetic pathways that lead to very long fatty acids. Science remains challenged, however, in that not all animal models would produce the same lipid mixtures as humans, and because the energy needs and hormonal stimulation needed for lipid production are not fully understood. Enormous amounts of energy are required to add two carbons to a growing lipid chain (1ATP, 2NADPH; 16C palmitic acid requires 7ATP plus 14NADPH). For this to occur, the meibocytes need both a sufficient supply of oxygen and a reliable carbon source. Although the basal cells in the acini have access to oxygen from capillaries, as the cells mature, they continually plump up with lipids, and they distance themselves from the capillaries (Fig. 4). Both glucose (the typical carbon source) and oxygen do not diffuse well through lipids—hence, the conundrum as to how these resources are supplied to the maturing acinar cells. In mature meibocytes according to Jester et al. \(^{32}\) “the mitochondria are shrunk and electron-dense.” In contrast, Gorgas and Völk \(^{34}\) have not reported any degeneration of mitochondria in mature meibocytes but only the presence of osmophilic, dense inclusion bodies that occur in all stages of meibocyte differentiation. Despite this, the cells fill with lipids; therefore, the resources for producing the lipids must be accessible to the central acinar cells. It remains unclear whether this process occurs via transport or diffusion.

There are also questions about hormonal regulation of the lipid production process, because insulin and glucagon are typically involved with fat and sugar metabolism. They are water soluble and would have little access to the maturing acinar cells. By contrast, steroid hormones (particularly androgens) are known to influence the acinar cells, and their lipid solubility would give them access to the maturing acinar cells. In addition to the synthesis of meibomian lipids, the ultrastructure of meibocytes indicates that there are special features of stacked membrane arrays and peroxisomes, which means that these cells must also have the machinery to synthesize polar lipids, such as phospholipids and cholesterol, for synthesis of their internal membranes. There remains the possibility, however, that some of these polar lipids and others, such as \((\text{O-acyl})-\omega\)-hydroxy fatty acids, are specifically synthesized for secretion. Before the puzzle of how the synthesis of these lipids is controlled versus those specifically destined for secretion can be systematically considered, a consensus must be reached on the composition of normal meibomian secretions. It is also highly likely that some of the enzymes associated with the lipid synthesis are membrane bound, which makes them difficult to study. Immunohistochemical studies may resolve this dilemma, because they will help determine the compartmentalization of the enzymes—an important factor, in that enzyme location is an indication of the end destination of the lipids. Unfortunately, no easily accessible primer text about the biochemical pathways has been published to date.

The most abundant components of the meibomian lipids are wax and sterol esters, consisting of fatty acids and fatty alcohols, long-chain (＞20C) fatty acids and alcohols, and sterols, particularly cholesterol. The most abundant fatty acid is oleic acid, which has 18C and is monounsaturated (18:1(\text{cis})9), which means it has 18C with the ninth bond being a \(\text{cis}\) double bond. Wax esters are formed by condensation of fatty acids with fatty acids, and sterol esters are formed by condensation of sterols with fatty acids. \(^{103,116}\) Theoretically, these lipid components could either be synthesized de novo in the acinar cells or taken up from the bloodstream or both. The evidence for de novo synthesis is supported because the synthetic enzymes for the components and the transesterases to form the final products have been detected either directly or indirectly (mRNA) in the acinar cells. \(^{77,81,117}\) There is no direct evidence to date that the lipids are taken up from the bloodstream (this does not refer to steroid hormones, which are lipids, being taken up by the acinar cells as part of their hormonal action) and this is an area that warrants further investigations. Such uptake could show variations in lipid composition with change in diet, and the plasma cholesterol levels warrant further investigation relative to the cholesterol \(^{118}\) or cholesterol esters in tear fluid or meibomian secretions. Recently, patients with blepharitis and taking flaxseed oil (omega-3) showed no difference in omega-3 fatty acids, omega-6 fatty acids, total monounsaturated, poly, or monounsaturated fatty acids, despite levels of omega-3 fatty acids being higher in the blood. \(^{119}\) And if diet is important, it would not explain how the meibomian lipids of koalas, which have a very restricted and specific lipid dietary intake (sole diet of eucalyptus leaves), have a lipid profile similar to other mammals (Butovich IA, Millar TJ. IOVS 2009;50:ARVO E-Abstract 2545). However, a correlation has been shown between polar lipid profiles and variations in diet in women with Sjögren’s syndrome; that is, those with a single polar lipid peak after HPLC analysis had about double the intake of omega-3 fatty acids compared with those who had multiple polar lipid peaks. \(^{120}\) Therefore, it is most likely that all the lipids secreted by the meibomian glands are synthesized by the gland and we must understand fatty acid and cholesterol synthesis, the key enzymes involved in these pathways, and in which cellular compartments they are located.

**A Skeletal Overview of Lipid Synthesis**

Fatty acid synthesis catalyzed by fatty acid synthase occurs in the cytoplasm, but the carbons come from the mitochondria (Fig. 9). Therefore, mitochondria are necessary, not only for generating the large amounts of energy needed for lipid synthesis, but also for the carbons of lipids. This fact is enigmatic in the context of meibomian glands, where the more mature acinar cells continue to plump up with lipids, but at the same time their mitochondrial number decrease, and they are further displaced from their oxygen source.

**Fatty Acid Synthesis**

The carbon chain of the fatty acids is built two carbons at a time from repeated enzymatic cycles. As part of this process, the acetyl-CoA has to be activated to a higher energy level. This activation is induced by adding carbon dioxide to it to form malonyl-CoA (Fig. 10). With each cycle of fatty acid synthesis, two new carbons from malonyl-CoA are added to the chain, and the third carbon is released as CO2 (Fig. 11). With each cycle, through several different reactions catalyzed by different domains of the enzyme fatty acid synthase, two reductions
occur, each of which converts one NADPH to NADP. Each of these steps is about the same as consuming three ATPs. The result is an even-numbered fatty acid, typically 16C long (palmitic acid), which is cleaved from the enzyme by a thiolase. Many of the fatty acids in the meibomian gland are much longer than 16C, and the further elongation requires different enzymes. If an odd-numbered straight-chain fatty acid is to be synthesized, propionyl-CoA (4C) is initially used as the carbon source instead of malonyl-CoA, and when CO2 is displaced, three carbons are added to the chain, resulting in an odd-numbered fatty acid.

**Straight-Chain Fatty Acids.** For chain elongation, the palmitoyl component of palmitoyl ACP is transferred to coenzyme A (CoA-SH) to form palmitoyl-CoA. Then, elongation (C18–C28) occurs in the endoplasmic reticulum,121 and some of these are converted by additional enzymes into fatty alcohols. The elongation process occurs in the same way as described above, except that in this case, CoA is the carrier. Two new carbons are loaded from malonyl-CoA onto palmitoyl-CoA, followed by reduction, dehydration, and reduction to form the new saturated chain extended by 2C. In forming the most prominent fatty acid in meibomian gland secretions, oleic acid, the palmitic acid (16C) has to be elongated and then desaturated. Fatty acyl-CoA desaturase catalyzes the introduction of a double bond into the acyl chain (bond 9 of stearic acid), to form oleic acid. This oxidation occurs on the inner face of the endoplasmic reticulum and involves coupled electron transfers through cytochrome-b5, FADH2, and NADPH, which is converted to NADP+.

FIGURE 9. Transfer of carbons for lipid synthesis from the mitochondria to the cytoplasm. When the tricarboxylic acid (TCA) cycle in the mitochondria is blocked due to an excess of the high-energy molecule NADH, there is a buildup of mitochondrial acetyl-CoA that indicates to the cell that it has a surplus of energy and therefore does not need to oxidize carbons to obtain more energy. Instead, it is more desirable to store the carbons as fats until the energy is needed. The acetyl group (2C) of acetyl-CoA is passed to oxaloacetate (4C) to form citrate (6C), and citrate is transferred across the mitochondrial membrane into the cytoplasm. It is then lysed (citrate lyase) and coupled to cytoplasmic CoA to form cytoplasmic acetyl-CoA, which is used for fatty acid synthesis, and oxaloacetate, which is cycled back (indirectly) to the mitochondrial matrix. Figure courtesy of Tom Millar.

FIGURE 10. Formation of malonyl-CoA. Figure courtesy of Tom Millar.
leic acid (18:2; all-cis) and linolenic acid (18:3; all-cis) are normally obtained from the diet. These fatty acids have been detected in human meibomian gland secretions, albeit in small amounts. One possibility is that there are additional desaturase enzymatic activities in the meibomian gland that are able to desaturate ω-3 or ω-6 bonds in C18 fatty acids. One example of such enzymatic activity is the product of the gene fat-1, which has been found in animals. These desaturases are membrane bound and probably require other membrane-linked cofactors, such as cytochrome-b5 for their activity. The presence of large numbers of ordered peroxisomes in more mature cells in the meibomian gland acini may be the location of these enzymes, but this still has to be demonstrated. That these enzymes are membrane bound means that they are very difficult to purify, and therefore searching for their gene expression is more likely to reveal their presence. Another important highlight of plant membrane-bound desaturases is that they use glycerolipids as their substrates and not acyl-CoA. The presence of this substrate is also likely to be the case for similar desaturases (e.g., FAT-1), if they exist, in meibomian glands. This notion implies that the carbon chain source would be from the membrane lipids of the endoplasmic reticulum.

**Branched-Chain Fatty Acids.** Some branched-chain fatty acids have been detected as components of meibomian lipids. It appears from studies of rabbit meibomian glands, with the use of radioactive labeled precursors, that the branched carbon chain is supplied from branched side-chain amino acids. In rabbits, isoleucine has been used predominantly in vivo, whereas, in vitro, valine has also been used. In neither case has leucine been used as a precursor. For these, the incorporation must have been at the initial loading of the acyl carrier domain of fatty acid synthase, because the branched fatty acids were either iso- or anteiso-branched (at the omega end of the carbon chain). Since the new chain grows from the carboxyl end, if incorporation were not at initiation, there would be multiple branching along the length of the chain. Similarly, if it were incorporated after the initial synthesis of palmitic acid, the extension phase for longer chain fatty acids, they would also occur randomly and possibly as multiple branched methyl groups near the carboxyl end, but this is not the case. Whether the preference order for the amino acid precursors for branched-chain fatty acids is the same in other animals as it is in rabbits still has to be established.

**Fatty Alcohols.** Fatty alcohols are synthesized from their corresponding fatty acids. The fatty acid component of acyl CoA is reduced to form the corresponding fatty alcohol (acyl-CoA reductase). Whether the cofactor in the meibomian gland is NADPH or NADH is not known, but two high-energy compounds are consumed in the reaction. In the meibomian gland, there appears to be a preference for long-chain fatty acids, because long-chain fatty alcohols seem to be the predominant species found to be associated with cholesterol and wax esters. This association may come about because of an enzymatic preference for longer chain fatty acids or because the shorter chain fatty acids are preferentially used for the acid component of wax esters or (O-acyl)-ω-hydroxy fatty acids.

**Cholesterol Synthesis.** An overview of cholesterol synthesis is that all 27C compounds come from acetic acid, in the form of acetyl-CoA. As above, the acetyl component of acetyl-
CoA is transferred from the mitochondria to the cytoplasm via citrate (Fig. 9). It is then converted to HMG-CoA (Fig. 12), which is converted to mevalonate (Fig. 13). This reaction is catalyzed by HMG reductase, which is activated by insulin and inhibited by glucagon. Therefore, it would be of interest to examine the composition of meibomian lipids from patients with uncontrolled diabetes, as in theory, this critical enzyme would have decreased activity, and hence less cholesterol would be synthesized. Mevalonate is converted to a 5C compound, isopentenyl pyrophosphate (Figs. 13, 14). Three of these are joined to form a 15C compound—farnesyl pyrophosphate—and two farnesyl pyrophosphates are joined to form a 30C compound, squalene (Fig. 15). Squalene is cyclized to form lanosterol, and three methyl groups are then removed to form cholesterol.

The mRNAs for the enzymes associated with this pathway have been identified in mouse meibomian gland extracts, and most of them are increased by testosterone, which is an indication that this pathway is upregulated by testosterone. This suggestion is supported by the histochemical detection of various hydroxysteroid dehydrogenases in meibomian gland acinar cells. These enzymes were located in developing and mature, but not basal, acinar cells of the meibomian gland. These enzymes are not found in all sebaceous glands, but are associated with sebaceous glands of the face and neck. In sebaceous glands, the levels of these enzymes decrease with age, which does not seem to be the case with meibomian glands. Perra et al. did not mention this, even though samples were taken from 18- to 60-year-olds. In terms of function, the enzymes are located in the endoplasmic reticulum and are able to catalyze the conversion of androgens into their potent metabolic forms, particularly dihydrotestosterone, which is associated with upregulation of gene transcription for enzymes associated with fatty acid and cholesterol synthesis, as well as other functions associated with lipid metabolism.

### Other Synthetic Pathways

Triglycerols have also been reported to be components of meibomian lipids. The pathway for synthesis of triglycerols involves having one of the intermediates of glycolysis, dihydroxyacetone phosphate, converted to glycerol 3-phosphate (glycerol 3-phosphate dehydrogenase), which uses NADH as the hydrogen source. Acyl transferases sequentially catalyze the transfer of acyl groups from acyl-CoA to C1 and then C2 of the glycerol 3-phosphate to form phosphatidic acid. Phosphatidic acid phosphatase catalyzes the dephosphorylation of phosphatidic acid to form diacylglycerol, which is then converted to triacylglycerol by transfer of an acyl group from acyl-CoA (acyl transferase).

The suggestion and, more recently, the detection of ω-hydroxy fatty acids means that ω-hydroxy fatty acids must be synthesized in the meibomian glands. The enzymatic pathway for ω-hydroxy fatty acids means that ω-hydroxy fatty acids must be synthesized in the meibomian glands. At this stage, only long-chain ω-hydroxy fatty acids have been detected, which tends to indicate that the hydroxylation occurs at the end of acyl chain synthesis, not at the beginning. Hydroxylation of an inert terminal methyl group would be very unusual and normally needs several intermediates. Such intermediates have yet to be detected. However, many of these compounds, which may be in low amounts, would be undetected unless specifically sought.
Cholesterol esters tend to have long-chain (>C20) fatty acids attached to them,\textsuperscript{132} which indicates that the cholesterol transacylase(s) involved prefer long-chain acids. This preference differs from that of the wax acyl-CoA:alcohol transacylases, which do not appear to be specific for chain lengths.\textsuperscript{125} The (\(O\)-acyl)-\(\omega\)-hydroxy fatty acids tend to have long-chain hydroxyl fatty acids (C30:1, C32:1, and C34:1) acetylated through their \(\omega\)-hydroxyls by a \(C_{18}:1\)-FA,\textsuperscript{132} which indicates that the transacylases may be very specific.

**Comments on the Synthesis of Lipids in Meibomian Glands**

**Vascular Supply.** Fat synthesis needs both energy and an excellent blood supply as the source of its oxygen. This necessity is reflected in adipose tissue development, where lipocyte density correlates positively with capillary density, and the cells tend to cluster around large blood vessels.\textsuperscript{133} Examination of the published literature on the structure of the meibomian gland and structural studies of meibomian gland development have not paid particular attention to capillary size and density. Although this may not be particularly essential in development, a study of the capillaries could be of benefit in understanding the aging process, in which gland atrophy has been noted. Local swelling, such as occurs when there is a blockage of the meibomian gland duct, may also lead to poor blood flow and atrophy of the glands. In other fat cells, angiogenesis factors such as PGE2 (ubiquitous) and \(\beta\)-butyrylglycerol (specific to fat cells) are synthesized and secreted by adipocytes. To date, secretion of these factors by meibocytes has not been investigated.

The predominant source of energy in these synthetic pathways is NADPH, typically produced by a side path of glycolysis, the pentose phosphate pathway. Key enzymes associated with this pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) have been detected in the differentiating and degenerating cells of human meibomian glands, but not in the basal cells. The need for this pathway means that, to produce lipids, meibocytes require a rich supply of glucose and therefore depend heavily on insulin and a rich blood supply for glucose uptake. Insulin also stimulates lipid synthesis (many kinases that control activation of lipid synthetic enzymes are sensitive to insulin). Similarly, glucocorticoids have been long known to increase adipose tissue mass via their hypertro-
A clinical method of testing this hypothesis would be the assessment of the meibomian glands that are altered in patients who have Cushing’s syndrome or are receiving cortisol treatment.

The membranes between acinar cells are particularly and unusually interdigitated and could be a region of cytoplasmic exchange. On the positive side, published micrographs also show, on casual inspection, that there is a concentration of mitochondria around lipid droplets that is consistent with the molecules that come from the mitochondria being a carbon source for the new lipids. Similarly, some lipid droplets are surrounded by organized concentric layers of endoplasmic reticulum. This arrangement is consistent with the enzymes associated with fatty acyl chain elongation and oxidation (formation of double bonds) occurring in these regions. It may also mean that, at this stage, different lipids are in different lipid droplets, but it probably has no impact at the end, when the cells disintegrate as part of the holocrine secretion.

**Proteins and the Meibomian Gland**

There is emerging evidence that specific lipoproteins or other proteins (Thangavelu M, et al. *JOVS 2010;51:ARVO E-Abstract 2375*) may be associated with meibomian lipids. There has been a study on adipose differentiation-related protein, a protein that marks the differentiation of adipose tissue. This protein seemed to localize adjacent to the lipid droplets and, in micrographs, appeared to be present at lower levels in the most mature cells in the follicle. The low magnification made this difficult to ascertain. Lipocalin has also been found in human meibomian gland secretions and hence it is possible that this lipocalin also sequesters lipids. It may originate from the meibomian gland, given that lipocalin mRNA has been identified in the mouse meibomian gland.

**Physical Properties of Meibomian Lipids**

The transition temperature from a solid to a liquid for meibomian lipids is actually a range. 28°C to 32°C, because of the mixture of lipids. This transition has been explained by the arrangement of lipids from their transconformation (ordered and rigid) to their gauche conformation (disordered and fluid), as evidenced by infrared spectroscopy. The temperature of the eyelids will therefore affect the liquidity of meibomian lipids and hence their viscosity. In liquids, viscosity (η) is a measure of resistance to flow at a particular temperature. In substances that exhibit Newtonian viscosity, if the force is doubled, the flow rate doubles, and hence the resistance to flow (viscosity) remains the same. Other substances alter their viscosity (non-Newtonian) depending on the force applied. (A good example is toothpaste, which is very viscous when sitting on a toothbrush, but flows easily out of a tube when force is applied.) This phenomenon is called shear thinning. Tiffany and Dart have reported the viscosity of human meibomian lipid samples to vary between 9.7 and 19.5 Pa·s (cf. boney, 10 Pa·s; glycerine, 1 Pa·s; olive oil, 0.1 Pa·s; and water, 1 mPa·s), depending on the force applied. Therefore, the viscosity of meibomian lipids exhibits non-Newtonian behavior. The temperature at which the viscosity was measured in these experiments was not indicated, but considering the data, it is likely that it was below the transition temperature (30°C). In a practical sense, these data represent the viscosity of the meibomian secretion sitting in the ducts. Blinking would apply shearing force that would lower the viscosity, making the lipids easier to eject from the meibomian orifices. This shearing force would be increased by having narrow openings in comparison to the diameter of the duct (as anatomic studies indicate is the case), and this effect would also reduce the viscosity. A lower viscosity would also occur because the temperature of the eyelid (35°C–37°C) would be above the transition temperature. In the same experiments, the viscosity of lipids extracted from chalazion secretions were so high that only one measurement, despite heating to 70°C, could be made, and that was 69.9 Pa·s. This material contained more phospholipids, free fatty acids, cholesterol, and triglycerides than do normal meibomian lipids.

Viscosity measurements on thin films are complex, and several different experimental parameters, such as frequency and amplitude of shear forces and surface pressure of the film, are varied to come up with appropriate measures of viscosity. Films made from human meibomian lipids show increasing viscosity (complex viscosity) with surface pressure and attain a viscosity of 1 mPa·s at a surface pressure of approximately 25 mN/m, dropping to 0.1 mPa·s at 35°C, at a shear frequency of 6.2 rads. The refractive index of meibomian lipids varies between 1.46 and 1.53 per the visible spectrum, with a small, relatively linear decrease over a temperature range from 25°C to 45°C. The refractive index of human skin lipids is approximately 0.013 less. This high refractive index probably has little overall refractive influence over a pure air–water interface because the lipid layer is very thin. Although there seems to be an abundance of lipids available on the eyelid margins (300 µg) compared with the amount of lipids estimated to be in the lipid layer of the tear film (9 µg), we still do not know what enables them to spread from the reservoir across the ocular surface to form a film. There is indirect evidence that a bigger reservoir of lipids on the eyelid margins leads to a thicker lipid layer. The amount of lipids is higher on the eyelid margins just after waking, and there is a thicker oil film on the eye surface in the morning. A surfactant is needed for lipids to spread across an aqueous surface. In the absence of a surfactant, the lipids form lenses on the surface. One of the main questions about meibomian lipids centers on what enables them to spread. Holly proposed that this could be achieved by an initial spread of polar lipids over the aqueous surface followed by nonpolar lipids. However, it is most likely that the lipid film is not respread on each blink, but instead collapses and expands, as indicated by the same interference patterns seen over multiple blinks; only after this is a new layer formed. What conveys these properties is still not understood and whether proteins from the aqueous layer become part of this outer layer of the tear film, as some models suggest, still has to be demonstrated. Conversely, there is also no evidence they are not part of this outer layer.

**Regulation of the Meibomian Gland in Health and Disease**

Sebaceous glands are present throughout the body and are classified into two major types: pilosebaceous, which are associated with hair follicles, and free (i.e., preputial and meibomian), which occur in the transitional zone between the skin and mucous membranes. An extensive amount of information is known about sebaceous glands, and a review of their physiological regulation in health and disease would be challenging. More than 6000 articles have been published about these glands since 1904, and numerous factors are known to modulate the development, proliferation, differentiation, maturation, lipogenesis, and secretion of sebaceous glands throughout the body (Table 1). These factors include sex steroids, corticosteroids, hypothalamic and pituitary hormones, insulin, retinoids, thyroxine, melanocortins, neurotransmitters, growth factors, and peroxisome proliferator-activated receptor ligands (Table 1). The control points for sebaceous gland regulation often involve effects on...
gene expression, protein synthesis, and lipid production. There appear to be considerable differences in the control mechanisms, however, as well as in the lipid composition, of sebaceous glands between species and between different types of sebaceous glands.\(^{145}\)

In contrast to sebaceous glands in general, there is relatively little information about the physiological regulation of the meibomian gland. Fewer than 850 articles about the meibomian gland have been published in the past 106 years (Table 2), and fewer than 50 of these papers, including reviews, address the topic of physiological control. The paucity of studies is astonishing, given that recent research has demonstrated the presence of more than 270 receptor mRNAs in the mouse meibomian gland alone (Table 3). Yet it is unknown whether most of these transcripts are translated and functional. Of particular interest is the lack of knowledge about the neural influence on the meibomian gland. This tissue is the only sebaceous gland in the human body that has rich sensory, sympathetic, and parasympathetic innervation,\(^{145}\) including contact with nerve fibers reactive for acetylcholinesterase, substance P, vasoactive intestinal peptide, dopamine \(\beta\)-hydroxylase, nitric oxide synthase, tyrosine hydroxylase, somatostatin, neuropeptide Y (NPY), and CGRP.\(^{39,56,57,59,81,157–169}\) Further, the meibomian gland contains mRNAs for serotonin, adrenergic, CGRP, cholinergic, dopamine, \(\gamma\)-aminobutyric acid, glutamate, NPY, neurotensin, and somatostatin receptors (Table 3).\(^{81}\) It is entirely unclear, however, whether neurotransmitters are released in the vicinity of the meibomian gland, act on glandular receptors, or induce a physiological effect.

Almost all of our understanding of the physiological, as well as pathophysiological, regulation of the meibomian gland originates from research exploring the effects of androgens, estrogens, progestins, all-trans retinoic acid, and growth factors on this tissue and/or its epithelial cells. This topic is discussed in the following sections.

### Androgens

**Androgen Regulation of Sebaceous Glands.** Androgens exert a significant impact on the meibomian gland.\(^{81}\) This influence is not surprising, given that androgens control the development, differentiation, and lipid production of sebaceous glands throughout the body.\(^{40,81,145,146,148,151,155,156,170–188}\) Androgens act primarily on acinar epithelial cells in sebaceous glands, and these cells contain both androgen receptor mRNA and protein (in their nuclei). Acinar cells respond to androgens by increasing the transcription of multiple genes and synthesizing proteins that augment both the elaboration and secretion of lipids. Sebaceous gland activity and secretion may be inhibited by orchectomy or topical antiandrogen treatment.\(^{182,189–192}\)

Of particular interest, sebaceous gland function declines with age.\(^{193}\) This aging-associated dysfunction has been correlated with a reduction in androgen levels in the surrounding skin.\(^{193}\) Indeed, the age-related cellular shrinkage in certain sebaceous glands has been directly correlated with a reduction in androgen levels in the surrounding skin.\(^{193}\)

Androgen activity in sebaceous glands is significantly influenced by the activity of certain enzymes, particularly 5α-reductase (converts testosterone into the potent androgen 5α-dihydrotestosterone), aromatase (converts testosterone to 17β-estradiol, androstenedione to estrone), and 17β-hydroxysteroid dehydrogenase (HSD); regulates the interconversion of 17-ketosteroids with their corresponding 17β-hydroxysteroids and is necessary for the intracrine formation and/or inactivation of all active androgens and estrogens in sebaceous glands.\(^{194–200}\) These enzymes are vital, given that most of the androgens and estrogens in humans are synthesized in peripheral tissues (e.g., sebaceous glands) from adrenal sex steroid precursors (i.e., dehydroepiandrosterone [DHEA] and DHEA-sulfate) and that these enzymes regulate the critical steroidogenic pathways (Fig. 16).\(^{194–197}\) Of interest, the activity of these enzymes may vary according to sex, tissue location in the body, or cellular position within a pilosebaceous unit and may also be induced by microenvironmental factors (e.g., proinflammatory cytokines).\(^{200–203}\)

Androgens also regulate numerous pathways of lipid metabolism. For instance, depending on the tissue, androgens control:

**Table 2. Articles with the Phrase “Meibomian Gland” Cited in** PubMed from 1903 through November 2009

<table>
<thead>
<tr>
<th>Topic</th>
<th>Number of Articles</th>
<th>% of Articles</th>
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<tbody>
<tr>
<td>Clinical assessment and treatment</td>
<td>405</td>
<td>48.0</td>
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<tr>
<td>Cancer</td>
<td>141</td>
<td>16.7</td>
</tr>
<tr>
<td>Lipid analysis, properties and synthesis</td>
<td>99</td>
<td>11.7</td>
</tr>
<tr>
<td>Literature reviews (partial)</td>
<td>52</td>
<td>6.2</td>
</tr>
<tr>
<td>Anatomy and histochemistry</td>
<td>50</td>
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<td>Physiological regulation</td>
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<tr>
<td>Pathology (experimental)</td>
<td>25</td>
<td>3.0</td>
</tr>
<tr>
<td>Neural innervation</td>
<td>17</td>
<td>2.0</td>
</tr>
<tr>
<td>Culture systems</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>Stem cells</td>
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<td>0.4</td>
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A total of 844 articles were evaluated. The category Literature Reviews (Partial) does not include topics related to lipid analysis, neural innervation, or physiological regulation. Those reviews are placed in their specific topic areas.
<table>
<thead>
<tr>
<th>Receptor mRNA Present in the Mouse Meibomian Gland</th>
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<tbody>
<tr>
<td>5-hydroxytryptamine (serotonin) receptor 1a</td>
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<td>5-hydroxytryptamine (serotonin) receptor 1c</td>
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<tr>
<td>5-hydroxytryptamine (serotonin) receptor 7</td>
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<tr>
<td>7 transmembrane g protein coupled receptor</td>
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<tr>
<td>Activin a receptor, type 1</td>
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<tr>
<td>Activin a receptor, type 1a</td>
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</table>
| Activin a receptor, type ii-like |}

(continues)
The induction of genes involved in fatty acid and cholesterol metabolism, including fatty acid synthase, ATP-citrate lyase, malic enzyme, acetyl-CoA-carboxylase, 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) synthase, HMG-CoA reductase, glycerol 3-phosphate acyl transferase, farnesyl diphosphate synthase, malic enzyme, acetyl-CoA-carboxylase, 3-hydroxy-3-methylglutaryl synthesis, including fatty acid synthase, ATP-citrate lyase, among others,184,204–219 has been the focus of recent research. For example, androgen receptors appear to be dependent on the presence of functional androgen receptors within the meibomian gland. Testosterone, for example, regulates the expression of more than 1580 genes in the male mouse meibomian glands.77,117,222,224,225 These findings suggest that the human meibomian gland harbors the enzymatic machinery necessary for the intracellular regulation of lipogenic enzymes.215–221,223,228–229

**Androgen Regulation of the Meibomian Gland.** Androgens regulate the meibomian gland and modulate gene expression and lipid production within this tissue.77,81,117,222–225 These hormonal actions appear to be mediated, at least in part, through binding to classic nuclear receptors. Sex steroid receptors typically bind their specific hormone, and the activated hormone–receptor complex then associates with a response element in the regulatory region of target genes and controls gene transcription and eventually protein synthesis.226–229 The meibomian glands of male and female rats, rabbits, and humans contain androgen receptor mRNA and androgen receptor protein within the acinar epithelial cell nuclei.225,227,230 Further, androgens regulate the expression of numerous genes in mouse, rabbit, and human meibomian glands.77,117,222,224,225,231 These genomic actions appear to be dependent on the presence of functional androgen receptors.224,225

The effects of androgens and estrogen on the human meibomian gland may be exerted predominantly after the local formation of sex steroids from adrenal precursors. Human meibomian glands contain all the following steroidogenic and metabolic enzyme mRNAs: steroid sulfatase, 3β-HSD-Δ5-isomerase type 1,17βHSD types 1 and 3, types 1 and 2 5α-reductase, aromatase, glucuronosyltransferase, and sulfotransferase.78,229 Moreover, at a minimum, 3α-HSD, 3β-HSD, and 17β-HSD are known to be translated in epithelial cells of the human meibomian gland.129 These findings suggest that the human meibomian gland harbors the enzymatic machinery necessary for the intracellular regulation and metabolism of sex steroids.

Androgens exert a significant influence on gene expression in the meibomian gland. Testosterone, for example, regulates the expression of more than 1580 genes in the male mouse meibomian gland.77,81,117,222,225 Many of the upregulated genes are related to lipid metabolism (Fig. 17), lipid transport, sterol biosynthesis, fatty acid metabolism, intracellular protein transport, oxireductase activity, peroxisomes, mitochondria and early endosomes.77,81,117,222,225 Moreover, some of the proteins and pathways encoded by these upregulated genes have been the focus of recent research. For example:

- ATP-citrate lyase, acetyl-CoA-carboxylase, acetyl-CoA-carboxylase,-acetoacetyl-CoA synthase, fatty acid synthase, HMG-CoA synthase, HMG-CoA reductase, mevalonate kinase, phospho-

**Figure 16.** Major biosynthetic and inactivation pathways of androgens and estrogens in humans. Direction of enzymatic action is shown by arrows. Abbreviations include Sulfatase, steroid sulfatase; ST, sulfotransferase; Sulf Met, sulfated metabolites; HSD, hydroxysteroid dehydrogenase; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; Estrone S, estrone sulfate; DHT, dihydrotestosterone; 5-diol, 5-androstene-3β,17β-diol; ADT-G, androstenedione-glucuronide; 3α-diol-G, androstane-3α, 17β-diol-glucuronide. Reproduced from Schirra F, Suzuki T, Dickinson DP, Townsend DJ, Gipson IK, Sullivan DA. Identification of steroidogenic enzyme mRNAs in the human lacrimal gland, meibomian gland, cornea, and conjunctiva. Cornea. 2006;25:438–442 with permission from Wolters Kluwer/Lippincott Williams & Wilkins.
mevalonate kinase, mevalonate pyrophosphate decarboxylase, isopentenyl pyrophosphate isomerase, geranylgeranyl pyrophosphate synthase, squalene epoxidase, lanosterol demethylase; and Δ7-sterol reductase are all key enzymes involved in the initiation and progression of cholesterol, fatty acid, sex steroid, and/or lipid synthesis. Fatty acid synthase is also known to be regulated by androgens in other tissues and is expressed in meibomian gland epithelial cells (Richards SM, et al. J OVS 2002;43: ARVO E-Abstract 3150).

- Fatty acid transport protein 4 facilitates the cellular up-take and metabolism of long- and very-long-chain fatty acids.
- The elongation of very-long-chain fatty acid-like types 1 and 3 enhance the tissue-specific synthesis of very-long-chain fatty acids and sphingolipids. These proteins could be involved in the androgen-induced increase of long-chain fatty acids in the total lipid fraction of rabbit meibomian glands. Orchiectomy causes a marked change in the lipid pattern of rabbit meibomian glands, whereas the topical or systemic administration of 19-nortestosterone (versus placebo) for 2 weeks begins to restore the lipid profile toward that found in intact animals. Further, researchers speculate that androgen signaling machinery regulates the expression of secretoglobin in human meibomian gland. Many of the genes modulated by testosterone in female tissues are identical with those controlled by androgens in male meibomian glands. Some genes, however, are regulated in a sex-specific manner.

Androgens also affect the lipid, and possibly protein, composition within the meibomian gland. Orchiectomy causes a marked change in the lipid pattern of rabbit meibomian glands, whereas the topical or systemic administration of 19-nortestosterone (versus placebo) for 2 weeks begins to restore the lipid profile toward that found in intact animals. Further, researchers speculate that androgen signaling machinery regulates the expression of secretoglobin in human meibomian gland epithelial cells. This protein may be secreted and have a lipocalin-like function in the tear film. Whether all effects of androgens on the meibomian gland are mediated through classic receptors is unclear. It is possible that the action also involves binding to glandular membrane receptors, stimulation of signal transduction cascades, and consequent alteration of gene transcription. In addition, androgens may act indirectly, by regulating the secretion of the hypothalamic and anterior pituitary hormones that influence the meibomian gland.

**Influence of Androgen Deficiency and Treatment.**

Given the influence of androgens on meibomian gland function, researchers have hypothesized that androgen deficiency, such as occurs during menopause (decrease in ovarian and adrenal androgen secretion), aging in both sexes (decline in the total androgen pool), autoimmune disease (e.g.,
Sjögren’s syndrome, systemic lupus erythematosus, and rheumatoid arthritis,201,252,253 complete androgen insensitivity syndrome (CAIS; women with dysfunctional androgen receptors),254,255 and the use of antiandrogen medications (e.g., for prostatic hypertrophy or cancer)256 all lead to MGD, altered lipid profiles in meibomian gland secretions, decreased tear film stability, and evaporative dry eye.257–260

When compared with control groups, it has been found that subjects taking antiandrogen therapy have significant changes in their meibomian glands, including orifice metaplasia (a condition defined as an abnormal growth and keratinization of the duct epithelium),261 reduced quality of secretions, a striking alteration in the neutral lipid profile of secretions, and a morphologic appearance consistent with severe disease.258,264 Many of the lipid changes are all or none, identical in both eyes, and feature characteristic shifts in fatty acid patterns.262

In addition, patients have a significantly greater frequency of tear film (i.e., debris, abnormal menisci, and instability), conjunctival (i.e., tarsal injection, and inferior staining), cornal (staining), and lid (i.e., irregular posterior lid margins, sleeves, and collarettes) abnormalities, as well as an increased appearance of ocular surface symptoms (i.e., light sensitivity, painful eyes, and blurred vision).258 These findings add to another that determined that leuprolide acetate administration to reduce testosterone levels is associated with ophthalmic problems and blurred vision in some patients.262 These results may help explain the significant association between the use of medications to treat benign prostatic hyperplasia and dry eye disease.253

Researchers have also discovered that androgen receptor dysfunction in patients with CAIS is associated with a significant increase in dry eye signs and symptoms. This particular group of patients have a significantly higher frequency of meibomian gland orifice metaplasia and irregular posterior lid margins, as well as a decreased quality of meibomian gland secretions, when compared to normal, age-matched males and females. Patients with CAIS also have striking alterations in the neutral and polar lipid patterns of their meibomian gland secretions, relative to those of normal male and female controls.257,259 The normal aging process is also associated with a significant reduction in the quality of meibomian gland secretions and a significant increase in the frequency of metaplasia of meibomian gland orifices.105,264 Both the polar and neutral lipid profiles of meibomian gland secretions are significantly altered with aging.105,264 These findings were observed when comparing 37- and 70-year-old patients, and the period between the fourth and eighth decades coincides with a dramatic decline in androgen levels in both sexes.251

In addition to these observations, investigators have found that patients with nonautoimmune dry eye and MGD are androgen-deficient,205 and others have observed that the topical administration of DHEA, an androgen precursor, to humans, rabbits, and dogs stimulates the production and release of meibomian gland lipids and prolongs the tear film breakup time (TBUT).266 Further, studies have reported that reduced serum levels of testosterone are more prevalent in women with dry eye and correlate with the subjective severity of ocular symptoms267,268 and that serum testosterone concentrations correlate positively with meibomian gland secretion volume and orifice diameter in pre- and postmenopausal women, respectively (Suzuki T, et al. IOVS 2007;48:ARVO E-Abstract 434; Suzuki T, et al. IOVS 2008;49:ARVO E-Abstract 92). However, the meaning of the results in the latter studies is unclear, given that serum testosterone levels represent only a very small fraction (<0.2% in women) of the total androgen pool in humans268 and have little or no value except as an index of ovarian activity.251,268,269 The majority of or all androgens (75% before and 100% after menopause) in women are synthesized in peripheral tissues from adrenal sex steroid precursors (DHEA and DHEA-sulfate).269 Perhaps the only valid and reliable estimate of the total androgen pool in humans is the serum concentration of conjugated dihydrotestosterone metabolites (androsterone glucuronide and androstane-3α 17β-diol-glucuronide)251,270,271 that reflect the total intracrine production and metabolism of androgens in peripheral tissues throughout the body.269

Overall, these findings suggest that the meibomian gland is an androgen target organ, androgens promote lipogenesis and suppress keratinization within this tissue, and androgen deficiency may lead to MGD and evaporative dry eye. This apparent interrelationship of androgen deficiency with MGD and evaporative dry eye may help to explain why topical or systemic androgen administration has been reported to alleviate the signs and symptoms of dry eye in women and men.260,272–275 Efforts directed at alleviating this endocrine imbalance (e.g., topical androgen application) may prove beneficial in the treatment of MGD and the associated evaporative dry eye, in androgen-deficient individuals. Consistent with this possibility are clinical trial results that suggest that treatment of MGD with topical testosterone would improve the quality of meibomian gland secretions and reduce ocular discomfort (Schiffman RM, et al. IOVS 2006;47:ARVO E-Abstract 5608).

Estrogens

Estrogen Regulation of Sebaceous Glands. Estrogens cause a significant decrease in the size, activity, and lipid production of sebaceous glands in a variety of species.145,181,192,279–283 Indeed, estrogen was once termed the prototype agent for the suppression of sebum production,282 and for several years, estrogen treatment was used to reduce sebaceous gland function and sebum secretion in humans.180,181,260,261,284,285

One mechanism proposed for this hormone action is that estrogen induces the release of lysosomal enzymes within sebocytes, leading to premature cellular destruction and decreased sebum output.282,286 Additional suggested mechanisms are that estrogens reduce testosterone uptake, interfere with testosterone’s conversion to dihydrotestosterone, and antagonize androgen action in the sebaceous gland.279,282,285 In fact, estrogens have been described as the mainstay of treatment to decrease the effects of androgens on the sebaceous gland.180 These antiandrogen actions of estrogens are dose-depended, and may be overridden by treatment with physiologic levels of androgens.181,260

Of interest, androgen treatment causes a significant decrease in the number of estradiol-binding sites,177,286 and both hormones antagonize each other’s modulation of their own receptors286 in sebaceous glands. Further, some androgen effects are believed to be dependent on low levels of estrogen.286

Estrogen Regulation of the Meibomian Gland. The meibomian gland contains estrogen receptor mRNA and protein250,266,289 and estrogen administration to ovariectomized mice appears to result in characteristic alterations in glandular morphology.290 Estradiol-17β also regulates the expression of almost 200 genes in mouse meibomian glands,291 including those related to tyrosine kinases (fibroblast growth factor receptor 1) immune factors (interleukin 1 receptor, type II), extracellular matrix components (matrix metalloproteinase 3), steroidogenesis (17β-hydroxysteroid dehydrogenase 7, which converts estrone to biologically active estradiol292), prolactin activity (prolactin receptor), and lipid metabolism, to name a few.

Estrogen increases the expression of selected genes associated with lipid dynamics, such as phosphatidylcholine transfer protein,293 which replenishes the plasma membrane with...
phosphatidylcholines, and downregulates others including carboxyesterase 3, a lipase. These hormone actions suggest that 17β-estradiol promotes lipid production in the meibomian gland; however, researchers have found that most of estrogen’s effects are not consistent with this conclusion. Rather, 17β-estradiol seems to have an overall negative influence on lipid generation. For example, estrogen stimulates the expression of several genes involved in lipid and/or fatty acid catabolism (the anti-lipogenic STAT5A and STAT5B) and suppresses genes involved with lipid biosynthesis, mobilization, processing, and membrane trafficking.

Given these latter antagonistic effects, as well as the impact of estrogens on sebaceous glands in general, it is logical to presume that estrogen treatment reduces lipid synthesis in the meibomian gland and promotes both MGD and evaporative dry eye. The following studies support this statement:

- An epidemiologic evaluation of 25,665 postmenopausal women found that those who receive estrogen replacement therapy had a significantly higher prevalence of severe dry eye symptoms and clinically diagnosed dry eye syndrome than did women who never received the treatment.
- An assessment of 44,257 women with dry eye showed that one of the highest prevalences of comorbid conditions was the use of estrogen replacement therapy.
- Estrogen treatment of women in two studies led to tear film instability, foreign body sensation, contact lens (CL) intolerance, and ocular surface dryness.

Other genes in the meibomian gland are suppressed by 17β-estradiol, but stimulated by testosterone. Some of these genes encode for secreted acidic cysteine-rich glycoprotein (regulates cell growth), vascular endothelial growth factor A (promotes cell migration), cathepsin K (degrades extracellular matrices), and matrix metalloproteinase 3 (degrades fibronectin, laminin, gelatins, and collagens). These genes could be involved in cell maturation, migration, and holocrine secretion in the meibomian gland. If so, these hormone responses would be consistent with an antisebaceous gland effect of estrogens and a prosebaceous gland effect of androgens.

Progestins

Progestin Regulation of Sebaceous Glands. It was once believed that progestereone was the trophic hormone that regulated sebaceous gland secretion in women analogous to androgens in men, because progestin treatment significantly increased sebum production. However, earlier and later studies disagreed, finding that progestin administration had no effect on sebaceous gland output, and still others reported that these hormones reduce sebaceous gland function by inhibiting local androgen metabolism and activity. One explanation of these conflicting findings is that progestin’s effect on different types of sebaceous glands seem to be significantly influenced by the dose, endocrine environment, and the subject’s sex.

Progestin Regulation of the Meibomian Gland. The meibomian gland contains progestosterone receptor mRNA and protein and responds to progestosterone exposure with an apparent change in morphology. The addition of progestin to estrogen hormone replacement therapy also causes a significant decrease in the estrogen-related symptoms of dry eye, which may reflect a positive influence on meibomian gland activity. A definitive link between the action glandular progestin and ocular surface symptoms has yet to be shown.

Researchers have demonstrated recently that progesterone has a significant influence on gene expression in the mouse meibomian gland. Most genes are downregulated by progesterone, including those associated with immune processes, gluconeogenesis, and energy transduction. The most striking effect is the downregulation of all genes related to ribosome biogenesis, assembly, and structure, suggesting that progesterone has an overall suppressive impact on protein, macromolecule, and cellular biosynthesis in the meibomian gland.

Combined administration of progesterone with estradiol-17β also has a significant effect on the expression of more than 300 genes in the mouse meibomian gland. Most molecular biological responses duplicate those of estradiol or progesterone treatment alone. However, some do not, including a unique upregulation of genes involved with the localization ontology. The explanation of this combined progestin/estrogen response is unclear.

Sex Steroid Involvement in Sex Differences in the Meibomian Gland

In summary, sex steroids have a significant impact on meibomian gland function and, depending on the specific steroid, may prevent or promote MGD and evaporative dry eye. In addition, the differential action of sex steroids, as with other sebaceous glands, may well mediate the sex-related differences known to occur in the morphologic appearance, gene expression, neutral and polar lipid profiles, and secretory output of the meibomian gland. Consistent with this proposal is the observation that androgens appear to mediate almost 30% of the sex-associated variations in gene expression of the mouse meibomian gland. Moreover, it is possible that sex-specific aromatase activities also play a role in the sex-related differences of the meibomian gland (Liu S, et al. IOVS 2007;48:ARVO E-Abstract 5657).

Effect of all-trans Retinoic Acid on the Meibomian Gland

The compound all-trans retinoic acid decreases sebocyte growth, development, and lipid production and causes blepharoconjunctivitis. It is also known to be converted in sebocytes to 13-cis retinoic acid (isotretinoin), which leads to thickening and keratinization of meibomian gland ducts, degeneration and necrosis of meibomian gland acinar cells, periacinar fibrosis, and reduced lipid content of meibomian tissue in animal models. Isotretinoin is the agent that has revolutionized the dermatologic treatment of severe acne over the past several decades.

In humans, the administration of 13-cis retinoic acid results in blepharoconjunctivitis, abnormal meibomian gland secretions, meibomian gland atrophy, decreased TBUT, increased tear film osmolarity, and dry eye signs and symptoms. In effect, the retinoic acid derivatives promote MGD and evaporative dry eye.

Major mechanisms involved in the action of retinoic acid include the suppression of androgen receptor mRNA and protein and the inhibition of retinol dehydrogenase-4, which leads to a decrease in the local production of dihydrotosterone. Understanding the influence of retinoic acid on the meibomian gland is very important, given that this compound is the key ingredient of many antiaging cosmetics for use around the eye, and as people age they become more susceptible to the development of dry eye.

Influence of Growth Factors and Other Agents and Conditions on the Meibomian Gland

Various other components and conditions are known to influence the physiology and pathophysiology of the meibomian gland. These include:
• epidermal growth factor and bovine pituitary extract, which promote the proliferation and possibly the differentiation, of immortalized human meibomian gland epithelial cells (Liu S, et al. IOVS 2009;50:ARVO E-Abstract 3669). Epidermal growth factor has also been shown to stimulate the differentiation of rabbit meibomian357 and human sebaceous glands155 gland epithelial cells in vitro. Treatment of cancer patients with epidermal growth factor inhibitors is associated with the appearance of MGD (Joshi J, et al. IOVS 2008;49: ARVO E-Abstract 2365);
• topical epinephrine, which induces hyperkeratinization of the duct epithelium and leads to plugging and dilatation of the meibomian gland350;
• ω-3 fatty acid, the intake of which has been correlated with variations in the polar lipid profile120 and saturated fatty acid content119 of human meibomian gland secretions. In addition, a reduced intake of ω-3 fatty acids has been found in women with Sjögren’s syndrome,559 and these women typically have MGD340 (Krenzer KL, et al. IOVS 1999;31:ARVO Abstract 2864);
• hypothalamic hypogonadism, which was found to be associated with obstructive MGD in a 55-year-old male patient.162 The clinicians proposed that this lid condition was due to androgen deficiency162;
• aldosterone, possibly, given that mineralocorticoid receptors are present in human sebaceous glands.341 However, a potential aldosterone target in human meibomian gland tissue is unknown. Aldosterone has no effect on the neutral lipid or ganglioside composition of rabbit meibomian glands342;
• multiple endocrine deficiency (Addison’s disease and hypoparathyroidism), which was found to be associated with severe MGD.343 Given this finding, it is of particular interest that parathyroid hormone receptor mRNA is present in the meibomian gland (Table 3), but whether this receptor mRNA is translated and is functional has yet to be determined.

It is also intriguing that the human meibomian gland, like other sebaceous glands,200,544-547 secretes not only lipids, but also proteins.154 Such proteins may significantly influence the stability of the tear film as well as the appearance of the ocular surface.154 However, the regulation and role of this protein secretory process await clarification.

Clearly, further studies are needed to delineate the nature and extent of the endocrine, neural, nutrient, and growth factor (among others) influence on the meibomian gland, to increase our understanding of the physiological mechanisms controlling this tissue in both health and disease.

Pathophysicsology and Pathology

Hyperkeratinization

Hyperkeratinization is a major reason for obstructive MGD and causes degenerative gland dilatation and atrophy without inflammation. That this is a typical pathology of the meibomian glands comes as no surprise in view of the glands’ embryologic development. Hyperkeratinization could represent the removal of a developmental block of full keratinization (cornification) of the ductal epithelium that occurs because of various internal and external factors, rather than a de novo acquirement. Factors increasing epithelial keratinization and meibomian gland obstruction range from advancing age50,87,548 and hormonal disturbances,547,291,349 to the toxic effects of medication and chemicals50,551 and the breakdown products of meibomian lipids.41,352,553 or influences of external factors such as epinephrine eye drops,554 to CL wear.555

Obstructive MGD due to hyperkeratinization was first described by Korb and Henriquez2,555 in patients who had only minimal or transient symptoms suggestive of ocular dryness, but became clinically symptomatic because of CL intolerance. Manual expression of their meibomian glands verified an obstruction of the orifices and revealed hyperkeratotic clusters consisting of desquamated epithelial cells and thickened meibum. After expression and removal of the plugs, the tear film normalized and CL intolerance disappeared. Histology of obstructed glands verified dilatation of the central duct by cell debris and sebaceous material.3,556

Later histologic examinations of meibomian glands from patients with symptomatic dry eye disease who had inspissation of duct orifices and expressible highly viscous meibum, verified signs of obstruction of the excretory duct by increased keratinization. Inside the gland, this resulted in obstruction and dilatation of the ducts as well as cystic degeneration and loss of secretory meibocytes (Figs. 18, 19) that were replaced by a squamous metaplasia of the acinar epithelium.557 These alterations occurred without the presence of inflammatory leukocytes. It can be assumed that the observed acinar degeneration and atrophy that follows dilatation of the ductal system and results from obstruction of the gland leads to a later secondary hyposecretion due to the loss of secretory meibocytes. A similar pathology was observed in glands with cystic dilatation that were obstructed by surgical procedures or by neoplasia. Also in these cases, dilatation of the ductal system was reported, together with atrophy of the acini.51 The dilatation of the ductal system appears more pronounced in this condition when compared with findings in obstructive MGD.557 A large histopathologic study of the meibomian glands in 72 autopsies confirmed the morphology of cystic dilatation of ducts and acini and reported these pathologic alterations in 34.7% (25) of the cases.558 At present, obstructive MGD (Figs. 18, 19) appears to be the disease’s most common form.3,4,10,557-562

Animal Models of MGD

Several naturally occurring or induced animal models of MGD have been identified or developed. These models, in turn, may be very useful in exploring the pathophysiological mechanisms underling this condition. Rabbit,520,358,354,365,364 and monkey models that feature hyperkeratinization have been produced by the topical application of epinephrine,538,354,365,364 and polychlorinated biphenyl poisoning.565 As shown in Figure 20A, a common histopathologic finding in these rabbit and monkey models is an abnormal dilatation of the ducts, which show lumina filled with keratinized materials. In addition, the epithelium of the ductal orifices is typically hyperkeratinized and obstructed (Fig. 20B). It is currently believed that hyperkeratinization of the orifice itself, as well as the ductal epithelium, contributes significantly to the luminal plugging observed in obstructive MGD.

Mouse models of MGD and meibomian gland hyperplasia (MGH) are also available. These are either natural or have been generated by immunization, mutation, or transgenic or knock-out technologies (Table 4). The resulting strains may present a variety of phenotypes, such as ductal hyperkeratinization, acinar cell loss, and progressive glandular atrophy; or no meibomian gland; or glandular enlargement (Table 4). Some of these models may well have utility for studies of evaporative dry eye and corresponding ocular surface sequelae. Consistent with this proposal are the findings that the meibomian gland atrophy in acyl-CoA:cholesterol acyltransferase-1– knockout mice is associated with corneal erosions,366 and that the meibomian gland’s absence in X-linked anhidrotic–hypohidrotic ectodermal dysplasia is often paralleled by corneal defects (e.g., neovascularization and keratinization) and ocular surface inflammation.567 The ocular surface problems in these models, however, may not solely depend on glandular impairment.567
FIGURE 18. Comparison of the structure of a normal and an obstructed human meibomian gland. (A, B) A histologic section through a normal meibomian gland at the inner lid border. (A) The terminal part of the central duct (cd) and the terminal acini are encircled by fibers of Riolan’s muscle (riol), which represents the marginal inner part of the orbicularis muscle (orb) and is split by the downgrowth of the ciliary (c) hairs (compare with Fig. 5). The free lid margin is covered by the keratinized epidermis (ep), which transforms at the inner lid margin into the conjunctival mucosa (conj). The section does not pass through the orifice of the central duct (cd). (B) In a magnification of (A), it is seen that the connecting ductules (de) from the acini (a) of a normal gland are typically narrow and enter the central duct in an oblique direction. (C–E) Section through a meibomian gland with obstructive MGD. (C) The orifice (open arrow) is in the typical position, still within the keratinized epidermis, which extends for about half a millimeter into the central duct and forms an excretory duct. Even though the obstruction is not very advanced, as judged from the moderate dilatation of the central duct (cd), there are distinct alterations of the gland structure. The cd is already partly dilated, the epithelium of the wall is thinner than in the normal gland, and the wall is partly undulated. (D) The orifice is obstructed by numerous keratin lamellae (small arrows). (E) The secretory acini (a) are distinctly smaller and more roundish than in a normal gland, whereas the ductules (de) are dilated and enter the central duct (cd) at about right angles (small arrows). An atypical lumen (asterisk) has formed within the acini, and the secretory meibocytes are reduced in number and form only a few remaining cell layers. In one location, the residual meibocytes of a presumably disrupted acinus appear integrated into the wall of the central duct (double arrowhead). Inflammatory leukocytes are not apparent. Taken together, these findings indicate atrophy of the dilated meibomian gland. Light microscopic images of paraffin-embedded sections stained with hematoxylin and eosin (H&E); size markers are shown in the images. Reprinted from Knop E, Knop N, Brewitt H et al. [Meibomian glands, Part III: meibomian gland dysfunction (MGD)—plaidoyer for a discrete disease entity and as an important cause of dry eye.] Meibom-Drüsen, Teil III: Meibomdrüsen Dysfunktionen (MGD)—Plädoyer für ein eigenständiges Krankheitsbild und wichtige Ursache für das Trockene Auge. *Ophthalmologe.* 2009;106:966–979 with the kind permission of Springer Science and Business Media.


FIGURE 20. Epinephrine-induced MGD in rabbit. (A) The lumina of the dilated ducts are filled with keratinized material, representing keratin lamellae that are shed from the hyperkeratinized ductal wall. (B) The epithelium of the orifice was also hyperkeratinized and obstructed. Figure courtesy of Hiroto Obata.
Additional mouse models that display significant alterations in sebaceous gland structure and function (Table 4) may also serve as MGD or MGH models, but this possibility has not yet been evaluated.

**Cytology of Meibum: Meibomian Secretion**

It is believed that normal meibomian gland secretion (i.e., the meibum), is clear; however, it may have turbid, inspissated, or toothpaste-like consistency in MGD (Fig. 21A).

To examine the cytologic features of turbid meibum, impression cytology of the meibum was performed in a large study by Obata et al. (IOVS 2002;43:ARVO E-Abstract 60) in 50 elderly patients at least 60 years of age. The results demonstrated that keratinized materials were present in almost all cases, similar to the material first described by Korb and Henriquez and later analyzed by Ong et al. with biochemical and immunologic methods. Inflammatory cells were not detected in most cases (Fig. 21B), which may serve as a supportive indication that inflammation, at least based on the immigration of inflammatory leukocytes, does not represent a major etiologic factor in obstructive MGD (Obata H, et al. IOVS 2002;43:ARVO E-Abstract 60), as previously observed in histol-

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### Table 4. Possible Mouse Models of MGD, MGH, and Evaporative Dry Eye Syndrome

<table>
<thead>
<tr>
<th>Condition</th>
<th>Factor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene knockout</td>
<td>Stearoyl-coenzyme A desaturase 1 (3 types)</td>
<td>No meibomian gland</td>
</tr>
<tr>
<td></td>
<td>Ectodysplasin-A (3 types)</td>
<td>No meibomian gland</td>
</tr>
<tr>
<td></td>
<td>Ectodysplasin-A receptor (3 types)</td>
<td>No meibomian gland</td>
</tr>
<tr>
<td></td>
<td>Acyl-CoA:cholesterol acyltransferase-1</td>
<td>Meibomian gland atrophy, corneal erosions</td>
</tr>
<tr>
<td></td>
<td>Melanocortin-5 receptor</td>
<td>Decreased production of sebaceous lipids</td>
</tr>
<tr>
<td></td>
<td>Smad4</td>
<td>Ectopic row of hair follicles in place of meibomian glands (distichiasis)</td>
</tr>
<tr>
<td></td>
<td>Aire</td>
<td>T cell infiltration in meibomian glands</td>
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<tr>
<td></td>
<td>Blimp1</td>
<td>Enlarged meibomian glands</td>
</tr>
<tr>
<td></td>
<td>Tumor necrosis factor receptor-associated factor 6</td>
<td>Modified meibomian glands</td>
</tr>
<tr>
<td>Transgenic or gene overexpression</td>
<td>Human apolipoprotein C1</td>
<td>Meibomian gland atrophy</td>
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<tr>
<td></td>
<td>Biglycan overexpression, under control of the keratocyte-specific keratanan promoter</td>
<td>Meibomian gland aplasia</td>
</tr>
<tr>
<td></td>
<td>Rat erbB2 overexpression in basal layer of mouse epidermis, under control of the bovine keratin 5 promoter</td>
<td>Sebaceous gland enlargement</td>
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<tr>
<td></td>
<td>Smad7 or parathormone-related protein overexpression</td>
<td>Sebaceous gland hyperplasia</td>
</tr>
<tr>
<td></td>
<td>c-Myc overexpression</td>
<td>Enhanced sebum production</td>
</tr>
<tr>
<td></td>
<td>Ki-14-noggin</td>
<td>Formation of ectopic pilosebaceous units at the expense of meibomian glands</td>
</tr>
<tr>
<td></td>
<td>Ectodysplasin-A</td>
<td>Sebaceous gland hyperplasia</td>
</tr>
<tr>
<td></td>
<td>Ectodysplasin receptor</td>
<td>Enlarged meibomian glands</td>
</tr>
<tr>
<td></td>
<td>Keratin 5-glucocorticoid receptor</td>
<td>No meibomian gland</td>
</tr>
<tr>
<td>Mutation</td>
<td>Rhino</td>
<td>Meibomian gland ductal hyperkeratinization, acinar cell loss and eventual atrophy</td>
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<tr>
<td></td>
<td>Rough fur (ruf)</td>
<td>Sebaceous gland hypertrophy</td>
</tr>
<tr>
<td></td>
<td>Downless (dl) locus</td>
<td>Meibomian gland defects</td>
</tr>
<tr>
<td>Experimental systemic lupus erythematosus (SLE)</td>
<td>Murine immunization with a human monoclonal anti-DNA antibody, bearing a major Id 16/6Id</td>
<td>Hypertrophic meibomian glands and chronic eyelid inflammation</td>
</tr>
<tr>
<td>Natural</td>
<td>Crinkled</td>
<td>No meibomian gland</td>
</tr>
<tr>
<td></td>
<td>Bare skin</td>
<td>Sebaceous glands rudimentary</td>
</tr>
</tbody>
</table>

* See Refs. 82, 320, 338, 354, 363–384.

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**FIGURE 21.** Features of pathologic meibum. (A) Yellowish white, turbid meibum from a 72-year-old woman. (B) Impression cytology of yellowish white, turbid meibum from a 71-year-old man. An orange, keratinized material is seen on the nitrocellulose membrane. Cellular components such as inflammatory cells are not seen. Papanicolaou staining. Figure courtesy of Hiroto Obata.
Acinar Atrophy

In addition to the large number of reports that show the causative influence of hyperkeratinization and gland obstruction on the clinical picture of obstructive MGD as the most frequent pathology found in the meibomian gland, there is also evidence that the gland may undergo a degenerative atrophic process with progressive destruction of the tissue inside the gland.353,354,356,357,358 Atrophic degeneration can be explained by the increase in intraglandular pressure due to the stasis of continuously produced secretum. There is also evidence that atrophy occurs with advancing age, as in other organs of the body.355,356,357

Influence of Aging. A natural aging process is indicated, not only by increasing alterations of the posterior lid margin, as described by Hykin and Bron,358 but also by a decreasing number of active glands, as evidenced by vital stains.359 Diagnostic expression,360 and a decrease in visible gland tissue (gland dropout).361 Norn50 found that the number of active glands, as evidenced by vital stains,50 and a decrease in visible gland tissue (gland dropout). Norn50 found that the number of active glands, as evidenced by vital stains,50 and a decrease in visible gland tissue (gland dropout).50

The influence of aging on the mouse meibomian gland was investigated in a recent study by Nien et al.87 who observed several indications of a reduced gland function in the sense of atrophic changes due to an alteration of factors that are essential for the maturation of sebaceous glands. Immunohistochemical results showed that with increasing age, a significant decrease in mitosis in the proliferating cells, as evidenced by Ki67 staining, occurred in the basal meibocyte layer. This reduction was paralleled by a decrease in the size of the acini and a relocation of the meibocyte maturation marker PPAR-y from a cytoplasmic expression in young (2 months old) and young adult (6 months old) animals to a nuclear expression in old (2 years old) animals. Also, a decrease in lipid production, as verified by oil red O staining, from numerous smaller lipid droplets in young individuals to fewer and larger ones in old individuals, was observed. In addition, cells that were positive for BLIMP1 and for the bone marrow cell marker molecule CD45 were observed around the acini. It is not clear from these observations whether the increase in the number of these cells, which occurred in the old animals and was assumed to reflect increased infiltration of leukocytes, represented an event accompanying the atrophic alterations or whether it contributed to the development and progression of acinar atrophy as a causative factor.

Age-dependent alterations in humans were reported by Obata et al.,359 who described acinar atrophy without distinct dilatation as one of the pathologic findings in meibomian glands, which may suggest a primary acinar atrophy that leads to a decrease in the meibomian gland secretion with aging. Atrophic acini were observed as small and irregularly shaped acini, as opposed to normal round-shaped acini, Fig. 22). Age-dependent alterations in humans also were reported by Arita et al.355 who, using the technique of infrared meibography, showed a strong age-dependent increase in meibomian gland dropout (disappearance of the glandular tissue inside the tarsal plates) between the third and eighth decades of life. This increase was accompanied by a decrease in TBUT. At present, it is not clear whether gland dropout really indicates the physical disappearance of glandular tissue or whether it becomes invisible because it assumes the characteristics of the surrounding conjunctival tissue. Later, the same group observed that CL wearers also had a high meibomian gland dropout rate.355 Young CL wearers had a dropout rate comparable to normal individuals in their 80s. The dropout was dependent on CL wearing time in lifetime years but independent of the type of lens. The pathogenesis of gland dropout in CL wearers is not yet clear, as discussed by Arita et al.355 and may include obstructive events as well as chronic mechanical traumatization of the conjunctival and tarsal tissue. Inflammatory events, as observed in giant papillary conjunctivitis can also result in gland dropout.360 It can hence be assumed that inflammatory mediators find their way from the conjunctival tissue through the tarsus toward the meibomian gland and conceivably contribute to gland dropout and also potentially to acinar atrophy, as discussed by Knop and Knop.360

The age-dependent atrophic changes in the mouse meibomian gland361 were different from those typically observed in acinar atrophy due to obstructive MGD, because they did not show the well-known hyperkeratinization and dilatation of the ductal system and the acini.334,351,357,258 It is not clear at present how this may relate to the supposed primary atrophy occasionally observed in humans.358,359 Therefore, there is apparently a difference in the mechanisms of atrophy in murine age-dependent atrophy versus that based on pathologic obstruction. Both of these atrophic events, however, most likely result in a decrease in oil production by the meibomian glands, with downstream negative influences on the ocular surface and tear film.

While the possibility should be considered that there is a primary, age-dependent form of MGD that leads to a gradular decline of glandular function358 (Figs. 22), the predominant form of MGD apparently is due to hyperkeratotic obstruction of the gland, which presents the characteristic signs of dilatation (Figs. 18, 19).
Atrophic events occur also in other ocular glands, such as the acinar atrophy in the human lacrimal gland that was also predominantly found in aged tissues.\(^{390}\) Extended periods of autonomic denervation and transient denervation by intraglandular injection of botulinum toxin cause acinar atrophy in salivary glands.\(^{391,392}\) Straatsma\(^{31}\) speculated that obstruction of the orifices enlarges the ducts and acini, which encounter resistance from the dense connective tissue of the tarsus, and thus leads to cystic changes. Eventually, increasing intracystic pressure may inhibit cell differentiation. Turning our attention to other tissues, it is well known that obstruction by duct ligation, for instance, causes atrophy in the salivary gland,\(^{391}\) and it can be assumed that this effect is based on mechanisms similar to those in the meibomian glands. Ischemia or hypoxia causes atrophy in the prostate and pancreas.\(^{392,393}\) Such phenomena are well known as disuse atrophy, which is a common consequence of glandular obstruction.

**Basement Membrane Thickening of Acini.** Basement membrane thickening of acini is frequently associated with acinar atrophy and can be observed in hematoxylin and eosin (H&E) staining, but more clearly in periodic acid-Schiff (PAS) staining\(^{387}\) (Fig. 22B). It is unclear whether the basement membrane thickening is a primary or a secondary change after acinar atrophy. In any case, basement membrane thickening may interfere with blood supply from capillaries around the acini and may hence negatively influence the homeostasis of the meibomian gland.

**Influence of Blood Supply.** Large blood vessels are not present in the tarsus and are inconspicuous in routine H&E-stained paraffin-embedded sections. Blood vessels in the tarsus are best observed by immunohistochemistry with a marker of vascular endothelium. This labeling shows that capillary vessels surround the acini (Fig. 23) and that they contribute to the nutrition of the meibomian gland.

**Inflammation**

McCulley et al.\(^{394}\) stated that primary meibomitis did not appear to be a primarily infectious entity, but represents a facet of generalized sebaceous gland dysfunction. The definition and involvement of inflammation in MGD have been unclear in the past for several reasons. First, there have been several coexisting terms, such as posterior blepharitis, meibomitis, and MGD, used interchangeably and ambiguously in distinguishing definitively between the different disease entities. Second, there has been an ongoing discussion of how to define inflammation. It can be defined as an inflammatory cell infiltration in classic pathology or as the involvement of inflammatory cytokines in current molecular biology, even when no inflammatory cell infiltration is found by light microscopy. The latter would constitute a proinflammatory state, as opposed to overt inflammation, that would still be able to alter the differentiation of the tissue. Third, inflammation can be divided into two categories: infectious and noninfectious. Although it was found that the observed bacteria were commensal species that occur in normal individuals and, in a larger number, in those with blepharitis,\(^{41,42,353}\) it reflects an increased growth rather than an infection.\(^{42}\) Unifying definitions for these findings have been suggested.

Few reports are available that mention an inflammatory cell infiltration in the meibomian glands in MGD. In a histopathologic study of tarsal tissues obtained at autopsy,\(^{358,387}\) lipogranulomatous inflammation and granulation tissue showing inflammatory cell infiltration were observed. However, it is not certain whether these changes represent pathologic features of MGD. It is important to note that, in the presently available studies, an inflammatory cell infiltration was not observed in the present report.

**FIGURE 22.** Acinar atrophy of human meibomian gland. (A) Acinar atrophy: Atrophic acini show a small and irregular, not rounded, shape (arrows); the duct appears slightly dilated. No inflammatory cell infiltration is seen. Figure reprinted from Obata H, Horiuchi H, Miyata K, Tsuru T, Machinami R. Histopathological study of the meibomian glands in 72 autopsy cases (in Japanese). *Nippon Ganka Gakkai Zasshi*. 1994;98:765–771 with permission from the Japanese Ophthalmological Society. (B) Basement membrane thickening of the acini: Basement membrane thickening (arrows) is frequently associated with atrophy of acini. Periodic acid-Schiff (PAS) staining. Figure courtesy of Hiroto Obata.

**FIGURE 23.** Capillary vessels in a normal human meibomian gland. Immunostaining of factor VIII, a marker of vascular endothelial cells, reveals capillary vessels surrounding the acini. Figure courtesy of Hiroto Obata.
specimens of cystic dilatation and acinar atrophy of the meibomian gland. In contrast, in vivo confocal microscopy reported inflammation in the tarsal conjunctival epithelium and stroma in patients with blepharitis and meibomitis, suggesting that the presence of an inflammatory infiltrate enables the differentiation between MGD and meibomitis. In another in vivo confocal microscopy study, a periglandular inflammatory cell infiltration was observed in the eyelids of patients with obstructive MGD, and the infiltration was cleared by treatment with topical levofloxacin, topical 0.1% flurometholone, and oral minocycline. The differentiation between individual cell types is difficult in confocal microscopy, however, and frequently it is not possible to differentiate clearly between activated stromal cells and leukocytes. As such, in vivo confocal microscopy cannot identify the presence or absence of inflammation as clearly as has been achieved by histopathology.

If obstructive MGD increases the intraglandular pressure, ducal and acinar epithelia may undergo cell stress. It is speculated that this stress also triggers MAP kinase activity in the meibomian gland, with downstream release of chemokines and cytokines and ultimately the occurrence of inflammation, as observed in the epithelium of the conjunctiva and cornea. Further studies are needed to explore the involvement of inflammation in MGD.

**Infection and Therapy**

The pathophysiology of MGD is complex and whether bacterial infection is a cause of MGD remains controversial. It is well known that commensal bacteria such as coagulase-negative staphylococci (CNS), *Staphylococcus aureus*, and *Propionibacterium acnes* are related to and contribute to the pathologic course of chronic blepharitis. In contrast, Gutesell et al. described that the inflammation related to bacterial infection was not an important factor in obstructive MGD, because only a minimal or absent inflammatory cell infiltration was found in histopathology. Bacterial infection can, in principle, locally destroy the meibomian gland structure as seen in hordeolum. Such direct and active bacterial infection is reportedly not involved in the pathogenesis of MGD, bacterial products such as lipases and phospholipases could alter the lipid composition, influencing the tear film and causing evaporation. Lipase could alter the lipid composition, influencing the tear film and causing evaporation. Persistent accumulation of meibum inside the obstructed glands, and (3) was further verified by the presence of keratinized cell material in the opaque thickened meibum expressed from obstructed glands, and (3) was further verified by the presence of keratinized cell material in the expressed meibum of patients in molecular biological and immunologic assays (Obata H, et al. IOVS 2002;43:ARVO E-Abstract 60). Animal models of obstructive MGD also supported hyperkeratinization as a major causative factor.

When the delivery of meibum onto the lid margin and tear film is blocked by an obstruction, meibum accumulates within the ductal system of the gland due to the continuing secretion from the secretory acini. Persistent accumulation of meibum inside the obstructed glands conceivably results in a progressive increase in pressure inside the ductal system and thus in progressive widening of the ductal system. After a prolonged time, this increased internal pressure also extends into the secretory acini, via a widening of the small connecting ductules. The acini undergo atrophic changes, with a loss of secretory meibocytes and eventual squamous metaplasia that can result in full cornification of the epithelium of the ducts and acini. The consequence of acinar atrophy is a secondary hyposcrenation, and acinar atrophy is conceivably also the reason for the gland dropout seen on meibography. Acinar atrophy appears mainly as a pressure atrophy, because the dilatation of the meibomian gland is limited by the more rigid tarsal connective tissue, although other factors that interfere with cell differentiation may also contribute to it. It was pointed out in the earlier histopathologic description of meibomian gland dilatation in the human and verified by later studies that this process of obstructive atrophy of the meibomian gland occurs in the absence of overt inflammation and in the absence of inflammatory leukocytes in the glandular tissue. Although recent in vivo confocal investigations points to a potential presence of periglandular inflammatory cells in certain cases, this technique provides only limited information compared with histopathology. In light of the rapidly increasing knowledge of ocular surface immunology and tissue homeostasis, it can be hypothesized that at least subclinical inflammatory events contribute to the degenerative process. Inflammatory reactions are exerted by factors such as irritative lipid species (e.g., free fatty acids) or by proinflammatory downstream agonists (e.g., phospholipase A2).
Inflammatory cytokines, possibly triggered by MAP kinase 397 or therapy of obstructive MGD.6,389

Changes in the gland structure conceivably depends on the influence on cell differentiation.

This can be explained by the fact that the meibomian glands in the acini generates an increasing pressure inside the glands that leads to a gradual dilatation, first of the central duct. (D) Additional atrophy: After a prolonged time, the increased pressure inside the gland leads to dilatation of the connecting ductules and a pressure atrophy of the acini with rarefaction of secretory meibocytes. This effect causes shrinkage of the whole acini that may represent the histopathologic equivalent of the clinically detectable gland dropout and results in a presumed secondary hyposecretion. (E) Additional cornification of the glandular epithelium: In late stages the whole ductal epithelium can become cornified and the meibocytes replaced by a stratified squamous cornified epithelium. Reprinted from Knop E, Knop N. [Meibomian glands. Part IV: Functional interactions in the pathogenesis of meibomian gland Dysfunction (MGD).] Meibom-Dreüsen, Teil IV: Funktionelle Interaktionen in der Pathogenese der Dysfunktion (MGD). Ophthalmmologe. 2009;106:980–987 with the kind permission of Springer Science and Business Media.

A2, leukotrienes, or arachidonic acid359 and by induced inflammatory cytokines, possibly triggered by MAP kinase397 or other pathways. Such factors are also described in the obstructive alteration of the hair-associated sebaceous glands of the skin (acne).107 The extent of the dilatation and atrophic changes in the gland structure conceivably depends on the grade of obstruction and on the duration of the process, which points to the necessity of a timely diagnosis and appropriate therapy of obstructive MGD.6,389

Interacting Pathways in the Pathogenesis of MGD

Core Mechanisms. Many underlying factors such as age, sex, hormonal disturbances, and environmental factors, as well as changes in the composition of meibum, contribute to the pathogenesis of obstructive MGD. These factors appear to form different pathways that interact in an interrelated sequence of events (Fig. 25) and also give rise to several vicious circles that reinforce the process and can aggravate the dysfunction if they are not limited by effective and timely therapeutical interventions.

Obstruction. Obstruction of the meibomian gland as a core mechanism of MGD leads to two limbs of downstream consequences, both of which result in a decreased availability of meibomian lipids at the lid margin and tear film and hence in an evaporative dry eye condition: (1) directly via a low delivery of oil onto the lid margin and (2) indirectly via a stasis of meibum inside the gland that results in several downstream events, such as increased pressure, resultant dilatation, and, eventually, acinar atrophy, which causes low secretion.

Hyperkeratinization. Various endogenous and exogenous factors (Fig. 25) that can influence the pathologic course of MGD lead to hyperkeratinization of the epithelium at the lid margin and meibomian gland, either directly or by a possible influence on cell differentiation.

Hyperkeratinization appears as the main pathomechanism of MGD, as indicated by a large body of literature (Knop E, et al. IOVS 2009;50:ARVO E-Abstract 4833). This can be explained by the fact that the meibomian glands share strong similarities with the hair follicles of the cilia in embryologic development and structure.26–28 The meibomian glands have preserved an incipient stage of keratinization in the form of keratohyalin granules throughout the luminal layer of the ductal epithelium in animals52 and in the human.50 As mentioned earlier in the article, these observations have led to the statement that the meibomian gland can be regarded as a hair follicle without a hair shaft.24,431 The hyperkeratinization in obstructive MGD may therefore be caused by the removal of a developmental block that prevents progression of the natural incipient keratinization into full cornification. In disease, such as chronic blepharitis, the meibomian glands can still develop a hair, a condition known as distichiasis.432–433 Hyperkeratinization may also be influenced by an aberrant differentiation or migration of stem cells. The migration and differentiation of stem cells generally assumes a higher degree of plasticity due to wounding531 and probably also due to mechanical stress or to the occurrence of mediators of a subclinical inflammation. Such factors are shown to be influential in the development of acne in the hair-associated sebaceous glands.107 Other factors shown to increase keratinization of the lid margin and meibomian gland are topical or systemic medications and chemical toxins.305

With advancing age, degenerative changes, including hyperkeratotic events, generally increase at the posterior lid margin and also affect the orifices of the meibomian glands by orifice narrowing.518 Meibomian gland drop out, which conceivably reflects a loss of functional glandular tissue and represents an endstage of acinar atrophy, results in respective symptoms of ocular dryness that proceed strongly with aging.53

Hormonal influences on meibomian gland function are well described. Pathologic alterations (in particular of androgen action) result in hyperkeratinization of the epithelium at the lid margin, obstructive MGD with a lack of meibum on the lid margin and on the tear film, an altered lipid profile, and associated dry eye symptoms.105,257–260

Increased Viscosity. Besides hyperkeratinization, increased viscosity of meibum is the other most important pathogenetic
Figure 25. Pathways and proposed sequence of events that lead to self-enforcing vicious circles in MGD. Mechanisms and interactions (arrows) in MGD occur as a result of underlying causative factors (colored square boxes located in the periphery). The core mechanisms of gland obstruction due to ductal hyperkeratinization and increased viscosity of the meibomian oil (meibum) are shown in the center of the figure on a yellow underlay and result in two effector limbs (wide shadowed downstream arrows, also on yellow underlay). Associated functional complexes, such as progenitor cell differentiation, bacterial growth, inflammation, and seborrhea, are shown on color-shaded spherical zones around the core mechanisms. Dashed arrows depict likely interactions; functional complexes of likely but insufficiently clarified importance are shown in dashed circles. Vicious circles that result in a progressive process of dysfunction are indicated by red bent arrows. Hyperkeratinization of the epithelium of the excretory duct and orifice is the main factor that leads to obstruction of the meibomian glands. This effect is influenced by endogenous factors such as age, sex, and hormonal disturbances as well as by exogenous factors, such as topical medication. These may act, at least in part, via the release of a physiological inhibition of full keratinization and via an aberrant differentiation of progenitor cells. Increased viscosity of meibum through qualitative changes of its composition is the other important causative factor that contributes to the obstructive process. It may occur independently, because of the influence of endogenous or exogenous factors or a preexisting obstructive stasis of secretion. Obstruction leads on the one hand (left effector limb arrow) to the immediately clinically observable low delivery of meibum onto the lid margin and tear film that results in an evaporative dry eye condition. On the other hand (right effector limb arrow), obstruction also results in several consecutive negative effects directly inside the meibomian glands, because of an internal stasis of meibum. Stasis can be associated with increased viscosity of the meibum, which reinforces the obstruction in a vicious circle. The continuous secretory activity of the meibocytes leads to a progressive increase in pressure within the glands. This increased pressure can, in another vicious circle, induce an activation of the epithelial cells that reinforces hyperkeratinization. Pressure further leads to a dilatation, first of the ductal system and, after a prolonged time, also to atrophy of the acini, with rarefaction of their secretory meibocytes, and thus results in a secondary hyposecretion with low secretion of lipids. Atrophy may be the reason for the clinically detectable gland dropout, and CL wear is, by presently unknown mechanisms, associated with gland dropout. Stasis of meibum also promotes the growth of bacteria on the ocular surface and possibly inside the glands, usually pre-existing commensals, that produce lipid-degrading enzymes. Their action on the meibomian lipids leads to the production of toxic mediators, such as free fatty acids, that may initiate subclinical inflammatory reactions with release of inflammatory cytokines. Toxic and inflammatory mediators may promote subclinical inflammatory events inside the gland, in the periglandular conjunctiva, on the lid margin, and on the ocular surface, as suggested by observations in dermatology (e.g., in acne pathogenesis and skin irritation). Toxic mediators are also assumed to have negative effects on tear film stability. Furthermore, they can lead to qualitative changes in the composition of meibum that increase its viscosity or, through activation of epithelia on the lid margin and possibly inside the gland, they can reinforce keratinization. Altogether, these events can give rise to several vicious circles (red arrows) that increase the preexisting obstruction, if not limited by timely diagnosis and therapeutic intervention. There is evidence from a mouse model that acinar atrophy may also occur due to the aging process. If MGD occurs in conjunction with systemic skin diseases such as seborrheic dermatitis, possibly accompanied by blepharitis, an increased amount of oil (seborrhea) with decreased viscosity can be observed on the lid margin. Seborrheic blepharitis, similar to stasis, can be associated with increased bacterial growth and its downstream negative effects. The seborrheic oil has a different composition than that of normal meibum and thus may have negative effects on the tear film. All major mechanisms of the schematically depicted process are supported by findings in the literature. Reprinted from Knop E, Knop N. [Meibomian glands, Teil IV: funktionelle Interaktionen in der Pathogenese der Dysfunction (MGD). Ophthalmologe. 2009;106:980–987 with the kind permission of Springer Science and Business Media.

Increasing age, which brings about hormonal changes, is associated with increased frequency of changes in lipid composition, such as alterations of the polar and neutral lipid profiles. Qualitative lipid changes may result in an increased viscosity of meibum, as observed in the decrease in monounsaturated fatty acid, specifically oleic acid, in patients with chronic blepharitis. Since a decreased desaturation of lipid raises its melting point and hence leads to its thickening, this phenomenon can reinforce an obstructive process and explains an elevated rate of obstruction in blepharitis. A loss of the polar lipids that are assumed to maintain the adherence of the superficial nonpolar lipid layer to the aqueous tear film may contribute to an increase in tear film instability and evaporation in patients with MGD.

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factor in MGD. It is observed in all cases of obstructive MGD, also occurs in animal models and may be primary or secondary. A secondary change in obstructive MGD appears to be due to the stasis of meibum inside the ductal system of obstructed glands and to the potential influence of lipid degrading enzymes. Stasis of meibum thus can, in a vicious circle, further aggravate the obstruction. Highly viscous meibum is mixed with hyperkeratotic cellular material, as seen in expressed pathologic human meibum prepared as smears or in impression cytology (Obata H, et al. IOVS 2002;43:ARVO E-Abstract 60) (Fig. 21) and in histopathology. As verified by molecular biology and immunohistochemistry, increased viscosity has also been observed inside the obstructed glands of animal models (Fig. 20).
**Associated Functional Complexes.** Apart from the core mechanism of MGD as an obstructive process that is caused by hyperkeratinization of the meibomian duct and orifice, together with increased viscosity of meibum, there are also some associated functional complexes that interact with the pathogenesis.

**Altered Cell Differentiation.** An aberrant differentiation of stem cells and progenitor cells inside the meibomian glands or on the lid margin can result from alterations in the endogenous and exogenous underlying factors (such as age, sex, and hormone levels). In ductal cells, this change can lead to hyperkeratinization (as explained earlier) and may contribute to alterations in the lipid profile in the acinar cell. Alterations in cell differentiation can also generally be assumed to be involved in the aging process of the human and are shown in acinar atrophy of the mouse. Aberrant cell differentiation is also responsible for general pathologic alterations of the meibomian gland.

**Seborrheea.** For a long time, disease affections at the lid margin, including the meibomian gland, were simply termed blepharitis because a clear distinction between anterior and posterior blepharitis or MGD was not always made. In their blepharitis classification, McCulley et al. reported a large percentage of the seborrheic type of blepharitis. In most of these patients, it was associated with generalized skin disease, mainly seborrheic dermatitis, which affects areas of the skin rich in sebaceous glands. It frequently, but not always, presents with copious amounts of oil of low viscosity and is generally associated with hyperkeratinization. This helps explain why seborrheic dermatitis may be associated with hyperkeratotic obstructive MGD. Seborrheea is assumed to give rise to several feedback mechanisms that can reinforce qualitative and quantitative changes in meibum and epithelial hyperkeratinization. An increased sebum production is also known to promote the obstructive disease of the skin sebaceous glands in acne. In patients with chronic blepharitis and seborrhea, qualitative changes in lipid composition were observed, including a higher amount of monounsaturated fatty acids that lead to a decreased melting point and hence explain the fluid appearance of oil on the lid margin in this condition. Seborrheea, similar to obstructive MGD, is also associated with increased bacterial growth and hence shares the presence of bacterial lipid-modifying enzymes and respective alterations of the lipid composition as well as the downstream effects of toxic mediators and negative influences on the tissue and conceivably on the tear film. We can therefore link seborrhea with increased bacterial growth and subclinical inflammation in the pathogenesis of blepharitis. Definitions for the various subtypes of blepharitis and MGD can be found in the Definition and Classification Report.

**Influence of Bacteria: Commensal Bacterial Growth.** MGD can be associated with an increased growth of bacteria on the lid margin, but conceivably inside the obstructed ductal system as well, which represents an ideal undisturbed niche full of nutrients. Evaluation of bacteriology in patients with chronic blepharitis and commensal bacteria such as *P. acnes* as the most frequently isolated organism, found on 98% of lid margins. The same species were cultured from 52% of freshly expressed meibum samples after lid margin cleaning with a sterile swab. Even though this technique does not totally exclude the possibility of contamination of the freshly expressed meibum with bacteria from the lid margin, the findings in this study give a strong indication that commensal bacteria also may be naturally present inside the meibomian gland. It is important to note that this bacterial colonization does not represent an infection, but rather an increased growth of preexisting commensal species. The observation of a high degree of association of MGD with all the seborrheic groups of blepharitis led to the hypothesis that lipid abnormalities are a causative factor in the disease. In a later study, it was observed that the previously cultured bacterial species were able to degrade meibomian lipids by their lipid-degrading enzymes (lipases and esterases), which modify the normal meibomian lipids and lead to an altered lipid spectrum. This degrading influence resulted in significant changes in some free fatty acids. It is known that free fatty acids are irritants to epithelia, can penetrate the epidermal barrier, and can cause inflammation and hyperkeratinization. They are also recognized as factors that promote the obstructive disease in skin sebaceous acne. In particular, free fatty acids and fatty acid alcohols are thought to contribute significantly to chronic blepharitis, and it is assumed that they can irritate and activate the epithelium and stimulate keratinization. Increased availability of cholesterol from cholesterol esterase activity can further promote bacterial growth. The increased growth of commensal bacteria and the downstream presence of irritative lipid species and toxic mediators must be regarded as an influencing factor in MGD. These factors do not appear to present a primary cause of obstructive MGD, however, but rather a secondary phenomenon that becomes important due to a preexisting obstruction and stasis. Bacterial growth negatively reinforces hyperkeratinization and may play a role in qualitative and quantitative changes in the meibum.

**Inflammatory Mediators.** Increased bacterial growth is linked with subclinical inflammatory events through the release of lipid species, such as free fatty acids, that may irritate and act in a proinflammatory manner on the tissue and potentially on the tear film as well. Increased amounts of phospholipase A2 are found in the meibum of patients with blepharitis. Phospholipase A2 can induce the formation of arachidonic acid, an unsaturated fatty acid, from which prostaglandins and leukotrienes are synthesized. These factors have a central position in inflammatory processes. They can irritate and activate the ocular surface epithelium and it is speculated that they also result in tear film instability. Activated epithelial cells then produce inflammatory cytokines such as TNF- and interleukin- and promote a subclinical inflammatory microenvironment. Such inflammatory cytokines are also produced by normal and stressed sebocytes. The addition of IL-1 to isolated pilosebaceous units in vitro results in hypercornification, as discussed by Zouboulis. Similar events are described in the obstructive disease (acne) of the hair-associated sebaceous glands of the skin, as discussed by Kurokawa et al. In acne, three distinctive events occur. First, desaturated fatty acids, lipoperoxides, and commensal bacteria such as *P. acnes* that also occur at the lid margin can induce the production of inflammatory cytokines by epithelial cells. Second, inflammatory cytokines such as IL-1 activate epithelial cells and induce an alteration of normal epithelial differentiation toward increased proliferation and keratinization, which together result in obstructive sebaceous gland disease. Third, peroxides, such as the lipoperoxides that arise during the pathologic modification of meibomian lipids, can act as ligands for the transcription factor PPAR-γ, which promotes cell maturation and lipid production. These effects may contribute to the seborrhea in acne but also in the seborrheic blepharitis associated with MGD.

**Physiological Aging Process.** An age-dependent degeneration of the human meibomian gland that may reflect physiological age-related changes is indicated by various observations. In the human, degenerative changes, including hyperkeratosis
and meibomian orifice narrowing\textsuperscript{348} generally increase at the posterior lid margin with advancing age and are assumed to induce obstructive MGD. Aging also results in changes in the composition of meibum that are reflected by alterations in the polar and neutral lipid profiles,\textsuperscript{105} as explained earlier. Obstructive MGD may be responsible for the observed reduction by half in the number of actively secreting glands between the ages of 20 and 80 years,\textsuperscript{503} as well as for the drastic loss of functional glandular tissue (gland dropout)\textsuperscript{55} that is observed over the same age range and results in respective symptoms of ocular dryness. CL wear is found to cause alterations that resemble an increased aging process,\textsuperscript{555} although the exact pathomechanism that causes the gland dropout is not yet clear.

Apart from the changes in the meibomian glands that can occur secondary to obstruction, there are also indications that age-dependent alterations may affect the glandular tissue’s physiology directly.\textsuperscript{387} In a mouse model, Nien et al.\textsuperscript{87} observed several indications of reduced gland function in the sense of atrophic acinar changes due to an alteration of factors that are crucial for the differentiation and maturation of sebaceous glands. In old animals, a significant reduction in meibocyte mitosis is paralleled by a decrease in the size of the acini, by a relocation of the meibocyte maturation marker PPAR-γ, and by a decrease in lipid production. These alterations apparently occur without gland obstruction and hyperkeratinization. In contrast to the pathology in obstructive MGD, an increased number of bone marrow-derived cells occurs in the glandular tissue that are perceived as reflecting a cellular inflammatory reaction.

From these findings, it may be assumed that, similar to other organs in the body, the meibomian glands undergo a primary age-dependent form of degeneration that leads to a gradual decline in glandular function in contrast to a secondary pathologic obstructive MGD. In a mouse model\textsuperscript{87} the atrophic changes in the gland acini were apparently not accompanied by hyperkeratinization and obstruction of the ducts and orifices. These latter phenomena may represent useful criteria for differentiating between primary age-dependent degeneration and pathologic atrophic destruction of the glands due to obstructive MGD. On the other hand, since these atrophic changes in the mouse do not resemble the typical human pathology they may also represent species-specific differences compared with the typical human situation.

\textit{CL Wear.} CL wear is a widespread environmental factor associated with MGD.\textsuperscript{454} Although obstructive MGD was first described by Korb and Henriquez\textsuperscript{3} as causative in patients with CL intolerance, several later studies did not provide an unequivocal opinion\textsuperscript{356, 454 - 459} on the association of obstructive MGD with CL wear compared with non-CL wear.\textsuperscript{889} On the other hand, MGD therapy by lid hygiene was found by Paugh et al.\textsuperscript{456} to be effective in improving CL intolerance in such patients. Ong and Larke\textsuperscript{458} reported that the onset of CL wear results in an increased rate of MGD, independent of lens type, with block lenses and meibomian dysfunction (MGD)–plaidoyer for a discrete disease entity and as an important cause of dry eye. Meibomian, Teil III: Meibomian gland dysfunction (MGD)—plaidoyer for a ein eigenständiges Krankheitsbild und wichtige Ursache für das Trockene Auge. \textit{Ophtalmologe} 2009;106:966–979.


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The International Workshop on Meibomian Gland Dysfunction: Report of the Subcommittee on Tear Film Lipids and Lipid–Protein Interactions in Health and Disease

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Understanding the molecular composition (e.g., proteins and lipids) of the tear film (TF) and the contribution of the meibomian gland to the TF is critical in gaining knowledge about TF instabilities, dry eye syndromes, contact lens (CL) incompatibilities, and other eye diseases. Among its functions, the lipid layer of the TF slows evaporation of the aqueous component, preserves a clear optical surface, and forms a barrier to protect the eye from microbial agents and organic matter, such as dust and pollen.1 The TF contains a complex mixture of proteins, enzymes, lipids, mucins, and salts that allows the TF to perform its functions (Fig. 1). Researchers believe the outer lipid layer is 5 to 10 molecules thick and is composed primarily of wax and sterol esters, possibly intercalated with each other and with proteins rather than forming distinct repeating layers of molecules.2,3 Evidence from interferometric studies indicate that the TF lipid layer thickness ranges from 20 to 160 nm.4 If the size of a lipid molecule is approximately 2.2 nm (22 Å), then the calculated thickness for one layer would be 11 to 44 nm. The addition of polar and nonpolar layers would add to the lipid thickness, which indicates that the lipid component of the TF may be multiple layers thick or have other contributing sources to correspond with reported thickness measurements.5

While the signs and symptoms of TF instability are reasonably well characterized, we are only beginning to understand the specific molecular components of the TF and their relationship with disease and TF stability. The purpose of this review is to examine the meibomian gland’s contribution to TF lipids and lipid–protein interactions in health and disease.

**Review of the Tear Film Lipid Layer**

The meibomian glands are the main source of lipids for the human TF. The meibomian gland secretions consist of an extremely complex mixture of various polar and nonpolar lipids containing cholesteryl esters (CEs), triacylglycerol, free cholesterol, free fatty acids (FFAs), phospholipids, wax esters (WEs), and diesters.6–9 It was not until 1981 that the term meibum entered the lexicon, to describe these secretions.10 Current models of the TF originated in the 1950s11 and include three major, well-defined layers: the glycosalix layer, the intermediate aqueous layer, and the outermost tear film lipid layer (TFLL). The glycosalix layer, which covers the corneal epithelium, is believed to be relatively viscous because of the large amount of membrane-bound and secreted mucins. The aqueous layer is enriched in water-soluble proteins, mucins, and salts, whereas the TFLL is formed almost exclusively from lipids and attached and/or intercalated proteins.7 The TFLL is usually depicted as a two-layer structure: polar lipids form the lower sublayer and nonpolar lipids form the upper portion that is in contact with the air.11 This concept was first proposed by Holly12; Shine and McCulley later elaborated.13 Each sublayer is distinct in its responsibilities: The upper sublayer forms a thick blanket that seals the underlying aqueous portion of the TF. The outermost lipid component is believed to retard water evaporation, as lipid films have low water vapor transmissivity, depending on the lipid film thickness and composition.13 Nonpolar lipids are thermodynamically unstable when they are spread over an aqueous subphase; this allows them to collapse easily and form lipid droplets. When that happens, the aqueous portion of the TF is left unprotected and prone to rapid evaporation.13 Interestingly, the lower lipid sublayer is thought to create an interface that helps stabilize this upper portion. In this interface, the polar lipids are thought to be oriented perpendicularly, with their hydrophobic tails immersed in the nonpolar lipid sublayer, and their polar heads exposed to the aqueous layer. Shine and McCulley13 suggested that this polar lipid sublayer was one to three molecules thick. They further suggested that it is formed from phospholipids and other polar lipids, including phosphatidylcholine, phosphatidylethanolamine, sphingomyelin (SM), ceramides, and cerebrosides.13 More recently, another group of amphiphilic lipids—namely, very long chain (O-acyl)-o-hydroxy fatty acids, have been described as meibum components and are theorized to contribute to the polar lipid sublayer.14 Polar-to-nonpolar lipid layer thickness measurements have not been performed; however, it has been suggested that the polar lipid

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is 5% to 15% of the total lipid fraction. Therefore, this polar surfactant layer is hypothesized to be between 7 and 20 molecules thick and consists of more hydrophobic lipids above the polar lipid layer.6,15 This estimate does not consider a (quite possible) redistribution of the lipids of different classes between the sublayers and is based on an assumption that the overall lipid composition of TFLL is identical with that of meibum.2

It is likely that lipids are not the only class of molecules from which the TFLL is assembled. For instance, proteins are now considered an intrinsic part of TFLL.2 Many proteins are surface active compounds, meaning they will spontaneously populate the air–liquid interface, lowering the surface tension of water and creating a surface protein layer. This translocation of proteins from the bulk aqueous phase to the air–water interface is typically accompanied by protein denaturing (i.e., irreversible conformational changes—typically, unfolding—that, in the end, prevent proteins from submerging back into the depth of the aqueous layer). This results in the formation of a protein layer.2

In the presence of meibomian lipids, proteins have to compete for the available surface space. This competition results in either protein penetration (intercalation) in the lipid layer or protein attachment to (or association with) the lipid layer. Both result in surface property alterations of the TF and TFLL. Indeed, Saaren-Seppälä et al.16 demonstrated that TF lipocalin (Tlc) could actively interact with various artificial lipid membranes, regardless of overall charge and composition, and others have shown human Tlc binding to meibomian lipids organized in thin films. Similar experiments were conducted earlier to demonstrate that other tear proteins (such as lysozyme17 and mucins8) could also penetrate the lipid layers.

Thus, an update to the classic three-layer model (Fig. 1) of the TF and two sublayers of TFLL is warranted. This new model should incorporate proteins (lipocalin, lysozyme, mucins, and others) intercalated in and/or adsorbed to the TFLL and an addition of a novel class of lipids recently identified in human meibum, very long chain (O-acyl)-ω-hydroxy fatty acids, which may act in the formation of an intermediate surfactant lipid sublayer between the thick outermost nonpolar lipid sublayer and the aqueous layer of the TF.8,15,18

**FIGURE 1.** A proposed model of the precorneal tear film showing the relationship and interaction of lipid-binding proteins and the outer lipid layer.

### Analytical Methods for Lipid Evaluation

With the advancement in the lipid detection and identification technology, sample quality is critical. Modern instrumentation can detect a wide variety of compounds and provide accurate information on their structures. Thus, many of the analytes that previously had been impossible to detect and/or identify in meibomian lipids can now be discovered and categorized, even if present in minute quantities.

### Methods of Sampling and Storing Meibum and Tear Film Lipids

Handling and storage of lipid samples generally follows the recommendations provided in lipid chemistry textbooks and protocols available online from lipid chemical companies (see, for example, http://www.avantilipids.com, technical support, lipid storage, and handling).19 Care should be taken to minimize (or prevent) sample exposure or contact with any products made of plastic and silicone. Glass, stainless steel, noble metals, and Tellon (E.I. du Pont de Nemours and Co., Wilmington, DE) are the recommended collection and storage materials. The preferred conditions for storing lipid samples regardless of their origin are below temperature (−80°C), in an inert atmosphere (argon or nitrogen), and in a dark and dry state.

Meibum and TF samples can be collected using three main types of procedures: (1) soft or hard expression of meibum from the meibomian gland orifices10,20 –22; (2) microcapillary collection of aqueous TF samples and meibum directly from the meibomian gland orifices23 and (3) Schirmer test strips or similar tools to collect aqueous TF samples.2,24,25 Another technique, the surgical removal of eyelids and/or meibomian glands, has been implemented in animal and human cadaver studies,26 –28 but is unrealistic with living human volunteers.

Soft expression, or expression/pressure from the outside only of the eyelid, is possibly less likely to contaminate the samples with surrounding tissues due to the gentler handling of the eyelids. The hard expression technique is often described as a squeeze technique in which a conformer or device is used behind the lid while pressure is applied to the front of the eyelid. This technique yields a greater sample volume, yet may be more uncomfortable to subjects. Both techniques can
yield samples contaminated by the tears as well as surrounding tissues (cells and debris). The contamination may vary by patient and/or examiner.

The use of powder- and latex-free gloves worn by the examiner is highly recommended to avoid contamination of the samples by examiner skin secretions. The use of a slit lamp during sampling allows a better visualization of the meibomian gland orifices and reduces the chances of contamination with lid margin tears and debris.

Thin-layer Chromatography

Thin-layer chromatography (TLC) is an established method of quantifying global classes of lipids; however, TLC requires large amounts of sample that can make it difficult to study tear and meibum samples. Detection is visual, via a stain (bromo-thymol blue) or charring.

To identify individual lipid species, the region of interest is removed from the TLC plate, and the sample is immediately stored in a solvent. On average, approximately 15 mg of meibum is collected per eye by microcapillary collection, which is generally enough for downstream mass spectrometry analytical analysis, although protein extraction and/or additional analyses may require pooling.

Patients accept the Schirmer test strip technique as a general component of an ocular surface examination. However, the Schirmer test may be less comfortable for patients than the microcapillary technique. This is more than offset by the test's high safety profile, aided by the lack of sharp or hard objects used in sample collection. The Schirmer test cannot be used for collecting a pure meibum sample, however, because it is virtually impossible to avoid wetting the strips with aqueous tears. Consequently, it seems that expression and microcapillary tube collection or spatula collection is well suited for collecting samples of pure meibum, whereas microcapillary tube and Schirmer test strips are better suited for collecting aqueous tears. Additional research evaluating alternate and/or optimal collection and storage of lipid samples is warranted.

Mass Spectrometry

Mass spectrometry is a very sensitive analytical method that allows for both detection and structural determination from very small sample amounts. Many mass spectrometry-based meibum studies have been published using analysis techniques including GC-MS, liquid chromatography-mass spectrometry, atmospheric pressure chemical ionization (APCI) and MALDI. Phosphorylated lipids from rabbit and human tears have been detected and analyzed using a unique extraction procedure and sample preparation for MALDI-TOF (time of flight) analysis. No papers have been published on the detection of human meibum lipids using MALDI-TOF.

Tear Film Lipids and Lipid-Protein Interactions

Lipids are an extremely complex group of molecules, both structurally and functionally. One method of chemical property classification groups lipids into polar, amphiphilic and nonpolar lipids. By definition, polar lipids are relatively water-soluble. They include short chain fatty acids, hydroxylated fatty acids, hydroxy-ceramides (OH-Cer), monocacyl glycerols (MAGs), glycosylated lipids, phospholipids, and others.

These lipids tend to have relatively high hydrophilic-to-lipophilic balance (HLB), which is an objective physicochemical parameter used to describe partitioning of solubilized molecules between polar (aqueous) and nonpolar (oil) subphases. Thus, in a water-in-oil emulsion, polar lipids would concentrate in the aqueous subphase. Nonpolar lipids, on the other hand, do not dissolve in water. Typical members of this family are hydrocarbons, very-long-chain acyl-ceramides, WE, CE, and triacyl glycerols. Their hydrophilic-to-lipophilic balance is very
The formation of complex lipids happens through condensa-
tion reactions—mainly esterification and amida-
tion. The re-
actions of esterification are involved in the biosynthesis of
virtually every major complex lipid group (WE, CE, acylgly-
cerols, and liposaccharides), while amida-
tion is involved in the for-
mation of fewer lipids such as fatty acid amides (FAs), Cer,
SMs, cerebrosides.28,31,50–52 These reactions are reversible, which
means that in situ complex lipids may undergo enzymatic or
nonenzymatic hydrolysis, in the course of which they will
revert to a mixture of more simple lipids and other compounds
such as glycerol (in the case of acylglycerols) and carbohydrate-
ds (in the case of liposaccharides).45

The second major type of lipid transformation relating to TF
and TFL is lipid oxidation. One of the major prerequisites for
lipid oxidation is the presence of one or more double bonds in
the lipid structure. The double bonds may undergo enzymatic or
nonenzymatic oxidation. Many lipids can isomerize, either
induced by enzymatic transformation or spontaneously. High
temperature, UV light, and oxidation are typical causes of
isomerization. These reactions inevitably change the physical
and chemical properties of lipids and their mixtures because
(1) hydrolysis products are typically more water soluble than
the starting complex lipids; (2) lipid (per)oxidation products also
become more hydrophilic because of the addition of
oxygen and/or the formation of shorter, more hydrophilic
scission products; and (3) isomerization influences lipid pack-
ing and the physical properties of lipids (e.g., melting points,
boiling points, and density).35

For up-to-date, easily available information on lipids, the
following web sites are recommended: lipidmaps.org, lipidl-
library.co.uk, cyberlipid.com, hplc-ms.byrdwell.com, and lipid-
banks.jp, among others. An overview of lipidomics was also
recently published and may elucidate the molecular composi-
tion of these biomolecules.36

**LIPIDS OF THE TEAR FILM**

**Normal Meibum**

Biochemical characterization of meibum began in 1897,47
when Orlando Pes confirmed its lipid nature and suggested
that it was rich in fats, FFAs, and cholesterol. Several decades
later Linton et al.,30 Andrews,49 and Ehler49 each demonstrated
that meibum was rich in neutral fats, steryl, and WE. In sub-
sequent studies, acyl glycerols, ceramides, phospholipids,
and other polar lipids were reported to be present in
meibum, and most meibum components are nonpolar lipids of
different classes.32,51,50–52 The polar lipids are a minority,
though they have been implicated in playing a critical role in
the TF stabilization and disintegration. Several reviews on the
topic has been published,53–56 including the most recent
one by the International Dry Eye Workshop.57 Table 1 sum-
marizes all the lipids identified by a variety of techniques to
date.7–10,13–15,20,23,29,32–34,36,47–50,52,56,58–74

The most often detected classes of meibum lipids are probably ubiquitous WEs and CEs. Together, they are believed
to represent up to 60% of meibum lipids. WEs and CEs are
among the most hydrophobic members of meibum, whose
lipophilicity is rivaled only by hydrocarbons.

WEs from normal human meibum have been recently char-
acte rized using HPLC-MS.49,14,18,20 Numerous WE species
were detected, and the most prominent compounds were
C18,1 fatty acid based esters of very-long-chain saturated fatty
acids with C18 to C36 carbons.7,18,20 In a later study,17
additional types of WEs were described that included multiple
structures of C18:1. C18:3, and C18:4 fatty acid families. Their
fatty acids were esterified to the same very-long-chain fatty
acids, as were the C18:1 fatty acids. Compared with the
monounsaturated WEs, the polyunsaturated WEs were rela-
tively minor, but still noticeable, components of meibum. A
more detailed analysis of these polyunsaturated WEs revealed
that many of the individual members of these families were
present in several isomeric forms, which most likely differed in
the cis, trans geometry of their double bonds.17 For example,
a compound with m/z 641 (an ester of a C26:0 alcohol and a
C18:4 stearidonic acid, FALFA, C26:0: C18:4) was present as four
isomers, C26:0:C18:5 as three, and C26:0:C18:2 as two, whereas
C26:0:C18:1 was represented by just one isomer. These obser-
vations suggest that the overall number of individual WE spe-
cies in human meibum exceeds 100. Using direct-infusion ESI,
WEs were found to be mainly C16:1, but with a considerable
amount of C16:1.8 Meibomian lipids from another group
(namely, CEs) are equally complex.8,15 Unlike WEs whose
major fatty acids are of modestly long C18 to C30 variety, CEs
detected in meibum can have very long saturated and unsatu-
rated fatty acids, with their chains ranging between C14 and
C34. More than 20 individual CEs were observed in human
meibum. The dominant species were CEs with C22 to C26 FAs.
The molar ratio of the oleic acid–based C18:1 CE—one of the
common CEs in other tissues and organisms—was less than 5%
of all meibomian CE. Free cholesterol appears to be less than
0.5% of meibomian sterols and steryl esters.13

The relative amounts of phospholipids in human meibum are
very controversial and unresolved, but the amount of phospholipids
appears to be far less than previously thought.11–13,17,22,57,75 Dis-
crepancies in phospholipid quantitation and identification may
relate to (1) variations in sample collection techniques; (2)
varying degrees of contamination of meibum samples with
aqueous tears; and (3) differences in instrumentation and as-
associated techniques. However, given that the meibomian gland
secretes through a holocrine mechanism and that cell mem-
branes are enriched in phospholipids, it would seem that
phospholipids are, at least initially, secreted by the gland into
meibum. The ability to detect such polar lipids, and to assess
the nature and extent of possible variations between experi-
mental groups, may depend on the methods of data analy-
ysis.9,77–79 There are factors that can affect the sensitivity of an
individual phospholipid molecular species, such as the unsatu-
ration of fatty acyl substituent and chain length; however, the
most significant factor is ionization efficiency, which is de
dependent on the polar head group of the individual phospholipid
classes. Ion-suppression effects make it difficult to observe
minor components when a major class is also present in the ion
source. Finally, some phospholipids are more readily detected
in the negative-ion mode, whereas the opposite is true for
other classes of phospholipids that are more readily detected in
the positive ion mode. Those limitations complicate any direct
approach to accurate quantitation of phospholipids by mass
spectrometry.77

The lipid patterns of human meibum samples show many
similarities among individuals.9,56,79 For example, Joffre et al.75
and Souchier et al.80 evaluated the changes in the FA com-
position of normal donors and subjects with meibomian gland
dysfunction (MGD) or aqueous-deficient dry eye before and
after minocycline treatment. The FA composition remained
consistent in the normal subjects and did not differ much from
those in subjects with aqueous dry eye. The MGD patients,
however, produced a different, but a repeatable, pattern with
a lower ratio of saturated to unsaturated FA, and a higher ratio
of branched FA to straight-chain ones. Butovich et al.\textsuperscript{20,22} found that the composition of meibomian lipids collected from normal donors was reproducible intersubject from sample to sample when compared visually.

**Normal Tears**

Recently, researchers have reported that human meibum and aqueous tears differ somewhat in lipid compositions and the relative amounts of individual lipids. The most noticeable difference was an increase in the molar ratio of lower molecular weight WE-type compounds.\textsuperscript{7,9,20,79} Another difference is in the hydrocarbon chain ordering between lipids of meibum and aqueous tears: The former were less ordered at any tested temperature and had a lower phase transition temperature. This was explained by suggesting that both types of the samples had different lipid compositions.\textsuperscript{44} Of particular interest, aqueous tear samples show the spectrometric signals of organic phosphate esters, similar to that found in phosphatidylcholines and SMs.\textsuperscript{24,42,81}

**ANIMAL MEIBUM AND TEAR FILM LIPIDS**

Animal studies on meibum lipids have predominantly focused on bovines (castrated bulls) and rabbits, although other species such as the mouse, hamster, rat, and gerbil have also been studied. The major constituents of animal meibum have been identified as sterol and WEs,\textsuperscript{10,28,51,82,83} although the mouse may contain predominantly CEs.\textsuperscript{84} Cholesterol appears to be the major sterol in all animals tested,\textsuperscript{28,31,59,61,82,85,86} apart from the rabbit, in which it has been reported that 24,25-dihydro-8,9\textsubscript{9004} lanosterol is the major sterol.\textsuperscript{83} Other sterols identified have been 5\textsubscript{9251}-cholest-7-en-3\textsubscript{9252}-ol in the meibum of cows,\textsuperscript{28} cholestanol in that of rabbits,\textsuperscript{82} 3\textsubscript{9252}-hydroxy-5\textsubscript{9251}-cholestane and 3\textsubscript{9252}-hydroxy-\textsubscript{H9275}-7-cholestene in that of hamsters,\textsuperscript{85} lathosterol and perhaps methyl sterol in that of rats,\textsuperscript{58} and 3\textsubscript{9252}-hydroxy-5\textsubscript{9251}-cholestane in that of gerbils.\textsuperscript{86} Wax and sterol esters combined make up between 63% and 70% of the percent-weight of lipids in cow meibum\textsuperscript{30} and 78.5% of lipids in meibum of rabbit,\textsuperscript{82} with CE being 32% to 41% of cow meibum.\textsuperscript{10,31} Free cholesterol or other sterols make up only approximately 3% of cow or rabbit meibum.\textsuperscript{10,82}

The fatty acids and fatty alcohols have also been examined in detail. Using an isolated meibomian gland model, Kalattukudy et al.\textsuperscript{28} found that the glands of steers synthesize a high proportion of anteiso-branched chains of both the acids and alcohols. Some acids with very long carbon chains, as long as C\textsubscript{36}, have been found in the \textsubscript{H9275}-hydroxy fraction. Anteiso-C\textsubscript{25}, C\textsubscript{27}, and C\textsubscript{23} were the most highly labeled alcohols, confirming the findings of Baron and Blough,\textsuperscript{53} who detected that the fatty alcohol moiety of the WE in isolated meibum are branched chain C\textsubscript{23:0} to C\textsubscript{27:0}.

<table>
<thead>
<tr>
<th>Method of Analysis</th>
<th>Hydrocarbons</th>
<th>Monoacylglycerols</th>
<th>Diacylglycerols</th>
<th>Triacylglycerols</th>
<th>Free Sterols</th>
<th>Cholesteryl</th>
<th>Free Fatty Acid</th>
<th>Free Fatty Alcohol</th>
<th>Polar Lipids</th>
<th>Phospholipids</th>
<th>Sphingolipids</th>
<th>Squalene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical stains\textsuperscript{46}</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
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<td>PC\textsuperscript{38}</td>
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<td>Pos</td>
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<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
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<tr>
<td>TLC\textsuperscript{28}</td>
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<td>2.1</td>
<td>68.6</td>
<td>1.6</td>
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<td>10.4</td>
<td>5</td>
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<td>0-24</td>
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<td>0.8-5</td>
<td>0.7-7</td>
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<td>TLC/GLC/MS\textsuperscript{55,56}</td>
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<td>13-25</td>
<td>8-34</td>
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<td>Pos</td>
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<td>0.7-7</td>
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<td>TGL/GC/MS\textsuperscript{57}</td>
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<td>32.32</td>
<td>27.28</td>
<td>1.63</td>
<td>2</td>
<td>Pos</td>
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<td>14.83</td>
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<td>GLC/MS\textsuperscript{58}</td>
<td>3.1</td>
<td>45.2</td>
<td>39.4</td>
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<td>HPLC/ESI-MS\textsuperscript{59,60}</td>
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<td>Pos</td>
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<td>Pos</td>
<td>Pos</td>
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<tr>
<td>RP HPLC, MS, &amp; TLC\textsuperscript{71,72}</td>
<td>0.05</td>
<td>28</td>
<td>Pos</td>
<td>13</td>
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<td>Small</td>
<td>3</td>
<td>Pos</td>
<td>Neg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\* Numbers indicate percentages; PC, paper chromatography; TLC, thin later chromatography; GC, gas chromatography; MS, mass spectrometry; ESI-MS, electrospray ionization mass spectrometry; RP, reverse phase chromatography; HPLC, high pressure liquid chromatography; Pos, positive; Neg, negative.

† 35.7% of alcohol.
‡ C18 25% of fatty acids.
§ 81.84% of all esters.
∥ 18% of all esters.
¶ Normal chain accounts for 48–68% of fatty acids.
# PC and PE 38% and 16% of phospholipids, respectively.
** Ceramide and cerebroside 50 and 70% of sphingolipids, respectively.
†† PC > PE.
‡‡ (O-acyl)-\textsubscript{H9275}-hydroxy fatty acids (OAHFAs).
Nicolaides et al.\textsuperscript{10} reported that the fatty alcohols in total lipids and WEs of the steer range from C\textsubscript{18} to C\textsubscript{31}. The major synthesized fatty acids in the WEs are anteiso-C\textsubscript{15}, n-C\textsubscript{16}, anteiso-C\textsubscript{17}, and n-C\textsubscript{18:1}, whereas anteiso-C\textsubscript{25} and C\textsubscript{27} are the major labeled acids in the sterol esters. Steer fatty acids appear to be mostly composed of anteiso-branched and \( \text{-saturated} \) types.\textsuperscript{10} The triglyceride fraction which contained 8% of the total lipids is composed of labeled fatty acids similar to those found in both the sterol and the wax ester fractions.\textsuperscript{28} A group of \( \omega \)-hydroxy fatty acids have been identified from the meibomian glands of steers and humans. These acids comprise approximately 10% of all the acids of the steer lipid, are primarily monoenolic, and constitute three homologous series with members ranging from C\textsubscript{30} to C\textsubscript{38}.\textsuperscript{60,61}

For the rabbit, all chains of the fatty acids and alcohols are saturated. Fatty acids C\textsubscript{14} to C\textsubscript{22}, which are anteiso-branched. Three fatty alcohol types have been identified: anteiso-C\textsubscript{25}, C\textsubscript{27}, and C\textsubscript{29}.\textsuperscript{62} Only esters of dihydroxyanistearoic acid have been found that contain anteiso-C\textsubscript{15} to -C\textsubscript{19} fatty acids. The principal WE contain anteiso-branched C\textsubscript{15} to C\textsubscript{23} fatty alcohols and anteiso-branched C\textsubscript{14} to C\textsubscript{19} fatty acids in combination, making esters in the C\textsubscript{14} to C\textsubscript{40} range.\textsuperscript{83} FFAs makes up only 4.4% \( \pm \) 0.2% of the total lipids.\textsuperscript{82}

Harvey and Tiffany\textsuperscript{84} reported that the fatty alcohols in mouse meibum are predominantly iso-C\textsubscript{26} and anteiso-C\textsubscript{27}. Monounsaturated fatty acids in the omega 9 series, and saturated acids belong to the iso-, anteiso-, and n-series. Several 1,2-diols were also identified, with the least abundant of these being iso-C\textsubscript{16} and iso-C\textsubscript{20}. GC-MS studies on the intact WEs showed them to be composed of the branched-chain alcohols and both branched-chain and unsaturated acids.\textsuperscript{84} Studies on the meibum of hamsters have shown fatty acids with chain lengths from 10 to 32 carbon atoms are found, the most common being C\textsubscript{15} to C\textsubscript{26}. Fatty acids are mainly from the iso or anteiso series and tend to have longer chain lengths; the major alcohols have anteiso-C\textsubscript{24} and C\textsubscript{27} and iso-C\textsubscript{26} chains.\textsuperscript{85} The tears of Golden hamsters contain considerable amounts of the unusual lipid, 1-alkyl-2,3-dicarboxyglycerol.\textsuperscript{87} The rat has fatty acids in its meibum with chain lengths of between C\textsubscript{15} and C\textsubscript{34}, and a biphase distribution of maxima around C\textsubscript{16} to C\textsubscript{18} and C\textsubscript{24} to C\textsubscript{27}. The chains are straight-chain iso, anteiso, and monounsaturated. The unsaturated acids have double bonds in the \( \omega\)-7 and \( \omega\)-9 positions. The alcohols have corresponding structures.\textsuperscript{59} The gerbil has fatty acids in its meibum with chain lengths from C\textsubscript{12} to C\textsubscript{27} with, again, a biphase distribution with maxima at C\textsubscript{15} to C\textsubscript{18} and C\textsubscript{24} to C\textsubscript{27}. Chains are predominantly iso- or anteiso-branched. Unsaturated fatty acids are mainly C\textsubscript{16} and C\textsubscript{18}. Fatty alcohols are mainly branched, with chain lengths between C\textsubscript{24} and C\textsubscript{27}, although there were also several fatty alcohols, both branched and unsaturated, with chains up to C\textsubscript{32}.\textsuperscript{86}

The phospholipids of rabbits have been examined in a unique NMR study on animal tears and meibum. Greiner et al.\textsuperscript{27} reported that the phospholipid in meibum of rabbits is composed of 40% phosphatidyl choline, 18% phosphatidyl ethanolamine, 9% SM, 9% ethanolamine plasmalogen, 7% phosphatidyl serine, and 6% dihydroxyphosphatidylcholine. Ham et al.\textsuperscript{37} reported that species related to platelet-activating factor and/or lysophosphatidylcholine, phosphatidylcholine, and SM were found in the tears of normal rabbits and rabbits made to have dry eye through surgical procedures. The varieties and the concentrations of SM were greater in tears of rabbits with dry eye than in those of normal rabbits.\textsuperscript{77} In addition, the authors reported that they could not detect phosphatidylserine in the tears of normal rabbits, but this lipid was detectable in the tears of those with dry eye.\textsuperscript{57} The amount of polar lipids in the meibum of bovine (steers) has been reported to be 13.3% of the total lipids.\textsuperscript{10}

### MEIBUM LIPID CHANGES IN DISEASE

McCulley et al.\textsuperscript{88} demonstrated that various forms of blepharitis are associated with changes to the lipid composition of the meibomian gland secretions. Also, meibomian secretions from patients with meibomian keratoconjunctivitis (MKC) have shown lower levels of unsaturated fatty acids and alcohols of the wax and cholesterol esters and occasionally differences in triglyceride profiles.\textsuperscript{53,60-69}

There are generally low levels of phosphatidyl ethanolamine (PE) and SM in meibum of patients with blepharitis who also have dry eye symptoms.\textsuperscript{89} In vitro, SM can inhibit peroxidation of unsaturated fatty acids in phosphatidyl choline monolayers,\textsuperscript{90} and it has been shown that lipid peroxides can be significantly higher in tears of patients with severe-to-moderate dry eye \( (P < 0.05) \) or in those with good TF production but increased symptoms than healthy controls.\textsuperscript{91} Alternatively, the increase in oleic acid in the meibum of those with meibomian seborrhea may help explain the clinically significant burning symptoms that this group of people report.\textsuperscript{52}

Some of the differences seen in lipid types associated with different forms of blepharitis may be due to the presence of certain types of commensal lid bacteria that can hydrolyze lipids. People with meibomian seborrhea with a clinical appearance of Staphylococcus infection appear to harbor significantly more coagulase-negative Staphylococcus (CNS) strains capable of hydrolyzing cholesterol oleate than do normal individuals.\textsuperscript{65}

In addition, differences in subgroups have been demonstrated in people with androgen hormone deficiency, including males taking antiandrogen therapy, females with complete androgen insensitivity syndrome, and persons with Sjögren’s syndrome, all of whom show changes in the polar and nonpolar lipid components.\textsuperscript{34,38,64,79}

Obstructive MGD is characterized by an abnormal structure of the gland with characteristic changes in viscosity of the lipid expressed.\textsuperscript{64} An analysis of the lipid components in patients affected by MGD showed a significant decrease in triglycerides and cholesterol\textsuperscript{95} and a decrease in the amount of monounsaturated fatty acid, specifically in oleic acid.\textsuperscript{92} Decreased unsaturation of the nonpolar fatty acids tends to increase their melting point, leading to thickening of the meibum within the central duct. Infrared studies have shown that lipid order and phase transition temperatures are higher in meibum of donors with meibomian gland disease.\textsuperscript{14}

Recently, Joffre et al.\textsuperscript{71} demonstrated that the fatty acid profile of the excreta collected by Schirmer test strips in people with blepharitis is significantly different from controls. Total saturated fatty acids were 9.3% in those with blepharitis versus 24.6% in controls, with lower quantities of palmitic (C\textsubscript{16:0}) and stearic (C\textsubscript{18:0}) acids. Branched-chain fatty acids were present in greater proportion in MGD patients. Interestingly, small differences were observed in fatty acid composition between those with blepharitis and those with dry eye, with 50% more linoleic acid in the dry eye group.\textsuperscript{74}

The TF lipid layer changes secondary to MGD have a negative effect on the quality of vision measured as contrast sensitivity\textsuperscript{94} and on evaporation of tears from the ocular surface. Gilbard et al.\textsuperscript{95} demonstrated in rabbits that meibomian occlusion resulted in a rise of tear osmolarity, possibly as a consequence of an increased evaporation rate of water from the TF. Goto et al.\textsuperscript{96} showed an alteration of the lipid layer that results in a rise of tear osmolarity, possibly as a consequence of an increased evaporation rate of water from the TF.
could be responsible for the increase of hyperosmolarity, which, in turn, is able to produce an inflammatory response and damage of ocular surface epithelia. The biochemical changes in meibomian gland lipids may have a direct toxic effect on ocular tissues, since FFAs have been shown to be able to irritate the skin in acne vulgaris.76

The consistent finding of higher than normal levels of FFA in MGD offers a potential basis for symptoms associated with MGD. However, a study of the effect of branched-chain fatty acids on cultivated conjunctival human cells treated with iso-C\textsubscript{16} and iso-C\textsubscript{20} has shown no effects on the parameters of cytotoxicity. Only the mitochondrial dehydrogenase activity was significantly decreased in relation to the iso-C\textsubscript{20} concentration increase.74

Ocular rosacea has been associated with MGD.97 The concentration of triglycerides and FFA, especially the mono- and polyunsaturated forms, is increased and may be responsible for the activation of neutrophils and inflammatory mediators.69 Furthermore, the analysis of the ocular microbiota of patients with rosacea demonstrated bacterial growth in all patients,98 suggesting that rosacea may induce the production of lipases which in turn could disrupt the lipid layer of the TF and its protective role.

**Lipids on Contact Lenses**

**Types of CLs**

There are two major classes of CLs: the rigid lenses and the soft lenses. Rigid lenses were initially made from polymethyl methacrylate (PMMA). The newer generations of rigid CLs, (rigid gas-permeable [RGP] lenses), add fluorine and/or silicone to acrylic acid. The U.S. Food and Drug Administration classifies soft CLs into several groups (I to IV) based on their water content and overall ionic nature of the lens material. Table 2 summarizes the properties of the soft CLs.

Since 1999, so-called silicone hydrogel CLs have been commercially available. These lenses were designed to achieve the oxygen permeability given by silicone and fluorine but in a soft lens form (>10% water content material). These lenses are classified in the FDA soft lens classification system outlined above. However, due to their very different chemical nature compared to classic HEMA-based soft lenses, there are proposals to separately classify these lenses into their own group (group V) within the soft lens system (Table 3).101

**Lipid Deposition**

While lipids from the meibomian gland appear to be essential for case of lens wear,102 investigators have analyzed the deposition of lipids onto the surface of CLs due to the possible clinical consequences. In in vitro experiments, RGP lenses lipid deposition has been shown to be dependent on the lens matrix hydrophobicity.103 For the polymers siloxanyl alkyl acrylate and fluoro-siloxanyl alkyl acrylate (silafoco A and paflufocon B, respectively), lipid in an artificial tear solution enhanced protein deposition but that protein in the artificial tear solution decreased lipid deposition on only the siloxanyl alkyl acrylate lens.105

In addition, differential lipid deposition can be seen by group. Group IV hydrogel lenses bind more phosphatidylcholine (although at <1 µg/lens) than other lens groups, possibly reflecting an interaction between the positively charged choline residue and the negative surface of the lens.104 Hydrogel lenses made of poly(2-hydroxyethyl methacrylate)-poly(methyl methacrylate), poly(methyl 1 methacrylate)-poly(vinyl alcohol) or poly(2hydroxyethyl methacrylate)-poly(2vinyl pyrolidone)-poly(methacrylic acid) can all adsorb lipids from solution in vitro, and lenses made from poly(methyl 1 methacrylate)-poly(vinyl alcohol) tend to adsorb slightly more lipid.105 Adsorption of cholesterol to poly(HEMA) lenses may collapse/condense the hydrogel lens material and expel water, whereas lipid binding to PMMA lenses was simply an adsorptive process.106

Cholesterol adsorbs in greater quantities than phosphatidylethanolamine for silicone hydrogel lenses or group IV lenses (Table 4).107

In vitro, the polyvinylpyrolidone in both galyfilcon A and senofilcon A may be responsible for the increased binding to cholesterol or phosphatidyl ethanolamine. Silicone hydrogels bind cholesterol in relatively high levels and also bind squalene, CE, and WE.108 Similarly, the level of lipid binding was greater for galyfilcon A (group Vd) and balaofilcon A (group

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**Table 2. Summary of Soft Contact Lens Groups, as Classified by the FDA**

<table>
<thead>
<tr>
<th>Group</th>
<th>Water Content</th>
<th>Polymer Type</th>
<th>Lens Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>&lt;50% H\textsubscript{2}O</td>
<td>Nonionic polymer</td>
<td>Tetrafiloc A, Polymacon</td>
</tr>
<tr>
<td>II</td>
<td>&gt;50% H\textsubscript{2}O</td>
<td>Nonionic polymer</td>
<td>Lidoftilcon A or B, Alfaftilcon A, Omafilcon A, Netifilcon A, Vascularon A, Hioxifilcon A</td>
</tr>
<tr>
<td>III</td>
<td>&lt;50% H\textsubscript{2}O</td>
<td>Anionic polymer</td>
<td>Buflifon A, Phemfilcon A, Ocufilcon A</td>
</tr>
<tr>
<td>IV</td>
<td>&gt;50% H\textsubscript{2}O</td>
<td>Anionic polymer</td>
<td>Etafilcon A, Vilifilcon A</td>
</tr>
</tbody>
</table>

Source: U.S. Food and Drug Administration.

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**Table 3. Proposed Classification of Silicone Hydrogel Lenses**

<table>
<thead>
<tr>
<th>Group</th>
<th>Basis of Categorization</th>
<th>Examples of Polymer Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Va</td>
<td>Nonlinear relationship between Dk (oxygen permeability) and water content</td>
<td>Comfilcon A</td>
</tr>
<tr>
<td>Vb</td>
<td>Contain an ionic (anionic) component</td>
<td>Balafilcon A</td>
</tr>
<tr>
<td>Vc</td>
<td>Plasma or bonded surface modification</td>
<td>Lotrafiloc A and B, Asmfilcon A</td>
</tr>
<tr>
<td>Vd</td>
<td>“Released” wetting agent</td>
<td>Galyfilcon A, Senofilcon A</td>
</tr>
</tbody>
</table>
than for lenses from group Vc (again no group Va lens was tested). Indeed, levels of cholesterol, squalene, cholesterol esters or wax esters on the group Vb lenses (lotrafilcon A and lotrafilcon B) were similar to levels adsorbed to a group IV HEMA-based hydrogel lenses.

Initial in vivo studies demonstrated that lipid was present in deposits on CLs, with the principal lipid type being CE. A particular form of deposit on lenses, often called jelly bump deposits, was shown to be composed of long and intermediate sized CE, triglycerides, and WE. White spots, a similar particular type of deposits found on non-regularly replaced hydrogel lenses, are predominantly comprised of lipid. The lipid white spot deposits have a distinct structural stratification with a lipid layer providing the interface between the CL surface and the deposit superstructure. This initial interfacial layer has been shown to be made from cholesterol, cholesterol ester and unsaturated lipids. Of note, diet plays a part in formation of these white spots, and individuals who consumed larger amounts of alcohol, protein, and fat exhibited increased lipid deposition on their lenses. As hydrogel CLs tend to be replaced much more frequently today compared to 15 years ago (typically on a weekly, bi-weekly, or monthly basis today versus annually in the past), the incidence of these white spot deposits (Fig. 2) has reduced and they are now rarely seen.

In vivo, RGP lenses deposit more lipid than many soft lens materials, probably due to the hydrophobicity of the lens. Silicone-based RGP lenses also deposit more lipid than fluorine-containing RGP lenses, probably because silicone increases the hydrophobicity of the CL, whereas fluorine decreases hydrophobicity and thus decreases lipid deposition. The level of lipid deposition on group 1 (polymacon and tetrafilcon A) and group III (Balafilcon A) appears to be related to characteristics of the wearer rather than lens material per se. FDA group II lenses deposit the most lipid, and FDA group III lenses deposit the least. On group II lenses (containing polyvinyl pyrrolidone) lipid deposition appears to increase over time (from 1–28 days of wear; \( P < 0.0001 \)), whereas lipid deposition on the group IV lens reaches a maximum after 1 day and increases no further. Lipid levels on group II lenses containing polyvinyl pyrrolidone are approximately twice that on group IV lenses, and again, there was a significant intersubject variation in lipid deposition levels. Lipid deposition on lenses ex vivo is shown in Tables 4 and 5.

Overall lipid deposition increases with longer replacement schedules (3 months vs. 1 month). These studies did not characterize the lipid types, but measured lipid adsorption using spectrophotometric methods, the sulfo-phospho-vanillin reaction or estimation of total phosphate (for phospholipids). The lipid deposits on worn hydrogel lenses were chemically analyzed, and detected WEs, fatty sterols, fatty alcohols, FFAs, and monoglycerides, whereas cholesterol, CEs, and triglycerides were not detectable. However, cholesteryl oleate, cholesterol, oleic acid, oleic acid methyl ester, and triolein were detected in extracts from worn hydrogel and RGP lenses. The discrepancies may be due to the types of lenses being investigated, with polar lipids depositing preferentially onto the more hydrophilic lenses compared to nonpolar lipids. Oleic acid methyl ester appears to adsorb less to group III and IV hydrogels and RGP than other lens types.

For the silicone hydrogel lenses (groups Vb and Vc), the degree of lipid deposition in vivo appears to be substantially higher than that seen with conventional hydrogels. Cholesterol was the most commonly deposited lipid, although oleic acid and oleic acid methyl ester was also detected. Another study using balafilcon A, lotrafilcon A, and galyfilcon A lenses (groups Vb, Vd, and Vc, respectively) was also able to detect cholesterol in deposits, but found very low levels of oleic acid or its methyl ester (summarized in Table 6).

Overall the levels of lipid deposition on the silicone hydrogels lenses were lotrafilcon A < galyfilcon A < balafilcon A,

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**Table 4. Deposition of Lipids onto Contact Lenses**

<table>
<thead>
<tr>
<th>Soft Lens Type</th>
<th>Polymer Name</th>
<th>Cholesterol</th>
<th>Cholesterol Oleate</th>
<th>Phosphatidyl Ethanolamine</th>
<th>Dioleoyl Phosphatidylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Polymacon</td>
<td>0.5</td>
<td>0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Group II</td>
<td>Lidofilcon A</td>
<td>0.6</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Group III</td>
<td>Phemphilcon A</td>
<td>0.7</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Group IV</td>
<td>Etafilcon A</td>
<td>0.9</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Group Vb</td>
<td>Balafilcon A</td>
<td>7.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Group Vc</td>
<td>Lotrafilcon B</td>
<td>24.1</td>
<td>3.2</td>
<td>1.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Group Vd</td>
<td>Senofilcon A</td>
<td>23.2</td>
<td>4.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
</tbody>
</table>
similar to the ranking and amounts seen during in vitro experiments.107,108 The differences in the cholesterol deposition between the two studies (300–600 µg/lens), are not easily explained.123,124 Another study examining the deposition of cholesterol onto various silicone hydrogel lenses had a ranking of lens types in their deposition of cholesterol; lotrafilcon B (group Vc) < senofilcon A (group Vd) < galyfilcon A (group Vd) < balafilcon A (group Vb), and the study identified the use of various cleaning/disinfecting solutions as a significant modulator of cholesterol deposition.120 saville et al.111 found during soft silicone hydrogel lens wear (senofilcon A or balafilcon A) adsorption included a range of molecular type of both SM and PC, with SM C16:0 and PC C34:2.

### Clinical Changes of Lipids and CL

The literature is unclear whether the deposition of lipid on CLs affects comfort, or whether clinical testing can be used to detect changes in lipid profiles on lenses. Clinically, galyfilcon A lenses (group Vd) tend to have more grade 3 to 4 lipid deposits than group IV lenses.120 Soaking silicone hydrogel lenses (group Vc) in lanolin reduces the drying time of tears over the lens surface and TF appears thinner over lens surface.125

There is an apparent decrease in cholesterol levels in tears for around 10 hours after lens insertion occurs.126 In addition, the phospholipid concentrations in tears of patients wearing polymacon (group I) or etafilcon A (group IV) lenses were 186 ± 39 µg/mL and 162 ± 35 µg/mL, respectively, with the latter concentration being significantly lower than that observed in the same subjects when not wearing CLs (220 ± 35 µg/mL, P = 0.0023).127 This may be of significance as concentrations of these phospholipids in tears of patients with marginal and moderate dry eye have been reported to be significantly lower than those in subjects without dry eye,128 and CL wear is well known to cause dryness and discomfort sensations in significant proportion of wearers.129,130 Furthermore, two studies131,132 reported that hydrogel lens wear altered the TF lipid composition by decreasing the levels of polar lipids and increasing levels of nonpolar lipids. These studies also found low levels of tear polar lipids (phospholipids) were associated with increased levels of tear instability during soft CL wear. It is possible that phospholipids in tears are degraded by group IIa secretory phospholipase A2 (sPLA2) deposited on CLs; etafilcon A (group IV) lenses deposit statistically significantly more group sPLA2 than polymacon (group I) lenses.133,134 These changes to the biochemistry of the TF may manifest as overt changes to the clinical picture of the lipid layer on the surface of the TF.

It has been established that wearing most CL types results in a disruption to the lipid layer appearance of the TF.135–137 Lipid layer thickness assessed via interference fringes is generally classified into six different types on the basis of the fringe patterns seen via a slit lamp system. These patterns—none, meshwork, wave, amorphous, colors, and other—increase in thickness layers form none to colors. During lens wear, the lipid layer does not uniformly coat RGP or soft hydrogel lenses.135–137 There is, however, an increase in lipid layer continuity over high-water-content soft lenses.138 Another study comparing two high-water-content hydrogel lenses, filcon 4A (67% water content) and lidofilcon (70% water content), found no difference between the two materials in terms of lipid layer appearance, but did demonstrate that the lipid layer over a lens just after waking was thicker than during normal open-eye conditions and that this correlated with more stable TF after waking than during open-eye.139

When the well-formed TF, including a healthy lipid layer over the CL, is missing or abnormal, evaporation of the TF during CL wear can occur, which may then lead to ocular discomfort. Thai et al.140 demonstrated that wearing either soft hydrogel lenses or silicone hydrogel lenses leads to increased evaporation of tears from the eye when compared to nonwear. While there were no statistically significant differences between evaporation rates between lens types, individuals did show significant differences with different lens types.141 Omafilcon A lenses have been noted in other studies to create thicker lipid layers.140,141 The ability of these lenses to sustain a thicker lipid layer may be due to the biomimetic nature of the phosphorylcholine in the lens. In vivo, the galyfilcon A lenses (silicone hydrogel) have a thicker lipid layer than the alphafilcon A (group II HEMA), which may give the former a more stable TF than the latter.141

In the absence of lens wear, no difference has been found in the lipid layer thickness (lipid layer pattern) or TF stability in asymptomatic or symptomatic lens wearers.142 However, TF stability is decreased in those intolerant of lens wear when compared with those who are tolerant, even though the lipid layer appearance was not different between the two groups.143 Further, people intolerant of CL wear (defined as being unable

### Table 6. Amounts of Lipid Deposited on Lenses during Wear

<table>
<thead>
<tr>
<th>Soft Lens</th>
<th>Total Lipid</th>
<th>Phospholipids</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymacon</td>
<td>66.3, 62</td>
<td>2.1, 0.05</td>
<td>4.1, 3.9</td>
</tr>
<tr>
<td>Alphafilcon</td>
<td>127</td>
<td>1.8</td>
<td>4.1–2.1</td>
</tr>
<tr>
<td>Etafilcon A</td>
<td>44.1, 29</td>
<td>19 (ng/lens SM), 19 (ng/lens PC)</td>
<td>0.1–0.5</td>
</tr>
<tr>
<td>Senofilcon A</td>
<td>59 (ng/lens SM), 195 (ng/lens PC)</td>
<td>0.3–2.7</td>
<td>9.9</td>
</tr>
</tbody>
</table>

SM, sphingomyelin; PC, phosphatidylcholine.
to wear CLs for more than 6 hours throughout the day) have increased levels of malondialdehyde and 4-hydroxy-2(5E)-nonenal (degradation products of polyunsaturated fatty acid and related esters) in their TF. In addition, intolerant subjects had significantly more sPLA2 in their tears compared with tolerant subjects. No differences in the number of blocked meibomian glands were found between the two groups.144

Tear Lipid–Protein Interactions

The seminal paper on tear lipid–protein interactions was published in 1973.12 Holly showed that the spread of lipids is facilitated by mucins.12 With high surface pressure and low surface tension, the meibum lipid coalesces into a droplet and does not spread across the surface of water. When mucin is dissolved in water, such as with the mucin–aqueous gel gradient of the TF (Fig. 1), the surface tension is lowered allowing meibum lipids to spread across the aqueous surface.

Lipopcalin

Cholesterol, fatty acids, fatty alcohols, glycolipids and phospholipids in the TF are bound by lipocalin and the binding remains after several levels of chromatographic separation.145 Additional binding studies of tear lipocalin revealed that apo tear lipocalin has a high affinity for phospholipids and stearic acid ($K_i$) of 1.2 and 1.3 $\mu$M, respectively, and much less affinity for cholesterol ($K_i$) of 15.9 $\mu$M. For fatty acids, binding affinity correlates with the length of the hydrocarbon chain. Tear lipocalin binds most strongly to the least soluble lipids permitting these lipids to exceed their maximum solubility in aqueous solution. These data implicate tear lipocalin in solubilizing and transporting lipids in the TF.145,146 Lipocalin is a major tear protein comprising 33% of total protein in a tear sample.146 It is secreted by the lacrimal gland and has also been detected in meibomian gland secretions.148 The structure, function, and molecular mechanisms of action of tear lipocalin have recently been reviewed.149–151

Conformational changes in tear lipocalin are evident when lipid binds the protein.152 It has been proposed that lipocalin scavenges lipid from the corneal surface and may enhance the transport and equilibration of lipid in the lipid surface layer.153 Recently, the solution structure of tear lipocalin bound to a native ligand was elucidated and the entire binding energy landscape was clarified by using a modification of site-directed tryptophan fluorescence.154 Gasymov et al.154 describe the process in which lipids exploit multiple binding sites in nanoseconds before exiting the cavity of the protein. In Figure 3, the more intense red indicates greater static quenching or static binding. Subsequently, the tear lipocalin was crystallized in space group P2$_1$ with four protein molecules with bound artificial ligand 1,4-butanediol and its x-ray structure was solved at 0.026-nm (2.6 Å) resolution.155 Breustedt et al.155 showed that the loop region and adjoining areas of the $\beta$-barrel allow considerable conformational flexibility, which allows tear lipocalin to adapt to ligands that differ vastly in size and shape. This observed promiscuity in ligand recognition may be important in understanding the function of tear lipocalin and lipid–protein interactions on the TF.

Tiffany and Gouveia156 found an interactive role of lipids and proteins in their tear viscosity study. It has been suggested that lipocalin forms dimers when lipid is bound to the protein. However, more recent work demonstrates that it is likely that tear lipocalin exists mainly as a monomer in the TF and that the dimeric form is minimal.155,157

Tear lipocalin deficiency is associated with meibomian gland dysfunction158 and the studies above show that lipocalin sequesters lipids. Whether the lipocalin/lipid complex interacts with the lipid layer has been the focus of recent studies. When human meibum was used in a in vitro study by Millar et al.,159 lipocalin bound slowly to a human meibomian lipid film compared with lysozyme or lactoferrin. The adsorption of lipocalin to a human meibomian lipid film was very different from its adsorption to a bovine meibomian lipid film, indicating the nature of the lipids in the film is critical to the adsorption process.

Based on these studies, it seems likely that tear lipid–protein interactions occur in vivo and that these interactions change the physical properties of tears. There are several gaps in knowledge that when filled could facilitate the development of therapies to reduce dry eye and MGD symptoms.

Lysozyme

Lysozyme, a major protein found in tears, acts as a bacteriolytic protein that depolymerizes mucopolysaccharides. Lysozyme does not sequester lipids as lipocalin does,146 but it interacts with and binds in vitro to the phospholipids of membranes160 and meibum films.17,159,161,162 It is possible that lysozyme not only stabilizes the structure of the lipid layer but that loss of lysozyme in disease states disrupts this stability, causing an increase in the rate of evaporation.

Changes in the concentration of tear lysozyme by disease or drugs may disrupt the structure of the lipid layer of the TF. It would be useful to define interactions between lysozyme and tear lipids in vitro using spectroscopic approaches and to determine whether or not structural alterations in the lipid layer caused by a change in the concentration of lysozyme leads to increased rates of evaporation.

Apolipoprotein D

Apolipoprotein D (apoD) is a member of the lipocalin super family and has been shown to be produced in the lacrimal gland and has been found in the tears.163 Although the physiological function of apoD is currently unknown, it has the ability to bind phospholipids, cholesterol, and other lipids. The function of this protein in tears may be to interact with the meibomian lipids present in human tear fluid and perhaps contribute to the surface spreading of these lipids. Another possible function could be as a...
Phospholipid Transfer Protein

The presence of phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) in human tears was investigated using Western blot analysis and quantitated using ELISA. PLTP was found to be present in tear fluid, whereas CETP was not. ELISA indicated that the PLTP concentration in tear fluid, 10.9 ± 2.4 µg/mL, is approximately two times higher than that in human plasma. PLTP-facilitated phospholipid transfer activity in tears, 15.1 ± 1.8 micromoles mL−1 h−1, was also significantly higher than that measured in plasma. These results suggest that PLTP may be involved in the formation of the TF by mediating lipid transfer in tear fluid. However, the concentration of this protein is relatively small in tears compared to lipocalpin and PLTP has not been specifically shown to bind with lipids in tears.

The studies by Millar et al.5,162 show that mucin binds to meibum in vitro; however, mucin–lipid interactions have never been studied on a molecular level. Currently, there is no evidence that certain mucins may interact with the lipid layer; conversely, there is no evidence mucin–lipid interactions do not exist, either.165

Although the amount of fatty acid and cholesterol bound to lipocalpin has been quantified, the amount and type of phospholipid and lysophospholipid bound to lipocalpin has not. In addition, it can be hypothesized that most phospholipids in tears could be bound to lipocalpin. Although the interactions between various lipids have been studied extensively, those directly relating to the mucin–lipid relationship, if any, remain a mystery. Research has yet to quantify whether binding of lipocalpin, lysoenzyme or tear fluid components to meibum lipid cause molecular-structural changes to the proteins or lipids. Nor do we yet know whether compositional changes in meibum with age or MGD alter the binding of proteins to meibomian lipids.

Influence of Bacteria on Tear Film Lipids

The normal microbiota of the eye, including Staphylococcus aureus, Haemophilus influenza, CNS, Propionibacterium sp., and Corynebacterium sp., produces enzymes that can degrade the lipids of the TF. CNS, Propionibacterium sp. and Corynebacterium sp. produce lipolytic exoenzymes (cholesterol esterase, fatty wax esterase, and triglyceride lipase) that can hydrolyze cholesterol esters and WEs. A higher number of CNS, able to produce these enzymes, has been found on lids of patients with chronic blepharitis, which along with S. aureus strains, produce lipolytic exoenzymes (cholesterol esterase, fatty wax esterase, and triglyceride lipase) that can hydrolyze cholesterol esters and WEs. A higher number of CNS, able to produce these enzymes, has been found on lids of patients with chronic blepharitis, which along with S. aureus strains, produce lipolytic exoenzymes (cholesterol esterase, fatty wax esterase, and triglyceride lipase) that can hydrolyze cholesterol esters and WEs. A higher number of CNS, able to produce these enzymes, has been found on lids of patients with chronic blepharitis, which along with S. aureus strains, produce lipolytic exoenzymes (cholesterol esterase, fatty wax esterase, and triglyceride lipase) that can hydrolyze cholesterol esters and WEs.
functionality of the proteins and lead to completely new proteins at the functional level.

Conclusions

Understanding the molecular composition (proteomics, lipidomics) of the TF and the contribution of the meibomian gland to the TF is critical to understand and describe TF instabilities, dry eye syndromes, CL incompatibilities and other eye diseases.

Elucidation of the compositional components of meibomian gland secretion and the TF has been challenging in the past because of limitations to analytical and biochemical techniques. Most analytical techniques had low sensitivity and low resolution, required large sample amounts (requiring pooling), and chemical derivatization for detection. This limitation caused possible degradation of the sample because of prolonged exposure time; low sample recovery due to derivation, isomerization and/or decomposition due to sustained high temperature analysis; long analysis time; lack of information on the actual molecular composition of the lipids; and contamination. Recently, new advances in analytical and biochemical techniques have allowed researchers better methods to examine the meibomian gland secretions and TF components with the ability to identify the actual specific molecular composition of lipids, proteins, posttranslational modifications and protein-lipid interactions. Although the signs and symptoms of TF instability are reasonably well characterized, we are only beginning to scratch the surface of understanding the specific molecular components of the TF and their relationship with MGD and TF stability.

References


Debra A. Schaumberg,1 Jason J. Nichols,2 Eric B. Papas,3 Louis Tong,4 Miki Uchino,5 and Kelly K. Nichols2

Scientists have been interested in studying the secretions of the meibomian glands for many years,1–5 and diseases associated with the meibomian glands (e.g., cancers, posterior blepharitis) have been noted in the medical literature since at least the early part of the 20th Century.6–11 However, the term “meibomian gland dysfunction” (MGD) was only introduced by Korb and Henriquez in 1980.12 The terminology “meibomian gland disease” was later introduced by Bron et al.13 as an umbrella term to indicate any disease affecting the meibomian glands (see Definition and Classification).

Although the etiology of MGD may differ from that of aqueous-deficient dry eye disease (which is due to insufficient lacrimal gland production), the two conditions share many clinical features, including symptoms of ocular surface irritation and visual fluctuation, altered tear film stability, and potential ocular surface compromise. When MGD is of sufficient degree, it may give rise to the second major subtype of dry eye disease, evaporative dry eye.14 These subtypes are not mutually exclusive, as has been acknowledged.15

Methods of Assessment for Epidemiologic Studies

Epidemiologic investigation has been limited by the lack of agreement regarding definition or a standardized, clinician-based assessment that characterizes MGD. In light of that, it is useful to consider which methods of assessment, incorporating both objective and subjective outcomes, would be most valuable for future studies of MGD.

We consider a purely objective outcome to be one that is obtained without the influence of the examining clinician or the patient’s perceptions. In contrast, measures assessed by a clinician or patient each have components of subjectivity. For example, a grading assessment made by a clinician is associated with a subjective aspect and therefore has an inherent within and between-examiner variability that affects study design and planning. Such variability is also inherent in patient-reported subjective outcomes, such as symptoms and standard visual acuity measures. In general, this committee agreed that the most valuable outcomes for assessment of clinical disease demonstrate the attributes of validity, reliability (low variability), sensitivity (to differences between patient groups), responsiveness (to change in disease status over time), feasibility, and practicality.

There is a lack of clarity on the objective and subjective measures for classification and outcomes of MGD in both clinical care and clinical trials. In part, this ambiguity is due to the paucity of evidence on the time course of the disease and its symptoms or the actual processes that cause them—for example, when symptoms associated with MGD actually develop in the disease process. Is it at the onset of meibomian gland damage or altered meibum production and/or secretion or after a certain level of damage or alteration has occurred? Further, the symptoms may not be due to actual meibomian gland damage or altered meibum secretion at all, but instead may arise from subsequent damage to other ocular surface tissues associated with secondary alterations in physiological processes. Therefore, there has been no consensus on the use of patient-reported or clinician-based assessments in MGD or on the relationship between different measures.

In considering the various objective and subjective clinician-assessed approaches used in the evaluation of MGD, it is important to differentiate between those approaches that evaluate some aspect of the meibomian glands or their secretions (primary assessments) and those that assess other physiological consequences related to gland injury or secretory alteration (secondary assessments). We propose that such secondary assessments be considered surrogate markers of MGD.

Objective Approaches

At present, objective approaches require specialized scientific equipment and are currently applicable for small-scale studies, but are not feasible for use in large epidemiologic studies. For the most part, emerging technologies are being used in these small-scale studies. Primary objective assessments include biochemical analyses of the meibomian glands or secretions (e.g., assays, chromatography, mass spectrometry, and spectroscopy). These approaches evaluate the meibum directly in terms of lipid and/or protein components. Secondary objective approaches that might be considered in the evaluation of MGD include evaporimetry (a measure of the consequences of an altered lipid layer), lipid layer interferometry augmented with computerized assessment in lieu of clinician assessment, and osmolarity (a measure of the consequences of evaporation).
**Subjective, Clinical Approaches**

Subjective clinical approaches for the evaluation of MGD include biomicroscopy of the lid margins in terms of telangiectasia and overall lid margin injection (dilated blood vessels at the surface of the skin or mucous membrane) or lid margin keratinization; evaluation of capping or plugging of the meibomian gland orifices and evaluation of the expressibility and quality of the meibum from the glands; and in vivo analysis of the meibomian glands themselves (atrophy or loss) through meibography. The latter technique captures images of the lids illuminated by near infrared or infrared light, allowing visualization of the glands. To date, this method has been assessed subjectively by a clinician or reader, but may lend itself to more objective methods of computerized image analysis. Some secondary, subjective, clinician-assessed approaches include corneal and conjunctival staining (due to excessive evaporation and subsequent aqueous-deficient dry eye) and measures of tear film stability, such as noninvasive and invasive tear film breakup times.

**Subjective, Patient-Reported Approaches**

MGD may be associated with symptoms and signs of ocular surface discomfort, such as eye itching, eye burning, heavy/puffy eyelids, eye dryness, eye irritation, watery/teary eyes, crust on lashes (particularly in the morning), eyelids being stuck shut (particularly in the morning), and eyelid and ocular redness, among others. Notably, these symptoms are the same or very similar to those reported in dry eye disease and/or in anterior blepharitis. Based on available evidence, since there is broad overlap in these symptoms and those for aqueous-deficient and evaporative dry eye patients, we cannot be certain whether a symptom survey that is specific to MGD can be developed. A concerted effort is needed to identify specific symptoms or develop instruments that would separate patients with MGD from those with other ocular surface problems. This is particularly important because patient-reported outcome measures have been described for dry eye disease, but these were validated across dry eye subtypes and were not specific to MGD. As more is learned about MGD and dry eye disease, such an approach might gain a foothold if evidence emerges to link specific features or patient symptoms to objective measures of MGD, ocular surface damage, and measures of tear dynamics.

Currently, defining MGD based on symptoms alone is not ideal, and instruments that are specific for MGD are not available and will be challenging to develop, because symptoms that researchers use are associated with more broadly defined conditions such as dry eye disease and blepharitis. For example, eyelid symptoms that include puffiness and morning "stickiness," which have been used for assessment of MGD in some studies, may be common to both anterior and posterior types of blepharitis (see Definition and Classification of Meibomian Gland Dysfunction). Recently, a telephone survey of 5019 adults (at least 18 years of age) in the United States found that 15% of the respondents recalled at least one of the symptoms traditionally associated with anterior blepharitis (crust or flakes on eyelashes on waking, eyelids sticking together on waking, and redness of the eyes or eyelids on waking) at least half of the time in the last 12 months, with 1% having experienced all three symptoms in that same period. However, the authors noted that eye care practitioners did not examine participants, and so the reported symptoms cannot be extrapolated to diagnosis. Clinically significant cases of MGD can occur with or without significant anterior blepharitis or aqueous-deficient dry eye. Thus, making specific symptoms a prerequisite in the definition of MGD may underestimate the prevalence of clinically relevant disease.

The difficulty of specifically identifying MGD is supported by the fact that the same telephone survey found that 40% of the respondents who had a diagnosis of blepharitis also had dry eye disease, and the symptom responses were quite similar between these two groups, except that symptoms of "eyelids stuck together on awakening," "eyes or lids red on awakening," and "thinning of the lashes" were more frequent in the blepharitis group.

The clinical picture of patients with ocular surface disease is often complicated. Clinically significant cases of MGD may have anomalies of the lipid component of the tear film and symptoms resulting from evaporative dry eye. Symptoms may also arise from the lid disease itself, with accompanying inflammatory events, or ocular surface damage (e.g., secondary to the release of inflammatory mediators from the lid into the tear film). These symptoms and the associated functional difficulties that arise are a significant concern in people with MGD; the importance of their assessment is clear. That these symptoms cannot be distinguished from those of aqueous-deficient dry eye or other conditions complicates this task, however.

We must also consider that symptoms may vary by both frequency and severity, with most studies so far concentrating on the former. There may also be subjective, patient-reported factors other than ocular surface or eyelid symptoms that are important to quantify, such as climate, humidity, or activity level (e.g., computer use). Understanding and quantification of these subjective, patient-reported aspects of MGD, as well as the perceived impact of the disease on an individual's life, are needed, so that valid techniques can be used for assessment of MGD. Scientifically proven validation techniques should be used in the future development of these assessments.

To the best of our knowledge, there has been no attempt to determine whether ocular surface and/or eyelid symptoms can differentiate between cases of anterior blepharitis or aqueous-deficient dry eye disease and cases of MGD. Nor has the extent to which these conditions occur together, or whether they are separate entities, been well documented.

**Combination (Correlative) Approaches**

Combination approaches would entail the integration of symptomatic assessment of MGD with measures derived both from clinical evaluation and by purely objective means. We advocate that such an approach could hold the greatest promise for moving the field forward. The inclusion of a clinical assessment could alleviate problems encountered when trying to define MGD based on symptoms alone, and consideration of symptoms could help identify the most clinically relevant cases. Thus far, the Beijing Eye Study comes closest to this type of construct by reporting the prevalence of eyelid telangiectasia (as a clinical sign of MGD) in the presence of symptoms of dry eye. At 69%, the reported prevalence was relatively high when compared to other studies that did not include symptoms in the definition. Future studies using this type of approach would probably succeed best if a set of MGD-specific symptoms can be identified.

Since the severity of MGD can be graded by standard techniques, such measures can form the basis for identification of MGD symptoms and their correlation with clinical disease parameters. Natural history and/or treatment studies using such measures could then advise on which grade (symptomatic or nonsymptomatic) is most predictive of progressive disease, as well as how treatment affects various MGD parameters and how various symptoms and signs affect a patient's quality of life.

It is worth noting that an epidemiologic definition of MGD may not correspond to the threshold used by clinicians for
treatment. For example, whereas symptoms would be expected to develop during the progression of MGD, in some cases intervention may be more effective if initiated before symptom onset. Although worth the effort, it may prove impossible to identify a set of symptoms that are specific to MGD. Last, signs and symptoms of MGD may not themselves show a high correlation, as with dry eye. These problems are common to epidemiologic studies in general and so should not preclude efforts to study MGD in particular. Care should be given to the choice of definition in various settings, taking into account not only factors such as the sensitivity and specificity of a particular definition, but also its cost and feasibility and the burden on study participants when applied on a large scale. Definitions and diagnostic criteria should be documented in sufficient detail to permit comparisons with future work.

**PREVALENCE OF MGD**

**Population-Based Studies**

Most population-based studies that have estimated the prevalence of MGD have included a patient-reported symptom outcome that is developed for the study of dry eye disease, but is not specific to MGD. However, there are now several studies that have also evaluated concurrently measured clinical correlates including lid telangiectasia, gland orifice capping, gland dropout, gland expressibility, and tear breakup time. For analysis of these studies, the clinical correlates chosen were evaluated either independently or grouped together with patient symptoms, to serve as an indicator of MGD.

The prevalence of MGD reported in published studies varies widely, from 3.5% to almost 70% (Table 1). A striking feature in looking across these publications is that the prevalence of MGD appears to be higher in reports arising from Asian populations. The 46.2% found in the Bangkok study,27 60.8% in the Shihpai Eye study,28 61.9% in a Japanese study,29 and 69.3% in the Beijing Eye Study contrast sharply with 60.8% in the Shihpai Eye study, 61.9% in a Japanese study,29 and 69.3% in the Beijing Eye Study,30 to 19.9% in the Melbourne Visual Impairment Project.31 The Japanese study found a higher prevalence rate of MGD than did the Bangkok study, as would be expected if the prevalence of MGD increases with age. There has been no published report on the age-specific prevalence of MGD.

There are other methodologic discrepancies that are worth mentioning. For example, the Bangkok study invited 550 volunteers from the population (above 40 years of age) to undergo annual eye screening.27 This method differs considerably from the random sampling used in many other population-based studies, and as a result, subjects with more severe MGD may have been overrepresented because they may be more likely to volunteer for screening. Likewise, the Japanese study by Uchino et al. could be limited by a similar type of bias because of the disadvantage of recruiting a very small number of the targeted population.29 Of 12,000 letters sent out to retirees, only 113 consented to the protocol and were recruited. This low participation rate makes it unlikely that the result is representative of the actual population prevalence.

**Clinic-Based Studies**

Clinic-based studies with smaller sample sizes (Table 2) have also been conducted. As there are still relatively few population-based surveys available, these studies may provide a limited amount of information regarding the prevalence of MGD and the distribution of certain clinical signs and symptoms, but the accuracy with which such studies can estimate true prevalence is questionable. To illustrate, the prevalence of MGD cases ranges from 20% to 70% in the non-contact-lens (CL) wearers, and 60% in two Japanese studies of patients with or without Sjögren’s syndrome. It is clearly difficult to make comparisons between these studies, as they involve special, highly selected patient cohorts.

We conclude that the value of this type of clinic-based approach to estimating the prevalence of MGD in the population at large is quite limited. In the future, however, clinic-based approaches may be better suited for study of the risk factors for MGD. Provided that a suitable control population can be identified, such studies may be able to include more detailed clinical assessments and diagnose MGD with a higher degree of specificity than can large epidemiologic approaches.
CLINICAL CORRELATES AND POSSIBLE RISK FACTORS FOR MGD

Systematic, epidemiologic evaluation of candidate risk factors for MGD remains in its infancy and is an emerging area of research. Nonetheless, decades of experience, some clinical studies and case series, and expert clinical impressions have suggested several factors that may co-exist with MGD, as well as others that may contribute to its pathogenesis. Moreover, given the highly integrated nature of the ocular surface system and the key role of the meibomian secretions in its maintenance, it is worth considering the strong possibility that the same factors implicated in dry eye disease play a role in MGD as well. In the following section, we summarize some conditions or factors that have been suggested to occur at increased frequency in patients with MGD. Whereas the association be-

<table>
<thead>
<tr>
<th>Table 1. Population-Based Studies Providing Estimates of the Prevalence of MGD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
</tr>
<tr>
<td>Bangkok Study*</td>
</tr>
<tr>
<td>Beijing Eye Study</td>
</tr>
<tr>
<td>Japanese study</td>
</tr>
<tr>
<td>Shihpai Eye Study</td>
</tr>
<tr>
<td>Melbourne Visual Impairment Project</td>
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<tr>
<td>Salisbury Eye Evaluation</td>
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</table>

* Not a true population-based study because the sampling methods were inappropriate.

<table>
<thead>
<tr>
<th>Table 2. Frequency of MGD in Selected Clinical Populations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
</tr>
<tr>
<td>Austria</td>
</tr>
<tr>
<td>California</td>
</tr>
<tr>
<td>China</td>
</tr>
<tr>
<td>Japan Sjögren’s</td>
</tr>
<tr>
<td>Japan Non-Sjo¨gren’s</td>
</tr>
<tr>
<td>Kuala Lumpur</td>
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<tr>
<td>United Kingdom</td>
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</tbody>
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<table>
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<tr>
<th>Table 3. Population-Based Studies that Have Evaluated the Relation between Ocular Surface Symptoms and Clinical Signs of MGD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
</tr>
<tr>
<td>Bangkok Study (Lekhanont et al.)27*</td>
</tr>
<tr>
<td>Beijing Eye Study (Jie et al.)30</td>
</tr>
<tr>
<td>Shihpai Eye Study (Lin et al.)28</td>
</tr>
</tbody>
</table>

G1, Grade 1.

* Not a true population-based study because sampling methods were inappropriate.
between many of these and MGD may simply be correlative, others can reasonably be hypothesized to constitute risk factors for the disease.

We have organized the discussion by breaking risk factors down into the three broad categories: ophthalmic, systemic, and therapeutic. We separately summarize the available evidence relating CL wear and MGD, for which there have been a few investigations. Although we think it is a useful strategy to classify risk factors on the basis of the strength of the evidence, as was done in the 2007 report on the Epidemiology of Dry Eye Disease by the International Dry Eye Workshop, 40 at present, there are generally few studies available for any particular factor’s possible association with MGD. Table 3 identifies the population-based studies to date that have attempted to quantify the relationship (if any) between dry eye symptoms and MGD. Consequently, the evidence that can currently be called on is insufficient to reliably classify the strength or likelihood of the hypothesized associations using such an approach.

### Ophthalmic Risk Factors

Maintenance and protection of the smooth refractive surface of the cornea is the function of the ocular surface system, which includes the surface and glandular epithelia of the cornea and conjunctiva; the lacrimal, accessory lacrimal, and meibomian glands, together with their apical (tears) and basal (connective tissue) matrices; the eyelashes with their associated glands of Moll and Zeis, and those components of the eyelids responsible for the blink and the nasolacrimal duct. 42 All components of the system are linked functionally by continuity of the epithelia, innervation, and the endocrine, vascular, and immune systems. In theory, chronic insult to any component of the ocular surface system can lead to clinically relevant sequelae. Given the central role played by the meibomian gland, it is feasible that the development of problems in this tissue (i.e., MGD) could be influenced by factors acting elsewhere within the system. Indeed, such factors may underlie the difficulty encountered when attempting to define and classify chronic afflictions such as dry eye disease, blepharitis, and MGD and may help explain the very large degree of overlap observed among this group of disorders. Table 4 lists factors thought to be associated with MGD. Some of the studies identifying higher risk factors are discussed below.

### Table 4. Ophthalmic Factors Hypothesized to Correlate with MGD

<table>
<thead>
<tr>
<th>Factor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aniridia</td>
<td>Jastaneiah and Al-Rajhi 58</td>
</tr>
<tr>
<td>Chronic blepharitis (anterior or posterior)</td>
<td>Auw-Haedrich and Reinhard 59</td>
</tr>
<tr>
<td></td>
<td>Jackson 58</td>
</tr>
<tr>
<td></td>
<td>Mathers et al. 37</td>
</tr>
<tr>
<td></td>
<td>McCulley et al. 39</td>
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<tr>
<td></td>
<td>McCulley and Shine 49</td>
</tr>
<tr>
<td>Contact lens wear</td>
<td>Arita et al. 36</td>
</tr>
<tr>
<td></td>
<td>Marren 55</td>
</tr>
<tr>
<td></td>
<td>Molinari and Stanek 54</td>
</tr>
<tr>
<td></td>
<td>Ong and Larke 52</td>
</tr>
<tr>
<td></td>
<td>Czepeita et al. 50</td>
</tr>
<tr>
<td>Demodex folliculorum</td>
<td>Kheirkhah et al. 51</td>
</tr>
<tr>
<td></td>
<td>Kojima et al. 52</td>
</tr>
<tr>
<td>Eyelid tattooing</td>
<td>Gonnenging and Sonneland 55</td>
</tr>
<tr>
<td>Floppy eyelid syndrome</td>
<td>Mathers and Billborough 54</td>
</tr>
<tr>
<td>Giant papillary conjunctivitis</td>
<td>Martin et al. 53</td>
</tr>
<tr>
<td></td>
<td>Molinari and Stanek 54</td>
</tr>
<tr>
<td>Ichthyosis</td>
<td>Baden and Imber 56</td>
</tr>
<tr>
<td>Salzmann’s nodular corneal degeneration</td>
<td>Farjo et al. 57</td>
</tr>
<tr>
<td>Trachoma</td>
<td>Bron and Tiffany 56</td>
</tr>
</tbody>
</table>

For example, dry eye disease has traditionally been divided into the two main subtypes: aqueous deficient and evaporative. 16 Under this classification, the most common primary etiologic factor thought to underlie the classic evaporative dry eye subtype is MGD. More recently it has come to be recognized that patients are likely to have (or develop over the longer term) elements of both aqueous-deficient and evaporative dry eye. For example, in a case series of dry eye disease characterized by a primary deficit in aqueous secretion such as in Sjögren’s syndrome, MGD is frequently present as well. 43 The MGD in Sjögren’s syndrome may represent a second primary defect of the disease (i.e., in addition to the known effects on the lacrimal gland). However, even in the case of aqueous-deficient dry eye with no identifiable primary cause of MGD, MGD may develop as a consequence of long-term changes brought about in the ocular surface system. In this regard, research has shown tear film lipid layer defects in patients with severe, aqueous-deficient dry eye disease and progressive reductions in tear film lipid layer spreading with increasing severity of aqueous-deficient dry eye. Whether such disturbances of the lipid layer and evaporative dry eye are due to MGD specifically, or alternatively, occur in the presence of completely normal meibomian glands have yet to be elucidated. An excellent review of such concepts, including the phenotypes of dry eye, was recently published by Bron et al. 59

Blepharitis is a generic term used to indicate the presence of inflammatory changes with diverse etiology and presentation that affect the eyelid as a whole. It is one of the most common ocular disorders encountered in clinical practice, and it overlaps substantially with MGD, as MGD is considered to be one cause of posterior blepharitis. Attempts to classify this disorder have been difficult, at least in part due to the complex and incompletely understood mechanisms thought to underlie its pathogenesis, its heterogeneous presentation, and the lack of information on its natural history. Clinical and laboratory investigations of patients with chronic anterior blepharitis have suggested an increased frequency and heavier colonization with certain common bacteria (e.g., Staphylococcus epidermidis and Staphylococcus aureus). 40, 60 Posterior blepharitis is a term used to describe inflammatory conditions of the posterior lid margin, including MGD. Some forms of posterior blepharitis appear to have a seborrheic etiology that can initially be associated with excess meibomian lipid production. Pure subtypes of blepharitis are probably the exception rather than the rule. In one study of 57 patients with various clinical signs and symptoms of chronic blepharitis (presumably of various types), 42 (74%) had evidence of meibomian gland loss shown by gland expression and meibography, whereas only 4 (20%) of 20 normal patients had any gland dropout. 57

Another ocular factor worth considering for a role in MGD is Demodex infestation of the eyelids (see Anatomy, Physiology and Pathophysiology of the Meibomian Gland). Authors of a recent small study observed MGD in five of six patients with this condition. 51 However, additional studies have shown limited to no correlation. 50 Moreover, Demodex infestation in the facial skin has been implicated in causing rosacea, a chronic skin condition of presumed inflammatory origin that frequently affects the eye, discussed below. 61

### Aging and Other Systemic Risk Factors

Age-related and other systemic factors or processes may influence the structure and/or function of the meibomian gland. Regarding the possible effects of aging, Den et al. 62 reported a cross-sectional study in which evaluation of lid margin anatomy, meibomian gland, ocular surface epithelium, and tear function was conducted in 354 eyes of 177 subjects. These authors observed that whereas only a few patients aged 50
young and younger showed notable abnormalities in the lid margin or meibomian glands, the frequency of such abnormalities increased dramatically in those older than 50 years. Hykin and Bron63 have reported in a cross-sectional study with 80 subjects between 5 and 87 years old that an increase in eyelid margin vascularity, keratinization, telangiectasia, and opacity of meibomian gland secretions was observed with aging. Sullivan et al.64 also showed significant alterations in older versus younger individuals’ polar and neutral lipid profiles derived from meibomian gland secretions by high-performance liquid chromatography or mass spectrometry. Such findings appear to coincide with a documented increase in the incidence and prevalence of dry eye disease with aging.65 The clinical significance of such apparent changes and whether they result directly from aging, are secondary to other age-related biological effects such as the well-known decline in production of sex-steroid hormones or some other mechanism, all of which have yet to be determined.

Sex steroid hormones, such as androgens, are known to control the development, differentiation, and lipid production of sebaceous glands throughout the body, and there is evidence that they have similar effects on the meibomian glands.66,69 Accordingly, androgen–meibomian gland interactions may comprise an etiologic factor in the pathogenesis of MGD. Consistent with this idea, Sullivan et al.65 observed that androgen deficiency, in patients receiving antiandrogen therapy, is associated with MGD, tear film instability, and dry eye symptoms. In a further study in which mass spectrometry of meibomian gland secretions was used in patients with complete androgen insensitivity syndrome, the authors identified significant alterations in the appearance of numerous molecular species in the neutral and polar lipid fractions. These biochemical changes were associated with the observation of clinically apparent MGD and functional dry eye due to tear film lipid layer instability. Mathers et al.66 measured levels of several sex steroid hormones and performed tear function tests in a group of 110 pre- and postmenopausal women. They observed a positive correlation between higher testosterone levels and better tear function among postmenopausal women, but a negative association in the premenopausal group. Although measures of meibomian gland dysfunction were not reported, this may point to the importance of the balance of different hormones.

Sjögren’s syndrome (SS) is an autoimmune disorder that affects exocrine glands, including the salivary and lacrimal glands, and leads to aqueous-deficient dry eye. The annual incidence of physician-diagnosed Sjögren’s syndrome has been estimated at 3.9 per 100,000, with a significantly higher incidence in women (6.9/100,000) than in men (0.5/100,000).67

Epidemiology and Associated Risk Factors 1999

Aging

Androgen deficiency

Atopy

Benign Prostate Hyperplasia

Cicatricial pemphigoid

Complete androgen-insensitivity syndrome

Discoid lupus erythematous

Ectodermal dysplasia syndrome

Hematopoietic stem cell transplantation

Hypertension

Menopause

Parkinson’s Disease

Pemphigoid

Polycystic ovary syndrome

Psoriasis

Rosacea

Sjögren’s syndrome

Table 5. Systemic Factors Hypothesized to Correlate with MGD

<table>
<thead>
<tr>
<th>Factor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aging</td>
<td>Den et al.62 DEWS46</td>
</tr>
<tr>
<td>Androgen deficiency</td>
<td>Hykin and Bron63 Schaumberg et al.70</td>
</tr>
<tr>
<td>Atopy</td>
<td>Sullivan et al.71 Sullivan et al.64</td>
</tr>
<tr>
<td>Benign Prostate Hyperplasia</td>
<td>Bron et al.74</td>
</tr>
<tr>
<td>Cicatricial pemphigoid</td>
<td>Sullivan et al.65</td>
</tr>
<tr>
<td>Complete androgen-insensitivity syndrome</td>
<td>Sullivan et al.65 Bron and Tiffany84</td>
</tr>
<tr>
<td>Cicatricial pemphigoid</td>
<td>Cermak et al.73</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Sullivan et al.73</td>
</tr>
<tr>
<td>Menopause</td>
<td>Ena et al.76</td>
</tr>
<tr>
<td>Parkinson’s Disease</td>
<td>Kaercher77</td>
</tr>
<tr>
<td>Pemphigoid</td>
<td>Ogawa et al.78</td>
</tr>
<tr>
<td>Polycystic ovary syndrome</td>
<td>Schaumberg et al.70</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>Mathers et al.66</td>
</tr>
<tr>
<td>Rosacea</td>
<td>Sullivan et al.65</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>Tamer et al.79</td>
</tr>
<tr>
<td>Stevens-Johnson syndrome</td>
<td>Iovine et al.80</td>
</tr>
<tr>
<td>Toxic epidermal necrolysis</td>
<td>Yavas et al.81</td>
</tr>
<tr>
<td>Turner syndrome</td>
<td>Horwath-Winter et al.82</td>
</tr>
<tr>
<td>Androgen deficiency</td>
<td>Zuber87</td>
</tr>
<tr>
<td>Menopause</td>
<td>Zuber88</td>
</tr>
<tr>
<td>Rosacea</td>
<td>Zengin et al.93</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>Alpere and Mannis85</td>
</tr>
<tr>
<td>Stevens-Johnson syndrome</td>
<td>Sotozono et al.90</td>
</tr>
<tr>
<td>Toxic epidermal necrolysis</td>
<td>Sotozono et al.90</td>
</tr>
<tr>
<td>Turner syndrome</td>
<td>Bron and Tiffany86</td>
</tr>
</tbody>
</table>

* The largest study of 39,876 women showed no association between menopausal status and dry eye disease.71
dry eye secondary to chronic graft versus host disease (GVHD) are also likely to exhibit coincident severe MGD.

Ectodermal dysplasia syndrome is a group of fairly rare genetic disorders identified by the absence or deficient functioning of at least two derivatives of the ectoderm, such as teeth, hair, nails, and sweat glands. In a report by Kaercher, alterations in the meibomian glands were observed in 21 (95.5%) of 22 patients and included partial loss of the glands, coarsening of the acini, or complete absence of meibomian glands when observed with transillumination. Under the Foulks and Bron classification scheme published in 2003, as well as the classification scheme presented in the Definition and Classification Report, congenital meibomian gland disease is considered separate from MGD. It is important to confirm whether the glandular change in ectodermal dysplasia syndrome is MGD, as opposed to a variable degree of MG agenesis, as the gene responsible for ectodermal dysplasia controls development of the sebaceous glands, among others.

Medication Risk Factors

Studies have been conducted specifically to look into possible effects of drugs on meibomian gland structure and function have not, to our knowledge, been conducted, with the exception of studies assessing 13-cis retinoic acid (Accutane; Hoffman-LaRoche, Nutley, NJ; removed from the market in 2009) therapy for acne. Clinically, 13-cis retinoic acid administration has been shown to result in abnormal meibomian gland secretions, meibomian gland atrophy, decreased TBUT, increased tear film osmolarity, and dry eye symptoms and is further detailed in the Report on the Anatomy, Physiology, and Pathophysiology of the Meibomian Gland. In effect, the retinoic acid derivatives may promote MGD and evaporative dry eye; however, the small sample size and clinical nature of those studies warrants further investigation of 13-cis retinoic acid as an associated or causal risk factor for MGD and evaporative dry eye.

There have been several studies that have evaluated the effect of medications on the risk for dry eye in general, and this information may be germane, given the overlap between dry eye and MGD (see Table 6 for medications hypothesized to be associated with MGD). Postmenopausal hormone therapy (PMH) is associated with a higher prevalence of dry eye disease. A large cohort study of more than 25,000 women showed an approximately 70% increased risk among those who used estrogen alone, as well as an approximately 50% higher risk in women who used estrogen in combination with progesterone or progestins. The most biologically plausible explanation for this association involves a possible effect of PMH on the meibomian glands leading to MGD and evaporative dry eye. Results of other studies are mostly consistent with the suggestion that PMH exerts an adverse effect on the ocular surface. Erdem et al. conducted a prospective study on 40 postmenopausal women, including 20 with, and 20 without, dry eye, and evaluated its development and progression after initiation of PMH. After 5 months of PMH, all patients with dry eye at baseline still had dry eye, and the condition developed in a further 11 (61.1%) patients (P = 0.003). Although these findings cannot be viewed as conclusive, given the unmasked and nonrandomized design, the data support the hypothesis that PMH increases the risk of dry eye while simultaneously refuting the alternative hypothesis that PMH could be beneficial in this circumstance. Further supporting evidence comes from the Blue Mountains Eye Study of 3500 residents, which showed that current PMH use was associated with a statistically significant, 60% higher prevalence of dry eye.

Other medications may also impact the risk of dry eye, including evaporative dry eye. For example, in a recently analysis of data from men participating in the Physicians’ Health Studies, the use of medications to treat benign prostatic hyperplasia was observed to be associated with a significantly increased risk of dry eye (OR 1.35; 95% CI, 1.01–1.80). On the other hand, the Physicians’ Health Studies showed the statin and antihypertensive drugs were not associated with dry eye, and antidepressants may increase the risk of dry eye. Among 6034 participants in the Physicians’ Health Studies for whom information on medication use was available, there was a nearly twofold increased prevalence of dry eye among men who used antidepressants. An analysis from the Beaver Dam Eye Study (age range, 43–86 years, 5924 subjects) showed that antidepressant use was a risk factor for incident dry eye over 10 years of follow-up (OR, 1.54; 95% CI, 1.05–2.27). Similarly, in the Blue Mountains Eye Study, there was a significant increase in the prevalence of dry eye among people who used antidepressants.

Antihistamines are another class of medications whose use appears to be associated with ocular dryness. Systemic use of antihistamines has been associated with increased risk of dry eye in a prospective analysis from the Beaver Dam Eye Study, as well as in an open label, short-term trial of loratadine, 10 mg once daily, among 18 adults with seasonal allergic conjunctivitis. However, it should be noted that no changes in TBUT were observed in the latter study.

Research has shown that dietary intake of ω-3 fatty acids (FAs) and the ratio of their consumption to that of ω-6 FAs affects the overall amount of inflammatory activity in the body. Miljanovic et al. observed that a higher dietary intake of ω-3 FA was associated with a decreased risk of dry eye, whereas a higher ratio of ω-3 to ω-6 FA reduced the risk of dry eye in a large cross-sectional study of 39,876 women in the Women’s Health Study. Small randomized trials of ω-3 to ω-6 FAs, as well as animal data, also suggest beneficial effects of essential FAs on the ocular surface in dry eye. More recently, Macsai presented a randomized placebo-controlled double-masked trial of 38 patients with blepharitis and simple obstructive MGD. After 12 months of intake, the group assigned to ω-3 FA had an improvement in TBUT, Ocular Surface Disease Index (OSDI) score, and meibum score, when

### Table 6. Medications Hypothesized to Correlate with MGD

<table>
<thead>
<tr>
<th>Medication</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isotretinoin (13-cis retinoic acid) therapy*</td>
<td>Caffery and Josephson²⁹</td>
</tr>
<tr>
<td>Antiandrogens</td>
<td>Egger et al.⁶⁵</td>
</tr>
<tr>
<td></td>
<td>Mathers et al.⁹³</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>Chia et al.⁹⁰</td>
</tr>
<tr>
<td></td>
<td>Moss et al.⁹⁷</td>
</tr>
<tr>
<td></td>
<td>Schaumberg et al.⁷⁰</td>
</tr>
<tr>
<td>Antihistamines</td>
<td>Moss et al.¹¹⁰</td>
</tr>
<tr>
<td></td>
<td>Ousler et al.⁹⁸</td>
</tr>
<tr>
<td>Medications used to treat benign prostate hyperplasia</td>
<td>Schaumberg et al.⁷⁰</td>
</tr>
<tr>
<td>ω-3 Fatty acids (possibly protective)</td>
<td>Barabino et al.⁹⁹</td>
</tr>
<tr>
<td></td>
<td>Creuzot et al.¹⁰⁰</td>
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<td></td>
<td>Kokke et al.¹⁰¹</td>
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<td></td>
<td>Macsai¹⁰²</td>
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<td></td>
<td>Miljanovic et al.¹⁰³</td>
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<td></td>
<td>Pinna et al.¹⁰⁴</td>
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<td></td>
<td>Rashid et al.¹⁰⁵</td>
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<td></td>
<td>Viana et al.¹⁰⁶</td>
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<td></td>
<td>Chia et al.⁹⁶</td>
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<td></td>
<td>Erdem et al.¹⁰⁷</td>
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<tr>
<td></td>
<td>Lin et al.¹²⁸</td>
</tr>
<tr>
<td>Postmenopausal hormone therapy</td>
<td>Schaumberg et al.¹⁰⁸</td>
</tr>
</tbody>
</table>

* Accutane; Hoffman-LaRoche, Nutley, NJ; withdrawn from the market in 2009.
compared with the placebo group. Changes in meibum composition were observed in the ω-3 group (P = 0.04 compared with baseline); the level of meibum saturated FAs decreased when measured by chromatography.102 See Clinical Trials for more details on ongoing ω-5 studies.

Environmental Factors

Environmental factors such as geography, temperature, humidity, and visual task may play a role in MGD and/or its impact on patients. For example, as already noted, there may be an increased frequency of MGD in Asian populations, and this may be related to differences in geography (or temperature, humidity, and air quality). Likewise, computer users often complain of eye strain, eye fatigue, burning, irritation, redness, blurred vision, and dry eyes. This constellation of ocular complaints resulting from video display terminal (VDT) viewing and sustained attention to a computer monitor is frequently associated with a decreased blink rate and can be regarded as a type of repetitive strain disorder, often referred to as computer vision syndrome.112 Fenga et al.113 reported a clinical study of 70 VDT users and found that 52 (74.5%) had MGD. There was also a significant correlation between the severity of symptoms of ocular discomfort and hours spent on VDT work, both in the total population (r = 0.36; P = 0.002; 95% CI, 0.13–0.54) and in the group of subjects with MGD (r = 0.37; P = 0.009; 95% CI, 0.10–0.58). It remains unclear whether such factors might contribute to the development of MGD itself or just exacerbate symptoms in preexisting MGD.

MGD AND CL WEAR

There is a long-standing clinical impression that CL wear increases the risk of MGD. It is thus perhaps surprising to find that relatively few studies have addressed this question directly. The peer-reviewed literature relating to CL wear and MGD falls into three areas, discussed in turn in the following section (Table 7).

CL Wear as a Risk Factor for MGD

Korb and Henriquez14 and Henriquez and Korb114 elegantly described the tissue changes that accompany MGD. In these studies, they showed a series of micrographs illustrating how stagnation of the sebaceous meibomian secretion occurs due to obstruction of the excretory duct by accumulations of epithelial cells desquamated from the ductal lining. These keratotic clusters of material cause the duct to dilate, and its ability to deliver a normal secretion is impaired or obliterated. This is consistent with mechanisms of duct obstruction, atrophy, and secretion proposed in the Report on Anatomy, Physiology, and Pathophysiology of the Meibomian Gland.

Korb and Henriquez14 reported on 38 asymptomatic and 40 asymptomatic CL wearers. In the former group, 90.1% of eyes had some MGD on the basis of the ability to express meibum gland output on one or two expression attempts, with firm digital pressure on the lower lid margin under the lashes. Once again, the difference was not significant.

The largest study was that of Hom et al.35 who specifically compared the frequency of MGD among CL wearers and non-CL wearers. The criterion for MGD was cloudy or absent gland output on one or two expression attempts, with firm digital pressure on the lower lid margin under the lashes. Although there was a small excess of MGD in the CL wear (41%) versus non-CL wear (38%) group, it was not statistically significant or likely to be relevant clinically. Based on a much smaller sample, Marren53 was similarly unable to find a significant difference, although the actual overall frequency of MGD in that patient group was higher in both CL wear (60%) and non-CL-wear (57%) groups. Her definition of MGD was any blocked gland orifices on gentle digital pressure below the lower lid orifices. Ong15 reported that 45% of CL wearers in his sample had MGD, compared with 35% of non-CL wearers. Once again, the difference was not significant.

In an effort to form a consensus from the available literature, we conducted a subanalysis using data from the more completely characterized studies. To be included, studies had to have reported the total number of CL wearers and non-CL wearers, together with the number in each group displaying MGD. The result is summarized in Table 7 and yields an overall frequency rate estimate for MGD in CL wear of 37.7% ± 5.4% and 32.1% ± 4.3% in non-CL wearers (errors are 95% CIs). This difference is not statistically significant, suggesting that CL wear may not increase the risk for MGD. However, as noted, most of these studies have limitations in size, design, and analysis that preclude any sort of conclusive statements in this regard.

Table 7. Summary and Meta-analysis of Studies Reporting Prevalence of MGD in CL and Non-CL Wearers

<table>
<thead>
<tr>
<th>Study</th>
<th>Total Subjects</th>
<th>CL Wearers</th>
<th>Non-CL Wearers</th>
<th>CL Wearers with MGD n (%)</th>
<th>Non-CL Wearers with MGD n (%)</th>
<th>Difference in % MGD between CL and Non-CL Wearers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hom et al.35</td>
<td>398</td>
<td>162</td>
<td>236</td>
<td>66 (40.7)</td>
<td>89 (37.7)</td>
<td>3.0</td>
</tr>
<tr>
<td>Marren53</td>
<td>50</td>
<td>20</td>
<td>30</td>
<td>12 (60.0)</td>
<td>17 (56.7)</td>
<td>3.3</td>
</tr>
<tr>
<td>Ong and Larke32</td>
<td>140</td>
<td>70</td>
<td>70</td>
<td>21 (30.0)</td>
<td>14 (20.0)</td>
<td>10.0</td>
</tr>
<tr>
<td>Ong15</td>
<td>181</td>
<td>53</td>
<td>128</td>
<td>16 (30.2)</td>
<td>29 (22.7)</td>
<td>7.5</td>
</tr>
<tr>
<td>Aggregate</td>
<td>769</td>
<td>305</td>
<td>464</td>
<td>115 (37.7)</td>
<td>149 (32.1)</td>
<td>5.6</td>
</tr>
<tr>
<td>95% CI</td>
<td></td>
<td></td>
<td></td>
<td>(32.3–43.1)</td>
<td>(27.9–36.4)</td>
<td></td>
</tr>
<tr>
<td>Two-tailed P-value</td>
<td></td>
<td></td>
<td></td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A recent study by Arita et al. offers direct evidence that CL wear may affect the morphology of the meibomian glands. Using meibography to view the glands in the everted eyelid, they graded MG loss on an ordinal scale (0–3) referred to as the meiboscore. Higher meiboscores indicate more severe degrees of loss. Wearers of CLs of any type (rigid or soft) had significantly higher meiboscores (1.72 ± 0.24, mean ± 95% CI) than non-CL wearers (0.96 ± 0.23). The duration of CL wear was weakly associated with the meiboscore. Based on this result and the observation that the upper eyelid showed more of a difference between CL wearers and non-CL wearers than did the lower lid, the authors suggest that irritation of the glands through the eyelid by the lens may be responsible for the observed morphologic changes.

Reconciling the findings of Arita et al. with those of the studies in Table 1 requires further work. One obstacle is that differences in interpretation and definition of what constitutes MGD and/or gland loss exist across the various studies. For example, it is not clear to what extent subjects exhibiting low meiboscores, as defined by Arita et al., would respond to the diagnostic criterion, common in other studies, of gentle forcible meibomian gland expression. Also of interest is the relationship between the degree of meibomian gland loss and symptoms in CL wear, more generally.

MGD and Symptoms in CL Wearers

The question of the relationship between CL wear, symptoms, and MGD has received relatively little attention in the literature to date. Korb and Henriquez and Henriquez and Korb described a syndrome characterized by deficient or inadequate meibomian gland secretions, minimal or transient symptoms suggestive of ocular dryness, fluorescein staining of the cornea, and CL intolerance. Of 71 eyes of affected subjects, 36% showed no secretion from the lower lid glands on gentle expression. Only 2.5% of the 80 asymptomatic, control, CL-wearing eyes were similarly affected. Based on these findings they suggest that asymptomatic CL wearers are five times more likely to show normal meibomian gland expression than are those intolerant of CL wear.

Supporting evidence comes from Paugh et al. who studied the effect of lid scrubs and massage on TBUT and subjective comfort in 21 CL wearers with MGD. They defined MGD as an absent or cloudy meibomian gland secretion on repeated expression. Treatment was applied unilaterally for 2 weeks and showed a significant increase (4 seconds) in TBUT relative to pretreatment in the treatment eye and subjective reductions in discomfort and dryness, assessed on 10-point scales, of approximately 1.7 and 1.1 points, respectively. These latter assessments were made bilaterally, as the subjects could not, in general, distinguish differences in symptoms between the eyes. Control eyes did not change on average. No statistical tests are reported in the paper but, judging from the standard deviations quoted, these differences are probably near the level of statistical significance. These data suggest that discomfort and dryness symptoms in CL wear can be associated with MGD, since the application of treatment brings improvement in the symptoms. However, the study does not provide evidence to suggest that CL wear was a cause of the MGD in these patients.

A somewhat contrary view emerges from Nichols and Sinnott, who conducted an extensive study of 360 CL wearers to look for risk factors associated with CL-related dry eye (CLDE). They were unable to find any significant association between meibomian gland drop out and CLDE symptoms. They did show a reduced lipid layer thickness and corresponding faster pre-lens tear film thinning times and increased osmolarity in the symptomatic CL wearers, suggesting that the outcome of symptoms may be derived from mechanisms other than gland loss. The contrast between this finding and those of Paugh et al. and Korb and Henriquez is striking. The temporal relation between the factor of interest and disease status has not been properly determined. At the present time, we consider the prior studies as providing some evidence of concurrent factors or correlates of MGD, rather than as providing true risk factors for MGD. That being said, we reiterate that there appears to be some consistency for certain ophthalmic, systemic, and environmental factors associated with MGD. Possible demographic differences in MGD rates such as by age, sex, and race or ethnicity still need better delineation, especially relative to the underdetermined incidence of the disease. There is evidence that CL wear may be associated with certain aspects of MGD, but this, too, needs much better delineation. For instance, it is well known that approximately 50% of contact lens wearers have frequent dry eye symptoms, but it is not known how much of this may be due to MGD. The effects of CL wear on the health of meibomian glands (atrophy), the excretion of the meibomian glands, or function of the lipid layer itself in terms of retarding evaporation need further study.

MGD and CL-Related Papillary Conjunctivitis

The question of a link between CL-related papillary conjunctivitis (CLPC), or giant papillary conjunctivitis (GPC) as it is also known, and MGD has been addressed by only a few studies, with equivocal results (Table 4). Reporting on 42 contact lens wearers, Mathers and Billborough found that the 27 subjects with clinical signs of GPC had significantly greater meibomian gland dropout than the remainder. Martin et al. found evidence of MGD in each of their 42 subjects with GPC. Molinari and Staneck on the other hand, found that, although 23 of 105 subjects in their study had MGD, none had co-existing GPC.

In reconciling these findings, it may be that the link between MGD and CLPC/GPC is not causal. Rather, the factors in contact lens wear that produce the clinical presentation of CLPC/GPC can produce simultaneously manifesting MG effects without any substantial etiologic connection.

Future Directions for Understanding the Epidemiology of MGD

Although there are several studies that have provided frequency estimates for MGD, these studies have been limited in that they have provided simple frequency or prevalence, rather than incidence, estimates. Further, the studies have generally used nonstandardized definitions of MGD, making it difficult to directly compare the frequency estimates. Future population-based studies should be conducted with standardized classification criteria to better delineate the frequency of MGD, including both prevalence and incidence. Likewise, prior studies that have evaluated potential risk factors for MGD have been nonexistent, or limited by small size, cross-sectional design, and other methodological shortcomings. Virtually none of the studies has evaluated incident (new) cases, and therefore, the temporal relation between the factor of interest and disease status has not been properly determined. At the present time, we consider the prior studies as providing some evidence of concurrent factors or correlates of MGD, rather than as providing true risk factors for MGD. That being said, we reiterate that there appears to be some consistency for certain ophthalmic, systemic, and environmental factors associated with MGD. Possible demographic differences in MGD rates such as by age, sex, and race or ethnicity still need better delineation, especially relative to the underdetermined incidence of the disease. There is evidence that CL wear may be associated with certain aspects of MGD, but this, too, needs much better delineation. For instance, it is well known that approximately 50% of contact lens wearers have frequent dry eye symptoms, but it is not known how much of this may be due to MGD. The effects of CL wear on the health of meibomian glands (atrophy), the excretion of the meibomian glands, or function of the lipid layer itself in terms of retarding evaporation need further study.
As summarized herein, there are several commonly used clinician-based assessment methods in addition to reporting symptoms that are generally used in the evaluation of MGD for outcome purposes. Each of these methods is limited by their subjectivity (and therefore, variability), which may lead to a lack of responsiveness as the disease progresses, with time or sensitivity between disease states (i.e., dry eye and MGD). Further, it is unclear how several of these outcomes truly relate to the nature of the disease. For instance, meibography is commonly used to image the meibomian glands (to determine atrophy), but it is unclear how this relates to the gland excretion or symptoms experienced by the patient. Yet, it is hard to argue that atrophy of the meibomian glands is not important in the disease process in some way. It is recommended that the community focus on the relation between the meibomian gland status (through meibography) and other clinical correlates and symptoms of MGD.

Similarly, it is well known that symptoms are a major component of MGD, but there is a paucity of data on the relative importance, including frequency and severity, of specific symptoms associated with the disease. Specific subjective outcomes measures for MGD have not been properly established or validated. Related to this, it is unclear what role MGD has in the overall quality of life of an individual. It is recommended that the community focus attention on these patient-reported aspects of outcome development.

It is not entirely understood how the truly objective, analytical measures associated with the assessment of MGD relate to the disease in terms of its incidence (a biomarker, perhaps), clinical correlates (meibomian gland plugging, expressibility, and meibum quality), or subjective outcomes. This uncertainty is particularly true of the biochemistry of the lipid excretion of the meibomian gland in relation to other outcomes. It is recommended that the community try to focus more attention on better understanding these relationships (e.g., the relation between tear osmolarity and symptoms of MGD), in addition to developing a better understanding of potential biomarkers in MGD that may either help diagnostically or track changes in MGD with time or with treatment.

Finally, it is critically important that studies be undertaken that begin to establish the natural history of MGD and associated risk factors. There are many questions that could be answered in this regard. For instance, the time course of disease progression is uncertain, including the relation between true etiologic factors and the development of symptoms of disease. As mentioned, the relation between meibomian gland atrophy (gland loss) and symptom development is uncertain; for instance, it could be that some atrophy of the glands is normal and may not lead to patient symptoms or ocular surface damage. In addition, the actual source of the symptoms of MGD is not known (e.g., do they derive from the meibomian glands or the ocular surface?), nor has the primary contributing factor leading to their development been identified. Once atrophy is present and the patient develops symptoms, it may also be possible for the glands to return to their normal state (for instance, if gland loss is due to CL wear and the individual discontinues CL wear), but this has not been studied to our knowledge. Further, associated morbidities that may occur after the onset of MGD have not been established with quantitative estimates. This includes, for example, correlates such as the visual impact of the disease or the potential susceptibility of patients with MGD to ocular surface infection. Even the relation and cross-correlation between MGD and dry eye disease is not well understood. For instance, is MGD a risk factor or cause of dry eye disease? Or might dry eye disease be a risk factor or cause of MGD? What is the time course (temporal relation) for the development of these common comorbidities? As noted by Lemp and Nichols in their study of those individuals who had been diagnosed with either MGD or dry eye disease, 40% had been diagnosed with both MGD and dry eye disease. Further, the patient-reported symptoms between those with MGD or dry eye disease were correspondingly similar, with limited exceptions.

**SUMMARY**

In summary, MGD appears to be a prevalent problem with potentially severe detriments to well-being. Nonetheless, even basic information regarding its prevalence, demographic and geographic distribution, risk factors, and impact on ocular health and quality of life are only beginning to emerge. The same was said of dry eye disease more than a decade ago, and since that time, research efforts have grown exponentially. We are confident that the time has now arisen to embark on the systematic study of MGD as well. It is through such efforts that a better understanding of the disease will be gained, and strategies for prevention and treatment will begin to be developed.

**References**


D iagnostic tests of meibomian gland dysfunction (MGD) and of MGD-related disorders are based on the demonstration of abnormal anatomy and physiology of the glands and the detection of specific pathologic events. For this reason, this subcommittee report is divided into two sections. In part I, those aspects of meibomian anatomy and physiology that are relevant to currently available tests are described; a fuller account of the anatomy and physiology is provided in the report of the Anatomy Subcommittee of this workshop. In part II, each test and its performance is described in detail. In part III, the practical application of selected tests is summarized and recommendations for future approaches are made. Additional recommendations and a summary of pertinent literature and concepts are presented in Appendices 1 to 17.

I. ANATOMY AND PHYSIOLOGY OF THE MEIBOMIAN GLANDS: CLINICAL IMPLICATIONS

The superficial location of the meibomian glands in the tarsal plates permits their anatomic features to be quantified by meibography and confocal microscopy (Appendixes 7, 8). In normal subjects, the meibomian orifices are disposed at regular intervals along the lid margins, just anterior to the mucocutaneous junction (MCJ). Biomicroscopically, they are surrounded by a characteristic ring-shaped architecture, reflecting the concentric arrangement of orifice, mucosa, distal acini, fibers of the muscle of Riolan, and the connective tissue sheath of the glands (Fig. 1).1,2 This configuration becomes less well-circumscribed in old age and is destroyed in advanced MGD.3 Loss of this architecture may be scored and is an important clinical sign of MGD.

The lipid secretion of the meibomian glands is liquid at lid temperature and is delivered to the skin of the lid margin as a clear fluid termed meibum.4 Here, it forms shallow reservoirs on the upper and lower lid margins from which the tear film lipid layer (TFLL) is formed and replenished. The amount of lipid present in the normal, lower lid reservoir may be gauged by the technique of meibometry5,8,9 and used to infer the content of the total lid reservoir. In meibometry, a linear sample of meibum is blotted from the central third of the lower lid, onto a loop of plastic tape, and the amount of lipid present in the defined zone is gauged by the change in optical density (Appendix 9). In normal adults, the total amount of lipid contained in the upper and lower reservoirs has been estimated to be roughly 300 μg.5,8 This calculation was based on comparisons against a standard lipid with the assumption that the meibomian reservoir is shared equally between the upper and lower lids. However, a comparison of basal levels on the upper and lower lids has not yet been made. The technique may be used to quantify meibomian gland obstruction,7 but in the presence of MGD, the reading cannot be extrapolated to estimate the total extent of obstruction on both lids, because of the variability of the disease along the lid length.

As detailed in the Report on Tear Film Lipids, the meibomian secretion is a complex mixture of cholesterol, wax and cholesterol esters, phospholipids with small amounts of triglycerides and triacylglycerols, and hydrocarbons.10–13 The phospholipid content has been promoted as the basis for the interaction between the TFLL and the aqueous subphase of the tear film, necessary for tear film spreading17,44, however, recent studies have reported negligible levels of phospholipids in meibomian lipid, so that it may be necessary to seek an alternative candidate for this interaction.20,22,24 This is currently debated. The presence of hydrocarbons and to a lesser extent, triglycerides, has been interpreted in part as due to contamination by sebum and environmental chemicals.

The lipid mixture has a melting range in the region of 19.5°C to 40°C, which ensures lipid mixture fluidity at the surface of the lid.13 The melting range of the lipid mixture also influences its stability in the TFLL, since the temperature of the cornea is cooler (approximately 33.5°C)46 than that of the lid margin. This temperature difference may also be the basis of the sustained integrity of the TFLL over a series of successive blinks (the pleating effect, described later), a normal feature of TFLL dynamics.17 The stability of the TFLL may be measured by static and dynamic interferometric techniques (see Appendix 10).

The manner of secretion and delivery of meibum has been examined by using meibometry to follow the recovery of lipid on the lower lid reservoir after total removal of lipid from the upper and lower lid margins with organic solvents.5 In normal adults undergoing surgery under general anesthesia, partial recovery occurred over periods of 3 to 40 minutes, indicating that secretion and delivery continues in the absence of blinking. Various studies have shown that, from time to time, aliquots of meibum are also jetted directly from some glands into the subphase of the tear film, necessary for tear film spread-
the TFLL, and on this basis, it is generally accepted that blinking plays a role in the delivery of meibomian lipid to the TFLL.

Recent studies have followed the secretory recovery of single meibomian glands after drainage by compression. In these studies, a standardized device was used to apply a standard force to individual glands located at the center of the lower lid, to drain them of their meibomian lipid. The glands selected for study in 12 subjects aged 18 to 25 years, were optimally secreting in the sense that expression could be initiated within 2 seconds of the application of pressure. The mean time to effect drainage, was 12.1 ± 3.5 seconds (range, 8–20) and the time to partial recovery of secretion was 2.2 ± 0.5 hours. Repeat expression after partial recovery cleared the ducts of contained secretion in about half the time taken to drain them initially.

Using meibometry, Chew found that the basal level of meibomian lipid in the lower reservoir was highest within the first hour after waking. This finding was interpreted to reflect a damming back of secreted lipid within the ducts during prolonged eye closure, in the absence of blinking, as in sleep, and the release of the accumulated lipid on eye opening. The latter hypothesis, however, neglected the potential influence of altered lipid excretion, which has been assumed to occur from the lid margin across the skin of the lids. It should be kept in mind that a reduced removal of meibomian lipid during prolonged eye closure would also lead to a rise in the recorded basal level shortly after waking. This question should be amenable to study using the compression and drainage approach.

Meibomian Gland Activity

A few authors have addressed the question of gland activity in the waking state, using the term activity to mean expressibility of meibomian oil. Norm, staining with Sudan black or applying digital pressure along the full extent of the lower lid, concluded that approximately 45% of the adult glands were active at a given time. Here, it was assumed that, in the natural state, those ducts receiving lipid from actively secreting glands would be filled with liquid lipid and that would be reflected by the ability to express their contained oil.

These findings have been supported by recent studies employing standardized meibomian gland expression. Korb and Blackie have developed a standardized technique for meibomian gland expression using a custom-made expression device (Appendix 6) that applies a standard force of 1.25 g/mm² to the lid, over an area of approximately 40 mm² (Fig. 2). This force was chosen to approximate between that applied by the lids to the globe during spontaneous blinking and that applied during deliberate, forced lid closure. In studies by Comberg and Stoewer, cited by Miller, a hard lid squeeze results in a rise of intraocular pressure in the region of 18 to 70 mm Hg, whereas the Korb expression device raises the pressure to between 30 and 40 mm Hg.

The device achieves simultaneous expression from approximately eight glands (occupying approximately one third of the lid length, i.e., 8/24 glands). Gland expressibility is scored according to the number of the eight glands from which a fluid secretion can be expressed, regardless of its qualitative appearance. This is the Meibomian Glands Yielding Liquid Secretion (MGYLS) score. In a small group of normal young subjects, the average MGYLS score for the whole lower lid was 10.6 ± 2.6. The range was 6 to 15.5 (25%–65% were active, presuming there are 24 glands along the lower lid), suggesting that there is marked variation in activity between individuals. Also, these studies have shown consistently that the nasal glands are the most active, followed by the central glands, and finally, the temporal glands.

In a more recent report by Blackie and Korb, the secrecy of individual meibomian glands was studied in young healthy individuals without dry eye symptoms or signs. It was found that if a meibomian gland yielded liquid secretion at 8 AM, then, depending on its location along the lower lid, there was a high likelihood that it would continue to provide liquid secretion throughout a 9-hour day. For example, 70% of the nasal glands, 30% of the central glands, and 20% of the temporal glands provided liquid secretion throughout a 9-hour day. If a meibomian gland did not yield liquid secretion at 8 AM, it would continue throughout the day or not. Assuming that meibomian glands on the upper lid function in a similar manner, it seems that the marginal lipid reservoirs are maintained by the activity of only a proportion of the total number of glands. It will be of interest whether individual glands that are inactive at one time become active days or weeks later.
diurnal fluctuations in meibomian gland activity may lead to their use in future MGD diagnosis.

While these observations have not yet been confirmed by other groups, they have potentially important implications for those tests of meibomian function that depend on determining the expressibility of a set of glands. Based on the proportion of expressible glands alone (without reference to either quality of expressed secretion, state of the orifices or presence of local gland dropout), it may be difficult to differentiate between glands that are not expressible for physiological reasons or for pathologic reasons (i.e., due to the presence of MGD). Observation of orifice disease at the slit lamp could be helpful. Also, where an investigator selects expressibility as a measure of disease, it may be appropriate to specify location for consistency (e.g., the nasal third of the lid).

These studies also raise important questions about the temporal characteristics of meibomian gland secretion. It may be that the glands are engaged in a cycle of activity that changes from gland to gland over time across the length of the lids. This notion implies that each gland has periods of activity when secretions are released, followed by periods of quiescence, when their role is taken over by other glands. This hypothesis would fit in with the holocrine mode of meibomian lipid secretion. The studies cited earlier suggest that this does not occur in the short term (i.e., over a 24-hour period), but there may be a slower cycle in the long term, and this could be relevant to the conduct of clinical trials.

### The Tear Film Lipid Layer

The reported thickness range of the normal TFLL is approximately 20 to 160 nm and occupies the most anterior part of the tear film where it performs a major role in reducing evaporative water loss from the exposed surface of the eye. The layer can be observed by interferometry in which the predominant spectral color represents the TFLL thickness (Fig. 3; Appendix 10). By interferometry (or by recording the movement of particles in the film) the lipid layer can be seen to spread upward in the upstroke of the blink and to become comparatively stable after approximately 1 to 2 seconds. Owens and Phillips give a value of 1.05 ± 0.39 seconds, whereas Goto and Tseng using a different approach, report a value of 0.36 ± 0.22 seconds in healthy eyes, but 3.54 ± 1.86 seconds in eyes with lipid tear deficiency. King-Smith et al. show a time constant associated with exponential decay of lipid drift in the upward direction of 0.564 second and total upward movement of 3.23 mm. Prolongation of the lipid spread time may be an indicator of aqueous tear deficiency, but this has not yet been converted into a formal test for general clinical use (Yokoi N, et al. IOVS 2010;51:ARVO E-Abstract 5201). The duration of the normal blink is approximately 200 to 300 ms. The direction of movement of the horizontal wavefront suggests that the TFLL is delivered to the tear film primarily from the lower reservoir. To explain the ability of the meibomian lipid to spread over the aqueous subphase of the tear film, it has been proposed that the TFLL has a lamellar structure with an internal polar, phospholipid layer that spreads over the aqueous phase of the tear film. As noted earlier, in view of current reports suggesting a low meibum phospholipid content, it may be necessary to seek an alternative lipid layer structure. The more superficial lipid layers are hypothesized to be composed of nonpolar lipids, such as cholesterol and sterol and wax esters, which spread over the polar phase. It should be emphasized that when the spread of the TFLL is observed by interferometry, it is the full thickness of the TFLL that is visualized; the polar lipid layer, which is postulated to run in advance of the nonpolar layer, may be too thin to generate an interference pattern. Thinning of the TFLL has been noted in lipid tear deficiency.

In normal subjects, the interferometric pattern of the TFLL is relatively constant in appearance over several blink cycles, implying that its architecture is conserved to some extent from blink to blink. This preservation occurs despite the expectation that, at the end of the downstroke of a complete blink, the lid margins will be apposed and the lipid reservoirs combined. To explain this phenomenon, it has been proposed that, over this period of stability, the TFLL folds up concertina-wise in the downstroke of the blink and is restored by unfolding during the upstroke. However, it should be noted that subtle or more marked changes in pattern can be observed from blink to blink, which implies some kind of molecular reorganization within the film, either by local movements of lipid within the layer or an exchange across the apposed folds of the lipid layer. At some point, after several blink cycles, an abrupt and complete change in the interferometric pattern occurs, implying a mixing of the TFLL with the combined meibomian reservoirs. This results in a complete restructuring of the TFLL and the cycle begins again. The stable pattern is likely to be influenced by the temperature of the surface of the open eye, influencing fluidity of the lipid mixture, and by the composition of the meibomian lipid, which will influence its melting range. The cycle of stability is shortened in the presence of MGD, and this has been proposed as a measure of MGD-related disease in the Dynamic Lipid Layer Interference Pattern (DLIP) test.

With this background, the physiology of the meibomian glands may be summarized as follows: The glands are under neural and hormonal control and secrete their oil into shallow reservoirs on the lid margins. Secretion is intrinsic to the glands and delivery is aided by the blink. Only a fraction of the glands are active at a given time, with the possible inference that each gland goes through a cycle of activity followed by a period of quiescence, when acinar stores are replenished. There is an uneven distribution of gland activity along the length of the lid, with the least distribution temporally and the greatest distribution nasally. During sleep, it is hypothesized that secreted oil accumulates in the glands and that the excess is discharged on waking, with the resumption of blinking. The marginal lipid reservoir as well as direct expression from the meibomian gland are the sources of the TFLL. At the upstroke of the blink, lipid spreads from the lower reservoir onto the tear film to form the TFLL, with polar lipids, or some other surfactant component of the TFLL, interacting with the water phase of the tear film. Once formed, the TFLL maintains relative stability from blink to blink until it is reconstituted abruptly by a mixing of lipid from both reservoirs with that of the TFLL, and the cycle begins again.

![Figure 3. Spreading of a normal tear film lipid layer image by interferometry (courtesy of N. Yokoi).](image-url)
Many details of this account have yet to be filled in, but this summary may serve for the selection and interpretation of diagnostic tests. Whether MGD occurs on its own, or is part of a wider constellation of diseases, diagnosis requires that its manifestations be distinguished from other, unrelated ocular surface disorders.

**Ocular Surface Disorders**

Several symptomatic disorders affecting the conjunctiva, cornea, and the lids may be conveniently grouped together in the category of ocular surface disorders (OSDs). They include lid and conjunctival disorders and those disorders responsible for aqueous-deficient and evaporative dry eye. There is a certain overlap, since a disorder in one category may be associated with a disorder in another category. MGD is a good example, since it may exist in its own right, give rise to ocular surface damage, or cause evaporative dry eye. These disorders correspond to those referred to as dysfunctional tear syndrome (DTS) by Berens et al. In that report, the term DTS was offered as an alternative to the term dry eye, where DTS may be reasonably considered to describe any cause of symptomatic ocular surface disease, including dry eye.

In attempting to differentiate a particular disorder from other members of this large group, diagnostic tests must discriminate, not only between that particular disease and the unaffected normal state, but also between that condition and other members of the wider group of OSDs. This report is focused on MGD, and as such a description of selected tests of lacrimal function is given, since, in relation to the diagnosis of dry eye, normal lacrimal function must be demonstrated as part of the diagnostic work-up of evaporative dry eye. Tests of meibomian and lacrimal function and of evaporative water loss considered by the diagnostic group are listed in Appendices 3 and 5 through 14.

**Meibomian Gland Dysfunction**

This report as a whole deals chiefly with MGD. Other diseases of the meibomian glands are listed in Table 1 and are also discussed in Report on Definition and Classification. The term MGD has been widely used in the literature, as if it were synonymous with posterior blepharitis, and has been used in contrast to the term anterior blepharitis. However, as discussed by the Definition and Classification Subcommittee, MGD is but one of several causes of posterior blepharitis. Therefore, for clarity, only the term MGD is used herein.

MGD can be an asymptomatic, subclinical condition detectable only by gland expression or meibography. Alternately, it may be symptomatic and accompanied by specific clinical signs (Table 2). It may be primary and unassociated with other local or systemic disease, or it may be secondary to a range of systemic disorders, in particular, some common skin diseases, such as acne rosacea, atopic dermatitis, and seborrhea sicca and also, the cicatrizing conjunctival disorders (trachoma, Stevens-Johnson syndrome, pemphigoid; acne rosacea, atopy). It may also be caused clinically by exposure to drugs and toxins. There are several experimental models for MGD.

MGD may be focal, when it affects scattered glands, or diffuse, when it affects all glands to some degree. Since the natural history of MGD has not been studied, it is not known whether focal disease is always a precursor of diffuse disease. It may also be cicatricial or noncicatricial (simple) and inflammatory (meibomitis) or noninflammatory. Characteristic signs of MGD include the release of cloudy meibum or more viscous material on expression of the glands or by an absence of expressible secretion. Occasionally, the meibomian orifices may be capped by a lipid globule covered by an intact skin (meibomiana), or cap, which is hypothesized to be oxidized lipid and epithelial material.

A diagnosis of MGD may be made by the demonstration of a single affected gland, but clinically relevant disease is due to the involvement of multiple glands. For this reason, diagnosis demands both a qualitative and a quantitative approach.

MGD may be symptomatic in its own right or give rise to symptoms through its contributions to ocular surface damage or to dry eye. The mechanism of primary MGD is not known, but the pathologic events of noncicatricial MGD include hyperkeratinization of the terminal ductules, accumulation of cellular and lipid material within duct lumina, duct obstruction; cystic dilatation of the ducts and acini and secondary, disuse atrophy of the meibomian acini; and, at least in some instances, periglandular inflammatory changes.

The clinical features of MGD may be intrinsic when they involve the meibomian glands alone or the lid tissues in their immediate vicinity, or extrinsic, when they affect neighboring lid structures. Intrinsic features include orifice plugging, duct obstruction, and dilatation, gland atrophy and dropout and qualitative changes in expressed secretions. Extrinsic features represent secondary changes caused by the presence of MGD.

**TABLE 1. Classification of Diseases of the Meibomian Gland**

<table>
<thead>
<tr>
<th>Category</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced number of glands</td>
<td>2009</td>
</tr>
<tr>
<td>Congenital deficiency</td>
<td></td>
</tr>
<tr>
<td>Replacement of glands</td>
<td></td>
</tr>
<tr>
<td>Distichiasis</td>
<td></td>
</tr>
<tr>
<td>Distichiasis lymphoedema syndrome</td>
<td></td>
</tr>
<tr>
<td>Metaplastic disease of the meibomian gland</td>
<td></td>
</tr>
<tr>
<td>Meibomian gland dysfunction</td>
<td></td>
</tr>
<tr>
<td>Hypersecretory</td>
<td></td>
</tr>
<tr>
<td>Meibomian seborrhea</td>
<td></td>
</tr>
<tr>
<td>Hyposecretory†</td>
<td></td>
</tr>
<tr>
<td>Retinoid toxicity</td>
<td></td>
</tr>
<tr>
<td>Obstructive</td>
<td></td>
</tr>
<tr>
<td>Subclinical</td>
<td></td>
</tr>
<tr>
<td>Cicatricial or noncicatricial</td>
<td></td>
</tr>
<tr>
<td>Focal or diffuse</td>
<td></td>
</tr>
<tr>
<td>Primary, or secondary to:</td>
<td></td>
</tr>
<tr>
<td>Local disease</td>
<td></td>
</tr>
<tr>
<td>Anterior blepharitis</td>
<td></td>
</tr>
<tr>
<td>Cicatricial conjunctivitis (e.g. Trachoma; Stevens-Johnson syndrome</td>
<td></td>
</tr>
<tr>
<td>pemphigoid; acne rosacea, atopy</td>
<td></td>
</tr>
<tr>
<td>Chemical burns</td>
<td></td>
</tr>
<tr>
<td>Systemic disease</td>
<td></td>
</tr>
<tr>
<td>Seborrheic dermatitis</td>
<td></td>
</tr>
<tr>
<td>Acne rosacea</td>
<td></td>
</tr>
<tr>
<td>Atopy</td>
<td></td>
</tr>
<tr>
<td>Ichthyosis</td>
<td></td>
</tr>
<tr>
<td>Psoriasis</td>
<td></td>
</tr>
<tr>
<td>Anhidrotic ectodermal dysplasia</td>
<td></td>
</tr>
<tr>
<td>Ectroductaly</td>
<td></td>
</tr>
<tr>
<td>Fungal disease</td>
<td></td>
</tr>
<tr>
<td>Turner syndrome</td>
<td></td>
</tr>
<tr>
<td>Toxicity</td>
<td></td>
</tr>
<tr>
<td>PCB exposure; retinoids</td>
<td></td>
</tr>
<tr>
<td>Other (ocular)</td>
<td></td>
</tr>
<tr>
<td>Internal hordeolum</td>
<td></td>
</tr>
<tr>
<td>Chalazion</td>
<td></td>
</tr>
<tr>
<td>Concretions</td>
<td></td>
</tr>
<tr>
<td>Neoplasia</td>
<td></td>
</tr>
</tbody>
</table>

*Although there is evidence for an accumulation of meibomian oil within the glands, there is none yet for overproduction, as opposed to excessive release on expression.

† Hypothetical: Evidence is not available for a condition of primary hyposecretion.
but are encountered in other forms of OSD. They include lid margin hyperemia and telangiectasia.

II. DIAGNOSIS AND QUANTIFICATION OF MGD

A. Clinical Subtypes and Associations with MGD

Clinically, MGD can be categorized into four subtypes, which are described in detail:

1. MGD alone
   Asymptomatic
   Symptomatic (noncicatricial, cicatricial)
2. MGD with associated with ocular surface damage
3. MGD-related evaporative dry eye
4. MGD associated with other ocular disorders.

Characterization of these subtypes requires diagnosis and quantification of MGD itself first, followed by the inclusion or exclusion of other OSDs. Diagnostic tests are referred to briefly in the following account. Details of each test are provided in the appendices.

**MGD Alone. Asymptomatic MGD (Preclinical).** Although MGD is a symptomatic disorder, it does, like other disorders, go through an asymptomatic preclinical stage, when its presence may not be obvious to the clinical observer.49,50,80 – 82 At this stage it may be diagnosed by meibomian gland expression, with the demonstration of an altered quality of expressed secretions and/or decreased or absent expression. With progression, MGD is likely to become symptomatic, and additional lid margin signs (e.g., hyperemia) may be detected with the slit lamp. At this point an MGD-related “posterior blepharitis” may be said to be present.

Korb and Henriquez80 studied meibomian gland expressibility in patients with or without contact lens intolerance, by using both gentle and forceful meibomian gland expression. In

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**TABLE 2. Grading of MGD According to Clinical Features and Gland Expression**

<table>
<thead>
<tr>
<th>Classification and Grading System</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eyelid Margin</strong></td>
<td></td>
</tr>
<tr>
<td>Thickness (measured posterior margin to the posterior lash line)</td>
<td>0–5</td>
</tr>
<tr>
<td>Rounding of posterior margin</td>
<td>0/1</td>
</tr>
<tr>
<td>Irregularity; notching of margin</td>
<td>0/1</td>
</tr>
<tr>
<td>Vascularity of lid margin: telangiectasia</td>
<td>0/1</td>
</tr>
<tr>
<td>Lash loss</td>
<td>0/1</td>
</tr>
<tr>
<td>Trichiasis or distichiasis (state)</td>
<td>0/1</td>
</tr>
<tr>
<td>Malaposition</td>
<td>0/1</td>
</tr>
<tr>
<td>Anterior blepharitis</td>
<td>0/1</td>
</tr>
<tr>
<td>Mucocutaneous junction</td>
<td></td>
</tr>
<tr>
<td>Anteroplacement</td>
<td>0–3</td>
</tr>
<tr>
<td>Retroplacement</td>
<td>0–3</td>
</tr>
<tr>
<td>Ridging</td>
<td>0/1</td>
</tr>
<tr>
<td>Mucosal Absorption</td>
<td>0/1</td>
</tr>
<tr>
<td><strong>Orifices</strong></td>
<td></td>
</tr>
<tr>
<td>Number present (central 1 cm)</td>
<td></td>
</tr>
<tr>
<td>Number patent (central 1 cm)</td>
<td></td>
</tr>
<tr>
<td>Pouting or plugging</td>
<td>0/1</td>
</tr>
<tr>
<td>Narrowing</td>
<td>0/1</td>
</tr>
<tr>
<td>Loss of cuffing definition</td>
<td>0/1</td>
</tr>
<tr>
<td>Opaque/skarred</td>
<td>0/1</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>0/1</td>
</tr>
<tr>
<td>Other: (state)</td>
<td>0–3</td>
</tr>
<tr>
<td><strong>Main Duct</strong></td>
<td></td>
</tr>
<tr>
<td>Exposure (1 = &lt;1 mm exposed; 2 = ±1–2 mm; 3 = ±2 mm)</td>
<td>0–3</td>
</tr>
<tr>
<td>Cystoid dilatation</td>
<td>0–3</td>
</tr>
<tr>
<td><strong>Acini</strong></td>
<td></td>
</tr>
<tr>
<td>Visibility (1 = clusters; 2 = yellow stripes; 3 = not visible)</td>
<td>0–3</td>
</tr>
<tr>
<td>Concretions (1 = deep; 2 = subepithelial; 3 = extruding)</td>
<td>0–3</td>
</tr>
<tr>
<td>Chalazia</td>
<td>0–3</td>
</tr>
<tr>
<td><strong>Expressed Secretions</strong></td>
<td></td>
</tr>
<tr>
<td>Foam</td>
<td>0/1</td>
</tr>
<tr>
<td>Volume: (score the diameter of the largest pool expressed)</td>
<td>mm</td>
</tr>
<tr>
<td>Quality: (0 = clear; 1 = cloudy; 2 = granular; 3 toothpaste)</td>
<td>0–3</td>
</tr>
<tr>
<td>Expressibility: (1 = light; 2 = moderate; 3 = heavy pressure)</td>
<td>0–3</td>
</tr>
</tbody>
</table>

the asymptomatic group, they found that gentle expression generally released clear oil and rarely expressed inspissated material the consistency of toothpaste. There was a higher frequency of expressible glands in the asymptomatic group. With forceful expression, the number of expressible glands increased and in addition, more secretion was expressed from individual glands. An important observation was that in some asymptomatic subjects with apparently normal lids on simple clinical inspection, expression yielded either a creamy or an inspissated material from some glands, indicating the presence of MGD. Evidence of asymptomatic MGD was reported by Hykin et al.85 who first documented an increase in clinical features of MGD with increasing age, but free of lid-related symptoms, and Mathers et al.45 also recorded meibomian gland dropout in historically normal subjects. The preclinical stages of MGD with apparently age-appropriate normal lid margins may require expression or meibography for clinical diagnosis.

It will be important to identify which preclinical features are likely to be predictive of progressive disease, as the question arises whether early treatment might delay progression or reverse pathologic events. Treatment for early-stage disease is relatively simple, and there may be good reason to offer treatment at an early, preclinical stage of the disease. This suggests the need to perform meibomian gland expression to detect the presence of asymptomatic MGD.

Symptomatic MGD. Meibomian gland dysfunction has both subjective and objective features. Symptoms are a prominent feature of the disease.

Symptoms of MGD. In the 1995 International Dry Eye Workshop, symptoms were included in a list of global features of dry eye, each of which was an essential component of the dry eye, but did not link the association of the feature to either aqueous-deficient or evaporative dry eye.85 Global features included symptoms, ocular surface damage, tear instability, and tear hyperosmolarity. This approach was reiterated in the 2007 DEWS report.85 No attempt was made to identify symptoms that distinguished aqueous-deficient dry eye from evaporative dry eye.

MGD is a common disorder1,47,57,86–89 and is associated with evaporative dry eye. It has also been suggested that evaporative dry eye is the most common form of dry eye disease (Castillanos E, et al. J O V S 2008;49:ARVO E-Abstract 2371), although the evidence is not strong. MGD is a symptomatic disorder in its own right, with symptoms generated by the lid disease and associated ocular surface consequences. Where MGD occurs as the basis of evaporative dry eye, it must be asked whether the associated symptoms are distinct from those of the dry eye itself. However, current dry eye symptom questionnaires are not designed either to distinguish the symptoms of MGD from those of dry eye (e.g., in a separate domain) or to differentiate between aqueous-deficient and evaporative dry eye (Appendix 1). This deficiency should be remedied, and it is possible that questions could be identified that would characterize MGD and distinguish it from aqueous-deficient dry eye.

While MGD is a symptomatic disease of the lids, distinct from MGD-based evaporative dry eye, the diagnostic watershed between them has not been explored. Nonetheless, in those reported studies in which evaporation rates have been compared between normal subjects who lack features of ocular surface disease and symptomatic patients with MGD,89–92 it may be presumed that MGD patients whose evaporative rates fell within the normal range (i.e., below the cutoff for evaporative dry eye) may represent patients with symptomatic MGD alone or MGD-associated ocular surface disease. The evidence from recent meta-analyses of dry eye disease in which evaporation (and tear turnover rate) was considered in groups subdivided by phenotypes of evaporative dry eye and aqueous-deficient dry eye suggest a generally mixed etiology for both.

Individuals with a “pure” MGD phenotype represent an interesting group for further study, with the purpose of identifying an MGD-specific symptom set. It would be of particular interest to discriminate MGD from anterior blepharitis, another cause of lid-related symptoms. At the present time, no coherent effort has been made to identify symptoms that are specific to MGD itself.

With the use of currently available symptom questionnaires, one issue that arises is whether pure MGD, in the absence of dry eye, may masquerade as dry eye and therefore decrease the specificity of the test, when used as the sole identifier in dry eye diagnosis. A false-positive patient may be one with MGD, symptoms of discomfort, ocular surface staining, altered tear film lipid layer indices, but no tear hyperosmolarity. One hope would be that an MGD domain, consisting of a small number of selected questions, could be added to an existing questionnaire, which would allow the diagnosis of MGD (or at least of “blepharitis”) as a contributor to symptoms. An alternate hypothesis is that it is impossible to differentiate MGD from other ocular surface diseases on the basis of survey data alone; and therefore, combinations of subjective and objective measures may be necessary to fully differentiate the disease.

Some symptomatic features that might be anticipated to characterize MGD include personal habits related to the condition, such as lid rubbing to relieve itching and irritation; morphologic features, such as visible lid margin changes (e.g., redness and swelling) in the absence of crusts or flakes; and the presence of sensory symptoms referable to the lid margins (itching, irritation, and soreness).

Clinical signs of MGD. The key signs of MGD are as follows: meibomian gland dropout, altered meibomian gland secretion, and changes in lid morphology. Each is described in turn, including existing grading schemes for each parameter.

Meibomian gland dropout. Meibomian gland dropout refers to the loss of acinar tissue detected by meibography43–94 (Figs. 4, 5). It implies the partial or total loss of acinar tissue. In the original technique, the meibomian glands are observed in silhouette, by transillumination through the everted lids. The light source is applied to the skin side of the lid, and the disposition of the glands is viewed and recorded from the everted mucosal side. The detailed architecture of the glands is seen well in younger people, but becomes less well demarcated with age. The scope of this technique has been greatly increased by the introduction of noninvasive meibography in which the glands are documented, after eversion of the lids, by infrared photography7–9 (Fig. 4; Appendices 7, 8).
Meibomian gland dropout increases with age in normal subjects, not necessarily in response to the presence of obstructive MGD. Obata has suggested that gland dropout also occurs as an age-related atrophic process. It is hypothesized that measurable dropout is a feature of MGD and increases with MGD severity. Loss may be proximal (at the attached border of the lid), central, or distal (at the free margin of the lid) or may involve the whole gland. Extensive dropout is associated with increasing evaporative water loss from the eye. It will be important in the future to identify whether total loss of meibomian gland mass and/or number of affected glands and/or site of dropout (e.g., proximal versus distal) has the greatest effect on the other meibomian indices, including clinical lid characteristics, size of the marginal lipid reservoir, spread and integrity of the lipid film, lipid composition, and the evaporation rate. No study to correlate the location of dropout with the presence of plugging or the expressibility or quality of expressed material has yet been conducted. It could be anticipated that distal dropout, close to the orifices, would have the most profound functional effect and may correlate most closely with a diagnosis of MGD. It is also unclear whether lipid composition would be altered in the gland with partial dropout.

**Altered meibomian gland secretion**: In young normal subjects, digital pressure applied to the tarsal plate expresses meibomian secretions resident in the ducts and possibly in proximal acini, as a pool of clear oil. The secretion is also referred to as meibum. In MGD both the quality and the expressibility of the expressed material is altered. This material, which is made up of a mixture of altered secretions and keratinized epithelial debris, is also referred to as meibomian excreta. It must be recognized that expressibility and secretory activity are not the same; it is merely assumed that where meibomian oil is freely expressible, secretion is “normal.”

In MGD, the quality of expressed lipid varies in appearance from a clear fluid, to a cloudy fluid, to a viscous fluid containing particulate matter and a densely opaque, inspissated, toothpaste-like material (Figs. 6–8). These qualities have been incorporated into various grading schemes. Alternatively, the expressibility of glands during digital expression has been graded and expressibility from single or multiple glands, during the application of a standardized force, has also been measured by Korb and Blackie (Appendix 6).

**Changes in lid morphology**. Several additional morphologic features occur and have been incorporated into grading schemes. These are summarized below and in Appendix 5.

**Plugging of the meibomian orifices**. The meibomian orifices may exhibit elevations above the surface level of the lid, referred to as plugging or pouting, which are due to obstruction of the terminal ducts and extrusion of a mixture of meibomian lipid and keratinized cell debris (meibomian excreta; Fig. 9). This is a pathognomonic clinical sign of MGD.

The meibomian orifices and the mucocutaneous junction. Further important changes occur, affecting the location of the meibomian orifices in relation to the MCJ and the anteroposterior position of the MCJ itself. This junction is important because it forms the watershed between the lipid-wettable skin of the lid margin and the water-wettable mucosa.

**Noncicatricial MGD**, previously referred to as “simple MGD,” is a form in which, initially, the orifices retain their position anterior to the MCJ. In this situation, restoring the meibum delivery will allow oil be taken up once again into the TFLL. However, as Yamaguchi et al. observed in studying Marx’s line, the MCJ migrates forward with age, causing the orifices to lie behind the junction, within the mucosa. This process has been called conjunctivalization. Marx’s line is a line of conjunctival epithelial staining directly behind the MCJ, which is demonstrable with dyes such as rose bengal and lissamine green. It is present in all normal lids, in both sexes, and at all ages. Yamaguchi et al. demonstrated a forward move-

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**Figure 6.** Meibomian gland dysfunction. Cloudy expressed meibum (arrows) (courtesy of A. Bron).

**Figure 7.** Meibomian gland dysfunction: expression of opaque meibum (courtesy of D. Korb).

**Figure 8.** Meibomian gland dysfunction: strings of toothpaste-like opaque meibum expressed in response to forceful bimanual gland expression (courtesy of D. Korb).
ment of the line with age, at first encroaching on isolated meibomian orifices, then lying at the same level, and ultimately moving anterior to the gland orifices. As Marx’s line indicates the location of the mucocutaneous junction, this report demonstrates an anterior migration of the line itself. This was found to correlate with the presence of MGD. This aging process contrasts with the process, which draws the orifices posteriorly across the MCJ and into the conjunctiva in cicatricial disease. Both events result in the orifice location to lie behind the MCJ, but the mechanisms are distinctly different.

With progression, noncicatricial MGD can proceed to cause orifice stenosis or obliteration and periductal fibrosis (Fig. 10), so that meibomian oil can no longer be expressed by tarsal pressure. At this point, it is clinically noted that the condition is irreversible.

Cicatricial MGD may occur as an isolated, primary condition, in combination with noncicatricial MGD, but is most commonly found in association with the various forms of cicatricial conjunctivitis (e.g., trachoma, erythema multiforme, and pemphigoid). In this case, submucosal connective tissue scarring leads to a stretching and exposure of the terminal ducts of the glands and a thinning of the overlying conjunctival mucosa. This is termed ductal exposure and presents as a slightly elevated, riblike feature that is a telltale sign of the cicatricial process (Figs. 11, 12). Also, the affected orifices may be dragged posteriorly, across the MCJ, onto the tarsal plate, where they are ultimately lost to view or absorbed (Fig. 13). The affected ductules are frequently obstructed, but on occasion, pressure over the glands may express clear meibomian oil. Since affected orifices are located in the mucosa, any oil that they may deliver is released into the aqueous phase of the tear film and therefore is unlikely to contribute effectively to the tear film lipid layer (TFLL). The condition should be regarded as both structurally and functionally irreversible. Although therapy may suppress the inflammatory events, it cannot restore anatomic relationships. In this condition too, the MCJ may also be dragged posteriorly.

Cicatricial and noncicatricial MGD may occur together on the same lid margin in the absence of conjunctival scarring disease.

Additional features of MGD include rounding, notching, dimpling, telangiectasia, increased vascularity of the posterior lid margin, epithelial ridging between gland orifices (Figs. 14, 15), loss of orifice architecture, cystoid changes in the gland, formation of concretions within the acini and, possibly, the formation of chalazia (Appendix 5). The natural history of these changes and their clinical disease associations have not yet been explored.

B. Methods of Clinical Assessment of the Meibomian Glands: Grading Scales

Of those techniques described in the literature, the most consistently reported are those that quantify gland dropout and grade the quality or expressibility of meibum. Although the volume of expressed secretions has been proposed as an additional gradable parameter, the technique is not widely recommended, as this is a measurement of volume expressed, recorded as the diameter of expressed meibum, and is dependent both on the force applied and the duration of the force. Quantification of MGD is important, to assess its severity and monitor the response to therapy. It is also essential for application in clinical trials and in tracking its natural history. The diagnostic criteria for obstructive MGD proposed by the Japanese MGD Working Group can be seen in Appendix 17.

Meibomian Gland Dropout: Grading Scales. Meibomian gland dropout implies partial or total gland loss or atrophy and can be quantified by meiboscopy, meibography, and confocal microscopy (Table 3).
Meiboscopy is the quantification of meibomian gland dropout by using lid transilluminatiion and involves clinical observation alone. Meibography is the same technique, but using photodocumentation. Most current studies employ gland photography. Meibography is useful in providing a permanent record, which permits masking of scoring and therefore provides greater objectivity. Such records can be handled at a reading center, to provide improved standardization in clinical trials. The transillumination technique is relatively time-consuming, is challenging in patients with thickened tarsal plates, and may have limited general use. Arita et al. developed a noncontact method of meibography in which the glands of the everted upper or lower lids are imaged from the mucosal side with infrared photography. The technique is said to be more rapid and less disturbing for the patient than standard transillumination meibography. More recently, Matsumoto et al. have measured meibomian gland density per square millimeter and the diameter of intact glands, using in vivo confocal microscopy on the everted tarsal plate (Appendix 8). Table 3 summarizes studies of gland dropout in MGD, using the methods of meiboscopy and meibography, along with confocal microscopy.

Pflugfelder et al. used meiboscopy to estimate partial or total gland loss in the nasal and temporal halves of each of lid, using a 0 to 3 scale in which 0 was no gland dropout and 1 was 1% to 33%, 2 was 34% to 66%, and 3 was ≥67% dropout. Mathers et al. used meiboscopy to examine the frequency and degree of MGD in patients with chronic blepharitis. The total number of glands lost in the central portion of the lower eyelid (of eight adjacent glands) was measured. A score of 0.5 was assigned for half gland loss. Shimazaki et al. adopted a relatively crude scale of 0 to 2, in which 0 is no gland dropout, 1 is gland loss involving up to half of the lower lid, and 2 is more than half of the lower lid. de Paiva, also scoring the lower lid, used a 0 to 4 point scale, with 0 as no dropout and 1 as 0 to 25%, 2 as 25% to 50%, 3 as 50% to 75%, and 4 as ≥100% dropout.

The study by Nichols et al. has been particularly useful in validating the method of meibography (Table 4). Using a near infrared transillumination and capture system, imaging approximately 15 lower lid glands, the group reported the within- and between-observer reliability of two methods of grading. Image quality criteria were applied, and trained observers were used. In the gestalt system, they estimated the fractional, (partial or total) gland loss on a 1 to 4 scale, where 1 was no gland loss and 2 was 25%, 3 was 25% to 75%, and 4 was >75% gland loss in the image with partial glands. Alternatively, the number of intact glands in the region of interest was counted. It can be seen from Table 4, that for the gestalt system, using a weighted κ statistic, the method showed near perfect reliability within observers (κ = 0.91) and moderate reliability between observers (κ = 0.57). For the individual gland counting system, using the 95% limits of agreement method, reliability was judged to be moderate within observers and fair between observers. The two grading methods correlated highly (r = 0.96, P < 0.0001). The reader should consult the original article for details of the statistical treatment. However, overall this report appears to establish the method of meibography as a useful clinical tool.

Arita et al. quantified glands from a montage of images (described above). The scores for the upper and lower lid were summed to give a scale range of 0 to 6 for the two lids. The result was termed the meiboscore.

A body of evidence is beginning to indicate that meibomian gland dropout correlates with the clinical features of MGD, such as altered quality of expressed secretions and the consequences of gland obstruction, such as altered tear film lipid layer stability, increased evaporative loss, and ocular surface damage. The grading of dropout at baseline and subsequent examinations may provide information about long-term progression.

Concerning the mechanism of noncicatricial MGD-related disease, it is assumed that duct obstruction and increasing acinar loss (particularly distal loss) results in reduced meibomian lipid delivery. This effect would be measured by gland loss from the upper and lower lids, and the combined dropout score from the upper and lower lids would be needed to reflect this most accurately.

Scale ranges must be considered, to demonstrate the relationship between dropout and other parameters. Currently, there is no consensus as to the number of discrete increments that should be used in clinical grading. Bailey et al. have addressed the effects of scaling on clinical grading and have demonstrated an improved ability to detect clinical change when fine rather than coarse scale increments are used. This approach has been used effectively for the grading of corneal staining on a 0.1-step scale increment within a 0 to 4 scale and in the quantification of cataract. The small increment approach could be useful if applied to meibography.

At present, sometimes for ease of performance or for operational reasons, measurements are made on a limited region of one lid and from either the upper or lower lid alone. This may...
be because it is convenient to perform expression on one set of lids and meibography on the other. In a recent study of meibomian gland function in blepharitis, a high correlation between measures, including gland dropout, was found between the upper and lower lids, with the lower lid offering the most effective single measure. However, in a disease that can involve a focal portion of the lid, such measurements cannot reliably reflect events affecting both lids of both eyes. There is a need to develop approaches that can assess the full extent of each tarsal plate, to produce an aggregate score. Noncontact meibography and confocal microscopy appear promising from this point of view.

Meibography is attractive because it offers a permanent record and permits masking of scoring. In the future, for clinical trials, it is likely that digital imaging techniques will be developed that will document gland dropout more accurately and permit a focus of attention on the terminal ductule, a region of strategic importance.

**Meibomian Gland Expression: Grading Scales.** Meibomian gland expression is used in diagnosis and to obtain meibomian samples for lipid analysis (Appendix 6; Table 5). It is common to express the glands by applying digital pressure through the substance of the lids, but methods to standardize the application of force have also been developed. When the lids are normal, light expression may be expected to expel secretion contained in the ducts. It is possible that heavy expression releases presecretory lipids from the acini. Heavy expression is necessary to express the thicker grades of...
meibum associated with MGD or may be necessary therapeutically in the treatment of MGD. Expressibility is sometimes equated with functionality of the meibomian glands and they are likely to be closely related, but expression is not in itself a measurement of secretory activity, although it could be considered a surrogate measure of secretion.

In MGD, the quality of expressed oil varies in appearance between that of a cloudy fluid, a viscous fluid containing particulate matter and a densely opaque, toothpaste-like material. These qualities have been incorporated into various ordinal grading schemes75,96,97 (Table 5). The scores in these four-point systems are 0, clear (normal); 1, cloudy; 2, cloudy with particles; and 3, inspissated (like toothpaste).75 Similarly, in the Mathers scheme, 1 is clear, 2 and 3 are liquid but of decreasing transparency, and 4 is like toothpaste). When the expression of a fixed number of glands is assessed, there are two ways of generating a score. One way is to record only the highest grade encountered from any of the expressed glands. In this case, for a single zone, the score range is 0 to 3. The other is to record the sum of scores for each gland expressed, then the score range is 0 – (8 × 3) = 24. This approach is generally preferred and is recommended by this committee. However, a small caveat is that in long-term studies, inexpressibility encountered in normal lids is also a sign of total obstruction; an increase in the number of pathologically inexpressible glands with disease progression, would, paradoxically, lead to a fall in total score.
In addition, the *expressibility of glands* during digital expression has been graded, while expressibility from single or multiple glands, during the application of a standardized force, has been measured by Korb and Blackkie (Table 5). For multiple glands, the standard force is applied for 10 to 15 seconds with a specially designed instrument. The Shimazaki approach grades expressibility according to the response to different levels of digitally applied pressure and therefore brings an additional subjective element into the grading process. The approaches of Pfugfelder and of Korb relate to a fixed number of expressed glands, and the latter system clearly instructs the investigator to score only those glands that yield a liquid secretion (the MGYLS score), regardless of its quality (Table 5). To increase the scale range and reflect the status of the full length of the upper and lower lids, an aggregate score can be created from the summed expression grades from the nasal, central, and temporal regions of each lid. As noted earlier, even in young normal subjects, the expressibility, in terms of the fraction of glands from which fluid meibum may be expressed, varies for different regions of the lid and reduces progressively from the nasal to the temporal side. However, it may be reasonable to generate a composite score for the upper and lower lid by summing the nasal and central scores from each lid, not attempting to score the temporal region.

**Grading Morphologic Lid Changes: Grading Scales.**

The approaches to grading (Appendix 5) other morphologic features of MGD were discussed earlier and are presented in Table 2. Grading scales may be expanded by dividing each lid into quarters and grading the highest level of change in each region. Quantifying selected features in this way offers an opportunity to generate an aggregate MGD score that may then be used in conjunction with measures of gland expressibility and dropout. This approach was adopted by de Paiva et al. in a comparison of normal subjects with those who had ocular irritation. An aggregate score with a scale range of 0 to 11 was created by combining a meibographic grading (see Table 2) with a grading of lid changes, as follows: Orifices: metaplasia present is 1; absent is 0; and brush marks (linear vascular features): present is 1, absent is 0; Expressibility using digital pressure applied over five lower lid glands: 0 is all five glands expressible and 4 is none; 2 is three, 3 is two, and 4 is 0 glands expressible. Similarly, Arita et al. scored for the presence or absence of lid abnormalities, as follows: irregularity of the lid margin, 0=absent, 1=absent, 2=irregularity, 3=extensive; and expressibility of meibomian orifices, 0=absent, 1=slight, 2=moderate, 3=extensive.

**C. The Utility of Current Grading Scales.**

These various tests have been used to explore the prevalence of MGD and its relation to ocular disorders. Age-related data are available in normal subjects concerning morphologic lid changes, lipid levels at the lid margin, meibomian gland dropout, and expressibility of meibomian secretion. Mathers et al. used meibography to examine 72 normal subjects without dry eye and found that gland dropout remained, on average, below one gland per eight assessed, up to about age 50 years. After that, it increased to approximately two glands per eight assessed (25%). Similarly, using noncontact meibography, Arita et al. found a significantly positive correlation between the meiboscore (implying dropout) and age ($R = 0.428; P < 0.0001$). They found meiboscores up to about grade 1 (i.e., gland loss under a third of the total gland area) at age 50 in normal subjects and then increasing scores and gland dropout with advancing age.

In a study of asymptomatic, normal subjects, Hykin and Bron showed changes related to age, including increasing lid margin telangiectasia and cutaneous hyperkeratinization, increased narrowing and pouting (plugging) of meibomian gland orifices, and a decreased number of expressible glands. The *quality* (viscosity and degree of opacity) of expressed secretions did not change. In contradistinction, Mathers and Lane found that lipid viscosity increased with advancing age in normal subjects, a change that was highly significant for linear trend ($P = 0.0006$).

Chew et al. used meibometry in a large sample of normal subjects ($n = 421$) and found increasing lid margin levels of meibomian oil throughout life, with no differences found between the sexes after approximately age 50. These meibometry results seemingly contradict the finding that meibum is expressible from fewer orifices with advancing age. The paradox could be explained by a greater meibometry pickup from the lid margin with age.

Yamaguchi et al. assessed the disposition of Marx’s line in normal subjects by using fluorescein and other dyes and the following grading system: 0, Marx’s line runs entirely on the conjunctival side of the meibomian orifices; 1, parts of Marx’s line touch the meibomian orifices; 2, Marx’s line runs through the meibomian orifices; and 3, Marx’s line lies on the skin side of the meibomian orifices. Grading was performed in the inner, central, and outer thirds of the lower lid, giving a range of scores for the whole lid of 0 to 9. It was found that grading was reasonably consistent between observers. With age, the grade score increased, implying that Marx’s line (and the MCJ) moved anterior with time. The authors found a positive correlation between the regional meibography scores and quality of expressed meibum score (graded on a 0 to 4 basis), and the regional Marx’s line scores.

Several investigators (using the various methods discussed herein) have shown decreasing functionality of the meibomian glands with aging. Norn found that a maximum of approximately 14.5 lower lid glands could be expressed by digital pressure in normal subjects at the age of 20 years but that the number dropped to approximately seven glands beyond the age of 80 years. Hykin and Bron later confirmed these results.

Mathers et al. reported a prevalence of MGD of 20% in the normal population older than 20 years. In other studies the population prevalence of MGD has been reported to range between 3.5% and 68%. Arita et al. reported that positive meiboscores develop after the age of 20 years in men and after the age of 30 years in women. Meiboscores correlated with age in both sexes ($R = 0.428; P < 0.0001$) and there was also a positive correlation between the lid margin score (based on a cluster of features) and age ($R = 0.538; P < 0.0001$) and between the meiboscore and lid margin score ($R = -0.289; P = 0.0001$).

Several investigators have concluded that meibomian gland dropout is a useful index of obstructive MGD. Using meibography, Mathers et al. found meibomian gland dropout in 76% of their patients with chronic blepharitis. The dropout score in their normal group was $0.18 \pm 0.1$ (per eight lower lid gland surveyed) compared to $1.97 \pm 2.1$ in their blepharitis group, which likely contained patients with non-MGD forms of blepharitis. On the basis of a cluster analysis, they concluded that only gland dropout was useful in classifying dysfunction. Obstructive MGD was associated with a high level of dropout (mean $3.67 \pm 1.7$ glands missing per eight glands surveyed; nearly 50% versus normal subjects (mean $0.18 \pm 0.1$ glands missing; $\sim 2.2\%$). This study identified a group of patients with high levels of meibomian gland dropout, a high level of tear osmolarity, and high Schirmer values. This group would correspond well to that predicted by Bron et al. as an example of patients with evaporative dry eye during a phase of partial, reflex lacrimal gland compensation. A further group of patients was identified with high levels of
meibomian gland dropout, tear hyperosmolarity, and low Schirmer values. This group would correspond to a more advanced stage of evaporative dry eye, in which it is predicted that lacrimal compensation has failed and evaporative dry eye is accompanied by a functional, aqueous-deficient dry eye. An average dropout of $5.5 \pm 1.3$ glands ($\sim 69\%$ of eight glands) was found in this sicca group. This contrasts with much lower levels of gland dropout for subjects with seborrheic MGD and those with low Schirmer scores alone. Taken together, these results suggest that quantitative assessment of gland dropout is a valuable indicator of obstructive MGD.

Pflugfelder et al.\textsuperscript{55} used clinical meiboscopy to assess gland dropout in several dry eye subtypes, albeit with modest sample sizes ($n = 9-11$ subjects per subtype). These authors found mean gland dropout scores (graded on a $0-3$ scale based on percentage of gland (dropout) of approximately grade 2 for inflammatory and noninflammatory MGD subjects compared to a grade $<0.5$ for the controls. A significant finding in their report was that the degree of acinar loss in inflammatory MGD and atrophic MGD was roughly equivalent. Thus, gland dropout alone may not adequately discriminate these two clinical conditions. Khanal et al.\textsuperscript{118} found gland dropout to be effective in differentiating the evaporative dry eye subtype from those without dry eye, but was not effective in differentiating aqueous-deficient dry eye.

Matsumoto et al.\textsuperscript{79} using confocal microscopy, have shown a decrease in meibomian gland density in MGD patients ($47.6 \pm 26.6/ \text{mm}^2$, compared with $101.3 \pm 33.8/ \text{mm}^2$ for a control group). This group also introduced the measurement of meibomian gland diameter as a new parameter reflecting the health of the glands (Fig. 16). In their study, MGD was associated with an increase in residual gland width ($98.2 \pm 53.3 \mu m$ in MGD and $41.6 \pm 1.9 \mu m$ in controls) that was attributed to accumulated, inspissated debris within the acini. However, an alternative explanation may be that acinar enlargement is in part compensatory, due to the influence of a feedback loop.

This review of the current literature suggests that quantification of meibomian gland dropout provides a valuable baseline statement about the integrity of the meibomian glands. The dropout score appears to correlate with the presence of MGD diagnosed by other clinical criteria and to the effects of MGD on the surface of the eye.

MGD with Associated OSD. OSD is encountered in association with MGD and is found in its most advanced form in MKC. Various etiologies have been proposed for such damage, including the release of inflammatory mediators into the tear film and the mechanisms of evaporative dry eye. One source of such mediators includes the breakdown products of meibomian lipid, altered by the lipases of microbial commensals. A possible relationship has been reported between meibomitis and phlyctenular keratitis, a keratitis that is sometimes encountered in young females. In a small group of patients with phlyctenular keratitis, $57\%$ of whom had a history of chalazia, the location and severity of a meibomitis correlated well with the severity of the corneal nodules, and there was a possible association with specific HLA subtypes and with the presence of \textit{Propionibacterium acnes} in expressed meibum.\textsuperscript{119}

Ocular surface damage may be quantified by grading staining of the cornea and conjunctiva using selected dyes, by immunohistochemistry or flow cytometry on impression cytology specimens, and by the direct measurement of inflammatory mediators in the tears biochemically, with multiplex bead technology or using MALDI-TOF and proteomic techniques.\textsuperscript{120} These biochemical and clinical techniques have helped to describe the ocular surface phenotype in MGD and other OSDs and to monitor the severity of disease and response to treatment, but the events that they record are not specific to MGD, and they therefore have no unique role in its diagnosis. The precision of such tests was addressed in the 2007 DEWS Diagnosis report, and details of test sensitivity and specificity in the diagnosis of dry eye are summarized and incorporated both in the published templates and in additional materials available on the TFOS website (www.tearfilm.org). Intrinsic glandular inflammatory events may be recorded directly by confocal microscopy (Appendix 8).

MGD-Related Evaporative Dry Eye. In the presence of MGD, the amount of oil delivered to the reservoir is reduced, as a result of meibomian obstruction or gland atrophy or, in the case of cicatrical MGD, because the affected orifices are malpositioned, and the ducts are stretched and narrowed. A combination of mechanisms may often be at work when these forms of MGD occur together. With progression of MGD, it is assumed that a point is reached when the amount in the reservoir, or its distribution along the lid margins, is insufficient to maintain a normal TFLL, so that a functionally incompetent TFLL results. It is likely that compositional changes in meibum contribute to this disturbance, too. Abnormalities of the TFLL include abnormal (slow) spreading patterns,\textsuperscript{121} vertical interferometric patterning, and reduced TFLL stability. These are accompanied by an increased evaporative water loss (Fig. 17).

It is known that spreading of the TFLL is altered in the higher degrees of aqueous-deficient dry eye.\textsuperscript{62,66,122} This spreading has been attributed to thinning of the aqueous layer of the tear film.\textsuperscript{123} In a recent publication, it was suggested that this effect gives rise to a functional TFLL deficiency and a consequent increased evaporative water loss.\textsuperscript{117} Thus, it is proposed that a functional evaporative dry eye may occur in the presence of organic aqueous-deficient dry eye. This type of dry eye is predicted to occur in the absence of MGD, but would be compounded by it, if present. No TFLL spreading can be detected with a video-interferometer (DR-1; Kowa, Tokyo, Ja-
intolerance, 80,98 and there are several clinical reports of an seborrheic dermatitis, on its own (38.5%) or in combination with disease, such as seborrhea sicca, (11.5%), acne rosacea (34.6%), or cornea. In all cases, MKC was associated with some form of skin (SPK), preferentially affecting the lower interpalpebral globe and with conjunctival injection and superficial punctate keratitis patients exhibited both anterior and posterior blepharitis and Ong and Larke127 found an increase in the frequency of MGD in the meibomian gland by the release of inflammatory mediators. and GPC, it is also possible that the conjunctivitis initiates changes that the severity of GPC correlated with the severity of the MGD often encountered clinically in seborrheic blepharitis,125 in wear in the development of and/or progression of MGD. Further research is necessary to determine the role of CL functional meibomian glands, proportional to the duration of CL wear. Further research is necessary to determine the role of CL wear is associated with a decrease in the number of functional meibomian glands, proportional to the duration of CL wear. Further research is necessary to determine the role of CL wear in the development of and/or progression of MGD. Mixed Anterior Blepharitis and MGD. Mixed anterior blepharitis associated with MGD is not uncommon and is often encountered clinically in seborrhoeic blepharitis,125 in atopic blepharitis,126,125 and as a specific complication of systemic retinoid therapy.130

Documenting MGD in Different Clinical Situations. Quantification of MGD is important for diagnosis and treatment, but is also required in other clinical circumstances.

<table>
<thead>
<tr>
<th>Anterior blepharitis</th>
<th>Ocular surface damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crusting (61%)</td>
<td>SPK (100%)</td>
</tr>
<tr>
<td>Scales</td>
<td>Rose bengal staining (100%)</td>
</tr>
<tr>
<td>Lid loss (50%)</td>
<td></td>
</tr>
<tr>
<td>Lid margin irregularity (46%)</td>
<td></td>
</tr>
<tr>
<td>Posterior blepharitis</td>
<td>Ocular inflammation</td>
</tr>
<tr>
<td>Oil stagnation (100%)</td>
<td>Bulbar injection (100%)</td>
</tr>
<tr>
<td>Orifice abnormalities (23–53%)</td>
<td>Tarsal papillary change (100%)</td>
</tr>
<tr>
<td>Meibomian foam (62%)</td>
<td></td>
</tr>
<tr>
<td>Reduced tear secretion</td>
<td>General signs</td>
</tr>
<tr>
<td>Schirmer test &lt;10 mm (35%)</td>
<td>Concretions</td>
</tr>
<tr>
<td>Tear film instability</td>
<td>Clinical associations</td>
</tr>
<tr>
<td>Reduced BUT (100%)</td>
<td>Sеборрhea сicca; seborrheic dermatitis with or without atopy; acne rosacea</td>
</tr>
</tbody>
</table>

SPK, superficial punctate keratitis; BUT, break-up time.

pan) in the severest form of aqueous-deficient dry eye, but recovery can be confirmed, after punctal occlusion.66,124

MGD Associated with Other Ocular Disorders. There have been extensive reports of the association of MGD with other ocular and systemic disorders in the literature, including contact lens (CL) intolerance. The level of evidence associated with each ocular and systemic factor is discussed in detail in the report of the Epidemiology Subcommittee and is discussed briefly here for clinical significance.

Meibomian Keratoconjunctivitis. McCulley and Sciallis72 described a condition of tear film instability, ocular inflammation, and ocular surface damage in a group of patients with chronic blepharitis, which they called MKC (Table 6).7,2,125 In the study, patients exhibited both anterior and posterior blepharitis and some form of associated skin disorder. The features of MKC are summarized in Table 6. Signs of obstructive MGD were associated with conjunctival injection and superficial punctate keratitis (SPK), preferentially affecting the lower interpalpebral globe and cornea. In all cases, MKC was associated with some form of skin disease, such as seborrhea sicca, (11.5%), acne rosacea (54.6%), or seborrhoeic dermatitis, on its own (38.5%) or in combination with atopy (15.4%).

MGD and CL Wear. MGD is frequently associated with CL intolerance,86,90 and there are several clinical reports of an association between MGD and giant papillary conjunctivitis (GPC). Mathers and Billborough108 found significantly more gland dropout and greater viscosity of expressed secretions in CL wearers with GPC than without GPC, whereas Martin et al.126 found that the severity of GPC correlated with the severity of the MGD in a consecutive series of GPC patients. Although attention has been focused on the hypothesized role of MGD in CL intolerance and GPC, it is also possible that the conjunctivitis initiates changes in the meibomian gland by the release of inflammatory mediators. Ong and Larke127 found an increase in the frequency of MGD after 6 months of CL wear, and Arita et al.109 recently reported that CL wear is associated with a decrease in the number of functional meibomian glands, proportional to the duration of CL wear. Further research is necessary to determine the role of CL wear in the development of and/or progression of MGD. Mixed Anterior Blepharitis and MGD. Mixed anterior blepharitis associated with MGD is not uncommon and is often encountered clinically in seborrhoeic blepharitis,125 in atopic blepharitis,126,125 and as a specific complication of systemic retinoid therapy.130

Documenting MGD in Different Clinical Situations. Quantification of MGD is important for diagnosis and treatment, but is also required in other clinical circumstances.

Recruitment of Patients for Clinical Trials. Dry Eye. Certain considerations apply in the recruitment of patients for trials of drugs to treat aqueous-deficient dry eye. Since extensive MGD may be associated with dry eye, there may be reasons to exclude patients exhibiting the higher grades of MGD, which may exacerbate the dry eye and influence interpretation of drug efficacy. On the other hand, particularly in recruitment of patients with severe dry eye, it is unrealistic to exclude all patients with MGD. A compromise is to permit recruitment of patients with a low degree of MGD, based on meibum quality or expressibility. The MGD grade can be used for stratification in data analysis.

MGD. In clinical trials of drugs for the treatment of MGD or of MGD-related dry eye, a higher grade of MGD would be required at recruitment in order to demonstrate efficacy and permit responder analyses. The MGD grade, determined by one of the methods described above, would be recorded over the course of the study. An assessment of functionality, such as by the MGYLS score, would be an important inclusion.52 Measurement of gland dropout offers an objective way to stratify the baseline severity of the MGD. A detailed summary of existing trials is presented in the report of the Clinical Trials Subcommittee. Monitoring for MGD as an Adverse Event. MGD is a side-effect of systemic retinoid therapy, used in the treatment of acne vulgaris.97,131 In studies of the evolution of such changes it is necessary to recruit subjects with a low degree of MGD, in order that the development of pathological changes may be monitored carefully and detected quickly. This implies recruiting a relatively young, adult population and confining assessments to the nasal and possibly the central thirds of the lower lids, where normally, the percentage of active glands is relatively high.52

Natural History of MGD. While the natural history of MGD is not yet known, clinicians and researchers have the tools to address this in the future. Such studies would allow the evolution of primary MGD to be elucidated and could identify the chain of events leading to secondary forms of MGD.

III. A PRACTICAL APPROACH TO THE DIAGNOSIS OF MGD AND MGD-RELATED DISEASES

Standardization and accessibility are the keys to successfully performing any test. Standardization can be achieved in any clinic by performing examinations within a standard environment and ensuring, when auxiliary staff are involved, that the staff are well trained. The diagnosis of MGD, whether in isolation or associated with ocular surface damage or dry eye, should be viewed in the context of diagnosing any ocular surface disease, and tests should be performed in an order that minimizes the extent to which one test influences the tests that follow. The evidence base of tests used to define dry eye and its subtypes is summarized in Table 7. The effectiveness of these tests varies between 50% and 96%. However, the quality of evidence on which these statistics is based varies from study to study, dependent on the initial quality of the investigator’s definition of the condition, the presence of selection bias in the study design, and the size and sample of the population studied. It can be seen from examining Table 7 that if a 70% level of sensitivity and specificity is accepted as appropriate for an effective test, several clinical and laboratory-based tests are effective in differentiating the normal from a generic dry eye. On the basis of the evidence in Table 7, however, when evaporation rate is used as the gold standard, only two types of tests, tear secretion measured by fluorophotometry and the fluorescein clearance rate, are able to differentiate evaporative- from aqueous-deficient dry eye.
eye, at the second stage of diagnosis. However, a diagnosis of evaporative dry eye can be reinforced by positive findings from meibography, meibometry, scoring the functional severity of the MGD, and measures of TFLL dynamics.

With this background in mind, a series of recommended tests to be used in the diagnosis of MGD and in MGD-related disorders, including evaporative dry eye, is presented as follows (Table 8).  

1. Tests for the Diagnosis of MGD  
   a. In asymptomatic adults it is appropriate to include gland expression (e.g., by the application of moderate digital pressure to the central lower lid) to the routine work-up of the patient, to detect asymptomatic, nonobvious MGD.

   i. A diagnosis of MGD may require that the patient be further assessed for ocular surface damage and dry eye, by using appropriate diagnostic techniques.

   ii. In patients with ocular surface symptoms or morphologic lid signs of MGD (e.g., orifice plugging and other orifice or lid margin signs), meibomian gland functionality should be assessed by digital pressure over the central (± nasal) third of the lower/upper lids, to deter-

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**Table 7. Diagnostic Efficacy of Tests for Evaporative and Aqueous-Deficient Dry Eye**

<table>
<thead>
<tr>
<th>Test Measure</th>
<th>Normals vs. Dry Eye (Sens %/Spec %)</th>
<th>Normals vs. EDE (Sens %/Spec %)</th>
<th>EDE vs. ADDE (Sens %/Spec %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom questions</td>
<td>DE &gt;14.5 (82/36; vs. RB, SCH, TBUT); McMonnies</td>
<td>DE &gt;15; OSDI (80/79 vs. Lissamine, Sch, Symp) (60/83, Dr. diagnosis); Johnson and Murphy</td>
<td></td>
</tr>
<tr>
<td>Tear stability</td>
<td>FBUT &lt;10 seconds (82/86); Mengher et al.</td>
<td>&lt;5.5 mm/5 min (85/83); Khanal et al.</td>
<td></td>
</tr>
<tr>
<td>Tear secretion: Schirmer I, Schirmer II</td>
<td></td>
<td>PRT &lt;12 mm (56/69); Sakamoto et al.</td>
<td></td>
</tr>
<tr>
<td>Index of tear volume, PRT</td>
<td></td>
<td>PRT &lt;20 mm (86/83); Patel et al.</td>
<td></td>
</tr>
<tr>
<td>Ocular surface damage</td>
<td>RB Stain &gt;3.5; van Bijsterveld</td>
<td>RB Stain &gt;4 (95% vs. 96%) (65/84); Vitali et al.</td>
<td></td>
</tr>
<tr>
<td>Lid (meibomian morphology)</td>
<td></td>
<td>Expression grade &gt;1.0*</td>
<td></td>
</tr>
<tr>
<td>Meibomian gland expression</td>
<td></td>
<td>EDE ≥ 3 (83.0/90.0); Arita et al.</td>
<td></td>
</tr>
<tr>
<td>Meibography</td>
<td></td>
<td>Unit density &lt;70/mm; Kobayashi et al.; Matsumoto et al. (81/8)</td>
<td></td>
</tr>
<tr>
<td>Confocal acinar unit density/diameter</td>
<td></td>
<td>Long diameter &lt;65 μm (90/81) Short diameter &lt;25 μm (86/96)</td>
<td></td>
</tr>
<tr>
<td>Meibometry</td>
<td></td>
<td>Meibomian physicochemistry</td>
<td></td>
</tr>
<tr>
<td>Interferometry</td>
<td></td>
<td>Tear secretion: fluorometry, fluorescein clearance</td>
<td></td>
</tr>
<tr>
<td>Evaporation rate</td>
<td></td>
<td>Tear volume: fluorimetry</td>
<td></td>
</tr>
<tr>
<td>Tear meniscus height/radius/volume</td>
<td></td>
<td>Tear meniscus height/radius/volume</td>
<td></td>
</tr>
<tr>
<td>Tear osmolarity</td>
<td></td>
<td>Tear osmolarity</td>
<td></td>
</tr>
<tr>
<td>Tear dynamics/indices/evap/total flow</td>
<td></td>
<td>Tear dynamics/indices/evap/total flow</td>
<td></td>
</tr>
<tr>
<td>Tear dynamics/indices/evaporation/TFI</td>
<td></td>
<td>Tear dynamics/indices/evaporation/TFI</td>
<td></td>
</tr>
</tbody>
</table>

The sensitivity and specificity of tests discriminating normals from dry eye and its subtypes are reported. DE, dry eye; EDE, evaporative dry eye; ADDE, aqueous-deficient dry eye; TFI, tear function index; FBUT, fluorescein break-up time; PRT, phenol red thread; RB, rose bengal; SCH, Schirmer; TBUT, tear breakup time; TTR, tear turnover rate; SENS, sensitivity; SPEC, specificity.

TABLE 8. Specialized and Nonspecialized Tests for MGD and MGD-Related Disease

<table>
<thead>
<tr>
<th>Testing Category</th>
<th>Specific Test(s)</th>
<th>Tests for a General Clinic</th>
<th>Tests for a Specialized Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td>Questionnaires</td>
<td>McMonnies; Schein; OSDI; DEQ; OCI; SPEED etc.</td>
<td>McMonnies; Schein; OSDI; DEQ; OCI; SPEED etc.</td>
</tr>
<tr>
<td>Signs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meibomian function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lid morphology</td>
<td></td>
<td>Tear film and calculation of blink interval (BI)</td>
<td>Tear film and calculation of blink interval (BI)</td>
</tr>
<tr>
<td>Meibomian gland mass</td>
<td></td>
<td>Slit-lamp microscopy</td>
<td>Slit-lamp microscopy</td>
</tr>
<tr>
<td>Gland expressibility</td>
<td></td>
<td>—</td>
<td>Meibography</td>
</tr>
<tr>
<td>Expressed oil: quality</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Expressed oil: volume</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lid margin reservoir</td>
<td></td>
<td>—</td>
<td>Slit-lamp</td>
</tr>
<tr>
<td>Tear Film Lipid Layer</td>
<td></td>
<td>—</td>
<td>Meibography</td>
</tr>
<tr>
<td>Thickness</td>
<td>Interferometry</td>
<td>Interferometry</td>
<td>Interferometry</td>
</tr>
<tr>
<td>Spread time</td>
<td>Slit-lamp</td>
<td>Slit-lamp</td>
<td>—</td>
</tr>
<tr>
<td>Spread rate</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Evaporation</td>
<td>Evaporimetry</td>
<td>Evaporimetry</td>
<td>Evaporimetry</td>
</tr>
<tr>
<td>Tears</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osmolarity</td>
<td>Osmolarity</td>
<td>TearLab device, other</td>
<td>TearLab device, other</td>
</tr>
<tr>
<td>Stability</td>
<td>Tear film</td>
<td>TFBUT; OPI</td>
<td>TFBUT; OPI</td>
</tr>
<tr>
<td></td>
<td>TFFL</td>
<td>Spread time</td>
<td>Interferometry; spread rate; pattern</td>
</tr>
<tr>
<td>Indices of volume and secretion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tear secretion</td>
<td>Schirmer 1</td>
<td>Fluorophotometry/FCR</td>
<td>Fluorophotometry/FCR</td>
</tr>
<tr>
<td>Tear volume</td>
<td>Not available</td>
<td>Volume by fluorophotometry</td>
<td>Volume by fluorophotometry</td>
</tr>
<tr>
<td>Tear clearance</td>
<td>Meniscus height</td>
<td>Meniscus radius of curvature; meniscometry</td>
<td>Meniscus radius of curvature; meniscometry</td>
</tr>
<tr>
<td>Ocular surface inflammation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocular surface staining</td>
<td>Oxford scheme; NEI/Industry scheme</td>
<td>Flow cytometry; bead arrays; microarrays; mass spectrometry: cytokines and other mediators; interleukins; MMPs</td>
<td>Flow cytometry; bead arrays; microarrays; mass spectrometry: cytokines and other mediators; interleukins; MMPs</td>
</tr>
</tbody>
</table>

Tests of meibomian gland function are presented first followed by those for related disorders such as dry eye. See text for a recommended sequence of performance: DEQ, Dry Eye Questionnaire; FCR, fluorescein clearance rate; MMPs, matrix metalloproteinases; OCI, Ocular Comfort Index; OPI, Ocular Protection Index; OSDI, Ocular Surface Disease Index; SPEED, Standard Patient Evaluation of Eye Dryness; TFBUT, tear film breakup time; TFI, tear film index; TFFL, tear film lipid layer.

mine the extent and severity of the MGD (expressibility and secretion quality). This procedure should be performed using moderate digital pressure or a standardized technique, in the manner outlined in the previous sections. The patient should be further assessed for evidence of ocular surface damage and dry eye.

2. Tests for the Diagnosis of MGD-Related Dry Eye

The Committee recommends a two-tiered approach:

a. The first step is one in which normal subjects are discriminated from patients with dry eye of any type (generic dry eye).

b. The second step involves the differential diagnosis of MGD-related evaporative dry eye from aqueous-deficient dry eye.

Two approaches are proposed: one suitable for practitioners working in a general clinic and the other for investigators working in specialized units. The evidence base of the tests proposed varies according to the clinical setting needs.

A. Diagnosis of MGD-Related Disease within a General Clinic

A suitable sequence of tests to perform in a general clinic, in patients presenting with symptoms of ocular surface disease is as follows:

1. Administration of a symptom questionnaire.
5. Instillation of fluorescein and measurement of the tear film breakup time (TFBUT). Measurement is facilitated by viewing with a blue exciter filter and a yellow barrier filter. The diagnostic cutoff value for dry eye will be influenced by the volume instilled. The Ocular Protection Index (OCI) can be calculated as the ratio of TFBUT/BI (blink interval). A value of <1 is pathologic and implies that tear breakup is occurring in the waking state. The lower the value the greater the degree of tear film instability.

6. Immediately after measurement of the TFBUT, fluorescein staining can be graded on both the exposed cornea and conjunctiva. When a yellow barrier filter has not been used, it will be necessary to grade conjunctival staining independently by using lissamine green. This grading may be performed after the Schirmer test.

7. Schirmer test or alternate (phenol red thread test). A positive result (abnormal) from tests described in 1, 4, 5, and 6 provides partial evidence of the presence of generic dry eye, without specifying whether it is aqueous-deficient or evaporative. Evidence of aqueous-deficient dry eye may be obtained by measuring tear flow or an assessment of aqueous volume on the basis of tear meniscus height or Schirmer test.

8. If MGD has not been characterized (symptomatic/ asymptomatic) at a previous visit, then it can be assessed at the end of this sequence as follows:

a. Quantification of morphologic lid features.

b. Expression: quantification of meibum expressibility/quality.

c. Meibography: quantification of dropout.

If testing suggest the diagnosis of a generic dry eye and tests of tear flow and volume are normal, then evaporative dry eye is implied, and quantification of MGD will indicate the meibomian gland’s contribution.

This test sequence also permits a diagnosis of symptomatic MGD, with or without ocular surface staining and with or without dry eye, to be made. The graded scores for each test can be used to monitor the disease during treatment.
B. Diagnosis of MGD-Related Disease within a Specialized Unit

An “ideal” or comprehensive test series is proposed for corneal specialists or for investigators engaged in clinical trials, in which they have access to a wider range of diagnostic equipment. Some of the tests listed are alternatives. It is suggested again that the diagnosis be made in two steps: First, diagnose generic dry eye and then subtype it with the grade of MGD.

This test series consists of a symptom assessment (Appendix 1; e.g., the OSDI,146 DEQ,147), a measure of tear osmolarity (Appendix 15), a tear secretion test (fluorophotometry or fluorescein clearance rate; Appendix 13), a measure of the volume of the tears in the eye (by fluorophotometry and meniscometry; Appendix 14), a stability test (the TFBUT or noninvasive TBUIT, Appendix 2; or interferometry, Appendix 10), and a measurement of tear evaporation (by evaporationometry, Appendix 11). Tests of ocular surface damage, such as corneal and conjunctival staining (Appendix 4), are also included in the test series (Table 9). Tests of inflammatory mediators and the presence of inflammatory cell markers and other proteomic and lipidomic mass spectrometry analyses (Appendix 12) can also be assessed to provide information regarding overall ocular surface inflammatory status, although the link to MGD specifically is not known at this time. Specific measures of tear production (Appendix 3) for the diagnosis of aqueous-deficient dry eye are also recommended.

A Severity Scale for MGD and MGD-Related Disease, Including Dry Eye

It is critical to understand the severity of any disease to assess its burden to the patient, the efficacy of therapy, and the prognostic implications (Appendices 5–7). Assigning severity levels to a disease is difficult, because the various elements that comprise the disease complex are of different weight and may not move in parallel as the disease progresses. The committee acknowledges that information of this kind is not yet available to inform the development of a severity rating for MGD and related disease. However, it was considered to be important to offer a provisional framework that could be assessed in the future as described below.

In the preamble to this section, we suggested that MGD may be a symptomatic disorder in its own right, a disorder that causes ocular surface damage and one that causes evaporative dry eye, which in turn may cause surface damage. Because these disease components may progress at different rates, separate severity levels have been generated for MGD and for MGD-associated disorders, using symptoms as a bridge between the two.

Severity levels for the parameters discussed above are presented in Tables 9 and 10. Treatment aspects are dealt with briefly, and a fuller account can be found in the report of the Management and Therapy Subcommittee.

Overall Recommendations. The recommendations of the Diagnostic Subcommittee are as follows and are summarized in Table 11:

MGD is a common disorder that may be asymptomatic or give rise to symptoms, either confined to the affected lids or arising from MGD-related ocular surface disease, including evaporative dry eye. It can also exacerbate aqueous-deficient dry eye.

The natural history of MGD is not precisely known; for practical purposes it should be regarded as a progressive but treatable disease in which therapy may prevent irreversible changes. This approach is a safe one that can be modified as further information becomes available.

Therapy is based on diagnosis and a decision to treat depends on the severity of disease. While simple diagnosis is straightforward, quantification of the degree and severity of MGD, which is the basis for treatment, is more complex.

A two-step approach to diagnosis is recommended in symptomatic patients. The assessment of meibomian gland function is based on lid morphology, gland dropout, meibum expressibility, TFFL appearance, and tear evaporation. A diagnosis of dry eye is established from measures of tear production and clearance, tear osmolarity and tear film stability, and the presence of ocular surface changes by tissue staining and perhaps further characterized by the presence of inflammatory biomarkers. Patients with symptoms of ocular surface disease should be assessed for ocular surface damage and for abnormalities of tear dynamics characteristic of dry eye (Table 8).

Quantification of MGD is based on grading meibum quality and expressibility. When the presence of MGD is more than trivial and treatment is instituted, the score should be noted and repeated periodically at follow-up. An aggregate score derived from the expression of upper and lower, central, and nasal lid zones should be considered as a method of monitoring the response to treatment. Newer, quantitative methods of expression may make grading more accurate in the future.

Although such grading approaches have been used to differentiate mild from severe disease, their repeatability is unknown, and therefore their value in demonstrating small changes in disease severity is unknown. There is good evidence that meibomian gland dropout is closely associated with MGD severity. It is therefore recommended that, when possible, baseline measurements of gland dropout be made by using meibography. Baseline measurements can be used for stratification purposes in clinical trials, but when such trials are extended, or in natural history studies or where meibomian gland damage occurs as an adverse event, they may provide a record of change over time.

IV. APPENDICES

Method of Working

Each reviewer used the following format when analyzing the diagnostic tests:

1. Identify the test.
2. Provide rationale for use.
3. Describe each of the techniques used in full detail.

Wherever available, test values for normals, MGD and dry eye were identified, together with the sensitivity and specificity of the test and recommended or reported diagnostic cutoff values. In those cases in which published papers included the diagnostic effectiveness of the test, these values were included in the reviewer’s report. Throughout the appendices, in the tables the following abbreviations are used: N, normal subject (i.e., no dry eye); DE, dry eye; EDE, evaporative dry eye; ADDE, aqueous-deficient dry eye.

APPENDIX 1

Test Identification: Symptom Questionnaires

A wide range of questionnaires have been used to assess the symptoms of ocular discomfort associated with dry eye conditions.148–152 Extensive reviews of the utility of these questionnaires have been published elsewhere.153,154 Despite the numerous questionnaires available, those most commonly used show good agreement.155

Rationale

Questionnaires allow the assessment of a range of symptoms associated with ocular discomfort. However, due to the
<table>
<thead>
<tr>
<th>Severity Level</th>
<th>Level 0</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
<th>Level 4</th>
<th>Level 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom frequency and severity</td>
<td>Normal</td>
<td>Subclinical</td>
<td>Symptomatic Minimal</td>
<td>Symptomatic Mild</td>
<td>Symptomatic Moderate</td>
<td>Symptomatic Severe</td>
</tr>
<tr>
<td>OSDI grade range (0–100)</td>
<td>0</td>
<td>0–12</td>
<td>13–22</td>
<td>23–32</td>
<td>33–100</td>
<td></td>
</tr>
<tr>
<td>MGD Grade</td>
<td>Clear</td>
<td>Minimally altered quality of expressed meibum from scattered glands; None to minor gland loss</td>
<td>Mildly altered meibum quality; occasional lid margin signs; mild gland loss</td>
<td>Moderately increased opacity and viscosity of meibum; plugging; increased marginal vascularity; loss of orifice definition; moderate gland loss</td>
<td>Markedly increased opacity and viscosity of meibum; plugging; increased marginal vascularity; loss of orifice definition; severe gland loss</td>
<td></td>
</tr>
<tr>
<td>Quality of expressed meibum grade range (0–3)</td>
<td>0</td>
<td>1–5</td>
<td>6–10</td>
<td>11–15</td>
<td>16–20</td>
<td>21–24</td>
</tr>
<tr>
<td>Treatment of MGD based on symptoms and gland status</td>
<td>+ General advice about MGD, the potential influence of diet, home and work environment, + Hygienic measures, heat and massage</td>
<td>± Topical ATs, ± Emollient lubricant or liposomal spray, ± Topical azithromycin, ± Consider oral tetracycline derivatives</td>
<td>+ Oral tetracycline derivatives</td>
<td>± Anti-inflammatories</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table should be read in conjunction with Table 11, which provides a staging scheme for MGD-related ocular surface disease. Severity levels for each parameter are graded 1–5. A subclinical severity level has been introduced to accommodate asymptomatic MGD with normal lid margin features (nonobvious MGD) diagnosed only after gland expression. Note that this MGD scoring system does not provide a score for totally obstructed glands. Alternative systems for grading MGD exist and should be considered (see Appendices 5–7). Arita et al. graded meibomian dropout in the combined upper and lower lids, using noninvasive meibography, with a scale range of 0–6 (Table 3). de Paiva et al. used a composite system combining dropout, lid signs and meibum expressibility, with a scale range of 0–11 (Table 3). Korb and Blackie recorded the number of glands in a zone of 8, which yield a liquid secretion after standardized expression (MGYLS score 0–8). General treatment concepts, summarized here, are adapted from the Report on Management and Therapy. Recommended treatments are additive. At each clinical assessment, lack of response to treatment at the previous level moves treatment to the next level. ±, the decision to use this treatment is based on clinical judgment; +, treatment is recommended at this level. LL, lower lid; OSDI, Ocular Surface Disease Index.

The increase in severity of MGD with increase in grade is denoted by a reduced quality of expressed meibum. Meibum quality (clarity and consistency) is assessed in eight glands of the central third of the lower lid on a 0–3 scale for each gland: 0 = clear; 1 = cloudy; 2 = cloudy with debris; 3 = thick, like toothpaste (total score range 0–24).
### Table 10. Staging the Severity of MGD-Related Ocular Surface Disease

<table>
<thead>
<tr>
<th>Symptom frequency and severity</th>
<th>Level 0</th>
<th>Level 1</th>
<th>Level 2 Minimally Symptomatic</th>
<th>Level 3 Mildly Symptomatic</th>
<th>Level 4 Moderately Symptomatic</th>
<th>Level 5 Severely Symptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>No symptoms</td>
<td>Asymptomatic or symptoms</td>
<td>Some of the time.</td>
<td>Half the time</td>
<td>Most of the time</td>
<td>All the time</td>
</tr>
<tr>
<td>Subclinical</td>
<td>0-12</td>
<td>&lt;7 to 3</td>
<td>0-12</td>
<td>13-22</td>
<td>13-32</td>
<td>23-32</td>
</tr>
<tr>
<td>OSDI range (0-100)</td>
<td>0</td>
<td>Normal &gt;308</td>
<td>Normal &gt;308</td>
<td>Mildly increased &gt;308</td>
<td>Moderately increased &gt;314</td>
<td>Markedly increased &gt;317</td>
</tr>
<tr>
<td>TFBUT, s</td>
<td>≥10</td>
<td>Normal &lt;308</td>
<td>Normal &lt;308</td>
<td>to ≤13</td>
<td>to ≤317</td>
<td>≤1 or instant breakup</td>
</tr>
<tr>
<td>Tear osmolarity, mOsM</td>
<td>Normal &lt;308</td>
<td>Normal &lt;308</td>
<td>Normal &lt;308</td>
<td>Mildly increased &gt;308</td>
<td>Moderately increased &gt;314</td>
<td>Markedly increased &gt;317</td>
</tr>
<tr>
<td>Conjunctival hyperemia</td>
<td>Nil</td>
<td>Minimal</td>
<td>Mild</td>
<td>Moderate</td>
<td>Marked</td>
<td></td>
</tr>
<tr>
<td>CCLRU</td>
<td>Nil</td>
<td>Nil</td>
<td>CCLRU</td>
<td>CCLRU</td>
<td>CCLRU</td>
<td>CCLRU</td>
</tr>
<tr>
<td>Ocular surface staining</td>
<td>0</td>
<td>0-7</td>
<td>8-14</td>
<td>15-25</td>
<td>24-33</td>
<td></td>
</tr>
<tr>
<td>Oxford scale (0-15)</td>
<td>0</td>
<td>0-7</td>
<td>8-14</td>
<td>15-25</td>
<td>24-33</td>
<td></td>
</tr>
<tr>
<td>NEI Industry scale (0-33)</td>
<td>0</td>
<td>0-7</td>
<td>8-14</td>
<td>15-25</td>
<td>24-33</td>
<td></td>
</tr>
<tr>
<td>Schirmer score, mm</td>
<td>≥10</td>
<td>≥10</td>
<td>&lt;7 to 3</td>
<td>&lt;7 to 3</td>
<td>&lt;3 to 3</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Treatment of MGD-related ocular surface disease</td>
<td>No treatment</td>
<td>No treatment</td>
<td>+ Artificial tear substitutes</td>
<td>+ Alternative AT selection</td>
<td>+ Alternative AT selection</td>
<td>+ Alternative AT selection</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ Simple viscosity agents (preservatives allowable at low frequency of use)</td>
<td>+ Immune modulation</td>
<td>+ Gels and ointments</td>
<td>+ Gels and ointments</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This should be read in conjunction with Table 9 which provides a staging scheme for MGD. Increasing MGD severity is perceived to lead to impaired spreading and stability of the tear film lipid layer, increased evaporative water loss, increased tear osmolarity, and ocular surface damage, which leads to conjunctival hyperemia and symptoms. These events are accompanied by inflammatory responses in the lids and on the ocular surface. Each measured parameter scales from least to most severe disease in five levels of severity. The numerical divisions are literature based, but require further validation. In an individual patient, it is unlikely that stages will lie in register for each parameter, but a global score can be generated by summing grades within the levels. Extensive MGD can be a cause of evaporative dry eye rather than aqueous-deficient dry eye. However, the Schirmer test is included in the battery of tests, to allow for the coinciding occurrence of both conditions. Treatment is based on symptoms, ocular surface damage and disturbed tear dynamics. For details, see the Report on Management and Therapy. Recommended treatments are additive at each level. At each clinical assessment, lack of response to treatment at the previous level moves treatment to the next level. The decision to use this treatment is based on clinical judgement; treatment is recommended at this level. MGD, meibomian gland dysfunction; AT, artificial tears; CCLRU, Cornea and Contact Lens Research Unit (School of Optometry and Vision Science, University of New South Wales, Sydney, Australia); NEI, National Eye Institute; OSDI, Ocular Surface Disease Index.
commonality of symptoms across a range of disorders including dry eye and MGD, these questionnaires are unlikely to be able to differentiate between etiologically distinct disease entities. Despite this limitation, some studies have looked at the role of questionnaires in assessing symptoms in MGD.

Method and Description

Studies have shown that MGD (diagnosed by gland orifice plugging and lid margin telangiectasia) is present in 61.7% of symptomatic patients. This was in close agreement with other studies where 63.6% and 64.6% of symptomatic
subjects were found to have signs of MGD. A higher proportion of MGD sufferers were found (74.3\%) among symptomatic video display unit (VDU) users but this is likely to be due to the population studied. Interestingly, in this VDU population MGD sufferers did not exhibit more severe symptoms than subjects with no evidence of MGD.\textsuperscript{160} Further evidence for the role of MGD in producing symptoms comes from the observation that a statistically significant negative correlation was observed between the number of meibomian glands capable of yielding liquid secretion and symptom score.\textsuperscript{161}

It is clear from these studies that MGD is present in excess of 60\% of patients with ocular discomfort. Current questionnaires have not been optimized or tested in their ability to differentiate between MGD and other causes of ocular discomfort. Undoubtedly, further studies are needed, particularly to assess the sensitivity and specificity of symptom questionnaires in the diagnosis of robustly defined MGD patients. Because of the commonality of symptoms with other disorders and the lack of a pathognomonic symptom in MGD, it is likely that questionnaires, although useful, will have to be used in conjunction with other methods in the characterization and diagnosis of MGD.

Of interest, the Ocular Surface Disease Index (Allergan Inc., Irvine, CA) has been recently validated across ocular surface disease severity,\textsuperscript{162} and while the symptoms may be different in MGD, it can be hypothesized that the OSDI could be used to document disease progression. The ocular surface disease ratings are as follows across the scale of the questionnaire (0–100): normal, 0–12; mild, 13–22; moderate, 23–32; and severe, 33–100, with a seven-unit change noted as clinically significant.\textsuperscript{162}

**APPENDIX 2**

**Test Identification: Fluorescein/Noninvasive Breakup Time**

Breakup time is thought to be a surrogate measure of tear stability.

**Rationale**

Tear breakup time (TBUT) is generally regarded as a test for diagnosis of evaporative dry eye; however, as discussed herein, TBUT testing is relevant in the diagnosis of MGD. Tear film instability is one of the core mechanisms of dry eye and may be the initiating event.\textsuperscript{165} It is dependent on many factors, including an adequate tear film lipid layer,\textsuperscript{164–171} which in turn is dependent on meibomian gland function.\textsuperscript{166,172,173} There is strong evidence to suggest that both lipid quantity and quality correlate with meibomian gland function and dry eye states.\textsuperscript{165–169,172–179} Hence, low TBUTs imply a possibility of lipid layer compromise and thus meibomian gland dysfunction, whereas high TBUTs suggest a normal lipid layer and adequate meibomian gland function.\textsuperscript{164,168,170,171} Thus, whenever TBUT is low, meibomian gland function and expressibility should be investigated for diagnosis and in considering treatment.

**Description: Fluorescein Breakup Time**

Tear film stability is measured by a test of fluorescein breakup time (FBUT), defined as the time to initial breakup of the tear film \textsuperscript{179} after a blink.\textsuperscript{179} It has been proposed that fluorescein breakup can be caused by quenching of fluorescence related to the increase in fluorescein concentration caused by evaporation.\textsuperscript{180} The classic and usual method to determine breakup time utilizes fluorescein to stain the tear film (FBUT).\textsuperscript{181–183} The fluorescein may be applied by wetting a commercially available fluorescein-impregnated strip with sterile saline and applying to the inferior fornix or to the bulbar conjunctiva. After instillation, the patient is asked to blink several times and to move the eyes, to mix the fluorescein in the tears. Observation is with the slit lamp, a cobalt blue filter, a beam width of approximately 4 mm, and full height, and the beam is slowly moved from side to side to cover the entire cornea.\textsuperscript{182,185} A yellow barrier filter enhances observation of the fluorescent tear film.\textsuperscript{179,184} The patient is instructed to blink naturally and then, once homogeneous tear film fluorescence is confirmed, to keep the eyes open while looking straight ahead. The time from upstroke of the last blink to the first tear film break or dry spot formation is recorded as the FBUT measurement. Either one or the average of three consecutive trials is the final value.\textsuperscript{178,179,182,184,185} Optionally, a video camera may be used to record TBUT with various methods used to automate timing and permit masking of the measurement.\textsuperscript{179,184} An alternative to the use of fluorescein-impregnated strips is the instillation of liquid unpreserved fluorescein onto the bulbar or conjunctival conjunctiva with a micropipette. Concentrations of 2% to 5% and volumes of 1 to 5 \(\mu L\) have been advocated.\textsuperscript{179,186} (Welch D, et al. \textit{IOVS} 2008;49:ARVO E-Abstract 2485). The observation procedure is the same as for the fluorescein-impregnated strip.

In performing a series of clinical tests for dry eye, measurement of the TFBUT is usually followed by measurement of fluorescein staining. There is a distinct advantage in completing the series in the right eye, before instilling dye and performing the series in the left eye, since this avoids dilution of dye in the tear film and diffusion of dye taken up into the ocular surface of the second eye (Appendix 4).

**Variations in the Technique of Administering the FBUT Test.** Despite the acknowledgment of the value of quantification of tear film stability, FBUT has been criticized as being inaccurate and poorly reproducible.\textsuperscript{186–190} (Welch D, et al. \textit{IOVS} 2008;49:ARVO E-Abstract 2485). The inherent nature of a large fluorescein-impregnated strip and the lack of a standardized procedure for moistening and applying the strip to the tear film prevents control of the volume delivered to the tear film and must result in variability. There is no agreement as to whether the moistened strip should be shaken before instillation or whether the strip should be applied to the superior, inferior, temporal, or inferior temporal bulbar conjunctiva or to the tear meniscus.\textsuperscript{160,178,182,185–187} The greatest source of variability in FBUTs relates to the volume of fluorescein delivered. FBUT measurement reliability is increased when 2 \(\mu L\) or less of a 5% fluorescein solution is applied with a micropipette versus the conventional strip method.\textsuperscript{179,186} Although micropipettes offer a precise method of instillation of microliter quantities of fluorescein, the use of unpreserved fluorescein solutions in the clinical setting requires sterile procedures, and while these procedures are applicable to research studies, they are not readily adaptable to clinical practice. Further, FBUTs are altered by reflex tearing, and the use of a pipette frequently causes apprehension in the patient and reflex tearing. A novel approach to both reducing the volume of fluorescein and eliminating sensation and reflex tearing during FBUT measurements, the Dry Eye Test (DET; Nomax, Inc., St. Louis, MO) was developed to deliver 1 \(\mu L\) of fluorescein solution to the tear film by application to the superior temporal bulbar conjunctiva.\textsuperscript{184} The DET is applicable in either research studies or clinical practice.\textsuperscript{179,184}

**Recommendations for Conduct of the FBUT Test.** Either the micropipette or the DET strip is applicable for research studies. The micropipette method should be standardized for volume and concentration of fluorescein. Recommendations for volume have varied from 5 to 1 \(\mu L\) and for concentration from 1% to 5%.\textsuperscript{179,186} The DET strip provides a standardized method to deliver 1 \(\mu L\) of volume.\textsuperscript{185}
Estimated Values of FBUT in Normal, Dry, and MGD Eyes. There are no reported estimated values for subjects with only MGD specifically.

With traditional volumes of fluorescein, FBUTs in normal subjects are >10 seconds versus ≤10 seconds in those with dry eye. With micro volumes of fluorescein, FBUTs in normal subjects are >5 seconds versus ≤5 seconds in dry eye.

Sensitivity and Specificity. The sensitivity and specificity of the FBUT test are reported to be 72% and 62%.169

Method and Description: Noninvasive Breakup Time Measurement

Noninvasive breakup time (NIBUT) measurement utilizes a grid or other pattern projected onto the precorneal tear film for the observation of distortion and/or abnormalities in the image. The patient is instructed to blink normally while looking straight ahead. The time interval in seconds from the upstroke of a blink to the first change of the image after a blink is defined as the NIBUT. The result of either one or the average of three consecutive trials is the final value.179,180,181 Optionally, a video camera may be used to record TBUT with various methods to automate timing179 (Welch D, et al. IOVS 2008;49: ARVO E-Abstract 2485).

The NIBUT test eliminates physical disturbance of the tear film from the instillation of fluorescein, along with the possibility of inducing tactile reflex tearing.169,179,180,181 NIBUT would therefore appear to be an ideal theoretical method of evaluating tear film stability, because it overcomes the objections to fluorescein invasive FBUT measurement and can provide more reliable and reproducible results. The TBUTs obtained with the NIBUT test are significantly greater than those values found with the FBUT.169,179,182,193–195 which has been attributed to the destabilizing effect of the instilled fluorescein. A study advocating the use of the NIBUT test for the diagnosis of mucous layer deficiencies and for distinguishing between aqueous tear deficiency and MGD nevertheless stated that the NIBUT test did not replace the FBUT test as the test of choice for the evaluation of tear film stability.169

Estimated Values of NIBUT in Normal, Dry, and MGD Eyes. The normal range for NIBUT is typically 40 to 60 seconds193 compared with normal ranges of 10 to 34 seconds181,184 for the FBUT. For dry eye NIBUT ≤10 seconds. Reported values of NIBUT vary significantly between investigators and equipment used, with the values of NIBUT remaining higher than those for FBUT.179,180,181 It has been suggested that the two methods measure different phenomena.169 There are no specifically reported estimated values for subjects with only MGD.

Sensitivity and Specificity. The sensitivity and specificity of the NIBUT test are reported to be 82% and 86%.189

APPENDIX 3

Test Identification: Schirmer test (in the Diagnosis of MGD)
The Schirmer test is traditionally a measure of tear production when performed for the recommended 5 minutes, although some research indicates that the test, when administered for shorter durations, may be a measure of tear volume on the ocular surface.

Rationale
The Schirmer test may not be a direct test of MGD; however, it is useful in the differentiation of aqueous-deficient dry eye and evaporative dry eye, both of which may occur concurrently with MGD. Although MGD may be a causative factor in evaporative dry eye, aqueous-deficient dry eye can occur simultaneously.

Method and Description
The Schirmer test without anesthesia is a well-standardized test performed with the patient’s eyes closed. There is wide intrasubject, temporal, and visit-to-visit variation, but the variation and the absolute decrease in aqueous deficiency are mostly due to the decreased reflex response with lacrimal failure. When the cutoff value is set at <5.5 mm/5 minutes, the sensitivity and specificity of the testing are 85% and 83%, respectively.197

The diagnostic cutoff used at present is <5.0 mm in 5 minutes, the reason for which is still unclear. Lowering the cutoff decreases the detection rate (sensitivity), but increases the specificity of the test.196 The repeatability of the Schirmer 1 test appears to be better with lower values (i.e., in more severe aqueous deficiency).198 A significant correlation was shown between Schirmer 1 test, tear stability and fluorescein staining in a recent study by Nichols et al.199 Meliobion gland disease greater than grade 1 (according to the method of Bron et al.196) did not appear to correlate with the Schirmer 1 result, tear meniscus height, phenol red test, and staining with vital dye in that study. Nichols et al.200 reported a poor correlation between Schirmer 1 test and dry eye symptoms. Many studies showed that no significant differences existed in tear quantity (Schirmer 1 test values) between patients with simple MGD and healthy control subjects, suggesting the difficulty of differentiating MGD patients from normal subjects based only on tear quantity.201–204 Shimazaki et al.205 and Goto et al.206 found no differences in Schirmer 1 test scores between patients with Sjögren’s syndrome and those with non-Sjögren’s syndrome dry eye. However, the presence of MGD in association resulted in much more severe ocular surface disease characterized by higher fluorescein and rose Bengal vital staining scores or tear evaporation. Den et al.207 found that changes in the lid margin, including vascular engorgement, irregularity, plugging of MG orifices, and replacement of the MCJ were closely related to aging and that there was an age-related decrease in tear quantity scores assessed with Schirmer 1 test. Subjects with Schirmer test scores <5 mm had significantly decreased meliobion gland expressibility grades. Arita et al.208 found an age-related decrease in Schirmer 1 test scores and meibomian gland dropout grades in a recent study. Sterile Schirmer test strips were used to collect meibomian oil in healthy individuals (n = 20), dry eye patients (aqueous-deficient; n = 32) and MGD patients (n = 25) after gentle massage of the lid margin in another study.209 Meibomian fatty acids were directly transmethylated and analyzed by using gas chromatography (GC) and GC mass spectrometry. Meibomian fatty acids were similar in healthy individuals and in dry eye patients with aqueous deficiency, but were different in MGD patients, who showed significantly higher levels of branched-chain fatty acids (29.8% vs. 20.2%) and lower levels of saturated fatty acids (9.3 vs. 24.6%)—in particular, lower levels of palmitic (C16) and stearic (C18) acids. The increase in branched-chain fatty acids may reflect greater quantities of wax and cholesterol esters and triglycerides in meibomian gland excreta. It was concluded that meibomian fatty acid composition and particularly the increase in branched chains evaluated with Schirmer strips could be a marker for meibomian gland dysfunction. The methodology also proved to reflect treatment effects by oral minocycline treatment suggesting iso-C20 (extracted from Schirmer strips) to be a useful biomarker for the diagnosis of MGD.210
Test Identification: Phenol Red (Cotton Thread) Test

The phenol red test (PRT) test has been developed as an alternative to the Schirmer test and is another method of analyzing a patient’s lacrimal system.

Rationale

The PRT tear test represents another approach to the analysis of a patient’s lacrimal system. It was developed to overcome the disadvantages of the Schirmer tear test, including variable results, poor repeatability, and failure to measure basal secretion, even when used with anesthesia.

Method and Description

Although the PRT method is quite similar to Schirmer, there are distinct differences. There is little or no sensation of the thread, making anesthesia unnecessary. A test time of only 15 seconds is required in comparison to the 5 minutes per eye needed for the Schirmer test. This test is performed with the patient’s eyes open while blinking naturally. The length (in millimeters) of the wet portion of the thread is recorded as the result. Because of the short test time and minimal sensation of the thread, it is theorized that this test gives an indication of amount of residual tears located primarily in the inferior conjunctival sac of the eye. Using a cutoff value of 12 mm, the sensitivity and specificity of the PRT are 56% and 69%, respectively. Even if the agreement with the Schirmer 1 test is highly significant, 32% of patients have discordant results. These two methods of functional assessment of tear secretion seem to be complementary, and further studies remain necessary to better understand the correlation of both tests in clinical practice.

A recent study found a weak agreement between Schirmer test and phenol red thread tests and between each test and symptoms of dry eyes. To determine the clinical viability of a phenol red–impregnated cotton thread in differentiating between normal, aqueous deficient, and non–aqueous-deficient dry eyes, Patel et al. recruited subjects on the basis of subjective symptoms, tear stability, rose bengal staining, Schirmer test, conjunctival hyperemia, patency and number of meibomian glands, presence of mucus strands, and appearance of lower tear meniscus. Based on the outcome of the tests, the subjects were categorized as having aqueous-deficient dry eye, non–aqueous-deficient dry eye, or normal eyes. Subjects were randomized, and a thread was applied by inserting it into the lower fornix of the right eye and leaving the thread in place for 120 seconds. The mean thread-wetting values were 15.5 ± 4.7 mm in aqueous-deficient dry eyes (n = 35), 22.7 ± 5 mm in non–aqueous-deficient dry eyes, and 19.4 ± 5 mm in normal eyes (n = 38). For the aqueous-deficient and non- aqueous-deficient dry eyes only, when a cutoff value of 20 mm was used, the calculated sensitivity and specificity were 86% and 83%, respectively. PRT was found not to have any correlation with MGD (in patients with more than grade 1 MGD as classified by Bron’s grading scheme) by Nichols et al. The test was also found to have poorer repeatability than the Schirmer test. The test was removed from Japanese dry eye diagnostic criteria on the founder’s request due to low repeatability and wide variation in scores.

APPENDIX 4

Test Identification: Ocular Surface Staining

Rationale. Ocular surface damage is encountered in association with MGD and is found in its most advanced form in MKC. Various etiologies have been proposed for such damage, including the release of inflammatory mediators into the tear film and the mechanism of evaporative dry eye. One source of such mediators includes the breakdown products of meibomian glands altered by the lipases of microbial commensals.

Ocular surface damage may be quantified by grading staining of the cornea and conjunctiva by using selected dyes, by immunohistochemistry, or by flow cytometry on impression cytology specimens and the direct measurement of inflammatory mediators in the tears biochemically or with multiplex beads, matrix assisted-laser desorption ionization (MALDI)-TOF (time of flight), and proteomic techniques (Reinoso R, et al. IOVS 2009;50:ARVO E-Abstract 517; Topcu Yilmaz P, et al. IOVS 2009;50:ARVO E-Abstract 3592; Calonge M, et al. IOVS 2009;50:ARVO E-Abstract 2548); Nichols KK, et al. IOVS 2009;50:ARVO E-Abstract 541).

Additional approaches include confocal microscopy (Appendix 8). These techniques have helped to describe the ocular surface phenotype in MGD and other ocular surface diseases and to monitor the severity of disease and response to treatment, but the events that they record are not specific to MGD, and therefore they have no unique role in diagnosis. The precision of such tests was addressed in the 2007 DEWS report, where details of their sensitivity and specificity in the diagnosis of dry eye is summarized and incorporated, both in the published templates and in additional materials available on the TFOS web site. In addition to the DEWS Management and Therapy report, ocular surface staining was cited as an important diagnostic criterion by Behrens et al. in the clinical diagnosis of ocular surface disease.

Method and Description

Grading Ocular Surface Staining. Several grading schemes have been reported and are discussed below.

1. van Bijsterveld
2. NEI/Industry Schema
3. Oxford Grading System

van Bijsterveld. One drop of rose bengal 1% is instilled. Staining is graded 0 to 3 on the cornea and for two exposed conjunctival segments (range: 0–9).

Cut-off: ≤3.5
N vs. DE
N vs. EDE
ADDE vs. EDE
Sensitivity/specificity 95% vs. 96% NA NA

In the European/American criteria for the diagnosis of Sjögren syndrome a 2.5 μL solution of rose bengal is instilled in the lower fornix. Grading is according to van Bijsterveld 1969. The sensitivity/specificity of rose Bengal staining is as follows:

Cut-off: ≤4
N vs. DE
N vs. EDE
ADDE vs. EDE
Sensitivity/specificity in diagnosis of SS 65% vs. 84% NA NA

NEI/Industry Schema. Nichols et al. used a modified version in dry eye diagnosis. The grading proforma presents five corneal and 2 × 3 conjunctival zones. Grades are 0 to 3 per zone, including 0.5 steps. Fluorescein or rose bengal is instilled from impregnated strips in control and dry eye subjects. There is strong agreement between corneal and conjunctival staining.

A revised version of this test, incorporating features of the NEI/Workshop grading system and the Oxford system (dubbed
The Oxford version 2) was presented as a poster at a Tear Film and Ocular Surface meeting (Taormina, Italy 2006). The purpose was to provide a finer scale and to standardize the conduct of the test. The NEI/Industry system has been modified to (1) standardize the size and location of recording zones and (2) to create panels of random dots whose increasing density in numbers from panel to panel is mathematically defined. To do this (1) a series of 10 panels is generated, with the probability $P$ that a pixel would be black given by $P = \exp(0.9 \cdot a)/\exp(0.9 \cdot b) - k$, where $a$ is the number of the current panel, $b$ is the number of the last panel + 1, and $k = 0.00042$. So that the dot size on the printed panels approximates that of a staining point on the ocular surface, the panels are scaled in a word processing program (MSWord, Microsoft, Redmond, WA), so that the dot size reflects the apparent size of an epithelial cell at the magnification used at the slit lamp. The grading range for each zone is from 1 to 10. Therefore, since the number of zones scored is $2 \times 3$ conjunctival and five corneal, the grade range is from 0 to 110. The system has not yet been validated.

Oxford Grading System. In this system the cornea and two conjunctival zones are graded, with a grade score 0 to 5 per zone (total range, 0–15). In an intra-observer study, two trained ophthalmologists graded a series of standard slides, showing corneal and conjunctival fluorescein staining, on two separate occasions. In an inter-observer study the same two observers graded fluorescein (blue exciter; yellow filter) and separate occasions. In an inter-observer study the same two conjunctival zones are graded, with a grade score 0 to 5 yet been validated.

Corneal, the grade range is from 0 to 110. The system has not been validated.

Additional studies have been undertaken to explore the utility of batteries of tests, including symptom questionnaires, ocular surface staining, Schirmer test, fluorescein clearance test (FCT), and corneal sensitivity, in the diagnosis of dry eye. These are summarized under the heading of mixed tests in the dry eye templates published in the DEWS (2007) report. In a study by Afonso et al. in patients with irritative ocular surface symptoms, meibomian gland dropout or orifice metaplasia correlated significantly with reduced fluorescein clearance and inversely with the Schirmer result.

APPENDIX 5

Test Identification: Signs of MGD in Lid Morphology

Rationale. The classification of MGD is based on clinical findings. Some recent classifications have used a terminology based on functional and microbiologic associations. McCulley et al. have suggested that the clinical spectrum of chronic blepharitis has changed and that the relative prevalence of *Staphylococcus aureus*, alone or in combination with seborrheic blepharitis, has decreased. In the literature and clinically, it has been hypothesized that the relative prevalence of seborrheic blepharitis has increased, with or without associated excess meibomian secretions (meibomian seborrhea) or inflammation (meibomitis). The terminology used clinically has been inconsistent, and the The Report on Definition and Classification has the specific purpose of unifying terminology. Nonetheless, clinically, primary meibomitis appears not to be a primarily infectious entity but to represent a facet of generalized sebaceous gland dysfunction found in association with seborrheic dermatitis or acne rosacea. These entities are recognized as chronic diseases requiring control for which there is no cure.

Additional attempts have been made to incorporate morphologic features of meibomian abnormality occurring in the gland acinus, duct, or orifice. Before meaningful classification of MGD morphology can be performed, it is important to define the normal anatomy of the lid and meibomian gland apparatus and its associated age-related changes. The appearance of the normal lid then can be used to provide a basis for a morphologic classification of posterior blepharitis, enabling better correlation with MGD and the earlier recognition of the diseased lid. Clinical descriptions of lid margin changes across age (children to elderly) are summarized below.

Lid Margin. The lid margin thickness has a normal range for adults and children. The lid margin in adults is 2 mm thick at its free edge and has lashes on its anterior aspect. Lid margin thickness in children ranges between 1.45 and 1.65 mm in the upper lid and 1.41 and 1.61 mm in the lower one. From adolescence onward, lid thickness increases to between 1.88 and 2.02 mm in the upper lid and 1.81 and 1.93 mm in the lower lid. The lid thickening that apparently occurs after childhood may be related to enlargement of the orbicularis muscle. Hormone-induced enlargement of sebaceous glands at puberty and could affect the meibomian glands.

Lid Vascularity. The lids of children are typically less vascular, with no telangiectasia, cutaneous hyperkeratinization, or squamous blepharitis. The absence of lid margin vascularity in children is striking, and the increase from adolescence may be secondary to increased MGD. In the elderly, telangiectasia, and cutaneous hyperkeratinization are significantly more common in the lower lid. This perhaps reflects increased exposure of the lower lid to various insults, including ultraviolet radiation. The increased prevalence of upper lid margin rounding in the elderly has not been thought of as a physiological finding and is generally considered to be more common in the lower lid, in association with posterior blepharitis and subconjunctival fibrosis. Other factors, such as exposure to work dust particles, urbanization, and cosmetics may be important.

Cilia. The cilia count in the sagittal plane does not change significantly with age; however, it is a clinical impression that loss of cilia occurs in elderly patients, as does hair loss elsewhere in the body.

Muscocutaneous Junction. The MCJ is constant in position immediately posterior to the meibomian gland orifices. The MCJ lies at the junction of the anterior two thirds and posterior one third of the lid, but may run an irregular course in normal elderly persons. No significant age-related changes in the position or form of the MCJ have been noted. Changes are typically seen in disease states, particularly MGD, acne rosacea, and severe atopic eye disease.

Orifices. The meibomian gland orifices are situated just anterior to the MCJ. The orifices are round, are rarely narrowed or pouted, and no orifice obliteration or retroplacement occurs. They may be congenitally absent, in association with the underlying gland and have been described as plugged by keratin and desquamated epithelial cells, damaged, or patent in a nonsecretory, resting phase. Plugging may eventually lead to obliteration of orifices, with the meibomian gland orifice narrowing and pouting. Squamous blepharitis. Orifice narrowing and pouting probably represent hypertrophy of duct epithelium. Pouting of orifices in asymptomatic individuals may be a feature of the aging lid as well as an early sign of MGD. Narrowing of the orifices increases with age, and the associated change in the shape of the surrounding epithelial cuff suggests that there is uneven distribution of tissue stress in the coronal and sagittal planes of the lid margin. Orifice obliteration is significantly increased with age in the upper lid. It has been reported in MGD, acne rosacea, and severe mucous membrane disease, such as trachoma and cicatricial pemphigoid, in which secondary MGD occurs, but not in normal subjects.
Main Duct. The glands themselves can be seen as yellow streaks through the tarsal plate in young people. They have a main duct opening on the lid margin at a meibomian gland orifice and 50 to 60 lateral ductules leading to a single or composite acinus. They are modified sebaceous glands; the upper lid contains approximately 30 and the lower 20. The upper lid glands are longer (10 mm) than the lower (5 mm).

Acini. These will be described in the section on meibography.

Tarsal Plate. Lower lid conjunctival hyperemia occurs with increasing frequency in elderly patients.

Secretions. A significant decrease in the quantity of secretion occurs with age, with fewer orifices freely expressing meibomian secretions. However, the decrease is usually not accompanied by an increased opacity or viscosity of the secretions, suggesting that these may represent markers of disease and result in the typical plugging of meibomian gland orifices in MGD.

Clinical Anatomy in MGD

Lid Margin. Thickening of the lid is a common feature of meibomian gland disease, but is difficult to measure because of the rounded contour of the anterior margin. It is best measured from the posterior margin to the posterior lash line, which are relatively constant features of the lid. Rounding of the posterior lid margin is often associated with thickening and interferes with the normal apposition of lid to globe. Vascularization increases with age. In MGD, there is an exaggeration with invasion of the outer and then inner cuffs of the orifice. Hyperkeratinization is an eczematous appearance of the cutaneous margin, frequently encountered in atopes with facial eczema, but also in nonatopic subjects. Irregularity of the lid margin arises from absorption of tissue, often in the region of obliterated meibomian orifices, but will occur with more gross distortions of lid architecture in cicatricial and ulcerative lid disease.

Mucocutaneous Junction. The MCJ location and morphology may be altered in MGD. The MCJ is best identified by its specular reflection. Although the position of the anterior edge of the tear meniscus may correspond with it in health, in disease it may not be an accurate guide.

1. Anteroplacement. The junction becomes irregular in MGD. The mucosa may spread forward, so that the orifices appear to lie in mucosal tissue.

2. Retroplacement. There is a posterior movement of the MCJ, with a spreading, keratinizing, squamous metaplasia of the posterior lid margin that extends onto the tarsal plate. The meibomian orifices may or may not move with the MCJ, which will determine whether the tear oil is delivered onto the surface of the tear film. Retroplacement is more common than anteroplacement.

3. Mucosal absorption. This may occur without retroplacement of the MCJ so that the MCJ and orifices are still at the same distance from the lash line, but come to lie closer to a new posterior lid margin.

4. Ridging. There is a ridgelike elevation of the MCJ or of tissue between the orifices. It may also be a secondary effect of mucosal absorption.

Orifices. Orifices demonstrate several presentations in MGD.

1. Number. The orifices may be reduplicated, or reduced in number, congenitally, sometimes as part of a syndrome, or as an acquired feature of MGD.

2. Capping. Scattered orifices may be capped by a dome of oil with a tough surface, but may be pierced by a needle tip to release the oil. The underlying orifice may be ulcerated and the cap epithelialized. Capping usually affects only occasional orifices and may be found in otherwise normal lids.

3. Pouting. An early sign of MGD is the elevation or pouting of the orifice, which is no longer flush with the surface. The term is probably equivalent to plugging. The meibomian orifice may be dilated, and expression may demonstrate the terminal ductule plugged with inspissated secretion or other material.

4. Retroplacement. This term is used to describe the result of a cicatricial process involving the posterior lid margin and may be accompanied with more extensive cicatricial changes within the tarsal mucous membrane near the marginal mucosa. The orifices may become oval or elongated at right angles to the plane of the lid margin, and posterior movement may be accomplished by duct exposure.

5. Obliteration narrowing. The punctum of the orifice may not be visible. The appearance of narrowing is accompanied by absent expressibility of lipid. Loss of definition of the cuffs of the orifices is a feature that is seen with age and in early MGD. Vascular invasion may accompany the process of loss of definition.

6. Opaque orifices. The degree of opacity of the inner cuff becomes accentuated. Opaque orifices are far more visible at the lid margin than normal. Scarring of the region of the orifices may occur, with tissue loss and depression of the surface. It is often accompanied by a range of degenerative changes at the lid margin.

7. Duct exposure. Exposure of the terminal duct of the gland in varying degrees is a common feature of MGD, suggesting the presence of an irreversible cicatricial process in the adjacent submucosa. The duct, as it forms the orifice at the lid margin, is seen to turn on its side anteroposteriorly, so that it becomes visible at the surface of the lid margin. The outer cuff becomes lost from view, whereas the inner cuff (the epithelial lining) and the translucent zone (the presumed dermal layer) are seen in profile. In the early stages, the duct may be patent and functional; later it is not. The changes may extend over the lid margin for several millimeters, which raises the question of whether it is associated with duct elongation or absorption of the distal part of the tarsal plate.

8. Cystoid dilatation of duct. Cystoid expansion may be seen anywhere along the course of the duct as a dark round or ovoid region along the course of a meibomian gland. Sometimes there are extended, cigar-shaped structures that seem to occupy the position of one or more meibomian glands, but it is not easy to distinguish dilatation of the duct from that of the gland acini by routine methods. Enlarged, distorted and also shortened glands may be distinguished by meibography and confocal microscopy.

Acini. The acini are susceptible to age-related and disease-associated alterations.

1. Visibility. As mentioned earlier, congenitally absent or deficient glands are represented by deficient orifices. Although the presence of acini may readily be judged in young, uninflamed lids, the visibility of the acini, when viewed by diffuse illumination of the tarsal plate, decreases with age as well as in the presence of chronic conjunctival inflammation. Observation can be improved by meibography. Enlargement or reduction in size of the glands may be recorded and concretions and chalazia may be present.

2. Concretions may follow the line of the meibomian glands and are believed to be deposits of lime salts
within acini. The clinical features of chalazia are well known and start as a firm, circumscribed, painless elevation on the tarsal plate, visible and palpable through the skin, which evolves slowly with time. The lesion is in line with the tarsal gland that it replaces, and the corresponding ductile orifice is occluded, with no oil being expressible.

3. **Chalazia** occur more frequently under the upper than the lower lid and more commonly in adults than in the young. They may be single or multiple, and they may be confluent. The lid may be sufficiently thickened to prevent eversion. More than one lid may be affected. Multiple chalazia are said to be more frequent in young people, especially seborrheic subjects with a history of chronic blepharoconjunctivitis, but also occur in elderly people or those with rosacea.

**Secretions Expressed.** The secretory functions of the meibomian glands are assessed indirectly by compressing the tarsal plate locally in relation to individual groups of orifices. This procedure may be performed with finger pressure, a cotton tip, or a glass rod or with the Korb expression device, to produce, in normal lids, a dome of clear oil over the orifices. The quality of the expressed secretion that can be elicited in this way in MGD is as follows:

1. Clear (i.e., normal).
2. Cloudy: diffusely turbid fluid secretions.
3. Granular: usually turbid fluid secretions, but contains particulate matter. The color of these secretions varies from whitish-gray to yellow.
4. Inspissated: a semisolid plug or a substance of toothpaste-like consistency; may be extruded as a plug or curled thread. Expression is usually delayed or requires extra pressure. The material contains keratinized epithelial cells.

The classification scheme, while not complete, is comprehensive enough to permit a detailed assessment of meibomian and lid morphology for the purposes of natural history and therapeutic studies. The purpose of classifying the features of MGD is the opportunity it provides to quantify them.

**APPENDIX 6**

**Test Identification: Meibomian Gland Expressibility**

**Rationale.** Meibomian gland expression can be performed as an indicator of meibomian gland function. In the normal patient, a clear to light yellow oil (meibum) is excreted from the glands when digital pressure is placed on the glands. Changes in meibomian gland expressibility may be a valuable indicator of disease.

**Method and Description**

The only method to determine whether a specific meibomian gland is functional and capable of providing secretion is to observe the secretion expressed from that gland. Since it is not possible to observe the flow of secretion from an individual gland during blinking or forced blinking, assessment requires expressing the meibomian gland with a physical force applied to the outer surface of the eyelid, while simultaneously observing the orifice of the gland with adequate magnification and conditions to detect the outflow of meibomian gland secretion. There are four types of expression:

1. **Traditional diagnostic expression to determine habitual meibomian gland functionality,** usually described without specifying the quantification of the physical force or time of expression. The description of the force applied has been limited to gentle or forceful. The usual procedure is to digitally express the central glands with a force that does not require a rigid surface on the inside surface of the lid. The finger is usually used for the expression, although a spatula, glass rod, or paddle may also be used. It is suggested that the expression should be maintained for 10 to 15 seconds. It is imperative to note that obstructive MGD may not be accompanied by obvious lid inflammation and other signs of lid pathology, and thus masquerade as nonobvious to the usual slit lamp examination. Thus, despite a wide prevalence in the general population, nonobvious obstructive MGD is usually overlooked due to minimally observable clinical signs associated with this type of MGD. It is therefore recommended that diagnostic expression be performed when dry eye symptoms are present, even when there is no obvious blepharitis, since the most prevalent form of MGD occurs in the absence of obvious blepharitis, and can only be detected by physical expression. After diagnostic expression, expression to determine the likelihood of successful meibomian gland treatment should follow. Therapeutic expression may be instituted as indicated.
Estimated Values in Normal, Dry Eye, and MGD Eyes

There are only three studies in which the number of meibomian glands yielding secretion was correlated to symptoms, and no studies correlating to other ocular surface findings. These three studies examined lower lids only. With digital expression, if four or more of the central six to eight glands are open, there is a low likelihood of dry eye symptoms. Using the instrument for standardized force expression, if three or more of the central six to eight glands are open, there is a low likelihood of dry eye symptoms. For the entire lid, with digital expression, if 10 or more of the approximate 24 glands yield secretion, there is a low likelihood of dry eye symptoms. Using the instrument for standardized force expression, for the entire lower lid, if 6 or more of the ~24 glands yield secretion, there is a low likelihood of dry eye symptoms. Conversely if 4 or fewer of the ~24 glands yield secretion, there is a high likelihood of dry eye symptoms.

Sensitivity and Specificity

There are no sensitivity or specificity data for nonobvious obstructive MGD. There is one study in which sensitivity and specificity were determined for meibomian gland function in blepharitis. The study reported the sensitivity/specificity data as follows: meibomian gland expression of the upper lid, 86%/73%, and for the lower lid, 53%/66% (McCann LC, et al. IOVS 2008;49:ARVO E-Abstract 1532).

Volume and Quality

Lipid Volume. Lipid volume has been assessed semiquantitatively by measuring the average diameter of the dome of expressed lipid in millimeters, using the slit lamp after 5 seconds of digital pressure on the lower lid. However, this evaluation can only measure lipid secretion, which is of a viscosity permitting the formation of a dome. The desired lipid viscosity for standardized force expression, for the entire lower lid, if 6 or more of the ~24 glands yield secretion, there is a low likelihood of dry eye symptoms.

Lipid Quality. There are numerous studies analyzing the various components of meibomian oil, but this is a developing concept, since a defining study for determining precise characterization of an optimal lipid layer has not been published. Similarly, lipid viscosity has not been standardized, although viscosity qualifiers such as thick, toothpaste-like, or globular versus fluid can be useful clinically.

APPENDIX 7

Test Identification: Meibography

Rationale. Meibomian gland tissue can be visualized by using meibography. As such, gland atrophy can be assessed.

Method and Description

Meibography is a technique for observing and documenting the morphology of meibomian glands in vivo. In the first published report of meibography, white light from an illuminator was applied to the conjunctival side of the everted eyelid, and the images were documented on black-and-white film. In the most basic version, white light from a transilluminator is applied to the cutaneous side of the everted eyelid, which allows observation and documentation of morphologic changes in meibomian glands from the conjunctival side once the lid is everted. The images are documented on black-and-white film, infrared film, a near-infrared CCD video camera, or infrared CCD videocameras. In a recent variation of the technique a near infrared infrared light source is used. In a recent study involving an infrared filter and an infrared CCD videocamera, meibomian glands were observed without a light source applied onto the cutaneous side of the everted eyelid, which made the meibography a patient-friendly examination.

The observable morphologic changes include gland loss and gland shortening, which is quantified using scoring systems. Different authors used different scoring scales as follows.

- Sensitivity/specificity (83.0/90.0)
- Arita et al. scored partial or complete loss of the meibomian glands in the lower eyelid by using the following scale: grade 0 (no loss of meibomian glands), grade 1 (lost area 50% or less than the observed area), and grade 2 (lost area more than 50% of the observed area).
- Plufgenfelder et al. scored partial or complete loss of the meibomian glands in the lower eyelid by using the following scale: grade 0 (no loss of meibomian glands), grade 1 (lost area less than one third of the observed area), grade 2 (lost area between one third and two thirds of the observed area), and grade 3 (lost area more than two thirds of the observed area).

Volume and Quality

Cut-off Aqueous-deficient dry eye ≥5
(Sensitivity/specificity) (85.0/90.0)

APPENDIX 8

Test Identification: In Vivo Laser Scanning Confocal Microscopy

Rationale. Scanning confocal microscopy allows for in vivo microscopy of ocular surface morphology in health and disease.

Method and Description

Confocal microscopy is a novel emerging noninvasive technology that is useful as a supplemental diagnostic tool for the in vivo assessment of the histopathology of many ocular surface diseases and anterior segment disorders associated with dry eye disease, including the in vivo examination of the bulbar and palpebral conjunctiva and the meibomian glands. In
studies related to MGD, in vivo laser confocal microscopy was performed with a new-generation confocal microscope, the Rostock Corneal Software Version 1.2 of the HRTH-RCM (Heidelberg Retina Tomograph II-Rostock Cornea Module; Heidelberg Engineering GmbH, Dossenheim, Germany). Briefly, after the upper or the lower eyelid is everted, the center of the Tomo-Cap containing 2 mg carbomer gel preserved with cetrimide (Comfor Gel; Bausch & Lomb, Berlin, Germany) is applied onto the palpebral conjunctiva, and the meibomian glands are scanned while moving the applanating lens from the lids margins toward the fornix with minute vertical movements. The meibomian glands are also scanned while the applanating lens is moved along the entire lid length with minute horizontal movements. It is recommendable to scan the temporal, central, and horizontal lid with the side camera attachment and to make notes of which sequences belong to which anatomic location in the lid margin, for ease in the later analysis. The examination time for each eyelid takes approximately 5 minutes. To reduce patient discomfort from touch, a drop of topical anesthetic is applied. No patient discomfort or any adverse effect related to this examination has been observed or reported.

In the examination of the MGD, the longest and shortest acinar unit diameter, periglandular inflammatory cell density, and acinar unit density have been recommended and found to be efficient parameters to evaluate the morphologic changes in the meibomian glands. The density of glandular acinar units and inflammatory cell density can be measured with an internal software. Clearly visible acinar units are all counted in a 400 × 400-μm frame, and the acinar density is described as the number of units per square millimeter. The longest and shortest diameters in micrometers can be calculated by using Image J software (developed by Wayne Rasband, National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/index.html). Three randomized, nonoverlapping, high-quality digital images of the nasal, middle, and temporal lower eyelid were analyzed. Individually, each confocal parameter was observed to have acceptable sensitivity and specificity for the diagnosis of MGD, which appears to be an important observation. Further studies looking into the sensitivity and specificity of these parameters for the diagnosis of mild stages of MGD will provide invaluable information. Moreover, the parameters seemed to correlate well with tear stability, vital staining scores, tear evaporation rate, and clinical grading of meibomian gland expressibility and glandular loss.

When the cutoff value of MG acinar unit density (MGAUD) is set at less than 70 units/mm², the area under the curve (AUC) is 0.91, and the sensitivity and specificity of the parameter are 81% and 81%, respectively. The AUC is 1 when the cutoff value of inflammatory cell density is set at less than 300 cells/mm²; the sensitivity and specificity of the examination is 100% and 100%, respectively. The AUCs are 0.95, 0.97 when the cutoff values for MGDL (MG longest diameter), MGSAD (MG shortest diameter) in the diagnosis of MGD are set at less than 65 and 25 μm, respectively. The sensitivity and specificity of these parameters under these cutoff values are 90% and 81% for MGLD and 86% and 96% for MGSD, respectively.

It seems that the combination of acinar unit diameter (MGAUD) with tear stability examination employing 1 μL of 1% fluorescein solution applied with a micropipette or fluorescein staining results in higher specificity without considerable change in sensitivity. Combination of MGAUD with tear stability or fluorescein vital staining examination also shows a higher specificity without considerable changes in sensitivity.

<table>
<thead>
<tr>
<th>Confocal Parameters</th>
<th>Controls (n = 15 eyes)</th>
<th>MGDs (n = 20 eyes)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinar unit density, per mm²</td>
<td>101.3 ± 33.8</td>
<td>47.6 ± 26.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>Acinar unit diameter, μm</td>
<td>41.6 ± 11.9</td>
<td>98.2 ± 53.3</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

In vivo confocal microscopy has been reported to be useful in describing the phenotypic alterations in MGD, such as subepithelial fibrosis, obstruction of meibomian gland orifices, cystic dilatation of the ducts, and lipid/glandular secretory accumulations in the acinar units and the ducts. Inflammatory cell density also seems to serve as a new and promising diagnostic parameter of in vivo confocal microscopy for evaluation of treatment responses in advanced obstructive MGD as well. In another recent study, a few periglandular inflammatory cells were noted in the eyelids of healthy control subjects (20 eyes of 10 subjects; mean age, 66.4 ± 8.9 years; mean inflammatory cell density in in vivo confocal microscopy, 50 ± 30 cells/mm³). The number of inflammatory cells in the eyelids of patients with obstructive MGD before treatment was observed to be approximately 10 to 30 times higher than in those of healthy control subjects. These observations suggest the potential of this novel technology in differentiating inflammatory obstructive MGD from noninflammatory subtypes and the potential for evaluating the outcome of different treatment protocols. The caveat for this parameter is that the current resolution of in vivo confocal microscopy cannot differentiate between inflammatory cell subtypes, except for dendritic cells and polymorphs. In vivo and ex vivo observations made with this new technology have the potential to overcome this disadvantage. In testing the applicability of the aforementioned confocal microscopy-based parameters in the diagnosis of MGD with an expressibility grade ≥2 (Shimazaki grading) and a meibomian gland dropout grade of 2 (Shimazaki: loss of ≥50% of glands along the entire eyelid), the receiver operating characteristic curve technique has recently been used to delineate the sensitivity, specificity, and cutoff value for each parameter. In this study, 20 right eyes of 20 patients with simple MGD (11 women and 9 men; mean age, 65.5 ± 15.5 years; range: 50–99) and 26 right eyes of 26 healthy control subjects (13 women and 13 men; mean age, 53.2 ± 15.7 years; range: 32–78) were analyzed. Individually, each confocal parameter was observed to have acceptable sensitivity and specificity for the diagnosis of MGD, which appears to be an important observation. Further studies looking into the sensitivity and specificity of these parameters for the diagnosis of MGD will provide invaluable information. Moreover, the parameters seemed to correlate well with tear stability, vital staining scores, tear evaporation rate, and clinical grading of meibomian gland expressibility and glandular loss.

**APPENDIX 9**

**Test Identification: Meibometry**

**Rationale.** Casual lid margin oil level can be measured via meibometry.

**Methods and Description**

Meibometry was first reported by Chew et al. in 1993 as a method of indirect assessment of the steady state level of meibomian lipids at the lid margin (the casual level). In this
Correlation between Confocal Microscopy Parameters, Tear Functions, Meibomian Gland Status

<table>
<thead>
<tr>
<th>BUT</th>
<th>FS</th>
<th>RB</th>
<th>MG</th>
<th>TEROS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammatory cell density</strong></td>
<td>-0.55‡</td>
<td>0.52‡</td>
<td>0.48‡</td>
<td>0.79‡</td>
</tr>
<tr>
<td><strong>MG acinar unit density</strong></td>
<td>0.55‡</td>
<td>-0.50‡</td>
<td>-0.46‡</td>
<td>-0.70‡</td>
</tr>
<tr>
<td><strong>MG acinar shortest diameter</strong></td>
<td>-0.40‡</td>
<td>0.45‡</td>
<td>0.37*</td>
<td>0.75‡</td>
</tr>
<tr>
<td><strong>MG acinar longest diameter</strong></td>
<td>-0.34*</td>
<td>0.47‡</td>
<td>0.30*</td>
<td>0.61‡</td>
</tr>
</tbody>
</table>


BUT = break-up time; FS = fluorescein staining; RB = Rose Bengal staining; TEROS = tear evaporation rate measurements from the ocular surface.

Spearman’s correlation coefficient by rank test.
* $P < 0.05$, considered significant.
† $P < 0.005$, considered very significant.
‡ $P < 0.0001$, considered extremely significant.

examination, the meibomian lipids are blotted onto a loop of plastic tape from the central third of the lower lid margin, and the amount of lipids taken up is measured optically or scanned and measured by a computer equipped with commercially available densitometric software. In the first reports, it was shown that the casual lipid level at the lower lid increases with age, yet is lower in women in their 20s through 60s, and the lipid level is evaluated as highest in the first hour after waking, but settles to a constant level throughout the remainder of the day. In the original meibometry method, optical density was read with a clinical meibometer (MB 550; Courage & Khazaka Electronic GmbH, Cologne, Germany) that obtained a point reading at the center of the blot. After that, Yokoi et al. reported another method in which the sampled lipid is scanned and the increase in transparency is integrated over the length of the blot. A few years later, Komuro et al. reported an originally developed meibometer that included a laser device comprised of a laser diode (690 nm) and photodetection units (window size: 2.5 × 5 mm), and an ultrasonography probe was used as the mounting area for the plastic tape.

The latter studies found that the casual lipid level of meibomian gland dysfunction (MGD) is significantly lower than that in aqueous-deficient dry eyes and normal eyes. The present limitation of meibometry is that in normal subjects, the lipid blot is uniform, and results can be extrapolated to the total lid margin. However, in cases of MGD, focal gland obstruction may vary along the lid length, so that central readings may not truly reflect the overall picture. In future studies, calibrations are needed to convert densitometry readings into equivalent values for the sampled meibomian lipid. Furthermore, development of a system to integrate along the full length of the lid would be ideal, and cutoff values for the diagnosis of MGD are needed. A detailed explanation of the examination method is detailed in the following text.

In the standard technique of meibometry, a preformed loop of meibometry tape (8 mm wide) is placed in the reading head of the meibometer, to establish the 0 reading. The loop is formed by heat-sealing the tape at a predetermined point to give a loop length of 20 mm. The handle is clipped to the prism housing of a Goldmann applanation tonometer or an ultrasonography probe holder mounted on the slit lamp biomicroscope. This arrangement permits controlled placement of the probe on the lid margin under direct vision. The tonometer or ultrasonography probe is set at 0 for each impression. With the subject looking upward without blinking, the lower lid is gently inverted (stretching should be avoided, as it might express oil), and the loop is then pressed onto the central third of the lid margin with sufficient pressure to obtain an imprint across the entire width of the tape, yet without bending the handle of the loop. A line of contact is seen across the full width of the tape, and contact is maintained for 3 seconds. After the blot is obtained, the tape is kept in the air for 3 minutes, to allow evaporation of any tears picked up from the lid. The loop is placed in the reading head of the clinical meibometer, and a reading is taken in the standard way. The casual lipid level (expressed as arbitrary optical density units) is calculated as \((C - B)/A\), where \(A\) is the reading without tape, \(B\) is the reading before blotting, and \(C\) is the reading after blotting.

**APPENDIX 10**

**Test Identification: Interferometry**

**Rationale.** Interferometry utilizes optical principles to visualize the tear film lipid layer, which consists of the lipid secreted from the meibomian glands. At the time of eye opening, this lipid layer is repeatedly spread, by blinking, over the aqueous layer of the tear film. The layer is very thin, and thus the light reflected from the surface and back of the lipid layer produces interference images that can be observed as specular images.

**Method and Description**

Interferometers are instruments that allow visualization and analysis of the interference image from the lipid layer. Several types of interferometers have been developed to see the lipid layer. Among them, the DR-1 (Kowa) has successfully been able to give quantitative analysis. Even before the development of interferometers, the spreading of the lipid layer over the aqueous layer had been observed. Interferometers provided a clearer image, and thus a difference was noticed in the interference patterns between normal subjects and dry eyes. Based on these observed differences, grade classifications were made for precorneal tear film and precontact lens tear film. For example, for the DR-1 there are five grades ranging from normal (grade 1 or 2) to dry eye (grades 2 to 5, with a grade of 5 being the most severe). Despite the limitation of this classification system where grade 2 may be classified either as normal or dry eye, the noninvasive nature of this test makes it a valuable tool for screening dry eye and assessing severity. To determine the condition of the lipid layer, one may measure either the thickness or the spreading rate of the lipid layer over the ocular surface. Early methods of measuring the thickness of the lipid layer did not give a precise value for the thickness, since they relied on Newton’s color scale which provides a relatively rough and semiquantitative value. However, over time, several advances have been made in the ability to determine the condition of the lipid layer by quantitative values. The most sophisticated of these was developed by a colorimetric approach, in which a new tear interference color chart was developed to describe the thickness of the tear film lipid layer.

A different approach, aimed at assessing kinetically the rate of spread of the lipid layer over the ocular surface, is based on two methodologies: One is measuring the spreading time of the lipid layer (the time required to reach a stable lipid film after opening the eye). In a study conducted to evaluate tear lipid spread time and pattern, the lipid spread was found to be horizontal in healthy eyes but vertical in lipid tear deficiency (LTD); lipid spread time is greater in normal subjects than in those with LTD. The second methodology measures the lipid layer spread more directly by using the rheological model, and it was noticed that the rate of spreading of the lipid layer depends on the volume of the aqueous layer. Based on this method, one can assess the precorneal aqueous tear volume by measuring the spread rate of the lipid layer, and normal tear volume may be given to the higher rate of spreading. Even though these approaches are still under development, the techniques have promise in many clinical applications in the diagnosis and/or quantitative grading of the severity of dry eye.
Appendix 11

Test Identification: Evaporimetry

Rationale. Evaporimetry measures tear evaporation from the ocular surface. The evaporation is very effectively reduced by the lipid barrier of the tear film. In conditions of 52% humidity at temperatures of 22°C, the evaporation of water from an open bath is $100 \times 10^{-5} \text{g/cm}^2/\text{s}$. When measured under these conditions, the lipids of the ocular tear film reduce their evaporation by approximately 80% to 90% in the normal eye. Mishima and Maurice in 1961 were the first to establish that the lipid layer retarded evaporation in an animal model of the rabbit eye. Iwata et al. developed another in vitro rabbit model with a cornea covered with a chamber through which dry air was passed; from the weight of water collected, they determined the evaporative rate to be $10.1 \times 10^{-7} \text{g/cm}^2/\text{s}$. They found that a fourfold increase in evaporation occurred with the removal of the rabbit’s tear film lipid layer. A similar proportional increase in human tear film evaporation was measured by Craig and Tomlinson in patients with an incomplete or absent lipid layer, a situation commonly found in MGD. Tear film evaporation depends on a variety of parameters, including ambient air flow and interaction of the numerous components in the tear film, including the lipid layer.

Evaporation Rate Derived from Capture of Fluid Loss from the Ocular Surface

Evaporation of fluid from the ocular tear film has been measured by numerous investigators since the first report in 1980 by Hamano et al. and a range of different techniques have been used. Hamano et al. determined the evaporation from the corneal area enclosed in a capsule by a pressure gradient technique, Cedarstaff et al. measured the increase in electrical resistance of air passed over the eye with an increase in humidity measured with resistance hygrometer. Subsequently, they adopted the vapor pressure gradient technique, calculating relative humidity and temperature at two points above an evaporative surface. Others have measured the increases in humidity of the air in a sealed goggle over time. Recently a continuous recording device measured changes in the humidity of the air stream passing over the eye, by microbalance technology.

Tear film evaporation rate has been reported in different units by various researchers; most use units of $10^{-7} \text{g/cm}^2/\text{s}$, but others report values in grams per square meter per hour (g/m$^2$/h). This difference may be resolved and the values rendered to the same units by various researchers; most use units of $10^{-7} \text{g/cm}^2/\text{s}$.

Evaporation rates recorded by the measurement of fluid loss from the ocular surface for normal and dry eyes have been reported in the literature over the past 30 years by King-Smith et al. The rate is also reported in units of microliters per minute by some researchers. The evaporation rate in microliters per minute is numerically equal to a hundredth of the value of the evaporation rate stated in units of $10^{-7} \text{g/cm}^2/\text{s}$, when the area of the evaporating ocular surface is 167 mm$^2$. The use of different techniques for measurement of tear film evaporation makes it difficult to compare evaporative findings in normal and dry eyes among different studies, because the absolute values recorded are technique dependent. However, there is a pattern to the observations reported in the literature, with significant increases from normal tear film evaporation seen in patients with both aqueous-deficient dry eye and MGD and evaporative dry eye (McCann LC, et al. IOVS 2008;49:ARVO E-Abstract 1542). Strictly, these comparative differences within individual studies are of diagnostic significance only where values in normal and dry eyes are recorded by the same technique in the same laboratory. However such evaluations as meta-analysis of evaporative rate are very effectively reduced by the lipid barrier of the tear film. In conditions of 52% humidity at temperatures of 22°C, the evaporation of water from an open bath is $100 \times 10^{-5} \text{g/cm}^2/\text{s}$. When measured under these conditions, the lipids of the ocular tear film reduce their evaporation by approximately 80% to 90% in the normal eye. Mishima and Maurice in 1961 were the first to establish that the lipid layer retarded evaporation in an animal model of the rabbit eye. Iwata et al. developed another in vitro rabbit model with a cornea covered with a chamber through which dry air was passed; from the weight of water collected, they determined the evaporative rate to be $10.1 \times 10^{-7} \text{g/cm}^2/\text{s}$. They found that a fourfold increase in evaporation occurred with the removal of the rabbit’s tear film lipid layer. A similar proportional increase in human tear film evaporation was measured by Craig and Tomlinson in patients with an incomplete or absent lipid layer, a situation commonly found in MGD. Tear film evaporation depends on a variety of parameters, including ambient air flow and interaction of the numerous components in the tear film, including the lipid layer.

Evaporation Rate Derived from Measures of Tear Film Thinning

A new paradigm has recently been introduced into the field of measurement of human tear film evaporation by King-Smith et al. who infer rates of evaporation from observations of tear film thinning. The notion of the actual fluid loss from the tear film has been thrown into confusion by their recent suggestion that the primary thinning of the tear film observed by their imaging interferometer is due to evaporation. The values for evaporation inferred from tear thinning are of a different order, a factor of approximately four to five times that reported in studies of direct measures of the capture of fluid loss from the ocular surface.
tion in this meta-analysis, the values obtained from tear thinning measures are not included.

<table>
<thead>
<tr>
<th>Cutoff (Sensitivity/specificity)</th>
<th>DE &lt; 22</th>
<th>EDE &gt; 22.3</th>
<th>EDE &gt; 27.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>(51.1/89.9)</td>
<td>(61.2/90.6)</td>
<td>(45.5/79.8)</td>
<td></td>
</tr>
</tbody>
</table>

**APPENDIX 12**

**Test Identification: Tear Lipid Composition and the Diagnosis of MGD**

**Rationale.** Considerable work has been undertaken in investigating the composition of tear lipids. The research has been hindered by the small sample size, difficulty of collection, the danger of contamination with skin lipids and cosmetics, storage problems, and intrinsic complexity of the mixture of the lipids. The analytical techniques used therefore reflect this complexity. A review of the analysis and composition of human tear lipids in health and disease will be dealt with in detail elsewhere in this MGD report.

**Methods and Description**

Within the current state of knowledge several impediments hinder the adoption of lipid composition as part of the clinical diagnosis of MGD. As yet, no uniform method of sample collection has been adopted. Collection techniques have included meibum from forced expression collected with capillaries or spatula (Butovich IA, et al. *IOVS* 2007;48:ARVO E-Abstract 441), lipid extracted from whole tears extracted from Schirmer strips. Once collection is achieved, analysis of the lipids involves complex multistep analytical techniques such as FTIR, NMR, GC-MS, TLC-GC-MS, and HPLC-MS. Mass spectroscopy often involves exotic ionization techniques such as MALDI, ESI, and API. The latest techniques make use of multistage fragmentation of ions (MS^n), which does allow for the separation and identification of ions, but the development of a commercial instrument and analysis software helped standardize the procedure (i.e., the Fluorotron Master; Coherent Radiation Inc., Buffalo, NY). The decay of fluorescein concentration in the tear film is measured by these techniques over a period of 30 minutes after instillation of 1 μL of 2% fluorescein sodium into the lower fornix with a measuring pipette, with scans being performed every 2 minutes. The change in rate of decay of fluorescence is then calculated for the total measurement period, and a biphasic decay in fluorescence is observed. The measurements for the first 5 minutes show a rapid decay, thought to be due to the initial reflex tearing produced by the instillation of the fluorescein drop. The later part of the curve (from 5 minutes outward) represents the measurement of tear turnover under basal conditions of secretion. It is this part of the curve that is fitted using appropriate software, and the decay in fluorescence is calculated from the log of the curve obtained from the following formula, to obtain the basal tear turnover rate:

\[
T0i(t) = \frac{100(C_i(t) - C_i(t + 1))}{C_i(t) - C_i(0)} \times V_b \times (\text{ng/mL})
\]

where \(C_i(t)\) is the fluorescein concentration in tear film at time \(t\) (min).

Assuming a monophasic decay of fluorescence from 5 minutes after instillation with a decay time constant \(b\) (min^-1):

\[
C_i(t) = C_i(0) \cdot \text{ebt} \times (\text{ng/mL})
\]

the following is obtained:

\[
Tb(t) = 100 \times (1 - \text{ebt}) \times (\% / \text{min})
\]

This calculation gives a measurement of the tear turnover recorded in percentage per minute (%/min). To express the turnover value in terms of microliters per minute (sometimes called flow), it is necessary to either assume a value for the tear volume (typically 7 μL) or to measure the volume from the initial dilution of the instilled sodium fluorescein in the tears. Initial dilution is calculated by back extrapolation to time 0 of the initial fluorescence decay. In this technique, it is the monophasic decay of fluorescence in the first 5 minutes after instillation of the fluorescein that is determined.

Tear volume is derived from the formula:

\[
V_i = (C_i - C_m - 1 \cdot k - 1 \cdot V_d)
\]

where \(C_i\) is the fluorescein concentration in the drop, and \(C_m\) is the initial fluorescein concentration calculated by back extrapolation with the Fluorotron in nanograms per milliliter.

The turnover in microliters per minute is then calculated from the product of tear turnover in percent per minute and tear volume. Values have been reported for tear turnover (%/min) and tear flow (μL/min) in major studies in the literature for normal and dry eye subjects, obtained with a commercial fluorophotometer. The data reported for normal subjects in most studies ranges from 10% to 20%/min, which equates to a tear flow rate of just over 1 μL/min. In contrast, Mathers et al. found normal tear turnover on the order of 7%/min or 0.19 μL/min, values not dissimilar to those found
in dry eyes. It is possible that the values of TTR published by Mathers are in error, and later reports by this group suggest a difference in their calculations, producing higher values in the range of 0.34 to 0.49 µL/min.\(^{385}\) TTR in normal subjects averages 1.03 ± 0.39 µL/min (16.1% ± 5.10%/min) when Mathers’ values are excluded and for the dry eye in all its forms, the average is 0.54 ± 0.28 µL/min (9.26% ± 5.08%/min). In those cases subtyped as aqueous-deficiency dry eye, the mean TTR is 0.40 ± 0.1 µL/min (7.71% ± 1.02%/min) and in evaporative dry eye, the mean is 0.71 ± 0.25 µL/min (11.95 ± 4.25%/min). These results suggest that all dry eyes show a reduced production facility (TTR) relative to the normal by approximately 60% in aqueous-deficient dry eye and by 30% in evaporative dry eye. Diagnostic cutoffs for TTR offer promising sensitivity and specificity when normal eyes were compared with all dry eyes and with each of the dry eye subtypes in a recent meta-analysis.\(^{386}\) TTR has potential in differentiating both evaporative dry eye (resulting from MGD) from the normal, as well as from aqueous-deficient dry eye, being a sensitive measure in the former case and highly specific in the latter (i.e.: efficient in classifying evaporative dry eye).

### APPENDIX 14

#### Tear Volume by Fluorophotometry: Reported Values

Measurement of tear volume by the fluorometric technique has yielded little difference between the volume in normal or dry eyes (including MGD).\(^{378,386}\) This outcome is unlike the situation with measurement by meniscometry where differences have been found. Scherz et al.\(^{383}\) have also found correlations between tear meniscus height (TMH) and volume by fluorophotometry. Volume measures by fluorophotometry were not found to correlate with PRT by Tomlinson et al.\(^{386}\)

### Test Identification: Meniscometry

**Rationale.** Meniscometry provides a measure of tear meniscus height, radius, and volume.

**Methods and Description.**

There are many ways to evaluate tear meniscus parameters, such as measuring the height, radius, width, and cross-sectional area, because 75% to 90% of total tear volume of the ocular surface is estimated to be kept in the tear meniscus.\(^{387}\) Among those parameters, measurement of tear meniscus height is the most popular assessment method, and there have been numerous reports that have attempted to use meniscus height in the diagnosis of tear deficiency. However, those previous methods require fluorescein instillation to obtain clear visualization, and this may induce reflex tearing due to some invasiveness. Yet in a report on slit-image photography\(^{388}\) that compared tear meniscus parameters, including height, radius, width, and cross-sectional area, the height and radius of the meniscus were found to be the best parameters for the diagnosis of dry eye.\(^{389}\) That method did employ fluorescein instillation, however, thus introducing some invasiveness that may cause reflex tearing and may add some aqueous to the original tear volume. Based on that background, meniscometry was developed\(^{389,390}\). Today, there are two systems of meniscometry; one based on photography\(^{390}\) and one based on the use of video.\(^{391}\) In a newly developed video-meniscometer, a rotatable projection system with a target comprising a series of black and white stripes (four black and five white; each 4 mm wide) was introduced coaxially, using a half-silvered mirror. The coaxial alignment of the video-meniscometer permits the meniscus of either eye to be readily accessed and allows for real-time recording of meniscus behavior corresponding to a 1.1 × 1.5 mm rectangular area of the meniscus. For the purpose of calculating the radius of tear meniscus curvature, a selected meniscus image recorded on a digital video recorder is captured on the computer, and analyzing software is applied for the calculation of the radius according to the concave mirror formula:

$$R = \frac{2W}{I/T}$$

where $R$ is the radius of the tear meniscus, $W$ is the working distance, $I$ is the image size, and $T$ is the target size.

Using meniscometry, the $R$ values in normal eyes were calculated as 0.365 ± 0.153 mm ($n = 36$) by the photographic system. However, probably due to some invasiveness of the photographic system as it sought the image in a dim light, the calculated $R$ values were larger than those obtained by the video system\(^{391}\) (0.30 ± 0.10 mm, $n = 36$), but smaller than those obtained by slit-image photography (0.55 ± 0.26, $n = 15$).\(^{388}\) Those differences are due to the effect of reflex tearing or the instillation of fluorescein into the aqueous. In a recent advancement in optical coherence tomography (OCT), the $R$ values are reportedly the smallest yet obtained (0.239 ± 0.112 mm $n = 40$).\(^{393}\) It has also been reported that those normal $R$ values were smaller than those in dry eyes (0.17 ± 0.05 mm, $n = 38$; 0.22 ± 0.09 mm, $n = 29$).

Through research using a video-meniscometer, the radius of the tear meniscus at the central lower lid margin of the left eye was measured in 36 healthy volunteers, 38 dry eye subjects (diagnoses based on the Japanese dry eye criteria), and seven dry eye patients with punctal plugs in both upper and lower puncta. Among those groups, the respective tear meniscus radii ($R$, in millimeters) were compared. The results showed a significantly smaller meniscus in dry eyes ($R = 0.17 ± 0.05$ [SD]) compared with that in normal eyes (0.30 ± 0.11 mm; $P < 0.0001$), whereas a significantly larger meniscus was found in dry eye patients with punctal plugs (0.57 ± 0.23) than in normal eyes ($P < 0.0001$) or dry eyes ($P < 0.0001$).\(^{395}\) Fourteen subjects from the normal group and 31 patients from the dry eye group had undergone the Schirmer I test, so the correlation between the radii and values of the Schirmer I test was investigated in those groups. It was found that there was an excellent agreement between the radius of tear meniscus and the Schirmer I test. If normal is determined by the fact that both the tear and ocular surface examinations are normal and the cutoff value of the radius is determined as 0.25 mm, then the sensitivity and specificity for the radius were calculated as 88.9% and 77.8%, respectively, which is compatible with the measurement of meniscus height.\(^{395}\)

Considering that the radius measurement obtained by meniscometry is noninvasive and that there is a significantly good correlation between $R$ and total tear volume over the ocular surface,\(^{396}\) the tear meniscus may be the expectable parameter for the screening of tear deficiency. For other applications, the video-meniscometer enables real-time monitoring of tear volume and also allows tear turnover to be evaluated after the instillation of eye drops, where not only the turnover of tear substitute at the ocular surface is evaluated but also the efficacy of drainage of the lacrimal pathway.
### Test Identification: Osmolarity

**Rationale.** Tear film osmolarity indicates the balance of inputs and outputs of the lacrimal system.

**Methods and Description**

The osmolarity of a sample can be determined in several ways, both in situ and by sampling, using methods that measure the colligative properties of the tears. These properties, such as freezing-point depression and vapor pressure, depend on the number of dissolved particles in a solution but are not dependent on the identity of the particles. The freezing point depression nanoliter osmometer is at present the most commonly applied principle in osmolarity measurement.397,398 In this method, the temperature of the freezing point is directly proportional to the total number of dissolved particles in the solution. Therefore, the osmolarity can be calculated from the depression in the freezing point. The most frequently applied freezing point depression techniques in tear research use nanolitre samples,398–402 most commonly with the Clifton Nanolitre Osmometer (Clifton Technical Physics, Hartford, NY).400 Although used in the diagnosis of dry eye disease, this method requires significant expertise, takes considerable time, and is open to error due to evaporation of test samples.401 Other techniques using the freezing-point depression technique such as the Advanced Tear Osmometer (Advanced Instruments, Inc., Norwood, MA) and the Otago Osmometer (Otago Osmometers Ltd, Dunedin, New Zealand) are also available.

Vapor pressure techniques have also been used in the measurement of osmolarity.403 These work on the principle that the vapor pressure of a solution is lower than that of the pure solvent at the same temperature and pressure; the decrease in vapor pressure, like depression of freezing point is proportional to the number of dissolved particles in the solution. Thus, the osmolarity of a solution can be calculated from its vapor pressure. Original vapor pressure osmometers engaged a precision thermocouple hygrometer to measure dew point depression and required large sample volumes.402 This necessitated the collection of reflex tears which in turn could lower the osmolarity values obtained.403 More recently, vapor pressure osmometers, such as the Wescor (Wescor, Inc., Logan UT) have been used. However, although easier to operate and more streamlined than freezing-point depression osmometers, they are still not suitable for the quick, easy application required in clinical practice.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Cutoff, mm (Sensitivity/specificity)</th>
<th>FARRELL ET AL.595</th>
<th>OCUSENSE INC.400</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DE &lt; 0.25 (74.5/73.6)</td>
<td></td>
</tr>
</tbody>
</table>

There is a need for a new instrument to facilitate clinical application and the adoption of osmolarity as a diagnostic test in dry eye disease. Recently the OcuSense system (OcuSense Inc., San Diego, CA) has been developed.404,405 This new osmometer is based on electrical impedance and "laboratory-on-a-chip" technology, which allows the calculation of osmolarity. This technique allows osmolarity testing of a very small volume (less than 20 nL), is a quick and accurate measurement of the osmolarity of the tear film in a clinical setting, and reduces the evaporation of the fluid. However, although the device measures charged particles, corrections or assumptions are made with regard to the contribution made by noncharged particles in the tear sample. The OcuSense system has recently been approved as a medical device by the U.S. Food and Drug Administration. A recent study compared the new OcuSense osmometer with the Clifton Osmometer, to determine the comparability of results between the instruments. Osmolarity values for controls and dry eye were 308 ± 6 mOsm/L and 321 ± 16 mOsm/L, respectively (OcuSense) and 250 ± 7 mOsm/L and 235 ± 14 mOsm/L respectively (Clifton); the difference was significant. Significant correlation was found between OcuSense and Clifton measurements (r = 0.904; P = 0.006). Bland-Altman analysis revealed agreement between techniques; most of the points fell within the 95% confidence limits, and actual values differed by less than 1%.406

A previous meta-analysis was performed on published data for tear osmolarity in samples of normal subjects and various subtypes of dry eye and pooled estimates of the mean and standard deviations for normal and (all) dry eye subjects were determined.407 A diagnostic referent (cutoff) value was derived and tested for effectiveness of diagnosis on independent groups of normal and dry eye subjects. A referent value of 315.6 mOsm/L was derived from the intercept of the distribution curves, and 316 mOsm/L from the ROC curve. When applied to independent groups of normal and (all) dry eye subjects, a value of 316 mOsm/L was found to yield sensitivity of 59%, specificity of 94%, and overall predictive accuracy of 89% for the diagnosis of dry eye syndrome. Tear hyperosmolarity, defined by a referent value of 316 mOsm/L, was superior in overall accuracy to any other single test for dry eye diagnosis.

Osmolarity is used in differentiating evaporative dry eye from the normal, but is of limited ability in assigning the subtypes into categories of aqueous-deficient dry eye and evaporative dry eye; this outcome is not unexpected when osmolarity in the subtypes is reported as 330.01 ± 13.34 and 325.57 ± 14.76, respectively, and 308.39 ± 9.29 in the normal in a recent study.408 Utilization of easy-to-operate instrumentation with high levels of sensitivity alone or in addition to dry eye clinical testing will continue to provide valuable information about the fundamental underpinnings of osmolarity as it relates to ocular surface disease.409

### APPENDIX 16

#### Test Identification: Indices of Tear Film Dynamics

**Rationale.** It would be useful in the study of dry eye to be able to describe and quantify tear film dynamics—the balance of inputs and outputs of the lacrimal system (the combination

<table>
<thead>
<tr>
<th>Technique</th>
<th>Cutoff, mOsm/L (Sensitivity/specificity)</th>
<th>DE &gt; 315 (73%/72%)</th>
<th>(60%/59%)</th>
<th>ADDE &gt; 325</th>
</tr>
</thead>
<tbody>
<tr>
<td>N vs. DE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N vs. EDE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADDE vs. EDE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
of production and evaporative loss) by a single index that describes the balance of input and output of the system.

Methods and Description

Tear Function Index. An early index for tear film dynamics was the Tear Function Index (TFI) devised by Xu et al. This index combined values obtained for tear secretion (from the Schirmer test with anesthesia) with measurements for drainage (turnover as measured by the fluorescein clearance test) in the following formula:

\[
\text{TFI} = \frac{\text{Schirmer value with anesthesia}}{\text{Tear clearance rate}}
\]

This index includes measures of two of the three main factors that determine tear dynamics, secretion and drainage. It has been argued that tear secretion is the most important determinant of tear dynamics, but, as it could not be measured independently and directly, the Schirmer test result had to represent the production component of dynamics. The ability of the TFI to discriminate between normal and dry eye patients was found to be considerably better than the Schirmer test or the tear clearance rate values alone. A value of the log to the base 2 of the TFI below 96 gave a sensitivity and specificity in the diagnosis of dry eye of 67.4% and 60%, respectively. A value for TFI below 34, gave sensitivity and specificity calculations for Sjögren syndrome of 78.9% and 91.8%. The major deficiency of the TFI as an index of tear dynamics is that it fails to take into account the elimination of tear fluid from the eye through evaporation. Evaporation is a key variable in differentiating some groups of dry eye. A recent study determined the effectiveness in dry eye diagnosis of another Tear Function Index (the Liverpool modification of the TFI) test. This report showed high sensitivity at 83% in the diagnosis of all dry eye from the normal but poor specificity (40%).

Total Tear Flow

Ideally, any index of tear dynamics should define the imbalance that leads to the condition of dry eye. Under basal conditions, the majority of the input, and output, of the lacrimal system can be determined through measurement of tear turnover and fluid loss by evaporation. Mathers has suggested “total tear flow” as an index that captures the principal sources of elimination of tear fluid from the eye. As the drainage facility is not necessarily affected in dry eye states, the tear flow is determined from tear turnover rate (effectively a measure of drainage) and, combined with evaporation, it gives an estimation of the tear production facility of the eye. Therefore, dry eye may result when tear flow (turnover) is reduced due to a deficiency of tear production deficiency (aqueous-deficient dry eye) or a high level of evaporation occurs (evaporative dry eye).

<table>
<thead>
<tr>
<th>N vs. DE</th>
<th>N vs. DE</th>
<th>N vs. EDE</th>
<th>ADDE vs. EDE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFI Cutoff</td>
<td>DE &lt; 96</td>
<td>DE &lt; 240</td>
<td>EDE, NA</td>
</tr>
<tr>
<td>(Sensitivity/specificity)</td>
<td>(64.7/60)</td>
<td>(83%/40%)</td>
<td>(NA)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ratio of Evaporation/Total Tear Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutoff</td>
</tr>
<tr>
<td>(Sensitivity/specificty)</td>
</tr>
</tbody>
</table>

Evaporation and Tear Turnover

A similar but simpler index is derived from the proportion of the production, measured by tear turnover rate (TTR), lost through evaporation. For these analyses, TTR can be thought of as a measure of production, although TTR may also be considered a measure of drainage. A meta-analysis (with and without Mathers’ values for TTR) show that for this simple ratio, close to one eighth (13.6%) of the tear production (TTR) is lost through evaporation in the normal eye. In (all) dry eye, the level of evaporative loss rises to 38.9%. In the subtypes of dry eye, the evaporative loss for aqueous-deficient dry eye is 39.5% of TTR and for evaporative dry eye, the loss is similar, at 36.6% of the TTR.

The use of indices derived from evaporation and TTR measures, whether combined into “total tear flow” as a denominator or using a single turnover measure for production, makes little difference in the ability to distinguish dry eye states from the normal. In fact, the difference with the simpler index of evaporation/TTR is slightly larger than for evaporation/total tear flow index; 2.7 to 2.9 times compared with 2.2 to 2.4×. Both indices show that dry eye, in all its forms, has a greater loss of fluid by evaporation, compared with its production, than occurs in the normal eye. The dynamic imbalance of loss to production is slightly greater in aqueous-deficient dry eye than in evaporative dry eye, even though the eye with evaporative dry eye has a greater loss through evaporation. The combined, though moderate, increase in evaporation in the...
eye with aqueous-deficient dry eye, and the poorer production of aqueous tear is the primary cause of the greater imbalance in the eye with aqueous-deficient dry eye. It appears that a change in the balance between input (TTR) and output (evaporation) by a factor of more than two or three times (dependent on index) leads to dry eye. So that the output:input percentage changes from just over 12% in the normal to greater than 25% in dry eye states (26.8% in evaporative dry eye and 28.3% in aqueous-deficient dry eye with the total tear flow index; or 36.6% in evaporative dry eye and 39.5% in aqueous-deficient dry eye from the simple index).

The above analysis is based on considering one input to the tear film from the lacrimal gland, and two outputs: drainage and evaporation. Levin and Verkman have emphasized the importance of a second input—osmotic flow from the conjunctiva and cornea into the hyperosmolar tears. This osmotic flow helps to reduce the osmolarity increase caused by evaporation and could help to explain why the high evaporation rates proposed by King-Smith et al. may not necessarily lead to unreasonably high osmolarity.

APPENDIX 17

The following are the diagnostic criteria for obstructive MGD proposed by the Japanese MGD Working Group.

Obstructive Meibomian gland dysfunction is considered to be present when all of the following three signs/findings are present:

1. Chronic ocular discomfort.
2. Anatomic abnormalities around the meibomian gland orifices (presence of one or more of the following is positive).
   a. Vascular engorgement.
   b. Anterior or posterior displacement of the MCJ.
   c. Irregularity of the lid margin.
3. Obstruction of the meibomian glands (presence of both is considered positive).
   a. Obstructive findings of the gland orifices by slit lamp biomicroscopy (pouting, plugging, or ridge).
   b. Decreased meibum expression by moderate digital pressure.

These diagnostic criteria for obstructive MGD were published in Atarashii Ganka (Journal of the Eye) 2010;27:627–631, and are reprinted in this appendix with the permission of the publisher (Medical-Aoi Publications Inc.).

The Japanese MGD Working Group consists of the following members:

Shiro Amano, Reiko Arita (University of Tokyo), Shigeru Kinoshita, Norihko Yokoi, Chie Sotozono, Aoi Komuro, Tomo Suzuki (Kyoto Prefectural University of Medicine), Jun Shimazaki, Seika Den (Tokyo Dental College), Kohji Nishida, Naoyuki Maeda, Shizuka Ko (Osaka University), Yukichi Hori (Toho University), Hisayo Kubota (Tohoku University), Eiki Goto (Tsurumi University), Masahiko Yamaguchi (Ehime University), Hiroto Ohata (Jichi Medical University), Masakazu Yamada (Tokyo Medical Center), Dogru Murat, Yoko Ogawa, Yukihiro Matsumoto, Kazuo Tsubota (Keio University).

References


The goals of the subcommittee were to review the current practice and published evidence of medical and surgical treatment options for meibomian gland dysfunction (MGD) and to identify areas with conflicting, or lack of, evidence, observations, concepts, or even mechanisms where further research is required. To achieve these goals, a comprehensive review of clinical textbooks and the scientific literature was performed and the quality of published evidence graded according to an agreed on standard, using objective criteria for clinical and basic research studies adapted from the American Academy of Ophthalmology Practice Guidelines1 (Table 1). It should be noted that, in many of the clinical textbooks and previous reports, terminology is often interchanged and the management of anterior and posterior blepharitis and/or meibomitis is often considered concurrently. Thus, a broad scope of documents was reviewed in this process. Consistency in terminology and global adoption of the term “meibomian gland dysfunction” would significantly aid clinical research and clinical care in MGD going forward.

**Current Practice Patterns**

Although there is general agreement among the recommendations of major clinical handbooks concerning the management of MGD, there are significant differences in practice patterns across the world, in part because of the availability of therapeutics as well as the clinical manuals that are commonly used. Specifically, *The Moorfields Manual of Ophthalmology*2 and *The Wills Eye Manual*3 (Table 2) recommend:

- warm compresses and lid massage up to four times per day for 15 minutes,
- adjunctive use of lubricants in cases of additional dry eye disease,
- topical antibiotic ointments for moderate to severe cases, and
- systemic tetracycline derivatives (e.g., tetracycline 250 mg four times per day or doxycycline 100 mg two times per day) for 6 weeks to several months in recurrent cases, and/or
- to consider topical steroids in severe cases for a short term and incision and curettage with optional steroid injection in chalazion.

Both manuals in reference to the management of blepharitis and meibomitis also recommend cleansing the lid margins with mild (baby) shampoo and cotton buds and suggest to advise patients about the chronic nature of the condition with no known cure.

Recently Lemp and Nichols4 published a perspective on the management of blepharitis that was based on a survey of 120 ophthalmologists and 84 optometrists attending an informational seminar sponsored by an ophthalmic pharmaceutical manufacturer. Respondents reported their clinical perception that 69% of blepharitis patient visits result in some form of treatment, with approximately half of this group receiving prescription-based therapy. Treatment goals for anterior and posterior blepharitis varied slightly between ophthalmologists and optometrists with the latter stressing the importance of reducing symptoms and a high safety profile of the prescribed medication, whereas ophthalmologists emphasized the importance of reducing the bacterial load in anterior blepharitis and improving meibomian gland function in posterior blepharitis. These goals are, of course, not incompatible.

**Current MGD Treatment Practice Patterns**

Overall, treatment of MGD varies greatly among eye care providers on different continents. Underreporting makes it difficult to assess practice patterns accurately, but most practitioners agree that underdiagnosis is common and clinical follow-up irregular. Recommendations for the performance of lid warming and lid hygiene are commonly made, but the precise technique varies greatly, both in duration and frequency of lid warming and cleansing.2,3 Practitioners have noted widespread deficiencies in both the patient education provided to differentiate aqueous-deficient dry eye and evaporative dry eye, and perhaps more important, MGD; patients’ comprehension of these nuances, even when provided, are varied. Likewise, practitioners on all continents note that patients commonly develop their own methods of performing lid hygiene, regardless of instruction. As a result, suboptimal and...
outside of North America. Cyclosporine itself is also not widely available commercially with MGD combined with aqueous-deficient dry eye, although reporting the use of topical cyclosporine in patient groups been few outside the United States. There have been studies studies of its efficacy in treatment of MGD or blepharitis have exacerbated cases or for anterior blepharitis, although, because of confusion in clinical differentiation of anterior and posterior blepharitis, use patterns are difficult to assess. Topical azithromycin, a macrolide antibiotic with presumed anti-inflammatory antibiotic–steroid combination. It should be noted that antibiotic–steroid combinations are used clinically for acute exacerbated cases or for anterior blepharitis, although, because of confusion in clinical differentiation of anterior and posterior blepharitis, use patterns are difficult to assess. Topical azithromycin, a macrolide antibiotic with presumed anti-inflammatory effects, is available in some but not all countries. Further, studies of its efficacy in treatment of MGD or blepharitis have been few outside the United States. There have been studies reporting the use of topical cyclosporine in patient groups with MGD combined with aqueous-deficient dry eye, although cyclosporine itself is also not widely available commercially outside of North America.

**Evidence Supporting Available Treatment Options**

**Artificial Lubricants in the Treatment of MGD**

Understanding the role of artificial lubricants, popularly called artificial tears (AT), in the treatment of MGD requires a brief discussion of the pathophysiologic mechanisms at work in both MGD and aqueous-deficient dry eye. Although aqueous tear deficiency is not a central pathophysiologic mechanism in MGD, it is a concomitant disease in many patients with MGD. Although published estimates vary between 50% and 75% according to the type of clinical practice surveyed, it is likely that the coincidence of aqueous tear underproduction and MGD is even higher. As suggested by the mentioned practice patterns, MGD is perhaps the most underdiagnosed, undertreated, and underappreciated disease in eye care worldwide.

Many patients in whom dry eye has been diagnosed by symptoms and/or routine clinical tests may instead have—according to clinical experience—MGD alone or MGD in combination with dry eye. Because the symptoms of aqueous-deficient dry eye are so difficult to differentiate from those of MGD-related, evaporative dry eye, it may be impossible to truly separate patients into distinct groups. In fact, it may be that these two forms of dry eye disease are spread across a spectrum, with patients only rarely experiencing symptoms and exhibiting signs of one type exclusively. This notion makes pathophysiologic sense, as both increased evaporation of tears and reduced production (volume) of tears increase the osmolality of tears, believed to be a central mechanism of pathophysiology in dry eye.

This co-mingling of aqueous-deficient dry eye and MGD is very important in crafting the approach to treating patients with various degrees of both of these diseases. Supplementation of the tear film can address the “final common pathway” that mediates the range of ocular surface disease, including evaporative dry eye (with or without MGD) and aqueous-deficient dry eye. Increasing tear volume reduces hyperosmolarity and also reduces friction between the tarsal conjunctiva and more specifically the epithelium of the lid wiper, corneal epithelium, and palpebral conjunctiva. It also improves

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**Table 1.** Grading Level of Evidence of Clinical and Basic Research Studies

<table>
<thead>
<tr>
<th>Clinical Studies*</th>
<th>Level I</th>
<th>Evidence obtained from at least one properly conducted, well-designed randomized controlled trial or evidence from studies applying rigorous statistical approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level II</td>
<td>Evidence obtained from one of the following:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Well-designed controlled trial without randomization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Well-designed cohort or case-control analytic study from one (preferably more) center(s)</td>
<td></td>
</tr>
<tr>
<td>Level III</td>
<td>Evidence obtained from one of the following:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Descriptive studies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Case reports</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Reports of expert committees</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expert opinion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Meeting abstracts, unpublished proceedings</td>
<td></td>
</tr>
</tbody>
</table>

**Basic Science**

| Level I | Well-performed studies confirming a hypothesis with adequate controls published in peer-reviewed journal |
| Level II | Preliminary or limited published study |
| Level III | Meeting abstracts or unpublished presentations |

* Studies specific to MGD/management of MGD discussed in the text are identified by the level of evidence.

---

**Table 2.** Recommendations in Clinical Handbooks for Treatment of Posterior Blepharitis and Meibomitis

<table>
<thead>
<tr>
<th>Lid-heating, massage, and cleaning</th>
<th>Warm wet face cloth for 5 minutes once or twice a day; massage upper and lower lid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical medication</td>
<td>Antibiotic ointment twice a day for 5 weeks; short term topical steroids in severe cases</td>
</tr>
<tr>
<td>Systemic medication</td>
<td>With corneal involvement: doxycycline 100 mg once a day or erythromycin 250 mg four times a day for 8 weeks</td>
</tr>
<tr>
<td>Adjunctive treatment</td>
<td>Lubricants if dry; management of skin disease</td>
</tr>
<tr>
<td>Moorfields Manual</td>
<td>Warm compresses for 15 minutes four times per day; clean with wet cotton bud and mild (baby) shampoo</td>
</tr>
<tr>
<td>Wills Eye Manual</td>
<td>Antibiotics at night in severe cases</td>
</tr>
<tr>
<td></td>
<td>Tetracycline 250 mg four times a day or doxycycline 100 mg twice a day for 6 weeks</td>
</tr>
<tr>
<td></td>
<td>Lubricants four to eight times a day</td>
</tr>
</tbody>
</table>
spreading of the tear film lipid layer (clinical studies level II). In addition, the use of AT rinses the ocular surface of toxins and debris and may dilute the concentration of inflammatory cytokines and other proinflammatory molecules that have been found in the tears (clinical studies level II/III). Via all these mechanisms, the frequent use of AT serves to reduce proinflammatory stimuli (clinical studies level II).

This proposed explanation of a positive role in the use of AT must be regarded as speculative and unproven, as neither basic science nor clinical studies of the use of AT in MGD have been published to substantiate the hypothesis. Even in the absence of the evidence from a randomized controlled study, most practitioners rely on AT as a mainstay of treatment for aqueous-deficient dry eye and for most varieties of ocular surface disease across disease severity. Under the broad umbrella of ocular surface disease, the efficacy of AT in the management of ocular allergy perhaps relates to the rinsing and lubricating effects achieved from regular and repeated doses. Many clinicians apply the same reasoning to the treatment of MGD in recommending the chronic use of AT.

Evidence from studies of aqueous-deficient dry eye provides a basis for rational selection of artificial lubricants in MGD. Key concerns in the selection of an AT include the role of preservatives, the role of viscosity, and more recently, the supplementation of oil (lipid) to the tear film. The role of preservatives in ocular surface toxicity has received increasing attention over the past decade. Even with indisputable evidence of preservative-induced toxicity in epithelial cells in vitro, clinical studies do not provide data that determine how frequently a preserved AT can be safely used in MGD. Conventional wisdom has been that bottled (preserved) AT can be used from four to six times daily without significant clinically evident toxicity (uptake of fluorescein stain by the corneal epithelium). Most studies of preservative-induced epithelial toxicity have studied detergent-type preservatives, such as benzalkonium chloride (BAK). Whether this recommendation should be modified because of increased incorporation of oxidative- or so-called vanishing preservatives, such as sodium chloride or perborate and sodium perborate 1.5% (Purite, Irvine, CA) cannot be determined on the basis of the evidence from a randomized controlled study, most clinicians apply the same reasoning to the treatment of MGD in recommending the chronic use of AT.

Several published studies support the superiority of the higher viscosity artificial lubricants in the treatment of dry eye. Most clinicians choose from the dozens of available AT preparations, assuming that the ocular surface residence time of a more viscous product will be longer. Ointments last longest, gel drops last next longest, and thin lubricants remain on the surface of the eye for the shortest time. Surface residence time must be balanced with undesired blurring of vision, which tends to correlate directly with viscosity.

### Topical Lipid Supplements in the Treatment of MGD

Supplementation of tear film lipids has been attempted by the use of lipid-containing eye drops and sprays, emulsion-type eye drops, and ointments. Historically, lipid-containing lubricant eyedrops have not been used widely because of the induced blurring of vision after their use. In recent years, newer formulations have been better accepted, although the number of published studies is small. In patients with noninflamed, obstructive MGD, with and without aqueous-deficient dry eye, Goto et al. reported a small randomized controlled clinical trial (clinical studies levels I and II) in which a self-formulated low-concentration preparation of homogenized 2% castor oil eye drops was used six times daily. Subjective symptom scores ($P = 0.004$), tear interference image grades ($P < 0.0001$), tear evaporation rates ($P = 0.01$), rose bengal staining scores ($P = 0.007$), tear film breakup time (TBUT; $P < 0.0001$), and meibomian gland expressibility grades ($P = 0.002$) after the oil eye drop period showed significant improvement compared with the results after the placebo period.

An emulsion-based lubricant eye drop has been studied in normal subjects and patients with aqueous-deficient dry eye, with or without MGD (clinical studies level II). Compared with the control eyes, emulsion-treated eyes showed rapid restructuring of the preexisting tear lipid film in tear-interference image examination.

Lipid-containing eye drops are difficult to obtain in many countries. Thus, the use of conventional eye ointment as topical lipid supplements in evaporative dry eye or MGD treatment has been tested. As bulk application of eye ointment causes long-lasting visual blur, Goto et al. used a low-dose, 0.05% lipid-containing ointment applied across the full length of the eyelid margin in patients with dry eye and meibomian gland obstruction and in a second study of patients with severe MGD (clinical studies level III). Olofoxacin eye ointment was chosen, as it contains both polar and nonpolar lipids. This method of application was used three times daily in addition to the preexisting ongoing treatment. After the additional lipid treatment, the symptom scores of ocular dryness ($P < 0.0001$), lipid layer thickness measured with a tear-interference camera ($P < 0.0001$), TBUT ($P = 0.01$), and meibum expressibility grades ($P = 0.0005$) improved significantly. Tear film interferometry indicated a more uniform thickness of the tear lipid layer after application of the ointment. Such an improvement was also observed in the treatment of meibomian absence in EEC (ectrodactyly-ectodermal dysplasia-clefting) syndrome with meibomian gland dysplasia. The presence of the antibiotic together with lipid ointment in these supplementation studies introduces some uncertainty about whether the lipid or the antibiotic is responsible for the observed improvements. Confirmation of the efficacy of the lipid formulation alone would require a suitably designed, randomized controlled trial comparing the ointment base alone with an olofoxacin preparation.

A lipid-containing liposomal spray has been studied in two prospective randomized multicenter trials (clinical studies level II) in patients who have evaporative dry eye, as defined by low TBUT and inflammatory lid margin changes. Patients received hyaluronate AT, triglyceride gel, or a phospholipid-liposome eye spray, each for a minimum of 6 weeks. Phospholipid liposomal spray achieved a significantly greater reduction of the lid-parallel conjunctival folds, lid margin inflammation, and improvement in the break-up time than did hyaluronate eye drops or triglyceride gel.

**Comments.** The use of lipid supplements in clinical studies has been demonstrated to improve some signs and symptoms of MGD, perhaps by improving tear film stability. Further randomized controlled masked clinical trials of patients with well-defined MGD are needed to determine efficacy across disease severity.

### Lid Hygiene and Warm Compress or Heat Application

Lid hygiene is regarded as the mainstay of the clinical treatment of MGD. It usually consists of two components: application of heat and mechanical massage of the eyelids.

**Eyelid Warming.** The application of warmth, either with moisture or without has received frequent study in MGD. Obstructive MGD has previously been defined as being associated with decreased meibum secretion. Yokoi et
al., using meibometry, reported that MG function in patients with MGD was significantly reduced compared with that in healthy subjects (basic science level II). McCulley and Shine suggested that meibomian secretion with ester fractions of different composition can have different melting points and that MGD can cause a shift toward lipids with higher melting points, producing a stagnant and less dynamic tear film (basic science level II). Indeed, meibomian secretions from normal subjects have been shown to begin to melt at 32°C and 35°C in patients with obstructive MGD. Eyelid-warming therapies can be expected to improve MG secretion by melting the pathologically altered meibomian lipids. The warming can be achieved by many diverse methods, including simple warm compresses (e.g., hot wet towel, heated rice bag) or devices such as infrared or hot air sources (clinical studies level II/III).

Warm compress therapy is a commonly recommended but poorly standardized treatment for MGD that is performed by patients for variable durations of heat application and with varying compliance. Nagymihalyi et al. reported that eyelid temperature significantly influenced the delivery of the meibomian gland secretions in healthy human volunteers (clinical studies level III). The application of a 250-W infrared lamp from a distance of 50 cm increased the eyelid surface temperature and increased the meibomian oil delivery on the eyelid margin. Olson et al. reported that 5 minutes of treatment with warm towel compresses (40°C) applied to the skin of closed eyelids increased the tear film lipid layer thickness by more than 80% in patients with obstructive MGD, with an additional 20% increase after 15 minutes of treatment. There was no increase in tear film lipid layer thickness with 5 minutes of treatment with towel compress at room temperature (24°C) applied to the contralateral control eyes (clinical studies level II). The increase in tear film lipid layer thickness in that study was found to be significantly related to the reduction of symptom scores. A protocol to optimize warm compress treatment has been published by Blackie et al. and recommends the continuous application of 45°C hot compresses for at least 4 minutes with optimal contact between compress and eyelid, replacing the compress every 2 minutes with a new compress preheated to 45°C to achieve adequate warming to alter secretions (clinical studies level II).

Alternative sources of heat for warm compress therapy include eye warmer devices, delivering infrared irradiation or moist air or eye warmer masks. Goto et al. reported increased tear stability and decreased dry eye symptoms after 2 weeks of treatment with an infrared eyelid-warming device applied to the eyelids for 5 minutes twice daily in patients with obstructive MGD (clinical studies level III). The application also improved tear evaporation, ocular surface epithelial damage, and meibomian gland orifice obstruction. Mori et al. reported warming of the eyelids with a disposable (noninfrared) eyelid-warming device for 5 minutes once a day for 2 weeks, which improved dry eye symptoms, tear stability, and uniformity of the tear lipid layer in MGD patients (clinical studies level II-III).

Matsumoto et al. reported that warm moist air device use for 10 minutes twice daily for a period of 2 weeks provided symptomatic relief of ocular fatigue, improvement of tear stability and ocular surface epithelial damage in patients with MGD (clinical studies level II). The thickening of the tear film lipid layer after 10 minutes of device application was confirmed in both patients and controls in that study. Mitra et al. reported that treatment of MGD with a moist air device increased the lipid layer thickness in normal individuals, helped achieving a more stable tear film, and provided subjective improvement in ocular comfort (clinical studies level II).

Ishida and Matsumoto also reported that eye warmer masks (Orgahexa; Therath Medico Inc., Tokyo, Japan) applied for 10 minutes for 2 weeks improved both tear functions and ocular surface status, and decreased symptoms significantly in MGD patients (clinical studies level III). The application of these masks were found to be more effective in MGD patients, but not in normal controls, compared to the conventional eye masks applied for the same period.

Eyelid warming with warm compresses has also been reported to induce transient visual degradation due to corneal distortion, apparently resulting from the application of light pressure with warm compresses, as evidenced by the polygonal reflex of Fischer-Schweitzer (basic science level II). Further larger-scale prospective randomized comparative studies investigating the alterations of subjective and objective findings in healthy controls and MGD patients with such devices have not been performed and should be conducted.

**Mechanical Lid Hygiene.** Lid hygiene (i.e., scrubs, mechanical expression and cleansing with various solutions of the eyelashes and lid margins) is frequently recommended, together with lid warming in the treatment of MGD. Romero et al. reported in a nonrandomized, uncontrolled, prospective study that lid hygiene with a combination of heated saline solution and preservative-free AT significantly improved tear break-up time and relieved symptoms in patients with MGD (clinical studies level II). The MGD patients in this study were treated with the aforementioned regimen for 6 weeks but were not compared to normal subjects. In an additional study of lid hygiene, Key reported that the use of hypoallergenic bar soap, dilute infant shampoo, or commercial lid scrub is useful in the treatment of anterior blepharitis (clinical studies level III). The biomicroscopic features of the blepharitis improved after treatment, but this study also lacked a comparative control group. Paugh et al. also reported that lid scrub and massage increased the TBT in patients with MGD (clinical studies level II). In this study, 2 weeks of treatment was found to be effective in the resolution of clinical signs with no significant changes observed in the controls. Kavathas et al. evaluated the response to treatment including hygiene, topical steroid, and topical antibiotic in obstructive MGD using confocal microscopy, although hygiene alone was not assessed (clinical studies level II). Current literature seems to have no studies on the above topics with clinical studies level I of scientific evidence, and such studies are needed, to confirm the efficacy of this frequent clinical treatment option in the future.

Properly performing lid massage may help the patient’s therapy; proper instruction to the patient is therefore necessary. For example, patients may be told that after application of a hot compress to the eyelids, they should apply traction on the lateral canthus to immobilize the upper and lower eyelids, that should be followed by down- or upward mild compression of the eyelids with the finger of the opposite hand beginning at the nasal canthus and moving laterally toward the lateral canthus.

Physical expression of meibomian glands for therapeutic purposes is an in-office procedure with at least an 80-year history. It can be supplemented by the patient’s performing self-expression and massage at home. The reported techniques vary from gentle massage of the lids against the eyeball to forceful squeezing of the lids either against each other or between a rigid object on the inner lid surface and a finger, thumb, or rigid object (e.g., glass rod, Q-tip, or metal paddle) on the outer lid surface. The rigid object on the inner lid surface is used to protect the eyeball from forces transferred through the eyelid during expression and also to offer a stable resistance, to increase the amount of force that can be applied to the glands. The amount of force needed to express obstructed glands can be significant and is usually limited by the pain induced by the expression and not by the amount of force that can be applied. The amount of pain
increases rapidly as the force of expression exceeds 15 g/mm² (~5 PSI) with forces of 80 g/mm² (~25 PSI) and greater, frequently producing excruciating pain, thus considerably limiting clinical application.67,68 Regardless of the method of meibomian gland physical expression, the goal is to express the meibomian gland obstruction and other material from the gland, thereby facilitating normal gland function. Clinically it is recommended that treatment with physical expression should be continued until the dysfunction is resolved.

**Comments.** Lid hygiene is widely considered an effective mainstream therapy for MGD and blepharitis, despite the lack of standardization of the technique and the uncertainty about patient compliance. Studies comparing specific techniques of lid hygiene would allow evidence-based recommendations regarding this simple and presumably effective therapy. Studies comparing the efficacy of the many available methods for eyelid warming are also lacking. Nonetheless, given the near unanimity of support for this therapy among international experts and clinicians alike, patients should be instructed in lid-warming and hygiene and urged to remain compliant, to maintain long-term control of symptoms. Follow-up examinations are to be recommended as a means of ensuring the patient’s compliance, as many patients are unlikely to remain compliant with these methods from one annual examination to the next.

**Topical Antibiotic Agents in the Treatment of MGD**

The uncertain role of bacteria in the pathophysiology of MGD and the incompletely understood optimal balance of normal lid microbiota make the role of topical antibiotics in therapy indeterminate. No evidence suggests that bacterial infection is the primary pathophysiologic process in MGD; but numerous clinical findings often seen in MGD may be related to the effects of the bacteria that colonize the eyelids. Bacteria may have both direct and indirect effects on the ocular surface and on meibomian gland function. These include direct effects on the production of toxic bacterial products (including lipases) and indirect effects on ocular surface homeostatic mechanisms, including matrix metalloproteinases (MMPs),69 macrophage function, and cytokine balance (Jacot JL, et al. IOVS 2008;49:ARVO E-Abstract 1985). The complexity and uncertainty of the role of bacteria in the MGD process, characterized by both infectious and inflammatory processes, has implications for appropriate recommendations for therapy. In the absence of peer-reviewed studies, recommendations for the use of this class of therapeutic agent in MGD must be regarded as speculative, and readers should individually evaluate the applicability of the data reviewed.

The mere demonstration of the presence of bacteria on the lid margin of patients with MGD does not imply causality. It may be that the excessive colonization of the lids, demonstrated in patients with blepharitis,70,71 with coagulase-negative staphylococcus (Staphylococcus epidermidis), Staphylococcus aureus, Propionibacterium acnes or other microbes is an epiphenomenon, indicating the possibility that microbes find the altered eyelid environment in MGD more hospitable than that of the normal eyelid. Keratinization of the lid margin epithelium, the accumulation of keratinized cell debris, within and/or around the meibomian orifice, and the presence of abnormal lipids all provide a rich substrate for the resident bacterial microbiota. Thus, it is also possible that the subsequent release of toxic bacterial products such as lipases or the secondary production and release of proinflammatory cytokines is pathogenic. Excessive bacterial colonization may be pathogenic via preferential selection of certain microbial species. Quorum sensing has been proposed as a mechanism to explain how excessive colonization can trigger certain species of bacteria to release potentially toxic bacterial products.72,73

Normally, as a kind of feedback mechanism, signaling molecules called autoinducers, allows bacteria to monitor the relative number of their own and other species in the same environment, facilitating coexistence. Malfunctions in this system may be triggered by the appearance of new bacterial species in the environment and may result in the release of potentially toxic bacterial products.74,75

In theory, for an antibiotic agent to be beneficial in MGD, it must be effective against the pathogens most likely to be present in this condition. A complete review of antibiotics and their properties is beyond the scope of this report, but commonly used topical antibiotics, their dosages, and their advantages and disadvantages will be briefly reviewed.

**Bacitracin.** Bacitracin is a protein disulfide isomerase inhibitor that interferes with bacterial cell wall synthesis. It has been used primarily as a topically applied agent, since it can be highly nephrotoxic in systemic use. Poor aqueous solubility limits its use primarily to ointment formulations. Bacitracin has a spectrum of activity similar to that of penicillin and has also been used to treat anterior blepharitis.76

**Fusidic Acid.** Fusidic acid, a topical antibiotic with efficacy against Gram-positive organisms, has been in clinical use since 1962. It inhibits protein synthesis by blocking aminoacyl-sRNA transfer to protein in susceptible bacteria. Although not widely used to treat blepharitis, research indicates that this drug may be effective for patients with blepharitis and associated rosacea. Seal et al.77 (clinical studies level II) used a treatment of 1% fusidic acid and noted improvement in the symptoms in 75% of patients with concurrent blepharitis and rosacea. In comparison, oral oxytetracycline yielded improvement in just 50% of these patients. Treatment was much less successful in patients who had blepharitis without rosacea. These patients had no response to fusidic acid alone, although 25% did respond to oxytetracycline.

**Metronidazole.** Metronidazole, FDA-approved as a 1% dermatologic preparation for the treatment of rosacea,78,79 is bactericidal against susceptible bacteria. Its exact mechanism of action is not completely understood, but an unidentified polar compound breakdown product is believed to be responsible for metronidazole’s antimicrobial activity, by disrupting DNA and nucleic acid synthesis in anaerobic bacteria. Barnhorst et al.79 (clinical studies level II), in a study of 10 patients, found ocular rosacea lid hygiene combined with topical metronidazole gel applied to the lid margin for 12 weeks to be more effective than lid hygiene alone in the fellow eye in improving eyelid and ocular surface scores. No adverse effects of the metronidazole treatment were encountered in this study. Saccà et al.80 reported a 50% positive response to metronidazole therapy in patients with Helicobacter pylori culture-positive blepharitis, although the authors conclude that the causative nature of H. pylori in chronic blepharitis warrants further evaluation.

**Fluoroquinolones.** The availability of topical fluoroquinolone antibiotics has influenced prescribing habits in a wide range of ocular infectious diseases.81 These drugs have minimal ocular surface toxicity, provide excellent coverage of both Gram-positive and -negative organisms, and have become the treatment of choice in treating even serious corneal infections. Concerns about emerging bacterial resistance have, in part, limited widespread use of this highly effective class of antibiotics in patients with blepharitis.82,83

**Macrolides.** Macrolide antibiotics are products of actinomycetes (soil bacteria) or semisynthetic derivatives of them. Erythromycin, the first macrolide antibiotic, has been widely available since its discovery in the soil in the early 1950s. Erythromycin and other macrolide antibiotics inhibit protein
synthesis by binding to the 23S rRNA molecule (in the 50S subunit) of the bacterial ribosome blocking the exit of the growing peptide chain. Because of frequent use and high selection pressure, the extensive use of erythromycin may provoke resistance among Gram-positive organisms, and its overall efficacy for ophthalmic applications for ocular infection is now questioned. Ophthalmic use of erythromycin is also limited by its low aqueous solubility; therefore, it is most often compounded as an ointment for ocular use. An eye drop formulation is available in some European countries. Newer topical macrolides, such as azithromycin, clarithromycin, and roxithromycin, have become available and offer an expanded spectrum of coverage and better penetration than older macrolide antibiotics.\textsuperscript{84,85}

**Antibiotic Anti-inflammatory Efficacy.** Macrolide antibiotics exert immunomodulatory and anti-inflammatory effects that are separate from direct antibacterial actions. Many studies have been conducted in the past decade in an attempt to understand the various cellular and molecular processes involved in the inflammatory response affected by macrolide compounds. Most in vivo studies have involved patients with chronic inflammatory respiratory diseases (asthma and diffuse panbronchiolitis). These studies have documented clinical and functional improvement after treatment with subtherapeutic levels of macrolides in respiratory disease patients, sometimes within weeks of therapy.\textsuperscript{86–88} There are similarities in the nature of these respiratory diseases and MGD. Both involve elements of infectious and inflammatory pathophysiology on a mucosal surface with complex biofilm. Whether drugs found to be useful in treating respiratory disease could prove useful in treating MGD is worthy of investigation.

Although the specific molecular mechanisms which give rise to the above-mentioned benefits are not clear, several areas have been investigated. Macrolides’ effects on proinflammatory mediators have been studied in clinical settings and in vitro. In both cases, a significant reduction of cytokine release (particularly IL-8, IL-6, and TNF-\(\alpha\)) has been observed.\textsuperscript{89,90} Although the role of these cytokines in MGD is not well understood, their role in dry eye has been better studied.

In addition, macrolides have potent effects on neutrophil functions, including chemotaxis and phagocytosis.\textsuperscript{91,92} Macrolides’ effects on neutrophils may be mediated by downregulation of adhesion protein expression.\textsuperscript{93,94} Macrolides also have potent effects on the functions of phagocytic cells, including macrophages.\textsuperscript{11,15,95–97} Finally, macrolides have been reported to downregulate genes coding for MUC5AC production.\textsuperscript{98,99} The mechanisms at work in respiratory diseases such as asthma, cystic fibrosis, and autoimmune bronchiolitis have much in common with MGD.

Macrolides have the ability to break down and prevent further development of the biofilms protecting the mucoid *Pseudomonas aeruginosa* strains, relevant in pulmonary diseases. Of the 14- and 15-membered macrolides, azithromycin has been demonstrated to have the highest potency for these activities, further supporting its immunomodulatory potential in respiratory diseases\textsuperscript{99} and potentially, in MGD. Extensive research in these areas remains ongoing.

The large body of data briefly summarized herein has generated substantial interest in studying the role of macrolide antibiotics such as azithromycin in the treatment of MGD. After oral administration, macrolides show a low serum concentration, along with a high tissue concentration and, in the case of azithromycin, an extended tissue elimination half-life. In a rabbit model following the FDA-approved regimen of 1 drop twice daily for 2 days then 1 drop once a day for 5 days, topical azithromycin (Azasite; Inspire Pharmaceuticals, Durham, NC) produced a maximum azithromycin concentration in eyelids of 180 \(\mu\)g/g and a half-life of 125 hours (data on file from the manufacturer).\textsuperscript{100}

**Review of Published Studies of Antibiotics in the Treatment of MGD.** In a study (clinical study level II) in which topical metronidazole plus lid hygiene was compared to lid hygiene alone, the researchers observed a significant improvement in the combined eyelid and ocular surface scores in treated eyes, but not in control eyes.\textsuperscript{78}

Another single-center, open-label clinical trial demonstrated significant improvement in signs and symptoms of MGD after 2 and 4 weeks of treatment with topical 1% azithromycin solution. Resolution of in signs and symptoms correlated with spectroscopic analysis of expressed meibum demonstrating improvement in ordering of lipids and phase transition temperature of lipids in the meibomian gland secretion\textsuperscript{101} (clinical studies level III).

An open-label multicenter study (clinical studies level III) demonstrated effectiveness of topical 1% azithromycin in treatment of subjects with blepharitis. Four-week azithromycin treatment demonstrated significant decreases from baseline in investigator-rated signs of meibomian gland plugging, eyelid margin redness, palpebral conjunctival redness, and ocular discharge at day 29 \((P \leq 0.002)\), which persisted 4 weeks after treatment \((P \leq 0.006)\).\textsuperscript{102} In an additional recent open-label study (clinical study level III), patients with MGD blepharitis were treated with azithromycin plus warm compresses or warm compresses alone.\textsuperscript{103} After using azithromycin twice daily for the first 2 days followed by once daily for the next 12 days of treatment, the azithromycin-treated patients showed significant improvements in meibomian gland plugging, quality of meibomian gland secretions, and eyelid redness. Also, a higher percentage of patients in the azithromycin group rated their symptomatic relief as good or excellent. Data from spectroscopic analysis of pre- and posttreatment meibomian gland secretion demonstrates a restoration of order pattern of the lipids and a cumulative reduction in lipid phase transition temperature, which suggests that azithromycin alters the lipases acting on the meibomian gland lipids in MGD.\textsuperscript{101}

The efficacy of azithromycin may be attributable to several factors mentioned previously. Animal studies of systemic azithromycin have shown it to have anti-inflammatory as well as antimicrobial effects.\textsuperscript{87,104} Pharmacokinetic studies also have shown that topical ocular azithromycin was detectable in all tissues and fluids for 6 days after the dose of a single drop, suggesting that topical azithromycin is likely to have a sustained duration of effect.\textsuperscript{100}

**Comments.** Topical antibiotics offer both opportunities and challenges in management of MGD and are not yet completely understood as a treatment regimen for MGD. Several topical and systemic antibiotics with activity against lid-related bacteria are available, but solid evidence from randomized controlled clinical trials is lacking to conclusively guide antimicrobial management of MGD. Although a handful of comparative studies have been performed, additional research is needed to better define the role of topical antibiotics, including macrolides, thought to have anti-inflammatory effects in a chronic management scheme for MGD.

**Treatment of Demodex Mite Infestation in Blepharitis.**

Demodex mites are elongated mites with clear head–neck and body–tail segments, of which the former has four pairs of legs. There are more than 100 species of Demodex mites, many of which are obligatory commensals of the pilosebaceous unit of several mammals. *Demodex folliculorum* and *Demodex brevis* have been confirmed to be the most common ectoparasites in humans. In the eye, *D. folliculorum* is found preferentially in...
the lash follicles and *D. brevis* in lash sebaceous glands. A role for *Demodex* mites in the pathogenesis of MGD has not yet been convincingly established\(^\text{105,106}\) (clinical studies level III).

*Demodex* infestation is thought to be nonexistent in healthy children under the age of 10 years, increases in an age-dependent manner, and is very likely present in the skin of 100% of the elderly.\(^\text{10,107}\) Although *Demodex* mites have been implicated as a cause of many human skin disorders, their pathogenic role has long been debated,\(^\text{10,108,109}\) in part because some *Demodex* mites can be found in the skin of asymptomatic individuals.

There is evidence that *Demodex* infestation of the lash follicles contributes to the occurrence of anterior blepharitis and that cylindrical dandruff is a pathogenicomic clinical sign.\(^\text{110}\) Gao et al.\(^\text{111}\) and Keirkhah et al.\(^\text{111}\) reported that weekly lid scrubs with 50% tea tree oil (TTO) and daily lid scrubs with tea tree shampoo are effective in eradicating ocular *Demodex* infestation in vivo, as evidenced by the reduction of the *Demodex* count to 0 in 4 weeks in most patients. This was associated with improvement in previously refractory ocular surface inflammation. Because TTO also may exert antibacterial, antifungal, and anti-inflammatory actions, its therapeutic benefit may be independent of its effect of killing mites. It has been postulated that the mites act as vectors to bring in common skin bacterial microbiota\(^\text{106,112,113}\) and that symbiosis or commensalism between mites and microbes could be part of the pathogenesis of MGD. The positive correlation between facial rosacea and serum immunoreactivity to two proteins derived from *Bacillus oleronius*, a bacterium that lives symbiotically within the mites, has led to suggestions that rosacea manifestations, including lid margin inflammation, could be due to a strong host immune response to this organism.\(^\text{105,106}\)

**Comments.** It appears that *Demodex* mites have an etiologic role in some forms of anterior blepharitis and that treatment with TTO is merited in these cases. There is no evidence that these mites can cause MGD, and therefore the role of pharmaceutical eradication of mites in the treatment of MGD is uncertain.

**Tetracycline and Derivatives (Systemic)**

The tetracyclines are bacteriostatic antibiotics, developed in 1948 and first proposed for the treatment of the cutaneous manifestations of acne rosacea in 1966.\(^\text{114}\) In the management of rosacea and MGD, they are mainly used for their anti-inflammatory and lipid-regulating properties, rather than for their antimicrobial effects\(^\text{59,115-117}\) (clinical studies level I-III).

**Mechanisms of Action.** At the systemic doses currently used in the treatment of MGD and rosacea, the antimicrobial effects of tetracycline derivatives in the lids are probably limited. The exception is minocycline, which has been shown to reduce the population of lid flora in rosacea patients, at a dose of 100 mg.\(^\text{120,121}\) This finding may reflect differences in the lipophilicity and hence pharmacokinetics of these drugs. On the other hand, tetracycline is poorly lipophilic, and doxycycline, and to a greater extent, minocycline, are lipophilic.\(^\text{122}\) In this study, the concentration of oxytetracycline, tetracycline, minocycline, and doxycycline was measured in tears after 5 days of daily treatment by mouth. Although oxytetracycline and tetracycline do not achieve antimicrobial levels in the tears at standard doses, minocycline does so in normal subjects at a dose of 100 mg, and doxycycline reaches near inhibitory concentrations.\(^\text{122}\) The authors conclude that doxycycline and minocycline are clinically effective at lower doses than tetracycline or oxytetracycline. It is hypothesized that the lipophilicity facilitates the entry of doxycycline and minocycline into the central nervous system and presumably influences its delivery to ocular structures and lid tissues presumably including the meibomian glands.\(^\text{122}\) (basic science level I).

However, there is good evidence that the efficacy of tetracyclines in the management of MGD depends on the suppression of microbial lipase production and hence the release of proinflammatory free fatty acids and diglycerides at the lid margin and ocular surface\(^\text{125-126}\) (basic science level I and II). Thus, the production of lipases and esterases by lid commensals such as *S. epidermidis* and *P. acnes*, is highly responsive to low doses of tetracyclines. To a lesser extent, this applies to both sensitive and resistant strains of *S. aureus*. Minocycline therapy has the particular attraction of both reducing the resident lid flora and inhibiting their production of lipases.

**Effects on Lipids and Meibomian Gland Secretions.** Tetracyclines inhibit lipase activity and therefore decrease deleterious free fatty acids\(^\text{127-128}\) (clinical studies level II). Free fatty acids destabilize the precorneal tear film and promote inflammation (chemotaxis to neutrophils and reactive oxygen species [ROS] production, among others). Excessive lipase activity and alterations of lipid composition thus directly influence tear stability and cause inflammation, inside meibomian glands, in tears and most likely throughout the ocular surface. High oleic acid may also play a role in keratinization of the lid margin and plugging of meibomian gland orifices. Minocycline at 100 mg per day for 3 months showed marked decrease of diglycerides and free fatty acids\(^\text{125}\) (clinical studies level III).

**Inhibition of Inflammation.** Tetracyclines may have anti-inflammatory properties through multiple mechanisms and events demonstrated in ocular tissues or nonocular systems. Target cells may be neutrophils (migration and chemotaxis), lymphocytes (proliferation, transmigration, and activation), and, most likely, epithelial cells (corneal, conjunctival, and others). Antioxidative effects have also been found (anti-ROS inhibition and accelerated degradation of NO synthase), as well as inhibition of phospholipase A2 and metalloproteinases (MMPs)\(^\text{12,127}\) (basic science level I). Moreover, there is a strong interaction between MMPs and inflammatory cytokines, each one activating the other type of mediator from its respective inactive precursor. Collagenase-2 (MMP8) has also been found in elevated levels in the tears of rosacea patients and decreases with doxycycline treatment\(^\text{130}\) (clinical/basic science level II).

**Antiangiogenesis and Antia apoptotic Properties.** Antiangiogenesis and antiapoptotic properties have also been reported,\(^\text{14}\) through direct (inhibition of caspase-1 IL-1β) or indirect (collagenases and other MMPs for angiogenesis; MMP- or ROS-mediated apoptosis) effects.

**Clinical Effects.** The use of tetracycline derivatives has been reported to be efficacious in many clinical studies. Rosacea, with cutaneous and/or ocular manifestations, is the most studied application. Although most studies were not placebo controlled, significant effects on symptoms, lid margin, ocular surface inflammation, and tear film stability have been described, although the effects seem less prominent on keratitis and conjunctival staining\(^\text{115-118}\) (clinical studies level I).\(^\text{100}\) Overall tolerance is mostly good, with minor concerns, such as diarrhea, nausea, headache, photosensitization, and vaginal or oral candidiosis, which are reported to be less severe at lower doses. In most cases, these side effects do not result in discontinuation of treatment.

**Dosages and Routes of Administration.** Most studies have addressed tetracyclines given orally at doses considered subantimicrobial, ranging between 250 mg once to four times a day (tetracycline and oxytetracycline) and 50 to 100 mg once or twice a day (doxycycline and minocycline). It should be
noted that a subantimicrobial dose of doxycycline of 40 mg a day, chosen for its anti-inflammatory properties, is used for rosacea\(^9,11^9\) (clinical studies level I).

Topical administration has also been proposed with doxycycline and tested in experimental models with promising results, in terms of MMP activation, corneal barrier function and surface keratinization\(^12,12^9\) (basic science level I, clinical studies level III).

Clinical Trials and Methodological Issues. Tetracycline derivatives are widely used in rosacea and various cutaneous diseases. In MGD, the use of these compounds has been described in level II or III clinical trials,\(^11^5,11^6,11^8\) showing a significant improvement in symptoms and signs. However, fewer placebo-controlled studies (clinical studies level I) have been published, and they showed milder effects than did open trials comparing effects before and after treatment.\(^1^9,11^9\) In one interesting level I trial, lid hygiene alone was compared to lid hygiene plus minocycline and showed significant changes in fatty acid composition, together with improvement in some, although not all, clinical signs.\(^12^6\) Nevertheless, many clinical studies even when not placebo-controlled, reported objective biological criteria that were strongly supportive of a significant role of tetracyclines, such as decreased lipase activity,\(^12^6\) improved meibomian lipids,\(^12^5\) or decreased MMP-8.\(^1^6^0\) Moreover, proof of concept was often addressed in experimental models, mainly of dry eye, showing a decrease or normalization of MMPs, inflammatory cytokines, corneal barrier dysfunction, or keratinization.

Comments. Tetracyclines are widely used in a variety of ocular surface diseases, including ocular rosacea, blepharitis, recurrent erosions, corneal angiogenesis, and dry eye. These compounds may act through several modes of action, mostly related to inflammation control and lipase inhibition. Although the individual response of patients is variable and the protocols related to inflammation control and lipase inhibition. Although not all, many clinical studies even when not placebo-controlled, reported objective biological criteria that were strongly supportive of a significant role of tetracyclines, such as decreased lipase activity,\(^12^6\) improved meibomian lipids,\(^12^5\) or decreased MMP-8.\(^1^6^0\) Moreover, proof of concept was often addressed in experimental models, mainly of dry eye, showing a decrease or normalization of MMPs, inflammatory cytokines, corneal barrier dysfunction, or keratinization.

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specifically. The authors report that both treatments were effective in reducing signs and symptoms of eyelid eczema, with a near superior benefit for tacrolimus in terms of eczema (total skin score) signs ($P = 0.05$). The effect of tacrolimus on specific signs of MGD has yet to be evaluated.

**Comments.** The studies of topical cyclopentolate are somewhat challenging to interpret, as the influence of a reduced Schirmer score or the presence of ocular rosacea complicates the interpretation. All three studies, although small in sample size, were designed in a randomized, controlled manner with attempts at reducing examiner bias with some form of masking. Participant dropout, always a challenge, is a critical element in smaller studies. In addition, although these studies examined some component of the lids, a uniform criterion to classify the patient’s MGD was not used between the studies, making comparison difficult. In addition, the presence of moderate aqueous deficiency, which is improved in two of the three studies, creates a conundrum of whether the treatment improved the lacrimal gland status and thus the lid margin as an indirect result, or the other way around. In each case, the effect was not demonstrated until the 2-month visit, and should be considered in making management decisions in patients with combined (or mixed) aqueous deficient dry eye and MGD. Further studies in this area are needed, and the results of these studies are worth consideration. Additional studies of tacrolimus may be warranted.

**Sex Hormones**

Extensive basic science research has probed the relationship between androgen sex hormones and the meibomian gland. Androgens have been shown to influence gene expression in mouse meibomian glands, especially to suppress genes associated with keratinization and stimulate genes related to lipogenesis. Androgen receptor dysfunction has been associated with marked clinical abnormalities in meibomian gland function, and the use of systemic antiandrogen medications has been associated with clinical MGD. Despite these clues from basic science investigations, there is no level I or II published study showing a beneficial effect in humans for a topical androgen preparation. One case report (clinical studies level III) was published describing successful treatment of dry eye by means of an androgen containing eye drop in a 54-year-old male resulting in a restored lipid phase of the tear film.

**Essential Fatty Acids**

Dietary supplements of $\omega$-3 fatty acids have gained in popularity over recent years because of the beneficial effects on anti-inflammatory by-products of prostaglandin metabolism. Clinical studies level II epidemiologic and clinical trials have demonstrated an association between the use of oral supplements of $\omega$-3 and symptoms of dry eye.

Few studies have been published on the efficacy of dietary $\omega$-3 supplements for MGD. Pinna et al. (clinical studies level II) reported the superiority of $\omega$-3 supplementation over lid hygiene or placebo treatment in patients with MGD. A recently published study included a randomized controlled trial (clinical studies level I) of the use of dietary supplementation with $\omega$-3 fatty acids in patients with MGD. In this prospective masked randomized placebo-controlled trial, patients with simple obstructive MGD and blepharitis who had discontinued all topical medications and tetracyclines received oral $\omega$-3 dietary supplementation consisting of 2000 mg three times a day for 1 year. Outcomes included symptom severity assessed according to the Ocular Surface Disease Index (OSDI) and objective clinical measures, including tear production and stability, ocular surface staining, meibomian gland assessment, and meibum evaluation. The study reported efficacy in improving both symptoms and objective findings between baseline measurements and 1-year measurements in both the treatment group and the placebo group (to a lesser degree). For the key outcome measures of TBUT, meibum score, and OSDI, no statistically significant differences between groups were found (both groups improved).

**Comments.** Published data provide some evidence to recommend dietary modification or the inclusion of dietary $\omega$-3 supplements in a treatment plan for patients with MGD. Further large-scale clinical trials with more subjects are needed to determine whether $\omega$-3 supplements are beneficial when patients are classified as MGD, MGD with aqueous-deficient dry eye, or aqueous-deficient dry eye alone as part of the study design.

**Surgical Options**

Surgical options in the treatment of MGD are generally limited to treatment of the complications of the disease, rather than the primary disease. MGD can be associated with pathologic conditions, such as conjunctivochalasis, entropion, ectropion, or horizontal eyelid laxity, which may be treated surgically, and treatment of these conditions can improve control of MGD. Meibomian gland secretion may be facilitated by the mechanical pumping effect of lid movements. This method requires a certain amount of tension in the medial or lateral canthal tendon. Increasing horizontal lid tension may increase excretion of meibum. One published case report (clinical studies level III) describes a 41-year-old man with bilateral ocular irritation and floppy eyelid syndrome. Histology of the tarsus removed at surgical correction revealed cystic degeneration and squamous metaplasia of the meibomian glands, abnormal keratinization, and granuloma formation. These findings suggest that MGD may be associated with keratoconjunctivitis in floppy eyelid syndrome.

Other pathologic eyelid conditions, such as chalazion, trichiasis, and keratinization of the lid margin may be associated with MGD. The incidence of trichiasis, keratinization, and cicatrical entropion secondary to MGD remains unknown. Treatment of these conditions with appropriate surgical procedures may improve patient symptoms, but the effect on MGD specifically cannot be determined. Discussion of the surgical management of these co-morbid conditions is beyond the scope of this article, and the treatment of such conditions should occur independently but concurrent with the management of existing MGD.

Intraductal probing has recently been introduced as a treatment for MGD. One report (clinical studies level III) on this management approach for MGD describes a modified surgical procedure as a primary treatment non--end stage MGD. This study of 25 patients demonstrated a high frequency of short-term symptomatic relief (Maskin S, et al. IOVS 2009;50:ARVO E-Abstract 4636). Further study of this technique is in progress.

**TREATMENT RECOMMENDATIONS**

**Staged Treatment Algorithm**

Without generally accepted definitions for a staging system of clinical severity of MGD, it is problematic to propose a treatment plan based on disease stage. Nonetheless, in the hope of assisting eye care providers who are attempting to fashion a logical, evidence-based treatment approach, the following disease-staging summary (Table 3) and staged treatment algorithm (Table 4) are proposed.

In the staging of disease, it is recognized that it is difficult clinically to separate the effects of MGD and the effects of aqueous-deficient dry eye on the ocular surface. In addition,
co-morbid diseases are often present. Thus, Table 3 represents a clinical picture of staged disease. Co-morbid conditions, defined as “plus” disease may require concurrent management per standard-of-care protocols.

Table 4 reflects an evidence-based approach to the management of MGD. The staged diagnosis algorithm is similar, yet not exactly identical to the severity grading found in the Diagnosis Report. This algorithm represents a consensus of recommendations from the panel of experts participating in the preparation of this report, having considered the evidence-based review of published studies of treatments, with consideration of the clinician who encounters a hybrid of MGD and other co-morbid conditions on a daily basis. Detailed grading of individual parameters of the eyelid may be more appropriate for clinical trials; however, details of the grading are incorporated into the table to provide additional points of reference.

With every systemic medication, systemic side effects have to be considered. With the above treatment algorithm in mind, phototoxicity for systemic tetracycline derivative use and anti-coagulant effects of essential fatty acids (EFAs) may be of specific concern. EFAs are nutritional supplements that have received much attention, but only one published clinical study to date supports their efficacy in MGD. This lack of evidence is also true of the use of sex hormones. There is no clinical support of the efficacy of hormones in treating MGD, and no licensed product is available. Hence, although it is discussed in this article, the panel agreed not to assign this potential treatment modality to a grade of disease. The risks of prolonged topical corticosteroid therapy (e.g., induction of cataract and elevated intraocular pressure) are well known. Hence, the use of such medications should be reserved for the treatment of acute exacerbations in MGD and should not be used in long-term therapy. Regular monitoring of intraocular pressure is mandatory with the use of topical corticosteroids.

Additional Therapies for MGD and Co-morbid “Plus” Conditions

The 2007 DEWS report recommended moisture chamber goggles, autologous serum, and large-diameter scleral contact lenses for the more severe levels of dry eye disease. Although some of these therapies may be beneficial for patients with aqueous-deficient dry eye in combination with MGD, studies of these therapies for MGD alone have not been performed. It can be hypothesized that a reduction in airflow across the corneal surface in a patient with lipid abnormalities or aqueous-deficient dry eye (related to MGD) could reduce tear evaporation. Kimball et al. have demonstrated that goblet cell loss in normal subjects decreases the rate at which the tear film thins between blinks. Similar studies in MGD subjects could provide additional insight to the etiology behind tear film stability.

In theory, large-diameter sclera contact lenses and autologous serum augment corneal health, with the serum providing nutrients in the form of growth factors and other factors, while the contact lens protects the ocular surface from further damage. It is unclear what the benefit would be for patients with MGD.

“Plus” disease therapies should follow standard of care guidelines and should be considered independent of MGD.

**FUTURE DEVELOPMENTS**

Since the precise mechanism of MGD remains uncertain, it is unclear whether any of the current treatments reviewed are palliative, provide indirect effect, or address underlying disease pathophysiology. The development of new examination devices such as noninvasive meibography and confocal microscopy provide new hope for better understanding of the pathophysiology of the gland and ocular surface dysfunction. Several clinical observations provide clues for future investigations of risk factors, pathophysiology, and novel treatment approaches. The prevalence of MGD in contact lens wearers is higher than expected, suggesting a role for the interplay between the tear film and meibomian gland function. A recent study of topical α-3 fatty acid supplementation demonstrated preliminary therapeutic potential, and along with other therapies, raises hope for an approach to management and prevention of MGD in the future. Since aging is a recognized risk factor for MGD and dry eye syndrome, it is possible that antiaging therapies, such as antioxidants, will be developed in the future.

**Surgical, Mechanical, or Physical Treatment**

Because compliance with prolonged, time-consuming therapies is traditionally poor, there is interest in treatment approaches that could provide long-lasting improvement with minimal application. Such new approaches, including surgical probing of the duct, were recently reported as a treatment for symptomatic MGD. The insertion of small stainless-steel probes (2, 4, or 6 mm in length) into the meibomian gland orifices and ducts was reported to relieve lid tenderness, improve vision, and reduce other symptoms of posterior blepharitis. In addition, several in-office eye-warming devices, thought to assist in improving meibomian gland secretions, have been tested or are in development. Long-term efficacy and safety are yet to be demonstrated with these techniques.

**Pharmacologic Treatments**

The treatment options for dry eye have been greatly expanded in the past 10 years, in large part due to improved understanding of the inflammatory process within the ocular surface functional unit. This understanding contributed to the development of treatment options such as cyclosporine, cevimeline, and pilocarpine, and additional options for the treatment of dry eye continue to be investigated. In contrast, the limited understanding of the pathophysiology of MGD has hampered the development of pharmacologic treatment of MGD. Inflammation, hormonal effects, oxidative stress, lipid production, postsecretion lipid changes, and aging are all important therapeutic considerations for the development of pharmacologic treatments for MGD. It is likely that the current enthusiasm of researchers regarding the study of the pathophysiology of MGD and the unmet needs of patients who have symptoms of this disease will drive development of new therapies.

Existing therapies, either alone or in conjunction with one another, require further evaluation in well-controlled masked

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**Table 3. Clinical Summary of the MGD Staging Used to Guide Treatment**

<table>
<thead>
<tr>
<th>Stage</th>
<th>MGD Grade</th>
<th>Symptoms</th>
<th>Corneal Staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+ (minimally altered expressibility and secretion quality)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>++ (mildly altered expressibility and secretion quality)</td>
<td>Minimal to Mild</td>
<td>None to limited</td>
</tr>
<tr>
<td>3</td>
<td>+++ (moderately altered expressibility and secretion quality)</td>
<td>Moderate</td>
<td>Mild to moderate; mainly peripheral</td>
</tr>
<tr>
<td>4</td>
<td>++++ (severely altered expressibility and secretion quality)</td>
<td>Marked</td>
<td>Marked; central in addition</td>
</tr>
<tr>
<td>“Plus” disease</td>
<td>Co-existing or accompanying disorders of the ocular surface and/or eyelids</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 4. Treatment Algorithm for MGD

<table>
<thead>
<tr>
<th>Stage</th>
<th>Clinical Description</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No symptoms of ocular discomfort, itching, or photophobia</td>
<td>Inform patient about MGD, the potential impact of diet, and the effect of work/home environments on tear evaporation, and the possible drying effect of certain systemic medications. Consider eyelid hygiene including warming/expression as described below (±)</td>
</tr>
<tr>
<td></td>
<td>Clinical signs of MGD based on gland expression</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Minimally altered secretions: grade ≥2–4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expressibility: 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No ocular surface staining</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Minimal to mild symptoms of ocular discomfort, itching, or photophobia</td>
<td>Advise patient on improving ambient humidity; optimizing workstations and increasing dietary omega-3 fatty acid intake (±)</td>
</tr>
<tr>
<td></td>
<td>Minimal to mild MGD clinical signs</td>
<td>Institute eyelid hygiene with eyelid warming (a minimum of four minutes, once or twice daily) followed by moderate to firm massage and expression of MG secretions (+)</td>
</tr>
<tr>
<td></td>
<td>Scattered lid margin features</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mildly altered secretions: grade ≥4–≤8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expressibility: 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None to limited ocular surface staining: DEWS grade 0–7; Oxford grade 0–5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Moderate symptoms of ocular discomfort, itching, or photophobia with limitations of activities</td>
<td>All the above, plus</td>
</tr>
<tr>
<td></td>
<td>Moderate MGD clinical signs</td>
<td>Oral tetracycline derivatives (+)</td>
</tr>
<tr>
<td></td>
<td>† lid margin features: plugging, vascularity</td>
<td>Lubricant ointment at bedtime (±)</td>
</tr>
<tr>
<td></td>
<td>Moderately altered secretions: grade ≥8 to &lt;13</td>
<td>Anti-inflammatory therapy for dry eye as indicated (±)</td>
</tr>
<tr>
<td></td>
<td>Expressibility: 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mild to moderate conjunctival and peripheral corneal staining, often inferior: DEWS grade 8–23; Oxford grade 4–10</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Marked symptoms of ocular discomfort, itching or photophobia with definite limitation of activities</td>
<td>All the above, plus</td>
</tr>
<tr>
<td></td>
<td>Severe MGD clinical signs</td>
<td>Anti-inflammatory therapy for dry eye (+)</td>
</tr>
<tr>
<td></td>
<td>† lid margin features: dropout, displacement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severely altered secretions: grade ≥13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Expressibility: 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased conjunctival and corneal staining, including central staining: DEWS grade 24–33; Oxford grade 11–15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>† signs of inflammation: ≥moderate conjunctival hyperemia, phlyctenules</td>
<td></td>
</tr>
<tr>
<td></td>
<td>‘Plus’ disease Specific conditions occurring at any stage and requiring treatment. May be causal of, or secondary to, MGD or may occur incidentally</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1. Exacerbated inflammatory ocular surface disease</td>
<td>1. Pulsed soft steroid as indicated</td>
</tr>
<tr>
<td></td>
<td>2. Mucosal keratinization</td>
<td>2. Bandage contact lens/scleral contact lens</td>
</tr>
<tr>
<td></td>
<td>3. Phlyctenular keratitis</td>
<td>3. Steroid therapy</td>
</tr>
<tr>
<td></td>
<td>4. Trichiasis (e.g. in cicatricial conjunctivitis, ocular cicatricial pemphigoid)</td>
<td>4. Epilation, cryotherapy</td>
</tr>
<tr>
<td></td>
<td>5. Chalazion</td>
<td>5. Intralidional steroid or excision</td>
</tr>
<tr>
<td></td>
<td>6. Anterior blepharitis</td>
<td>6. Topical antibiotic or antibiotic/steroid</td>
</tr>
<tr>
<td></td>
<td>7. Demodex-related anterior blepharitis, with cylindrical dandruff</td>
<td>7. Tca tree oil scrubs</td>
</tr>
</tbody>
</table>

**Meibum quality** is assessed in each of eight glands of the central third of the lower lid on a scale of 0 to 3 for each gland: 0, clear; 1, cloudy; 2, cloudy with debris (granular); and 3, thick, like toothpaste (total score range, 0–24). **Expressibility** is assessed on a scale of 0 to 3 in five glands in the lower or upper lid, according to the number of glands expressible: 0, all glands; 1, three to four glands; 2, one to two glands; and 3, no glands. **Staining scores** are obtained by summing the scores of the exposed cornea and conjunctiva. Oxford staining score range, 1–15; DEWS staining score range, 0–33.
randomized clinical trials of adequate sample size. Several promising pharmacologic therapies are currently being evaluated, and with the renewed interest in MGD, the future is bright for new therapeutic options.

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The International Workshop on Meibomian Gland Dysfunction: Report of the Clinical Trials Subcommittee

Penny A. Asbell,1 Fiona J. Stapleton,2 Kerstin Wickström,3 Esen K. Akpek,4 Pasquale Aragona,5 Reza Dana,6 Michael A. Lemp,7 and Kelly K. Nichols8

The objective of this subcommittee was to summarize the evidence in clinical trials on meibomian gland dysfunction (MGD) and to use this information to make recommendations for best-practice clinical trial design for this condition. We conducted a PubMed and Medline literature review (through the end of 2009) to identify treatment or observational trials. Our search terms were those commonly used interchangeably with MGD, including (in addition to MGD) posterior blepharitis, meibomian gland disease, and tarsal gland disease. The level of evidence for each study was classified (Table 1) according to American Academy of Ophthalmology (AAO) Classification Scheme. In short, level I evidence includes evidence obtained from at least one properly conducted, well-designed randomized controlled trial. It could include meta-analyses of randomized controlled trials. Level II includes evidence obtained from well-designed controlled trials without randomization, preferably from more than one clinical center or from multiple-time series with or without the intervention. Level III includes evidence obtained from descriptive studies, case reports, or reports of expert committees/organizations (e.g., panel consensus with external peer review). Additional information on levels of evidence is found in Table 1 of The Report on Management and Therapy. In some cases, the trial designs were not sufficiently described to have more than a tentative grading. Further, recent publications (August 2009 and later) were purposely excluded from Table 1.

Articles were reviewed according to the key components that are necessary for protocol design in determining safety and efficacy of a new treatment: objectives, trial design and methodology, patient group, inclusion criteria, exclusion criteria, outcome measures, treatment, and statistical considerations. We also evaluated clinical trials that had been registered at ClinicalTrials.gov if they included a summary of key trial design features. Further, a summary of key design features of the registered trials plus recommendations for future trials are suggested.

The review and summary were also based on the committee’s personal expertise, including experience in clinical trials in ocular disease and in MGD. The initial search was performed in March 2009 and updated in July 2009. Twenty-six eligible papers1–26 were identified and reviewed.

During the review, committee members found that the study investigators in the published papers often had not been explicit in describing their methods. As a result, the members, who conducted their reviews independently of one another, often interpreted the available data differently. The various interpretations are included in this summary.

Few publications qualify as well-designed randomized controlled trials. Aside from the three studies graded level I, there are additional trials that were randomized and controlled. Some were open-label with very small sample sizes and seemed to be lacking information on the statistical planning of the study. We expect that these smaller open-label studies will continue to provide information that will lead to larger placebo-controlled double-masked randomized clinical trials.

KEY TOPICS TO BE ADDRESSED

Trial Objectives

Overview and Results. A summary of key trial objectives and design for the 26 studies1–26 reviewed is detailed in Table 2. Of the 26 published articles, 25 reported the use of a treatment for MGD. Of those 25, 24 were considered interventional studies. Twenty-two (84.6%) of the 26 studies had as their objective the assessment of efficacy of a therapeutic approach. Of the 26, 9 (34.6%) were noncomparative. Of the remaining studies, most compared the treatment approach of interest with a traditional or palliative treatment, such as use of hot compresses or artificial tears, whereas several studies used a nontreatment control group for comparison.

Trial Design and Methodology

Overview and Results. The MGD clinical studies primarily comprised trials with fewer than 40 participants and were of short (<3 months) duration. Although most were prospective, fewer than half used a randomized controlled design, and only three were double-masked.

Twenty-four (92.3%) of the reviewed studies were interventional. Only one of those evaluated a surgical intervention, two evaluated a medical device, and the remainder assessed the efficacy of a supplement, drug intervention, or warm-compress therapy.

The majority of the trials (21/26, 80.8%) had a prospective component; some of the studies also reported a preliminary retrospective evaluation. Of the 26 studies reviewed, 16 used either a control group (e.g., normal/healthy group) or a pla-
In most of the studies, adult patients between 18 and 70 years of age were included. The age of the sample was not reported in two studies (Table 2). Clinical testing for entry in the study or as an outcome was described in 23 of the 26 studies reviewed (Table 3). Of the 26 studies, 13 (50.0%) included symptoms as an entry criterion, diagnostic criterion, or an outcome. The symptoms described are those primarily associated with dry eye disease; only one (3.8%) study specifically examined MGD symptoms in the presence and absence of dry eye disease.

MGD was clinically defined most frequently through the evaluation of meibomian gland obstruction and/or gland dropout and abnormal gland secretions. Of the 26 papers, 12 (46.2%) reported meibomian gland obstruction, 14 (53.8%) contained assessments of secretions, and 8 (30.8%) involved transillumination of the glands or meibography. Descriptions of lid abnormalities including erythema, irregularity of lid margins, lid margin thickening, and/or telangiectasia were included in 10 (38.5%) studies. Four (15.3%) papers specifically examined patients with evidence of lid inflammation, but did not define the symptom further.

It was of interest to note that tests typically performed in dry eye clinical trials, such as fluorescein tear break-up time (FTBUT), conjunctival and corneal staining, and Schirmer’s test, were included in only approximately 5% of the studies in defining the MGD subjects. Most studies, however, did evaluate these signs at baseline and in follow-up visits, and such signs were often used as outcome measures (Table 3). FTBUT was the most frequently reported clinical test in the studies evaluated (14/26, 53.8%).

Nearly all studies excluded subjects who had recent eye surgery or were current contact lens (CL) wearers, except for one study on MGD in association with CL intolerance and one study in which contact lens wear was allowed. Three studies specifically targeted MGD with concurrent skin disease, such as acne rosacea.

Comments. In summary, past MGD clinical trials did not have a uniform way of defining the study population, although symptoms and changes in the lid, especially plugging and abnormal secretions, were the most common clinical characteristics used to define the clinical sample of patients. Of note, dry eye disease was not typically either specifically included or excluded in selecting patients other than in selecting subjects with symptoms. Signs of dry eye disease were not generally used as selection criteria, although they were frequently included in the study design as outcome measures.

Inclusion Criteria

Overview. In approximately half of the identified studies, adult patients with a known history of MGD (12/26, 46.1%) or chronic blepharitis were enrolled, whereas specific eyelid findings were reported as entry qualifications for others. In three of the studies, the patients had to have clinical evidence of facial acne rosacea to be included. A previously established classification system was used in three studies, whereas in the remaining studies, no specific published criteria were routinely used. Several studies reported no specific inclusion criteria other than a diagnosis of MGD or posterior blepharitis. How these diagnoses were determined was not disclosed. A list of the details related to inclusion in the 26 studies can be seen in Tables 4 and 5.

Results. In most of the studies, adult patients between 18 and 70 years of age with chronic signs and symptoms of blepharitis or MGD were enrolled. In three of the studies (two prospective and one retrospective case series), children were included. The age of the participants was not mentioned in two studies. In three studies, treatment with warm compresses and lid scrubbing had to fail for the patient to be included.
TABLE 2. Study Design and Descriptive Features

<table>
<thead>
<tr>
<th>Ref.</th>
<th>First Author</th>
<th>Evidence Level</th>
<th>Title</th>
<th>Journal/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Perry HD</td>
<td>I</td>
<td>Efficacy of commercially available topical cyclosporine A 0.05% in the treatment of meibomian gland dysfunction</td>
<td><em>Cornea.</em> 2006 Feb;25(2):171-175</td>
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<tr>
<td>15</td>
<td>Olson MC</td>
<td>II</td>
<td>Increase in tear film lipid layer thickness following treatment with warm compresses in patients with meibomian gland dysfunction</td>
<td><em>Eye Contact Lens.</em> 2003;29(2):96-99</td>
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<tr>
<td>16</td>
<td>Paugh JR</td>
<td>II</td>
<td>Meibomian therapy in problematic contact lens wear</td>
<td><em>Optom Vis Sci.</em> 1990;67(11):803-806</td>
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<tr>
<td>18</td>
<td>Pinna A</td>
<td>II</td>
<td>Effect of oral linoleic and gamma-linolenic acid on meibomian gland dysfunction</td>
<td><em>Cornea.</em> 2007;26(3):260-264</td>
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<tr>
<td>1</td>
<td>Albietz JM</td>
<td>III</td>
<td>Effect of antibacterial honey on the ocular flora in tear deficiency and meibomian gland disease</td>
<td><em>Cornea.</em> 2006;25:1012-1019</td>
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<tr>
<td>2</td>
<td>Blackie CA</td>
<td>III</td>
<td>Inner eyelid surface temperature as a function of warm compress methodology</td>
<td><em>Optom Vis Sci.</em> 2008;85(8):675-683</td>
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<td>3</td>
<td>Cetinkaya A</td>
<td>III</td>
<td>Pediatric ocular acne rosacea: longterm treatment with systemic antibiotics</td>
<td><em>Am J Ophthalbmal.</em> 2006;142(5):816-821</td>
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<td>4</td>
<td>Dougherty JM</td>
<td>III</td>
<td>The role of tetracycline in chronic blepharitis. Inhibition of lipase production in staphylococci</td>
<td><em>Invest Ophthalbmal Vis Sci.</em> 1991;32(11):2970-2975</td>
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<tr>
<td>5</td>
<td>Epstein GA</td>
<td>III</td>
<td>Combined excision and drainage with intralesional corticosteroid injection in the treatment of chronic chalazia</td>
<td><em>Arch Ophthalbmal.</em> 1988;106(4):514-516</td>
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<tr>
<td>7</td>
<td>Goto E</td>
<td>III</td>
<td>Treatment of non-inflamed obstructive meibomian gland dysfunction by an infrared warm compression device</td>
<td><em>Br J Ophthalbmal.</em> 2002;86(12):1403-1407</td>
</tr>
<tr>
<td>9</td>
<td>Ishida R</td>
<td>III</td>
<td>Tear film with &quot;Orgahexa EyeMasks&quot; in patients with meibomian gland dysfunction</td>
<td><em>Optom Vis Sci.</em> 2008;85(8):684-691</td>
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<td>11</td>
<td>Matsumoto Y</td>
<td>III</td>
<td>Efficacy of a new warm moist air device on tear functions of patients with simple meibomian gland dysfunction</td>
<td><em>Cornea.</em> 2006;25(6):644-650</td>
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<td>22</td>
<td>Shine WE</td>
<td>III</td>
<td>Minocycline effect on meibomian gland lipids in meibomianits patients</td>
<td><em>Exp Eye Res.</em> 2003;76(4):417-420</td>
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<td>Ref.</td>
<td>Treatment</td>
<td>Interventional</td>
<td>Efficacy Assessed?</td>
<td>Comparative?</td>
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<td>Y (clinical samples, no direct patient treatment)</td>
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<th>Randomized</th>
<th>Masked</th>
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<td>17</td>
<td>5 months</td>
<td>Prospective</td>
<td>Y</td>
<td>Y-double</td>
</tr>
<tr>
<td>21</td>
<td>3 months</td>
<td>Prospective</td>
<td>Y</td>
<td>Y-double</td>
</tr>
<tr>
<td>26</td>
<td>1 month</td>
<td>Prospective</td>
<td>Y</td>
<td>Y-patient</td>
</tr>
<tr>
<td>8</td>
<td>2 week washout, 1 month</td>
<td>Prospective, cross-over</td>
<td>Y</td>
<td>Y-double</td>
</tr>
<tr>
<td>20</td>
<td>3 months</td>
<td>Prospective</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>10</td>
<td>2 weeks</td>
<td>Prospective</td>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>12 weeks</td>
<td>Prospective</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>5, 15, 30 minutes following application (same day)</td>
<td>Prospective</td>
<td>Y (contralateral eye)</td>
<td>N</td>
</tr>
<tr>
<td>16</td>
<td>2 weeks</td>
<td>Prospective</td>
<td>Y (contralateral eye)</td>
<td>N-examiner</td>
</tr>
<tr>
<td>18</td>
<td>6 months</td>
<td>Prospective</td>
<td>Y</td>
<td>Y-examiner</td>
</tr>
<tr>
<td>19</td>
<td>6 weeks</td>
<td>Prospective</td>
<td>N</td>
<td>Y-photo grader</td>
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<tr>
<td>24</td>
<td>8 weeks</td>
<td>Prospective</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>25</td>
<td>3 months</td>
<td>Prospective</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>14</td>
<td>2 weeks</td>
<td>Prospective</td>
<td>N</td>
<td>N (only interferometry grader was masked)</td>
</tr>
<tr>
<td>1</td>
<td>5 months</td>
<td>Prospective</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>2</td>
<td>Same day study</td>
<td>Same day study</td>
<td>Y (contralateral eye)</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>12–36 months</td>
<td>Retrospective case series</td>
<td>N</td>
<td>N</td>
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<tr>
<td>4</td>
<td>24 hours</td>
<td>Retrospective, sample collection</td>
<td>N</td>
<td>N</td>
</tr>
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<td>5</td>
<td>6 weeks</td>
<td>Initially retrospective, second part prospective</td>
<td>N</td>
<td>N</td>
</tr>
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<td>6</td>
<td>Same day study</td>
<td>Same day study</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>2 weeks</td>
<td>Prospective</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>9</td>
<td>2 weeks</td>
<td>Prospective</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>11</td>
<td>2 weeks</td>
<td>Prospective</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>13</td>
<td>Up to 12 months</td>
<td>Prospective case series</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>22</td>
<td>5 months on Tx, 3 months off Tx</td>
<td>Prospective</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>23</td>
<td>Same day study</td>
<td>Same day study</td>
<td>N</td>
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(continues)
### Table 2 (continued). Study Design and Descriptive Features

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Placebo or Control</th>
<th>Subject Group (n, Group)</th>
<th>Age Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Y</td>
<td>n = 33 enrolled, n = 16 (12 completed) treatment (Tx), n = 17 (14 completed) placebo</td>
<td>18 and older, average age, not given</td>
</tr>
<tr>
<td>21</td>
<td>Y</td>
<td>n = 57, n = 21 Tx, n = 16 placebo</td>
<td>18 and older, average age, ~72.6 y</td>
</tr>
<tr>
<td>26</td>
<td>Y</td>
<td>n = 150 enrolled (n = 139 completed), n = 50 high dose, n = 50 low dose, n = 50 placebo</td>
<td>18 and older, average age, ~47.2 y</td>
</tr>
<tr>
<td>8</td>
<td>Y</td>
<td>n = 20, 10 per group</td>
<td>18 and older, average age, 52.1 y</td>
</tr>
<tr>
<td>20</td>
<td>N</td>
<td>n = 30, 15 per group</td>
<td>18 and older, average age, ~51 y</td>
</tr>
<tr>
<td>10</td>
<td>Y</td>
<td>n = 21, n = 11 Tx and n = 10 placebo</td>
<td>18 and older, average age, 63.7 y</td>
</tr>
<tr>
<td>12</td>
<td>Y</td>
<td>n = 27, n = 16 Tx, n = 11 control</td>
<td>18 and older, average age, ~65 y</td>
</tr>
<tr>
<td>15</td>
<td>Y</td>
<td>n = 20</td>
<td>range, 26–59 y</td>
</tr>
<tr>
<td>16</td>
<td>Y</td>
<td>n = 21</td>
<td>range, 22–33 y</td>
</tr>
<tr>
<td>18</td>
<td>Y</td>
<td>n = 57 (49 completed), 19 per group</td>
<td>18 and older, average age, 50 y</td>
</tr>
<tr>
<td>19</td>
<td>N</td>
<td>n = 37 enrolled (26 completed)</td>
<td>18 and older, average age, 57 y</td>
</tr>
<tr>
<td>24</td>
<td>Y</td>
<td>n = 20 patients, 10 per group</td>
<td>18 and older, average age, 66 y</td>
</tr>
<tr>
<td>25</td>
<td>Y</td>
<td>n = 40, n = 22 Tx, n = 18 control</td>
<td>18 and older, average age, ~43 y</td>
</tr>
<tr>
<td>14</td>
<td>Y</td>
<td>n = 25 (17 treated, 8 untreated)</td>
<td>18 and older, average age, 53.6 y</td>
</tr>
<tr>
<td>1</td>
<td>Y</td>
<td>n = 84 (49 completed), of those enrolled 15 MGD and 20 MGD with tear deficiency</td>
<td>18 and older, average age, ~59 y</td>
</tr>
<tr>
<td>2</td>
<td>Y</td>
<td>n = 32 normal patients, group A (n = 10), B (n = 10) and C (n = 12)</td>
<td>18 and older, average age, 54.7 y</td>
</tr>
<tr>
<td>3</td>
<td>N</td>
<td>n = 4</td>
<td>range, 4–12 y</td>
</tr>
<tr>
<td>4</td>
<td>N</td>
<td>MKC n = 2 samples (isolates), <em>Staphylococcus</em> blepharitis, 2 samples (isolates)</td>
<td>Not defined</td>
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<tr>
<td>5</td>
<td>N</td>
<td>n = 298, first 146 patients: 88 surgery only, 58 surgery and steroids in combination. Additionally 152 patients with combined treatment.</td>
<td>range, 6–88 y, most &gt;50 y</td>
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<td>6</td>
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<td>n = 6</td>
<td>18 and older, average age, 45.8 y</td>
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<td>N</td>
<td>n = 37</td>
<td>18 and older, average age, ~55 y</td>
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<td>Y</td>
<td>n = 42, n = 20 Tx, n = 22 control</td>
<td>18 and older, average age, 54.5 y</td>
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<td>n = 35, n = 15 MGD, n = 20 control</td>
<td>18 and older, average age, ~58.8 y</td>
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<td>n = 5</td>
<td>range, 4–9 y</td>
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<td>n = 10</td>
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<tr>
<td>23</td>
<td>N</td>
<td>n = 46, chronic blepharitis n = 36, controls n = 10</td>
<td>range, 30–40 y</td>
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</table>
Several parameters were used, including symptomatology, lid margin, and ocular surface findings by slit lamp examination and dry eye findings, as follows:

- **Symptomatology.** No specific MGD questionnaire has been developed or validated to date. Only seven studies (7/26, 26.9%) used single or multiple patient symptoms or questionnaires specifically as inclusion factors, whereas three studies cited failure of conventional therapy. (It is unclear how failure was assessed; some examples of therapy were provided.) Published studies generally report on main symptom types: discomfort, visual disturbance, and ocular appearance. The main symptoms reported by patients in questionnaires or interviews in the studies assessed included dryness (6/26, 23.1%) and discomfort or foreign body sensation (6/26, 23.1%). These symptoms were usually graded subjectively as mild, moderate, or severe. Few studies applied questionnaires (mostly questionnaires used in dry eye studies) at entry into as well as exit from the study, but in general they did not require a certain level of symptomatology as a specific entry criterion.

- **Lid margin findings.** Lid margin signs are the most frequently reported inclusion criteria. Signs included posterior lid margin erythema/hyperemia, lid margin thickening/irregularity, meibomian gland orifice plugging, turbidity of meibomian gland secretions, lid margin telangiectasia, and meibomian gland plugging. Meibomian gland plugging was the single most common lid margin finding to be used as an inclusion criterion (8/26, 30.8%).

- **Ocular surface findings.** Findings included, but were not limited to, corneal infiltrates, neovascularization, bulbar con-
junctival hyperemia, and tarsal conjunctival papillae. Studies that involved patients with ocular rosacea listed ocular surface findings as inclusion criteria.

● Dry eye findings. It is notable that dry eye signs were inclusion criteria in several of the papers. They were used either to define groups or as an indicator of tear film stability. Dry eye signs included a low tear film breakup time (TFBUT), presence and degree of corneal staining using fluorescein, presence and degree of conjunctival staining as determined with rose bengal or lissamine green, and Schirmer’s test for measuring aqueous tear production and flow.

Comments. Except for the lid margin findings determined by slit lamp examination, there seemed to be no specific and consistent inclusion criteria for blepharitis or MGD, which are different from the criteria commonly used in dry eye studies. This deficiency is perhaps not unexpected, as the overlap between MGD and dry eye has yet to be fully understood. In general, symptoms associated with MGD may be related to altered tear film stability and evaporative dry eye. The most commonly used inclusion criteria in MGD studies to date are symptoms of discomfort/foreign body sensation and signs of meibomian gland plugging, expressibility of the meibomian glands, and quality of gland secretions.

Exclusion Criteria

Overview. The exclusion criteria are reported in 17 (65.4%) studies (Tables 4, 5B). The exclusion criteria varied according to the objectives of each trial and the sample of patients included. It is therefore possible to classify the papers in four different types, divided according to the purpose of the trial, the terminology used by the authors, and the patients included:

### Table 3 (continued). Clinical Characteristics and Symptoms Assessed as Either Entry Criteria or Outcomes

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Transillumination (Obstruction and Dropout)</th>
<th>MG Obstruction</th>
<th>MG Secretions</th>
<th>Interferometry</th>
<th>Eyelid Temperature</th>
<th>Lid Debris</th>
<th>Lid Edema/Thickening</th>
<th>Irregular Lid</th>
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<tr>
<td>8</td>
<td>30.8%</td>
<td>46.2%</td>
<td>53.8%</td>
<td>19.2%</td>
<td>11.5%</td>
<td>3.8%</td>
<td>26.9%</td>
<td>14.4%</td>
</tr>
</tbody>
</table>

(continues)
1. Obstructive meibomian gland dysfunction (nine papers)
2. Posterior blepharitis (six papers)
3. Seborrhea with secondary meibomianitis (one paper)
4. Meibomian therapy in CL wearers (one paper).

Furthermore, it is possible to classify the exclusion criteria reported into three different categories: (1) ocular disease-related, (2) iatrogenic, and (3) systemic disease-related.

**Results.** The 26 papers were reviewed for exclusion criteria, and in the 17 papers that included a description of exclusion criteria, 39 distinct criteria were reported. The most frequently were CL use (10/17, 58.8%), history of ocular surgery (7/17, 41.2%), and eye disorders affecting the ocular surface (6/17, 35.3%). The entire list of exclusion criteria and their frequency of citation is shown in Table 5B.

Considering the different goals of the studies included and the terminology used by the authors the exclusion criteria can be grouped as follows:

1. **Papers about obstructive MGD treatment.** In this group, three studies included patients with noninflamed obstructive MGD, one included patients with obstructive MGD and lid inflammation, three included MGD patients, and two described “simple” MGD. Seven studies evaluated different types of warm compress and lid hygiene for treatment of MGD. In these, the exclusion criteria were anterior blepharitis of more than moderate severity; infectious conjunctivitis; meibomitis; seborrheic MGD, and excessive meibomian lipid secretion; ocular adnexal pathology interfering with warm compress application; CL use; diabetes; current use of treatments for blepharitis; eyelid surgery; presence...
of dry eye conditions other than MGD; history of Stevens-Johnson syndrome; chemical, thermal and radiation injury; topical drugs; and surgery or procedures that might create ocular surface problems.

2. Papers about posterior blepharitis (a term often used synonymously with MGD in the literature). This group included six studies that evaluated the effect of different types of antibiotic and anti-inflammatory pharmacologic treatment of posterior blepharitis. All patients had, to some extent, an inflammatory condition. The exclusion criteria in this group of studies were topical therapy within 2 weeks before the beginning of the study, systemic treatment with other antibiotic or anti-inflammatory agents, plugs, CL wear, active ocular diseases other than blepharitis, lid abnormalities, fungal or viral infections, ocular surface surgery or other inflammatory ocular surface diseases such as Sjögren’s syndrome and Steven-Johnson syndrome, and thermal, chemical, or radiation injury.

3. Seborrhea with secondary meibomianitis. One study is included in this group, a trial studying the effect of the α6 fatty acid γ-linolenic acid on MGD patients. The reported exclusion criteria were infectious keratoconjunctivitis; in-

<table>
<thead>
<tr>
<th>Ref.</th>
<th>First Author</th>
<th>Evidence Level</th>
<th>Title</th>
<th>Inclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>Perry HD</td>
<td>I</td>
<td>Efficacy of Commercially Available Topical Cyclosporine</td>
<td>Adult patients with slit lamp diagnosis of meibomian gland dysfunction, with an</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>A 0.05% in the Treatment of Meibomian Gland Dysfunction</td>
<td>OSDI score of 12.</td>
</tr>
<tr>
<td>21</td>
<td>Scheckter BA</td>
<td>I</td>
<td>Efficacy of Topical Cyclosporine for the Treatment of Ocular Rosacea</td>
<td>Adult patients with rosacea-associated eyelid and corneal changes.</td>
</tr>
<tr>
<td>26</td>
<td>Yoo SE</td>
<td>I</td>
<td>The Effect of Low-Dose Doxycycline Therapy in Chronic Meibomian Gland Dysfunction</td>
<td>Adult patients with newly diagnosed chronic meibomian gland dysfunction with grade 2 or worse meibomian gland destruction or meibomian gland orifice obstruction, and whose symptoms failed to improve despite warm compression, lid massage, lid scrub, and topical eye drops or ointment therapy for more than 2 months.</td>
</tr>
<tr>
<td>20</td>
<td>Rubin M</td>
<td>I–II</td>
<td>Efficacy of Topical Cyclosporin 0.65% in the Treatment of Meibomian Gland Dysfunction</td>
<td>Adult patients presenting with posterior blepharitis defined as lid erythema and MG telangiectasia.</td>
</tr>
<tr>
<td>10</td>
<td>Lucas J</td>
<td>II</td>
<td>Efficacy of Topical Azithromycin Ophthalmic Solution 1% in the Treatment of Posterior Blepharitis</td>
<td>Adult patients with a diagnosis of poster blepharitis by a qualified ophthalmologist; patients must have a grade of at least 2 of lid redness/swelling and gland plugging.</td>
</tr>
<tr>
<td>12</td>
<td>Matsamoto Y</td>
<td>II</td>
<td>The Evaluation of the Treatment Response in Obstructive Meibomian Gland Disease by in Vivo Laser Confocal Microscopy</td>
<td>Consecutive adult patients with severe obstructive MGD associated with lid inflammation.</td>
</tr>
<tr>
<td>15</td>
<td>Olson MC</td>
<td>II</td>
<td>Increase Tear Film Lipid Layer Thickness Following Treatment with Warm Compresses in Patients with Meibomian Gland Dysfunction</td>
<td>Consecutive adult patients with symptoms of ocular dryness were enrolled with the following criteria: (1) subjective dry eye status determined by a score of ≥6 on dry eye symptoms questionnaire; (2) meibomian gland obstruction determined by SIE; (3) THIETF baseline of ≥50 mm; as well as fluorescein tear breakup time and Schirmer results of ≤10 mm/5 min.</td>
</tr>
<tr>
<td>16</td>
<td>Paugh JR</td>
<td>II</td>
<td>Meibomian Therapy in Problematic Contact Lens Wear</td>
<td>Adult patients with chronic posterior blepharitis defined by redness, thickening, or irregularity of the lid margin, telangiectasia, reduced or no secretions, poor quality secretions, gland capping.</td>
</tr>
<tr>
<td>18</td>
<td>Punna A</td>
<td>II</td>
<td>Effect of Oral Linoleic and Gamma-Linolenic Acid on Meibomian Gland Dysfunction</td>
<td>Consecutive adult contact lens wearers with (1) minimal or transient symptoms of dryness, (2) cloudy or absent MG secretions, and (3) CL intolerance not related to lens or solution parameters.</td>
</tr>
<tr>
<td>19</td>
<td>Romero MJ</td>
<td>II</td>
<td>Conservative Treatment of Meibomian Gland Dysfunction</td>
<td>Group 4 and 5 of McCulley Classification system for MGD (McCulley et al. Classification of chronic blepharitis).</td>
</tr>
<tr>
<td>24</td>
<td>Souchier M</td>
<td>II</td>
<td>Changes in Meibomian Fatty Acids and Clinical Signs in Patients with Meibomian Gland Dysfunction after Minocycline Treatment</td>
<td>Adult patients with chronic posterior blepharitis; patients not clinically defined.</td>
</tr>
<tr>
<td>25</td>
<td>Yalcin E</td>
<td>II</td>
<td>N-Acetyllysine in Chronic Blepharitis</td>
<td>Healthy adult individuals.</td>
</tr>
<tr>
<td>14</td>
<td>Mori A</td>
<td>II–III</td>
<td>Disposable Eyelid Warming Device for the Treatment of Meibomian Gland Dysfunction</td>
<td>Rosacea patients younger than 12 years of age, with or without obvious skin involvement, who were having active inflammation with ocular discomfort, photophobia, and red eyes, despite topical steroid, antibiotic or isotopes from patients with meibomian keratoconjunctivitis and staphylococcal blepharitis; patients not clinically defined.</td>
</tr>
<tr>
<td>1</td>
<td>Albutz JM</td>
<td>III</td>
<td>Effect of Antibacterial Honey on the Occular Flora in Tear Deficiency and Meibomian Gland Disease</td>
<td>Adult and pediatric patients undergoing surgical excision of chronic chalazia; patients not clinically defined.</td>
</tr>
<tr>
<td>2</td>
<td>Blackie CA</td>
<td>III</td>
<td>Inner Eyelid Surface Temperature as a Function of Warm Compresses Methodology</td>
<td>Adult patients with MGD unresponsive to conventional treatment, which could include lid hygiene and topical artificial tears, antibiotics (oral/topical) and/or corticosteroids.</td>
</tr>
<tr>
<td>3</td>
<td>Cetinkaya A</td>
<td>III</td>
<td>Pediatric Ocular Acne Rosacea Long-Term Treatment with Systemic Antibiotics</td>
<td>Adult patients with MGD defined as (1) occluded MG orifices, (2) cloudy secretions, (3) keratinization and/or macrocystic dilation, obstruction, and (4) nonnalulmed lid margins.</td>
</tr>
<tr>
<td>4</td>
<td>Dougherty JM</td>
<td>III</td>
<td>The Role of Tetracycline in Chronic Blepharitis; Inhibition of Lipase Production in Staphylococci</td>
<td>Adult patients with acute blepharitis for more than 6 months, categorized into six blepharitis groups.</td>
</tr>
<tr>
<td>5</td>
<td>Epstein GA</td>
<td>III</td>
<td>Combined Excision and Drainage with Intraleseal Corticosteroid Injection in the Treatment of Chronic Chalazia</td>
<td>Adult patients with MGD defined as (1) occluded MG orifices, (2) cloudy secretions, (3) keratinization and/or macrocystic dilation, obstruction, and (4) nonnalulmed lid margins.</td>
</tr>
<tr>
<td>6</td>
<td>Goto E</td>
<td>III</td>
<td>Improvement of Tear Stability Following Warm Compression in Patients with Meibomian Gland Dysfunction</td>
<td>Adult patients with MGD defined as (1) occluded MG orifices, (2) cloudy secretions, (3) keratinization and/or macrocystic dilation, obstruction, and (4) nonnalulmed lid margins.</td>
</tr>
<tr>
<td>7</td>
<td>Goto E</td>
<td>III</td>
<td>Treatment of Non-Inflamed Obstructive Meibomian Gland Dysfunction by an Infrared Warm Compression Device</td>
<td>Children with chronic lid margin inflammation (lid redness and thickening).</td>
</tr>
<tr>
<td>9</td>
<td>Ishida R</td>
<td>III</td>
<td>Tear Film with ‘Orgahexa Eyelash’ in Patients with Meibomian Gland Dysfunction</td>
<td>Patients were selected based on clinical appearance and categorized as having acne rosacea with or without meibomianitis, or seborrheic blepharitis alone.</td>
</tr>
<tr>
<td>11</td>
<td>Matsamoto Y</td>
<td>III</td>
<td>Efficacy of a New Warm Moist Air Device on Tear Functions of Patients with Simple Meibomian Gland Dysfunction</td>
<td>Adult patients with resistance to conventional treatment; which could include lid hygiene and topical artificial tears, antibiotics (oral/topical) and/or corticosteroids.</td>
</tr>
<tr>
<td>13</td>
<td>Meider DM</td>
<td>III</td>
<td>Oral Erythromycin Treatment For Childhood Blepharokeratitis</td>
<td>Adult patients with anterior blepharitis for more than 6 months, categorized into six blepharitis groups.</td>
</tr>
<tr>
<td>22</td>
<td>Shine WE</td>
<td>III</td>
<td>Minocycline Effect on Meibomian Gland Lipids in Meibomianits Patients</td>
<td>(continues)</td>
</tr>
<tr>
<td>23</td>
<td>Song CH</td>
<td>III</td>
<td>Enhanced Scectory Group II PLA2 Activity in the Tears of Chronic Blepharitis Patients</td>
<td>(continues)</td>
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**Table 4 (continued). Inclusion and Exclusion Criteria**

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<tr>
<th>Ref.</th>
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<tr>
<td>17</td>
<td>Contact lens wear, active ocular disease other than blepharitis, surgery within past 3 months, active ocular allergy, use of isotinoin within the past 6 months, or have autoimmune disease requiring treatment.</td>
</tr>
<tr>
<td>21</td>
<td>Eyelid defects, lagophthalmos, active ingredient sensitivity, pregnant/nursing mothers.</td>
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<tr>
<td>26</td>
<td>Topical therapy within 2 weeks before the beginning of the study.</td>
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<tr>
<td>8</td>
<td>Anterior blepharitis of more than moderate severity, infectious conjunctivitis, MGD with acute inflammation, seborrheic MGD. No patients wore CLs; unclear if this was an exclusion criteria.</td>
</tr>
<tr>
<td>20</td>
<td>Current punctal plugs, doxycycline, steroids, women of childbearing age with no contraception.</td>
</tr>
<tr>
<td>10</td>
<td>Eyelid structural abnormalities, active inflammation, fungal or viral infection, ocular surgery in the past 90 days, including LASIK or glaucoma surgery.</td>
</tr>
<tr>
<td>12</td>
<td>History of Sjogren’s syndrome; Stevens-Johnson syndrome; chemical, thermal, or radiation injury; or any ocular surgery or procedure that would create an ocular surface problem. History of contact lens use.</td>
</tr>
<tr>
<td>15</td>
<td>Exclusions were not defined other than evidence of ocular disease.</td>
</tr>
<tr>
<td>16</td>
<td>History of ocular trauma or surgery, use of tear-influencing medications, systemic connective tissue disease, ocular conditions (blepharitis, meibomitis, or any anterior segment disease), contact lens intolerance due to poor lens fit, deposits, care system hypersensitivity.</td>
</tr>
<tr>
<td>18</td>
<td>Infectious keratoconjunctivitis, inflammatory disease unrelated to MGD. Schirmer I test result &lt;10 mm/5 min, concomitant ocular disease, previous ocular surgery, alterations of the lacrimal drainage system, concomitant topical ophthalmic medications, topical ophthalmic steroids taken during the 4 weeks before the study, treatment with systemic drugs affecting tearing.</td>
</tr>
</tbody>
</table>
| 19   | Current use of treatments for blepharitis, current use of topical or systemic steroids, topical or systemic antibiotics, or topical or systemic antimetabolites, history of contact lens wear, history of eyelid surgery, presence of any ocular disease provoking dry eye syndrome and |}

flammatary disease unrelated to MGD; Schirmer I test result <10 mm/5 minutes; concomitant ocular pathologies; a history of ocular surgery; alterations of the lacrimal drainage system; concomitant topical ophthalmic medications; topical ophthalmic steroids taken during the 4 weeks before the study; treatment with systemic drugs affecting tearing, pregnancy, or diabetes; and other systemic, neurologic, or dermatologic disorders affecting the health of the ocular surface.

4. Meibomian therapy in CL wearers. The single paper in this group discussed the treatment of MGD in CL wearers. The exclusion criteria in this article were a history of ocular trauma or surgery; use of tear-influencing medication (e.g., antihistamine, antianxiety, anticholinergic); systemic connective tissue disease; ocular conditions such as blepharitis, meibomianitis, and any anterior segment disease; CL intolerance related to lens fit; the presence of deposits; and known care system hypersensitivity or toxicity.

The 39 exclusion criteria can be divided into three categories: (1) ocular disease-related, (2) iatrogenic, or (3) systemic.
### Table 5. List and Frequency of Reported Criteria (All Studies Represented)

<table>
<thead>
<tr>
<th></th>
<th>A. Inclusion Criteria</th>
<th>Frequency (%)</th>
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<tbody>
<tr>
<td><strong>Age of Participants</strong></td>
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<tr>
<td>Adult patients</td>
<td>22/26 (84.6)</td>
<td></td>
</tr>
<tr>
<td>Pediatric patients</td>
<td>2/26 (2.7)</td>
<td></td>
</tr>
<tr>
<td>Adult and pediatric patients</td>
<td>1/26 (3.8)</td>
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</tr>
<tr>
<td>Not mentioned</td>
<td>1/26 (3.8)</td>
<td></td>
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<tr>
<td><strong>Symptomatology</strong></td>
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<tr>
<td>Ocular Surface Disease Index questionnaire</td>
<td>1/26 (3.8)</td>
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<tr>
<td>Symptoms fail to improve with conventional therapy</td>
<td>3/26 (11.5)</td>
<td></td>
</tr>
<tr>
<td>Discomfort or foreign body sensation</td>
<td>2/26 (7.7)</td>
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<tr>
<td>Eye redness</td>
<td>1/26 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Photophobia</td>
<td>1/26 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Ocular dryness</td>
<td>3/26 (11.5)</td>
<td></td>
</tr>
<tr>
<td>Contact lens intolerance</td>
<td>1/26 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Ocular Symptoms Scale</td>
<td>1/26 (3.8)</td>
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<tr>
<td><strong>Lid Margin Findings</strong></td>
<td></td>
<td></td>
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<tr>
<td>Previous or current diagnosis of MGD or posterior blepharitis</td>
<td>12/26 (46.1)</td>
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<tr>
<td>Posterior lid margin erythema or hyperemia</td>
<td>5/26 (19.2)</td>
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<tr>
<td>Eyelid edema</td>
<td>1/26 (3.8)</td>
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<tr>
<td>Lid margin thickening or irregularity</td>
<td>4/26 (15.4)</td>
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<tr>
<td>Meibomian gland orifice plugging</td>
<td>8/26 (30.8)</td>
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<tr>
<td>Cloudy, yellow, or frothy meibomian gland secretions</td>
<td>5/26 (19.2)</td>
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<tr>
<td>Lid margin telangiectasia</td>
<td>4/26 (15.4)</td>
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<tr>
<td>Meibomian gland capping</td>
<td>3/26 (11.5)</td>
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<tr>
<td>Meibomian gland loss/destruction</td>
<td>5/26 (19.2)</td>
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<tr>
<td>Chalazia</td>
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<tr>
<td>Eyelid inflammation (not defined)</td>
<td>2/26 (7.7)</td>
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<tr>
<td>Eyelid noninflammation</td>
<td>2/26 (7.7)</td>
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<td><strong>Ocular Surface Findings</strong></td>
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<tr>
<td>Rosacea-associated eyelid and corneal changes</td>
<td>3/26 (11.5)</td>
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<tr>
<td><strong>Tear Film Findings</strong></td>
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<tr>
<td>Tear interferometry</td>
<td>1/26 (3.8)</td>
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<td>TBUT&lt;10 seconds</td>
<td>1/26 (3.8)</td>
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<td>TBUT&lt;5 seconds</td>
<td>1/26 (3.8)</td>
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<tr>
<td>Schirmer’s test &lt;10 seconds</td>
<td>1/26 (3.8)</td>
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<td><strong>Existing Classification Scheme</strong></td>
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<tr>
<td>McCulley Classification system for MGD</td>
<td>2/26 (7.7)</td>
<td></td>
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<tr>
<td>B. Exclusion criteria</td>
<td>1,2,6-8,10,12,14-21,24,26</td>
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</tr>
<tr>
<td></td>
<td>Frequency (%)</td>
<td></td>
</tr>
<tr>
<td>Contact lenses use</td>
<td>10/17 (58.8)</td>
<td></td>
</tr>
<tr>
<td>History of ocular surgery</td>
<td>7/17 (41.2)</td>
<td></td>
</tr>
<tr>
<td>Eye disorders affecting the ocular surface</td>
<td>6/17 (35.3)</td>
<td></td>
</tr>
<tr>
<td>Meibomitis, seborrheic MGD, and excessive meibomian lipid secretion</td>
<td>4/17 (27.5)</td>
<td></td>
</tr>
<tr>
<td>Topical medical therapy (of any kind)</td>
<td>4/17 (27.5)</td>
<td></td>
</tr>
<tr>
<td>Infectious conjunctivitis</td>
<td>4/17 (27.5)</td>
<td></td>
</tr>
<tr>
<td>Systemic diseases affecting the ocular surface</td>
<td>4/17 (27.5)</td>
<td></td>
</tr>
<tr>
<td>Topical or systemic steroids use</td>
<td>3/17 (17.6)</td>
<td></td>
</tr>
<tr>
<td>Active ocular disease</td>
<td>3/17 (17.6)</td>
<td></td>
</tr>
<tr>
<td>Pregnancy or childbearing age without contraception</td>
<td>3/17 (17.6)</td>
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<tr>
<td>Stevens-Johnson syndrome</td>
<td>3/17 (17.6)</td>
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</tr>
<tr>
<td>Chemical, thermal, or radiation injury</td>
<td>3/17 (17.6)</td>
<td></td>
</tr>
<tr>
<td>Decreased reflex tearing (&lt;10 mm/5 min Schirmer test result)</td>
<td>2/17 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>2/17 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Current treatment for blepharitis</td>
<td>2/17 (11.8)</td>
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<tr>
<td>Topical or systemic antibiotics</td>
<td>2/17 (11.8)</td>
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<tr>
<td>Alteration of lacrimal drainage system</td>
<td>2/17 (11.8)</td>
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<tr>
<td>Drugs affecting tearing</td>
<td>2/17 (11.8)</td>
<td></td>
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<tr>
<td>Sjogren’s syndrome</td>
<td>2/17 (11.8)</td>
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<tr>
<td>MGD with acute inflammation</td>
<td>2/17 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Anterior blepharitis with more than moderate severity</td>
<td>2/17 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Active ocular allergy</td>
<td>2/17 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Inflammatory diseases unrelated to MGD</td>
<td>2/17 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Ocular adnexal pathology interfering with warm compress application</td>
<td>2/17 (11.8)</td>
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<tr>
<td>Lid structural abnormality</td>
<td>2/17 (11.8)</td>
<td></td>
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<tr>
<td>Topical or systemic antimetabolites</td>
<td>1/17 (5.9)</td>
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<tr>
<td>Eyelid surgery</td>
<td>1/17 (5.9)</td>
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<tr>
<td>Use of isotretinoin within the past 6 months</td>
<td>1/17 (5.9)</td>
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<tr>
<td>Autoimmune disease requiring treatment</td>
<td>1/17 (5.9)</td>
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<tr>
<td>Smokers</td>
<td>1/17 (5.9)</td>
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<tr>
<td>Punctal plugs</td>
<td>1/17 (5.9)</td>
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<tr>
<td>Anterior chamber inflammation</td>
<td>1/17 (5.9)</td>
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<tr>
<td>Glaucoma</td>
<td>1/17 (5.9)</td>
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<tr>
<td>Anterior segment diseases</td>
<td>1/17 (5.9)</td>
<td></td>
</tr>
<tr>
<td>Best corrected visual acuity &lt;1.0 logMAR</td>
<td>1/17 (5.9)</td>
<td></td>
</tr>
<tr>
<td>Lid skin disease</td>
<td>1/17 (5.9)</td>
<td></td>
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<tr>
<td>Cicatrical conjunctival diseases</td>
<td>1/17 (5.9)</td>
<td></td>
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<tr>
<td>Sensitivity to study medication</td>
<td>1/17 (5.9)</td>
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</table>
Table 6. Categories of 39 Exclusion Criteria in 17 Studies

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular Diseases (20/39; 51.3%)</td>
<td></td>
</tr>
<tr>
<td>Eye disorders that affect the ocular surface</td>
<td>6/17 (35.3)</td>
</tr>
<tr>
<td>Meibomitis, seborrheic MGD, and excessive meibomian lipid secretion*</td>
<td>4/17 (23.5)</td>
</tr>
<tr>
<td>Infectious conjunctivitis</td>
<td>4/17 (23.5)</td>
</tr>
<tr>
<td>Active ocular disease</td>
<td>3/17 (17.6)</td>
</tr>
<tr>
<td>Stevens-Johnson syndrome</td>
<td>3/17 (17.6)</td>
</tr>
<tr>
<td>Meibomitis and seborrheic MGD†</td>
<td>3/17 (17.6)</td>
</tr>
<tr>
<td>Highly decreased reflex tearing &lt;10 mm</td>
<td>2/17 (11.8)</td>
</tr>
<tr>
<td>MGD with acute inflammation†</td>
<td>2/17 (11.8)</td>
</tr>
<tr>
<td>Anterior blepharitis of more than moderate severity</td>
<td>2/17 (11.8)</td>
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<td>Cricatrical conjunctival diseases</td>
<td>1/17 (5.9)</td>
</tr>
<tr>
<td>Lid skin disease</td>
<td>1/17 (5.9)</td>
</tr>
<tr>
<td>Iatrogenic (13/39; 33.3%)</td>
<td></td>
</tr>
<tr>
<td>Contact lens use</td>
<td>10/17 (58.8)</td>
</tr>
<tr>
<td>Ocular surgery</td>
<td>7/17 (41.2)</td>
</tr>
<tr>
<td>Topical medical therapy (of any kind)</td>
<td>4/17 (23.5)</td>
</tr>
<tr>
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<tr>
<td>Use of isotretinoin within the past 6 month</td>
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</tr>
<tr>
<td>Smoking</td>
<td>1/17 (5.9)</td>
</tr>
<tr>
<td>Plugs</td>
<td>1/17 (5.9)</td>
</tr>
<tr>
<td>Systemic Diseases (6/39; 15.4%)</td>
<td></td>
</tr>
<tr>
<td>Systemic, neurologic, dermatologic diseases affecting ocular surface</td>
<td>4/17 (23.5)</td>
</tr>
<tr>
<td>Pregnancy/child bearing age without contraception</td>
<td>3/17 (17.6)</td>
</tr>
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<tr>
<td>Autoimmune disease requiring treatment</td>
<td>1/17 (5.9)</td>
</tr>
<tr>
<td>Sensitivity to study medication</td>
<td>1/17 (5.9)</td>
</tr>
</tbody>
</table>

* Refs. 1, 2, 6–8, 10, 12, 14–21, 24, and 26.
† Exclusion criteria directly addressing the lid margins and meibomian glands (3/39; 7.7%).

1. Exclusion criteria concerning the presence or history of ocular disease was the most frequently reported (20/39 criteria; 51.3%). Among these, three (7.7%) criteria described the presence of meibomian gland dysfunction, defined by the authors as seborrhea and/or acute or chronic meibomitis. These three exclusion criteria were reported in four papers regarding the treatment of obstructive MGD and in one paper about MGD therapy in CL wearers.

2. Exclusion criteria related to iatrogenic events was the second most common category, with 13 of 39 exclusion criteria (33.3%) falling in this category. Among these, medical therapy, surgical therapy, use of CLs or punctal plugs, and smoking are included.

3. The last group of exclusion criteria refers to the presence of systemic diseases, pregnancy or being of childbearing age without contraception, and known sensitivity to study drugs. This group of exclusion criteria included 6 of the 39 criteria identified (15.4%).

Comments. Inclusion and exclusion criteria define the patient sample of all types of studies. In studies of MGD, it is crucial to state how MGD is diagnosed, use consistent terminology, and carefully define clinical characteristics. Dividing exclusion criteria into three categories (ocular disease-related, iatrogenic, and systemic disease-related) can help in producing a logical list of eligible and ineligible subjects, suitable for each trial. Further consistency in entry and exclusion criteria in clinical trials related to MGD is needed.

Outcome Measures (Endpoints): Primary and Secondary

Overview. Many of the studies we evaluated were relatively small in sample size and were exploratory in nature. Therefore, several clinical variables were regarded as outcome variables, without identifying specific primary and
secondary outcomes. The outcomes, therefore, can be grouped on the basis of clinical appearance. The main clinical characteristics can be categorized as follows:

- Symptoms (dry eye or blepharitis) and visual disturbance (fluctuation)
- Eyelid assessment (lid margin injection/hyperemia, blocked meibomian glands, debris on lashes, tarsal and lid margin telangiectasia, and edema)
- Tear film parameters (TFBUT, interferometry, aqueous production, and osmolarity)
- Ocular surface involvement (corneal and conjunctival staining)
- Inflammation of the ocular surface (injection)
- Abnormal meibum (expressibility, quantity, and quality)
- Bacterial involvement.

Defining the characteristics in this manner allows the outcome measures to be better contextualized, given the diversity of the disease. The individual papers were categorized according to the characteristics, and the main outcome measures are described in Table 7. All relevant studies independent of their level of evidence were included.

In these studies, it can be assumed that all types of MGD are more or less chronic, although chronicity was not explicitly described in all cases (none were described as acute). Future studies may include more specific terminology on the basis of the terminology proposed in the Report on Definition and Classification.

**Results.** While several outcome measures were used, a likely reflection of the diversity of the disease, the methods used to grade change varied. In general, there appeared to be no distinction between primary and secondary endpoints. In studies in which scales were defined, categorical or ordinal scales were often used (e.g., yes/no; graded 0–4; or none, mild, moderate, or severe).

The changes in outcome measures can be summarized as follows:

- Symptoms (improvement in total ocular symptom score, specific dry eye symptoms, reduction of visual fluctuation, and increase in comfortable CL wearing time)
- Eyelid assessment (reduction in graded severity)
- Tear film parameters (increased aqueous production TFBUT and improvement in tear lipid layer interference)
- Ocular surface involvement (reduction in graded severity of staining)
- Inflammation of the ocular surface (reduction in graded severity injection)
- Abnormal meibum (improved expressibility, quantity, and quality)
- Bacterial involvement (reduction of bacterial load)
- Improvement/reduction of the recurrence of inflammation (chronic lid margin, corneal, and chronic granulomatous disease).

The most frequently reported outcome measures in the 26 papers included ocular symptoms (14, 53.8%), TFBUT (14, 53.8%), meibomian gland secretion and expression (9, 34.6%), Schirmer I (10, 38.5%), corneal staining (8, 30.8%), meibomian gland obstruction (6, 23.1%), eyelids (5, 19.2%), and lipid layer interference (5, 19.2%).

Outcome measures associated with signs and symptoms of dry eye and not necessarily specific to MGD were used in most of the publications. Parameters related to evaluation of the eyelids have been more frequently used in recent years, and direct assessments of the glands have increasingly been used as outcome measures in the more recent papers.

There were no major differences in the choice of outcome measures when the evaluation was limited to papers of evidence level I or II.

**Comment.** The importance of signs and symptoms of dry eye appears evident in the outcome measures described in the literature. Specific symptom surveys for MGD as well as uniform grading of eyelid margin findings are needed. It is somewhat surprising that the different outcome measures selected in different trials appeared not to be associated with the different manifestations of MGD, but instead were
evenly distributed, independent of how the disease was expressed.

**Treatment**

**Overview.** Twenty-three of the 26 eligible studies had sufficient information to be assessed under the following categories (Table 2):

- Treatment type (pharmacological, homeopathic, surgical, and external)
- Dose regimen
- Concurrent treatment
- Control treatment
- Duration
- Washout
- Follow-up.

**Results.** Pharmacologic test treatments used in 11 (42.3%) of the 26 studies included systemic or topical macrolide antibiotics (3, 11.5%), systemic tetracyclines (4, 15.4%, in one case with topical prednisolone and tobramycin), and topical anti-inflammatory/immunosuppressive drugs (4, 15.4%). Homeopathic test treatments were used in three (11.5%) of the studies, including two with topical agents (honey or oil drop) and one with a systemic agent (linoleic acid). External treatments were reported in nine (34.6%) studies, including heat (warm compresses or a warming device) in seven (26.9%), lid hygiene in one (3.8%), and both treatments in one (3.8%).

Systemic treatments were almost invariably used twice daily. Dose regimens for tetracyclines ranged from 20 to 200 mg of doxycycline twice daily, 50 mg daily or 50 mg twice daily of minocycline, or 30 to 350 mg of erythromycin twice daily.

Concurrent treatment was continued or instituted in 10 trials. Artificial tears were used in nine trials and lid therapy in five, of which four used only lid hygiene. One study allowed whatever treatment in use 1 month before the trial to continue throughout the trial. Most did not disclose whether concurrent treatment was continued.

Nine studies used some form of treatment in the control group. Lid hygiene was the most frequent treatment in the control group (three studies); one study used heat, one used conventional eye masks, and one used warm towels. Control groups were assigned to artificial tears in three studies; one of those studies also used lid hygiene. One study involved a placebo control for systemic doxycycline.

A single application was used in four eyelid heat trials. Three devices were tested using one 10-minute application and then a 2-week trial. Including this trial, there were seven trials involving 2-week treatments. The other most common length for a trial was 3 months (six studies). There were four trials of treatments lasting 2 weeks to 3 months and three trials with treatment lasting longer than 3 months.

No washout period (run-in period) was observed in the majority (n = 17) of the trials. Artificial tears were prescribed for the washout in two trials (specified for 2 weeks in one of these). One study discontinued the use of systemic doxycycline for 2 weeks before the study’s start. No topical therapy was specified before two of the trials, with trial duration of 2 weeks and of 3 months in the other. In the 3-month washout trial, artificial tears were allowed to be used, but no punctual occlusion or CL use was permitted.

To standardize treatment, some studies required all subjects to use lid scrubbing and artificial tears at entry. Standardizing treatment for lid disease may help decrease confounding variables when evaluating a new treatment. Using standard treatment for 2 to 4 weeks before randomizing subjects may help eliminate the placebo responders and provide better baseline information.

Five studies included a follow-up (to rule out relapse) after 2 to 3 months.

**Comment.** Most trials lacked a washout period and did not check for relapse; half allowed concurrent use of other treatments and a third allowed treatment in the control group. There was a large variability between duration of studies, but pharmacological trials tended to be of longer duration and were more likely to have a follow-up period than those using external factors and were more likely to have a follow-up period.

**TABLE 7 (continued).** Clinical and Symptom Outcomes

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Meibomian Glands</th>
<th>Inflammation</th>
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<td>Destruction (Melibography)</td>
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(continues)
Statistical Considerations

**Overview and Results.** There were a limited number of well-conducted, randomized controlled trials available for statistical review. None of these studies gave much detail related to the calculation of effect size, power, or required sample size. There was limited information on how missing data—for example, loss to follow-up and exclusion due to noncompliance—were handled.

**ADDITIONAL CLINICAL TRIALS**

Additional ongoing clinical trials from ClinicalTrials.gov were retrieved with the search term *meibomian*. The relevant ones are listed in Table 8.

**Comments.** Several of the ongoing clinical trials are randomized double-masked placebo-controlled studies with well-defined primary and secondary outcome measures. Results from these trials may add to the list of clinical trials on MGD with a high evidence grades. At the time of this compilation, however, none of those studies had published results.

**NECESSARY MGD TRIAL DESIGN IMPROVEMENTS**

Decisions concerning the design of future trials should be based on available data from reliable studies published in peer-reviewed journals. Such studies should be prospective randomized double-masked (when possible) and controlled with a sufficiently large MGD sample.

To date, very few trials have met those stringent criteria, although as already noted, several are under way. It is unknown when, if ever, the results of those ongoing trials will be published.

**Objectives**

Although generic clinical trial design recommendations are available, design recommendations specific to MGD should include trials with well-defined objectives. Those objectives should be clearly stated and allow for concise and specific questions to be answered. Important and basic questions to address in MGD are:

- Is there an association between MGD and dry eye disease?
- Can we distinguish between MGD and dry eye disease? How?

Our review of past clinical trials of MGD suggests that there is no clear consensus. Some researchers include subjects with dry eyes, others exclude them, and still others fail to evaluate dry eye status altogether. Given the current lack of sufficient reliable data, answers to this question can only be tentative; no conclusive recommendations are possible. MGD appears to be clinically associated with alterations in the quality and quantity of lipids secreted by the meibomian glands, which contribute to the preocular tear film. Many clinicians believe MGD is the most common cause of evaporative dry eye and that there is considerable overlap in the occurrence of MGD and aqueous-deficient dry eye states, both demonstrating typical signs and symptoms suggestive of dry eye disease. Studies that evaluate the possible role of MGD in aqueous deficiency, possibly through creating an inflammatory state on the ocular surface, would also be welcome.

- Given that there is considerable uncertainty between MGD and dry eye disease, trials that evaluate the association between MGD and dry eye would be beneficial, as would observational trials that assess the natural history of MGD. Of special value would be a standardized symptom questionnaire that could distinguish MGD lid disease from dry eye disease.

- Developing alternative or indirect ways of assessing and testing MGD would also be desirable. Accurate, repeatable measures of symptoms are of obvious value as outcome measures and are directly relevant to the patient’s health. Quantitative measures of disease may also be useful, especially if it can be shown that reversal improves long-term health. Examples include osmolarity, interferometry, high resolution OCT, tests that can measure visual function and interblink visual acuity decay, and techniques that identify differences in the meibum. To learn how to use such tools, researchers need standardized video and/or web-based training. Clinical studies demonstrat-
the correlation between the results of these tests and clinical findings, such as symptoms or signs, should be executed first.

**Design**

The most desirable clinical trials would be prospective, randomized, controlled, and double-masked, if possible. Considerations important in good clinical trial design should be incorporated into any MGD trial (e.g., Guidelines from International Conference on Harmonization [ICH] E6 Good Clinical Practice: Consolidated Guidance, ICH topic E8 General Consideration of Clinical Trials; ICH topic E9 Statistical Principles for Clinical Trials; and E10 topic Choice of Control Group and Related Issues in Clinical Trials, see www.ich.org). Other types of designs, such as epidemiologic or registry studies, entail other considerations.

**Selection of Subjects and Inclusion/Exclusion Criteria**

Past MGD clinical trials did not have a uniform way of defining the study population, although symptoms and changes in the lid, especially plugging and abnormal secretions, were the most common clinical characteristics that were selected. Of note, dry eye disease was not usually specifically included or excluded in selecting patients, other than in subject recruitment based on symptoms. Signs of dry eye disease were uncommonly used as selection criteria but were often assessed to determine improvement. Future studies should carefully consider inclusion of tests for dry eye disease.

A consistent, standardized classification system is important in measuring the effects of intervention, in establishing natural history, and in defining inclusion and exclusion criteria. Two approaches can be taken when grading or classifying patients: grading of individual clinical characteristics or classification based on global severity. Individual grading is discussed in the Report on Diagnosis, whereas the Report on Management and Therapy utilizes a clinical-staging approach to determining disease according to a uniform grading methodology and not exclusively by tear and ocular surface characteristics. Development of such a consistent grading and evaluation methodology across all research in MGD would facilitate comparisons between studies.

To emphasize continuity between graders and examiners, a training program for researchers both for diagnosis and grading could be developed, perhaps using web-based delivery. Such a training program may assist in ensuring concordance between investigators and improve data quality. It might also include reading centers such as those used in other vision-related studies (i.e., those of the retina, glaucoma, and keratoconus). An important aspect in MGD may be ethnicity. Ethnic differences may influence the choice of study population, as it may affect the study medication’s safety, efficacy, dosage and dose regimen. Since epidemiologic data indicate a substantially higher prevalence of MGD in people of Asian descent, one must consider that both extrinsic (e.g., culture, including diet and medical practice) and intrinsic (e.g., genetic polymorphism; ICH E5: Ethnic Factors in the Acceptability of Foreign Clinical Data) factors have a potential to influence the outcome of a clinical trial. The ability to generalize results will also reflect the homogeneity (or lack thereof) of the study population.

Appropriate inclusion and exclusion criteria are essential to ensuring the integrity of the trial. Previously published clinical studies have not adequately identified clinically relevant and specific inclusion criteria for MGD that differ from

<table>
<thead>
<tr>
<th>Ref.</th>
<th>General Ocular Scores</th>
<th>Progression of Post-Surgical Healing</th>
<th>Overall Disease Improvement</th>
<th>Safety</th>
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<tr>
<td>17</td>
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<td></td>
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<td>21</td>
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<td>15</td>
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<td></td>
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<tr>
<td>16</td>
<td>Yes (OSSE)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>19</td>
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<td>25</td>
<td>Yes</td>
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</tbody>
</table>

**TABLE 7 (continued). Clinical and Symptom Outcomes**
those for dry eye disease. In general, the use of CLs has been a major reason for excluding subjects from trials, followed by exclusion criteria related to general ocular surface conditions or past surgery. In early phases of drug development, the inclusion and exclusion criteria may be very stringent, to maximize the chance of observing specific clinical effects of interest. These restrictive criteria may result in selection of a sample from a very narrow subgroup of the total patient population for which a treatment may eventually be indicated. However, in later confirmatory trials, subjects should more closely mirror the target population. The inclusion and exclusion criteria should be relaxed as much as possible to allow researchers the ability to suggest generalizations for routine patient care.

### Selection of the Control Group

Control groups have one major purpose: to allow discrimination of patient outcomes (for example, changes in symptoms, signs, or other morbidity) caused by the test treatment from outcomes caused by other factors, such as the natural progression of the disease, observer or patient expectations, or other treatment. Therefore, the choice of control group is always a critical decision in designing a clinical trial.

In most cases, the primary choice is to use a concurrent control group. The test and control groups should be similar with regard to all baseline and on-treatment variables that could influence outcome, except for the study treatment. In MGD, such baseline factors could be related to age, ethnicity, systemic disease, concurrent medication, and environ-

### Table 8. Relevant Registered Clinical Trials

<table>
<thead>
<tr>
<th>Condition</th>
<th>Title</th>
<th>Interventions</th>
<th>Status</th>
<th>Outcome</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGD</td>
<td>A Single-Center, Double-Masked, Randomized, Vehicle Controlled Study to Evaluate the Safety and Efficacy of Testosterone 0.05% Ophthalmic Solution Compared to Vehicle for the Treatment of MGD</td>
<td>Testosterone ophthalmic solution vs. vehicle</td>
<td>R</td>
<td>Primary: MG secretion (128 days) Secondary: comfort (128 days)</td>
<td>Phase II, enrollment by invitation only</td>
</tr>
<tr>
<td>MGD</td>
<td>Efficacy of 0.05% Cyclosporine Ophthalmic Emulsion Compare with Tear in MGD</td>
<td>0.05% cyclosporine eye drop</td>
<td>R</td>
<td>Primary: NTBUT (0,1,2,3 month) Secondary: OSDI score, TIBUT, fluorescein/rose bengal staining, MG (0,1,2,3 month)</td>
<td>Phase IV</td>
</tr>
<tr>
<td>Blepharitis</td>
<td>Lipids of the Human Tear Film and Their Effect on Tear Stability</td>
<td>Doxycline; essential fatty acid; azithromycin</td>
<td>R</td>
<td>Primary: inflammation of eyelid (2 months) Secondary: character of MG secretion (2 months)</td>
<td>Phase IV</td>
</tr>
<tr>
<td>Dry eye syndrome</td>
<td>A Prospective Clinical Study Assessing the Effects of Tetracycline Antibiotic on Tear Film and Tear Lipid Composition within a Population of Patients Diagnosed with Blepharitis and Dry Eye Disease Condition</td>
<td>Tetracycline: doxycycline analog</td>
<td>T</td>
<td>Primary: evaporimetry; fluorophotometry; MG expression and lipid analysis Secondary: Schirmer’s, TIBUT, bacteriology, transillumination and meibography</td>
<td>Phase IV</td>
</tr>
<tr>
<td>Blepharitis</td>
<td>A Placebo-Controlled Double-Masked Clinical Assessment Study of Essential Fatty Acid Supplement and Its Effect on Patients with Apparent Aqueous-Deficient Dry Eye Syndrome Condition</td>
<td>Essential fatty acid supplement</td>
<td>R</td>
<td>Primary: lipid bio chemistry changes Secondary: evaporimetry and fluorophotometry</td>
<td>Phase IV</td>
</tr>
<tr>
<td>Posterior blepharitis</td>
<td>Topical IL-1-Ra for Treatment of Posterior Blepharitis</td>
<td>2.5% IL-1Ra, Placebo; 5% IL-1Ra</td>
<td>R</td>
<td>Primary: MG secretion/quality, TBUT, cornea and conjunctival staining, and OSDI questionnaire (12 weeks) Secondary: MG occlusion, Schirmer with and without anesthesia. (12 weeks)</td>
<td>Phase I/II</td>
</tr>
<tr>
<td>KCS</td>
<td>Efficacy and Safety Study of Nutritional Supplements for Treatments of Dry Eye Condition</td>
<td>Dietary supplement: Hydroeye; vs. inactive capsule</td>
<td>R</td>
<td>Primary: Schirmer, OSDI, TIBUT, corneal staining (screening at weeks 4, 12, and 24) Secondary: Corneal topography, MGD, facial expression subjective scale, artificial tear usage, HLA-DR staining of impression cytology (screening, at weeks 12 and 24)</td>
<td>Signs of MGD were inclusion criteria</td>
</tr>
<tr>
<td>Blepharitis</td>
<td>Treatment of Patients With Blepharitis and Facial Rosacea</td>
<td>Doxycycline vs. placebo</td>
<td>R</td>
<td>Primary: Change in OSDI, bulbar conjunctival hyperemia (baseline to end of study) Secondary: Change in Schirmer result, TIBUT, meibum character/fluidity, MG inspissation (baseline to end of study)</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

Source: Clinicaltrials.gov, accessed March 2010. R, recruiting; T, terminated; OSDI, Ocular Surface Disease Index; KCS, keratoconjunctivitis sicca.
mental factors, to cite just a few examples. Randomization reduces potential bias between the experimental and the control group. To further minimize the risk of bias, the study should be double-masked, so that both the subject and the examiner are unaware of the medication versus placebo assignment. When double-masking is not possible, which may be the case in MGD if, for example, lid scrubbing is part of one treatment regimen (the examiner could remain masked), efforts to identify which outcome measures can be masked to an independent evaluator should be made as well as efforts to minimize subject discussion related to the therapy.

Currently there is no well-defined, accepted standard of care in the treatment of MGD. Therefore, careful discussion with regulatory agencies may be needed for study design issues such as a control paradigm. In the absence of an established standard of care, it is important to define the control or comparator treatment—for example, placebo control (vehicle) and/or lid hygiene.

Although choosing a comparator in a superiority trial in MGD may be straightforward, in a noninferiority trial for MGD, there is no established treatment with which to compare. Further, a clinically relevant noninferiority margin of error or range has to be determined. Other types of studies may include a crossover design. In such a case, it would be critical to address whether there is interference between treatments and what would define a suitable washout period. In any clinical trial in MGD, it is critical to address potential confounders, such as the effects of concurrent treatment (in the current literature, the most common concurrent treatments include lid hygiene and artificial tear substitutes), washout of current treatment, and treatment during the run-in phase of a study. Standard operating procedures to manage these situations should be clearly defined in the study protocol.

Duration of the Trial

In MGD, there has been a large variability in the duration of the studies reviewed. Pharmacologic trials tended to be of longer duration than those assessing other nonepiphenological factors and were more likely to include a follow-up period after treatment discontinuation. As in any clinical trial, the treatment duration must be sufficiently long to obtain the desired outcome. A follow-up period to address recurrence after treatment termination would be desirable. In pharmacologic treatment trials, the trial duration should correspond with the proposed clinical care treatment duration to adequately address any safety issues that the treatment regimen might create in a real-world setting.

Sample Size

The power of the study, calculated on the basis of the primary outcome measure in a study—for example 80% (generally accepted as the recommended minimum value)—is the ability of a test to detect an effect, if any. For example, if a previous trial has demonstrated a clinically meaningful decrease in corneal staining, evaporation, or tear osmolarity with a certain treatment, such data would be used to calculate the number of patients needed to achieve (at least), with the desired probability, a similar magnitude of effect in the planned trial. Necessary components in calculating statistical power include effect size, variability, sample size, and significance level. Given that there is limited published information available to assist with sample size or power estimates in MGD trials, the calculations are likely to be based on preliminary and/or uncertain data and information. Data from exploratory or early-phase studies continue to be needed. Presently, data comparing specific characteristics in normal and diseased subjects should be used to assist in obtaining data on a potential effect of a treatment and therefore in calculating sample size. An interim check (on masked data) to adjust the sample size may also be useful, but must be performed with extreme caution and is best suited in pilot work. A revised sample size may then be calculated by using suitably modified assumptions (ICH E9). When estimating a sample size, additional subjects should be included to compensate for withdrawal or loss to follow-up. These additional subjects are especially important in longer term trials or trials with complicated or noxious therapies in which a higher withdrawal rate is expected. A sufficient sample size is also needed to appropriately address the safety of an intervention (ICH E1).

Outcome Measures

Primary outcome measures or endpoints, as well as secondary outcome measures or endpoints, should be clearly defined in MGD trials. The selected outcome measures should provide the most clinically relevant and convincing evidence directly related to the objectives of the trial. Generally, the primary endpoint is one that demonstrates a clear quantitative measure of benefit. In MGD, the choice of outcome measures related to efficacy of an intervention would probably be dependent on the classification and severity of disease. The classification recommended by the Definition and Classification Subcommittee is based on pathophysiological changes in which the main categories are low- and high-delivery states. Consequently, in a low-delivery state such as an obstructive condition, a reasonable endpoint could include assessment of lid margin inflammation and/or gland obstruction. Likewise, disease severity or disease progression may be variables of interest. MGD severity staging, such as the scheme found in the Report on Management and Therapy, may include several clinical characteristics within a disease severity stage and could be used in clinical trials, but may lack the sensitivity of an individual grading scheme for a unique clinical test. Therefore, outcome measures have to be carefully selected to address the hypothesis of the proposed study.

In many of the past clinical trials, outcome measures have reflected ocular surface rather than lid signs (tear status and ocular surface staining). Such measures may reflect the concurrence of MGD and dry eye disease, especially in evaporative dry eye disease, in which lipid abnormalities are thought to lead to changes in tear film stability. In such conditions, typical signs of dry eye disease (TBUT, vital staining of the cornea and conjunctiva, and Schirmer’s test result) would be appropriate outcome measures for MGD. In addition, as discussed in the Report on Diagnosis, in the evaporative dry eye state, significant differences in tear turnover rate, evaporation, and osmolarity may be seen between evaporative dry eye and normal subjects. The use of such clinical endpoints is promising, but must have further evaluation. Accordingly, MGD trials should include adequate information about tear film parameters (typically included in the description of tear-deficient states), in addition to descriptions that delineate the extent of the lid margin disease. Endpoints associated with lid findings may be selected as primary or as important secondary outcome measures and graded using a uniform grading methodology. Of note, it is unclear whether existing grading scales reflect linear progression in severity, and efficacy may therefore be difficult to demonstrate for more severe disease.

As in dry eye disease, it would also be essential to evaluate treatment effects on symptoms specific to MGD but also including foreign body sensation or irritation; itching; burning; swollen eyelids; a feeling of dryness; excessive tearing; and a crust on the eye lashes, especially in the morning. These symptoms are very similar to those reported in dry eye disease.
To better define and evaluate patient symptoms in MGD, a specific and validated questionnaire specific to MGD is highly desirable, not only to differentiate between dry eye and MGD, if possible, but also to address a response to treatment. The use of electronic symptom diaries may improve real-time data collection, data quality, and accuracy.

Depending on the etiology, manifestation, and severity of the disease, additional outcome measures such as tear and ocular surface characteristics may be highly relevant endpoints. The clinical value of commonly used endpoints such as (but not limited to) changes in lipid layer interference pattern, meibum expressibility, quality and composition, and tear evaporation rate should have further evaluation.

**Surrogate Endpoints and Biomarkers**

Besides moving science forward, the use of surrogate endpoints or biomarkers has potential benefits during drug development. For example, data may be obtained sooner or by more uncomplicated and less invasive methods and may be ethically preferable or less costly. However, in MGD, there is no information on the specificity or sensitivity of biomarkers, let alone knowledge about how they may change in response to therapy. From a regulatory perspective, the use of surrogate endpoints or biomarkers in clinical trials depends on which weight these are given and what claims would be associated with data relying on such endpoints. In exploratory trials during earlier development of a drug, a surrogate endpoint or biomarker may be used as a secondary, or even as a primary, endpoint. A surrogate endpoint could, for example, be used to obtain a proof of concept, to aid in dose selection, to give support on a mechanism of action, or for subgroup characterization. Also in confirmatory trials, surrogate endpoints or biomarkers may be included. Regulators are often liberal, or even encouraging, when such endpoints are used during early development or as exploratory endpoints in a confirmatory study. Again, it depends on which weight the results associated with these endpoints will be given. If, on the other hand, a surrogate is to be used as a primary endpoint, the link to and relevance of a clinical outcome, or an outcome that matters for the patient (short or long-term) must be established. Surrogate endpoints must be validated by using clinical trial data, with both the surrogate and true endpoint in a representative patient sample. In such validation, the following guidelines should be considered. The surrogate endpoint or biomarker should be:

- Mechanistically plausible
- Able to predict clinical outcome (earlier, or in parallel with the “true outcome”)  
- Able to measure efficacy, severity, and safety
- Able to change with intervention and to predict an effect of treatment on a clinical outcome
- Standardized and reproducible between investigators and clinical trial centers.

**Methods of Minimizing Bias**

In MGD, specific considerations should be given to masking, compliance to study treatment, washin/washout, concurrent treatment, and methods for handling missing data. The latter could be critical and should be handled differently; for example, based on whether the condition is expected to progress or improve during the study period without treatment or whether discontinuations are due to adverse effects of an active treatment.

**Treatment**

Treatment duration must be clearly defined. Most past clinical trials in MGD have lacked a washout period and did not monitor relapse after the study’s end. Other studies allowed concurrent use of other treatment or treatment in the control group. Omitting washout or allowing concurrent medication may affect the ability to perform a robust efficacy or safety evaluation. If no confounding effects are suspected with a certain concurrent treatment, allowed (as well as not allowed) medications should nevertheless be predefined and monitored, and any potential effects on the study outcome should be identified.

**Adherence to Study Protocol**

Adherence to some management measures, including the use of lid scrubbing and hygiene, may be difficult to maintain. When such measures are included in a trial, it is critical that adherence be monitored with patient diaries. In addition, it may be wise to increase the sample size of the study, since a higher dropout may be expected.

**Assay Sensitivity**

Given that limited information is available on the magnitude of treatment effects in previous clinical trials in MGD, additional information would be of value before confirmatory therapeutic studies are performed that have a high probability of showing the desired outcome. Such information includes the magnitude of clinically relevant effect or noninferiority margins and which magnitude of a placebo response to expect.

**Modifications of the Protocol**

As previously discussed, interim analyses to assist in adjusting the sample size may be useful. In earlier phases of clinical development in MGD, an adaptive study design involving design modifications based on the results of an interim analysis may also be used to speed up the process of drug development or to allocate resources more efficiently without lowering scientific and regulatory standards. Assay sensitivity is especially essential during noninferiority trials, so that the trial data are not compromised. In such a trial, one way to ensure this would be to include a placebo group as a third arm.

**Statistical Plan**

As in any clinical trial, the principal features of the eventual statistical analysis of the data should be predefined and described in the statistical section of the protocol, for example, methodology for handling missing data, perhaps due to loss to follow up, noncompliance, or withdrawal due to adverse events. The ICH Topic E9, Statistical Principles for Clinical Trials, should be considered.

Future studies would be well served by more clearly defining the study population, especially if a multicenter trial is planned. Including evaluation for dry eye disease will help in defining its association with MGD disease and determining the effect of treatment on signs and symptoms associated with dry eye disease.

**Summary**

We suggest the following main priorities in future clinical trials in MGD:

- Natural history of MGD
- The association between MGD and dry eye disease
- A specific and validated questionnaire for symptoms of MGD
- Standardized grading for lid and other signs in MGD
- Feasibility and clinical value of lipidomic and protein inflammatory mediators
- Validation of surrogate clinical outcomes related to MGD.
References