Review Article

In Vivo Laser Scanning Confocal Microscopy of Human Meibomian Glands in Aging and Ocular Surface Diseases

Vincenzo Fasanella, Luca Agnifili, Rodolfo Mastropasqua, Lorenza Brescia, Federico Di Staso, Marco Ciancaglini, and Leonardo Mastropasqua

1 Ophthalmology Clinic, Department of Medicine and Aging Science, "G. d'Annunzio" University of Chieti-Pescara, 66100 Chieti, Italy
2 Ophthalmology Unit, Department of Neurological, Neuropsychological, Morphological and Movement Sciences, University of Verona, 37126 Verona, Italy
3 Ophthalmic Clinic, Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy

Correspondence should be addressed to Luca Agnifili; l.agnifili@unich.it

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Meibomian glands (MGs) play a crucial role in the ocular surface homeostasis by providing lipids to the superficial tear film. Their dysfunction destabilizes the tear film leading to a progressive loss of the ocular surface equilibrium and increasing the risk for dry eye. In fact, nowadays, the meibomian gland dysfunction is one of the leading causes of dry eye. Over the past decades, MGs have been mainly studied by using meibography, which, however, cannot image the glandular structure at a cellular level. The diffusion of the in vivo laser scanning confocal microscopy (LSCM) provided a new approach for the structural assessment of MGs permitting a major step in the noninvasive evaluation of these structures. LSCM is capable of showing MGs modifications during aging and in the most diffuse ocular surface diseases such as dry eye, allergy, and autoimmune conditions and in the drug-induced ocular surface disease. On the other hand, LSCM may help clinicians in monitoring the tissue response to therapy. In this review, we summarized the current knowledge about the role of in vivo LSCM in the assessment of MGs during aging and in the most diffuse ocular surface diseases.

1. Introduction

Meibomian glands (MGs) are holocrine glands embedded in the tarsal plate of the eyelids. Each gland comprises multiple acini connected by a long common central duct running throughout the entire length of the gland [1]. The functional unit of a meibomian gland is the meibocyte, which synthesizes the meibum, a lipid complex forming the superficial layer of the tear film. Meibum permeates the tear surface where it serves several important functions: it prevents tear evaporation and desiccation of the ocular surface, acts as a physical and hydrophobic barrier to the inward movement of environmental and organic agents, lubricates the ocular surface preventing irritation, and promotes clear ocular vision due to its optic properties. Thus, tear physiology is dependent upon the proper functioning of the MGs [2, 3].

Meibography represented for many years the only diagnostic approach to observe the MGs morphology in vivo [4].

With the recent diffusion of the laser scanning confocal microscopy (LSCM) (HRT I–III Rostok Cornea Module (RCM), Heidelberg Engineering, Heidelberg, Germany), it was possible to study the microscopic anatomy of several adnexal and ocular surface structures such as eyelid, conjunctiva, cornea, limbus, tarsal plate, and MGs [5–12]. LSCM allows integrated high-resolution evaluation of the morpho-functional ocular surface unit in normal and pathological conditions [13]. Several conditions may affect the anatomy and function of MGs, such as the primary meibomian gland dysfunction, dry eye, ocular allergy, the use of contact lenses, and the long-term application of topical medications in chronic ocular surface diseases and in glaucoma. In this review, we discuss the main MGs modifications during aging.
and during the course of the most diffuse ocular surface diseases.

2. Methodology and Results

PubMed searches were performed on September 20, 2015, using the following phrases: "in vivo confocal microscopy" and "meibomian glands", which identified thirty-four unique publications. Six were excluded, since they did not concern the use of IVCM to study MGs.

Three of the considered publications were not published in English; however, they provided enough information in the English abstract to warrant inclusion.

2.1. Meibomian Glands in the Healthy and Aging Human Eye. The confocal images are captured from a coronal scan through a mass of acini posterior to a central, vertically oriented MG duct [14]. LSCM provides structural and functional information of the glandular status: MGs acini appear to be constituted by convoluted borders with large cells lining the acini and fine cellular material within the lumen [14].

The main structural modifications induced by aging were evidenced for the first time by Wei et al. [15] and afterwards confirmed by Villani et al. [16] in a heterogeneous population study, which revealed significant negative correlations between age and meibomian glandular acinar unit density [1]. LSCM permits indirect functional evaluation of MGs, by assessing the secretion reflectivity [4].

Besides the acinar density reduction, the normal aging induces also an evident decrease of the acinar diameter (Figure 1) and an increase of the acinar wall inhomogeneity, without significant modifications of the glandular orifice diameter. The overall interpretation of the structural modification suggests glandular dropout with qualitative changes of the meibum. Therefore, the authors speculated that acinar atrophy leads to a decrease in the MGs secretion with aging.

These results are in accordance with the main clinical changes observed during aging, represented by tear film break-up time (BUT) and Schirmer test scores reduction.

Even though several studies reported likely hormone-dependent changes of MGs structure [17–19], LSCM did not document significant structural and functional differences between genders during aging [19, 20]. Further studies are warranted to elucidate the clinical implications of the hormonal action on MGs age-related changes.

In summary, LSCM in healthy subjects provides information of the normal modifications of glands with aging. Normal aging induces atrophic involution of the glandular unit along with progressive dysfunction of the secretive activity (Table 1). This appears to be in line with the involution of most parts of secretive structures, such as exocrine glands and lymphatics, observed in several tissues of the human body during aging [21–23]. In the eye, the glandular unit involution may take part in the increased risk of dry eye related conditions observed in the elderly.

2.2. Primary and Secondary Meibomian Glands Dysfunction. The primary meibomian gland dysfunction (MGD), characterized by inflammatory changes of the lid margin structures and in the anatomy of the MG orifices, is one of the most common ocular surface disorders: Hom et al. [20] reported prevalence of 38.9%, whereas Stanek [41] reported prevalence of 71.7% in individuals above 60 years old. MGD is a major cause of dry eye and results in a qualitative alteration and/or a quantitative reduction of the lipid secretion, which leads to decreased tear stability, increased tear evaporation, loss of lubrication, and damage to the ocular surface epithelia. LSCM, which currently has a primary role in the ocular surface analysis [26, 27], allows studying MG at cellular level. In MGD, LSCM permits the analysis of the cellular density of the superficial and basal epithelium of the eyelid and the assessment of the mean acinar area and density of MGs.
the glandular orifice area, the meibum secretion reflectivity, and the inflammatory features of periglandular interstices and acinar unit wall (patterns of inhomogeneity) [40]. In the first confocal study on MG diseases, Messmer et al. [28] showed dilatation and obstruction of the meibomian gland ducts in twelve patients with blepharitis/meibomitis or MGD. On the other hand, in fifteen out of 19 patients with blepharitis/meibomitis, but not in MGD, intense inflammation was observed in the tarsal conjunctival epithelium and in the stroma. The inflammatory reaction was defined by an increase of hyperreflective roundish elements corresponding to immune cells. Matsumoto et al. [29] reported that the mean acinar unit density was significantly lower in MGD patients than in control subjects, whereas the mean acinar unit diameter was significantly larger in MGD patients than in controls. Both the density and diameter of MGs acinar units significantly correlated with the severity of MGs dropout and expression grades (Figure 3).

Other confocal parameters that presented acceptable sensitivity and specificity for the diagnosis of MGD were the longest and shortest MG diameters: in the study of Ibrahim et al., these parameters resulted to be significantly worse than those observed in the controls [30]. As stated above, LSCM can also provide information about the inflammatory status of MG during MGD. Matsumoto et al. [42] measured the mean inflammatory cell density (cells/mm²) from the periglandular site of the entire lower and upper eyelid, reporting inflammatory cell numbers approximately ten to thirty times higher than those found in healthy controls. Interestingly, these cells markedly reduced after medical therapy: the combination of lid hygiene, topical nonpreserved artificial tears with 0.1% sodium hyaluronate, topical 0.5% levofloxacin and 0.1% fluorometholone, and oral minocycline (100 mg twice a day for 12 weeks) significantly improved the tear stability, the fluorescein staining scores, and the lid injection and cleared periglandular inflammatory infiltrates.

In a very recent study, LSCM revealed an increased immune cell number in the palpebral conjunctiva of patients with refractory MGD, compared to patients with therapy-responding MGD [43]. In the therapy-responding group, the mean inflammatory cell density in the periglandular area reduced from baseline values of 1216 ± 328 to 700 ± 436 cells/mm² at the last follow-up. No statistically significantly difference was found in the group that did not receive any therapy, 882 ± 301 cells/mm² before the initiation of trial and 843 ± 321 cells/mm² at the final follow-up.

Interestingly, OSDI scores correlated with epithelial immune cells infiltrating the palpebral conjunctiva, suggesting that the inflammation of the palpebral conjunctiva may contribute to explaining the MGD-associated refractory symptoms.

Secondary MGD can develop as a complication of the use of topical medications in patients with glaucoma [44–46] and in contact lens wearers (CLWs) (Figure 2) [32].

In CLWs, a decreased basal epithelium cell density, reduced acinar unit diameters, higher glandular orifice diameter, greater secretion reflectivity, and greater inhomogeneity of the periglandular interstices were the main observed findings. Morphologic changes in the MGs shown by LSCM were interpreted by the authors as signs of MGs dropout, duct obstruction, and glandular inflammation caused by chronic mechanical contact lens irritation.

In summary, MGD has a huge impact on the daily clinical practice since it plays a major role in the development of dry eye related conditions. Confocal microscopy precisely depicts, at a cellular level, the main macroscopic and microscopic MGs changes in patients affected with both primary and secondary MGD. The main features are represented by the MGs dropout, which is the glandular unit loss, the increase of the acinar surface (except for glaucoma), which, along with the increased meibum viscosity, is the hallmark of the glandular malfunction, and the inflammation of the periglandular interstice and acinar wall. The dilation of the duct orifice and the increased acinar diameter are the compensatory mechanism adopted by the glands to overcome the meibum stagnation (Table 1). All these changes lead to a
Figure 2: LSCM of MGs in a contact lens wearer. The most evident modifications are represented by the inhomogeneity of periglandular interstices (asterisk) and MG wall (arrow), periglandular inflammation, and the reduction of MG duct diameters. Bar represents 50 μm.

Figure 3: Confocal microscopy features in a 47-year-old male patient with MGD: inhomogeneity of periglandular interstices (asterisk) and MG wall (arrow), dilation and obstruction of the MG ducts, and reduction of the density of MG acinar units are the most significant alterations. Bar represents 50 μm.

Figure 4: LSCM of MGs in a patient with dry eye: the image shows a decreased diameter of meibomian gland ducts, along with marked inhomogeneous appearance of the MG walls and interstices. Several punctate hyperreflective elements, which reflect a high degree of local inflammation, are recognizable in the interstice and within the MG wall (arrow and asterisk). Bar represents 50 μm.

LSCM documented a decreased acinar diameter and area, which was associated with an increased OSDI score [38].

2.3. Meibomian Glands in Dry Eye. Dry eye syndrome (DES) is one of the most common disorders of the eye, with prevalence of 10% to 20% in the adult population. The International Dry Eye Workshop (2007) [47] defined DES as a “multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface.” In this field, LSCM documented the fine and peculiar microscopic findings of the target tissues including the cornea, conjunctiva, and meibomian glands. While several reports studied the confocal corneal and conjunctival changes in DES, MGs were only partially studied. In patients with primary DES and MGD, Villani et al. [33] reported increased acinar dilatation compared to primary DES and healthy controls, higher meibum reflectivity, and decreased diameters of gland orifices. Moreover, very interestingly, the authors showed increased acinar density in primary DES (Figure 4). The reduced lipid amount induces tear film hyperosmolarity, which leads to tear film instability, increased evaporation, and ocular surface inflammation [46].

A new integrated laser scanning confocal microscopy approach recently found some differences in the MGs features in patients with primary Sjogren syndrome, non-Sjogren syndrome dry eye, and MGD [32]. The pattern of inhomogeneity of the MGs walls and interstices was markedly higher in all groups of patients than in controls, with features more pronounced in primary Sjogren syndrome compared to non-Sjogren syndrome dry eye. The inhomogeneous appearance varies with the level of inflammation that is significantly higher in eyes with Sjogren syndrome, because of the autoimmune pathogenesis of this
condition. The presence of confocal signs of inflammation with the absence of dilative morphologic changes supports the occurrence of an inflammatory/atrophic nonobstructive MGD in the primary Sjogren syndrome [24, 33, 48].

Ban et al. [34] studied the morphological changes of MGs in patients with dry eye due to chronic graft-versus-host disease (GVHD), a major cause of morbidity and mortality in patients undergoing allogeneic hematopoietic stem cell transplantation for hematologic malignancies. They showed that the acinar unit density and the longest and shortest diameters of MGs acini are significantly decreased. Patients with severe DES after chronic GVHD show glandular fibrosis with MGs atrophy.

LSCM was also used to evaluate MGs modification in allergic keratoconjunctival diseases, such as vernal and atopic keratoconjunctivitis (VKC, AKC), which may frequently lead to secondary dry eye [VZ, VW]. In VKC, extensive periglandular Langerhans cell infiltration, with blurred lumen contours and hyperreflective solid matter in the lumen, was described [35, 39]. Ibrahim et al. [36] reported severe MGs changes in patients with AKC, with extensive fibrotic changes much more evident than that observed in MGD. These patients present shrunken MGs with extensive periglandular fibrosis, which does not allow gland enlargement as what occurs in obstructive MGD [25].

In conclusion, in patients with dry eye, confocal microscopy revealed that the inflammation is the first and most important pathogenetic mechanism involved in the development of the MG alterations. The main findings were the increased acinar density and the reduced orifice diameter, which differentiated DES from primary or secondary MGD (Table 1). In this way, DES seems to lead to an incomplete inflammatory-induced MGD. Other particular conditions potentially leading to dry eye, such as VK, AK, and GHVD, present also various degree of periglandular fibrotic reaction, which finally causes MGs atrophy. In this wide scenario of dry eye syndromes, LSCM may help clinicians in the early diagnosis of each type of disease leading to dry eye and in monitoring the response to therapy.

2.4. Meibomian Glands in Ocular Allergy. AKC and VKC are two of the most common and important ocular allergies. AKC is a bilateral chronic hypersensitivity disease characterized by conjunctival papillary hypertrophy, acute and chronic conjunctivitis, keratitis and corneal ulceration, eyelid eczema, and blepharitis [49–53]. Tears proinflammatory cytokines are responsible for the conjunctival and corneal damage and also for the MG alterations. MG changes play a critical role in the development of the allergy-related dry eye [29].

VKC is a recurring seasonally inflammatory condition of the cornea and conjunctiva, characterized by giant tarsal papillae and pathological changes of the conjunctival epithelium and MGs. LSCM documented intense infiltration of eosinophils and multinucleated granulocytes in the superficial conjunctival epithelium, extensive periglandular lymphocytic cells infiltration. These inflammatory alterations lead to macroscopic modifications of MGs, represented by glandular atrophy, and ductal dilatation (Figure 5). The same morphofunctional changes were described in ACK patients, where there is an important decrease in size and density of MG acinar units. MGs appear small and irregular with periglandular fibrosis and a restricted and not well-defined lumen, occluded by hyperreflective dense meibum [16, 31, 37, 54].

In allergic patients, the fibrotic involution of MG acinar units is more extensive than that observed in obstructive MGD, where the pressure induced by the stagnating meibum induces enlargement of MG acini [29].

In summary, MG modifications in patients with ocular allergy diseases are in part different from those observed in MGD and dry eye, since the periacinar fibrosis, along with the inflammatory infiltration and the meibum stagnation, represents the main finding (Table 1). In these ocular surface diseases, probably because of the periglandular compression induced by the fibrosis and the intense inflammation, MGs seem to be unable to activate adaptive mechanisms and develop a progressive atrophy. At present, the small sample size of research studies and poor standardization of examination and interpretation are the most challenging issues.

2.5. Meibomian Glands in Glaucoma. In the last years, LSCM was also used to evaluate the impact of glaucoma therapy on ocular surface structures [5–7, 55], including MGs [40]. In these studies, the modifications induced by the different classes of topical medications, the impact of the preservative and active compounds, and the role of the number of daily instillations in patients in multitherapy have been investigated.

MGs presented a reduction of glandular density and area and increased reflectivity of the acinar secretion, more evident in patients treated with two or more drugs compared to patients in monotherapy. The reduced density and area were expressions of glandular loss and reduced meibum production, respectively. Higher secretion reflectivity indicated increased secretion viscosity.
In conclusion, the long-term antiglaucoma therapy has a strong negative impact on MGs functionality: the iatrogenic changes of MGs, in fact, lead to meibomian gland dysfunction very similar to primary MGD, with the exception of the reduced acinar area (Table 1). It is hypothesizable that toxicity induces secondary meibomian gland dysfunction in which glands are unable to activate processes that attempt to overcome the secretion blockage. As a consequence, the acinar size decreases and MGs progressively drop out. These features are shared with CLW’s, in which the mechanical trauma leads to a secondary MGD, with the exception of the reduced acinar area. One may suppose that when the inflammation plays the main role, MGs modify their features as in primary MGD, whereas when toxic or mechanic stimuli play the main role, MGs reduce also their size.

These modifications present evident clinical implications since they could strongly affect adherence and persistence of treatment.

3. Summary and Conclusions

Until recently, the microanatomy evaluation of the ocular surface structures was limited to the impression cytology, which is based on sampling the superficial epithelial layers. This methodology, despite being highly reproducible, does not allow exploring deep tissues and induces discomfort to patients.

The rapid diffusion of LSCM in the last decades permitted an in vivo biopsy of all ocular surface tissues, at different depths, and the tissue analysis at cellular and subcellular level. The meibomian gland is one of the ocular surface structures that mostly benefited from the confocal assessment, since the standard meibography can only give a macroscopic analysis of the entire gland. The possibility of imaging MGs at cellular level, in vivo, and in a noninvasive way permit studying these structures in the most diffuse and important ocular surface diseases such as dry eye, ocular allergy, and drug toxicity.

In this way, LSCM may allow a preclinical diagnosis of MG conditions, potentially before glands begin to malfunction. In addition, since confocal microscopy does not produce significant discomfort for patients, this technique offers the advantage to strictly monitor the disease over time, to anticipate the treatment when required, to follow the response to therapy, and to modify the therapy regimen accordingly.

Moreover, the fine microscopic assessment could also clarify the pathophysiology of these conditions. This is even more important considering that the incidence of ocular surface diseases is exponentially growing in the last years.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors’ Contribution

Vincenzo Fasanella and Luca Agnifili equally contributed to this work and share primary authorship.
References


