

MICROSTRUCTURAL PROOFS OF DRY EYE CHANGES

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ABSTRACT

PURPOSE: To evaluate and to demonstrate the morphological changes of the Meibomian glands in patients with evaporative “dry eye” compared to normal subjects by *in vivo* laser scanning confocal microscopy and to correlate these changes to the clinical observations and tear functions.

DESIGN: Prospective over controlled case series

METHODS: The study was based on trans-tarsal images (optical slices) of 30 normal and 19 diseased lids (patients with subjective complaints and objective symptoms of evaporative “dry eye”). Each participant was examined by *in vivo* laser scanning confocal microscopy (HRTII Rostock corneal module). The results were compared to histological findings of normal or pathologically changed Meibomian glands.

RESULTS: Patients with evaporative “dry eye” presented with destructive changes of the Meibomian glands as follows: occlusion of the lumen, impaired morphology of the acines, lack of normal structure and infiltration with inflammatory cells. Reported ocular surface and tear function abnormalities were correlated to the Meibomian glands dysfunction. In all cases the lid hygiene and anti-inflammatory treatment demonstrated tendency to restoration of the structure.

CONCLUSION: *In vivo* laser scanning confocal microscopy can effectively demonstrate the morphological changes of the Meibomian glands in patients with evaporative dry eye symptoms. This new noninvasive technology is useful as a supplementary diagnostic tool for *in vivo* assessment of the histopathology of many ocular surface disorders and monitoring of the therapeutic effect in patients with Meibomian glands dysfunction. Glandular acinar density and acinar unit diameter seemed to be promising new parameters of Meibomian glands *in vivo* confocal microscopy. The examination has the potential to change the evaporative dry eye treatment approach.

Key words: *dry eye, Meibomian glands, in vivo laser confocal microscopy*

INTRODUCTION

Dry eye syndrome (DES) is a common problem often underdiagnosed in the eye care practice.

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However, it is a difficult condition to treat as clinical signs do not always correlate with patient symptoms. DES is thought to be a product of tear film abnormality, aqueous deficiencies or evaporation of the tear film (1), although it may also be a result of lid closure abnormalities or environmental conditions (e.g. air conditioning) (2).

The dry eye syndrome (DES) includes a broad spectrum of signs and symptoms that makes its definition and classification highly complex. That is one of the reasons why there are various other terms associated with this condition, such as dry eye syndrome, chronic dry eye disease, dysfunctional

tear syndrome, keratoconjunctivitis sicca or keratitis sicca (3-6).

“Dry eye” is one of the leading reasons for patient’s visits to ophthalmologists (7–11). Up to 25% of patients consulting eye care practitioners present with dry eye symptoms (12). Dry eye syndrome can be divided into two types: evaporative and aqueous deficient (13). Evaporative dry eye may arise due to a Meibomian oil deficiency or lid inadequacies such as an incomplete blink or a low blink rate (inability to properly distribute tears) (13).

The sebaceous glands within the lids have been described by Heinrich Meibom and named after him – Meibomian glands (MG). They produce secretion via holocrine mechanism. Meibomian glands (MG) are embedded in the tarsal plate of the upper and lower lids. Each MG comprises multiple acini connected by a long common central duct running throughout the entire length of the gland. Lipids excreted by MGs form the superficial layer of the tear film and are thought to retard tear evaporation and function as lubricants for the eyelids during blinking (14).

In the past light scanning *in vivo* confocal microscopy allowed evaluation of transparent tissues only. The new emerging non-invasive technology - *In vivo* laser scanning confocal microscopy is useful as a supplementary diagnostic tool for *in vivo* assessment of the histopathology of many ocular surface diseases and anterior-segment disorders including the *in vivo* examination of the bulbar and palpebral conjunctiva and the Meibomian glands (14). In this study, we used *in vivo* laser scanning confocal microscopy, selecting new diagnostic parameters, to evaluate the morphological changes of the MG in patients with Meibomian gland dysfunction (MGD) and compared the results with those of healthy control subjects.

METHODS

The study included 30 normal and 19 diseased lids (patients with subjective complaints and objective symptoms of evaporative “dry eye”). Each participant was examined by *in vivo* laser scanning confocal microscopy (HRTII Rostock corneal module) in order to acquire trans-tarsal images of the Meibomian glands. The study excluded all subjects with local eye diseases, use of medications oth-

er than non-preservative artificial tears and patients wearing contact lenses.

The Tear Break Up Time test was performed as follows, a fluorescein strip (Flourescein Strips Chauvin, Lab. Chauvin, Aubenas, France) was applied in the lower eyelid fornix, and the patient asked to blink three times and look straightforward. The break-up time of the tear film observed under cobalt blue filtered light of the slit lamp microscope was recorded with a stopwatch. The mean of three consecutive tear break-up tests was taken into consideration, and that of 5 s or less was considered abnormal (18).

In vivo laser scanning confocal microscopy was performed on all subjects with a new generation confocal microscope, the Rostock Corneal Software Version 1.2 of the HRTII-RCM (Heidelberg Retina Tomograph II - Rostock Cornea Module, Heidelberg Engineering GmbH, Germany), based on a diode laser with a wavelength of 670nm. Gel (Corneregel, Bausch&Lomb GmbH, Berlin, Germany) was used as a coupling agent between applanating lens cap and the objective lens.

After the eyelid was anaesthezied (Alcain, 0,5% collyr, Alcon) it was everted, the Tomo-Cap was positioned onto the palpebral conjunctiva by adjusting the controller, and the digital images of the underlying MG were observed on the computer screen. When the first superficial conjunctival cells were visualized, the digital micrometer gauge was set at zero, and then by pressing on the foot pedal, sequence images were recorded by a charge-coupled device (CCD) camera (maximum 30 frames/s) while gradually moving the focal plane into the subconjunctival tissue until the glandular structures were visualized. MGs were scanned while moving the applanating lens from the lids margins toward the fornix with minute vertical movements. MGs were also scanned while moving the applanating lens along the entire lid length with minute horizontal movements. The length of a single confocal microscopy examination session was approximately 10 min. None of the subjects complained of discomfort nor any adverse effects were observed as consequence of examination.

RESULTS

In vivo laser scanning confocal microscopy images demonstrated the Meibomian glands normal

morphology and anatomy of the Meibomian gland (fig. 1A) and the total obstruction of the Meibomian gland ductus (fig. 1B).

In vivo laser scanning confocal microscopy images also disclosed morphological alterations in patients with “dry eye” including the enlargement of glandular acinar units due to inspissation of meibum

secretions. We could note the atrophy in the glands with extensive periglandular fibrosis (fig. 2 A,B).

The pictures of *in vivo* laser scanning confocal microscopy demonstrated that the “dry eye” Meibomian gland is with enlarged lumen diameter (fig. 3A and B).

Patients with evaporative “dry eye” presented with destructive changes of the Meibomian glands

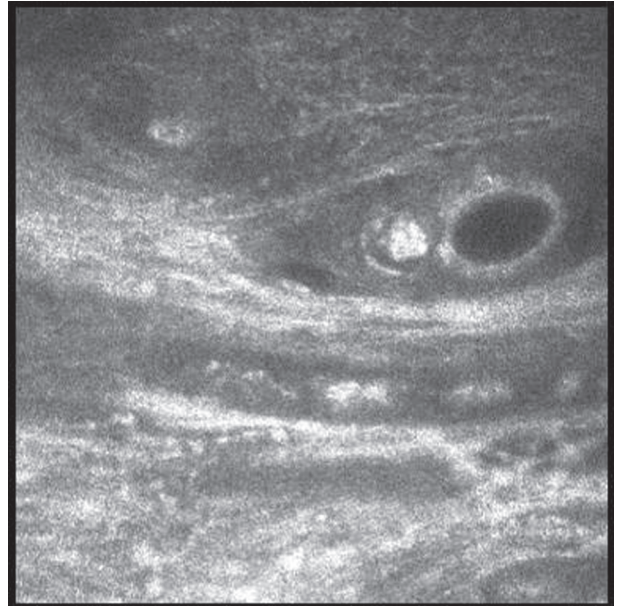
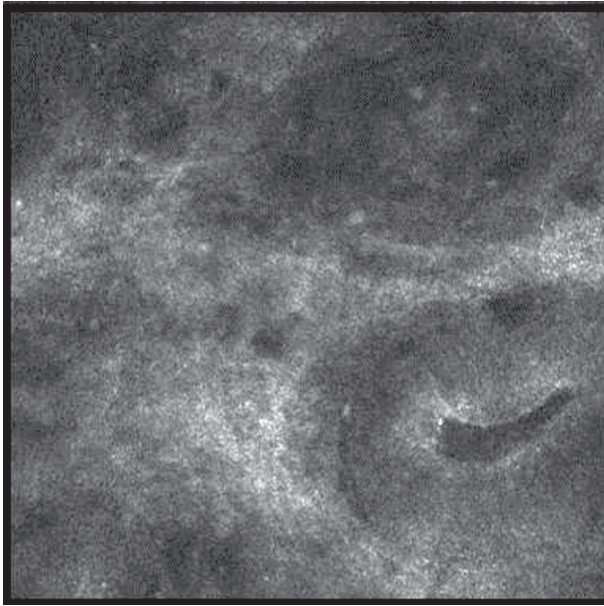


Figure 1 (A and B): Normal morphology and anatomy of the Meibomian gland (A) and total obstruction of the Meibomian gland ductus (B).

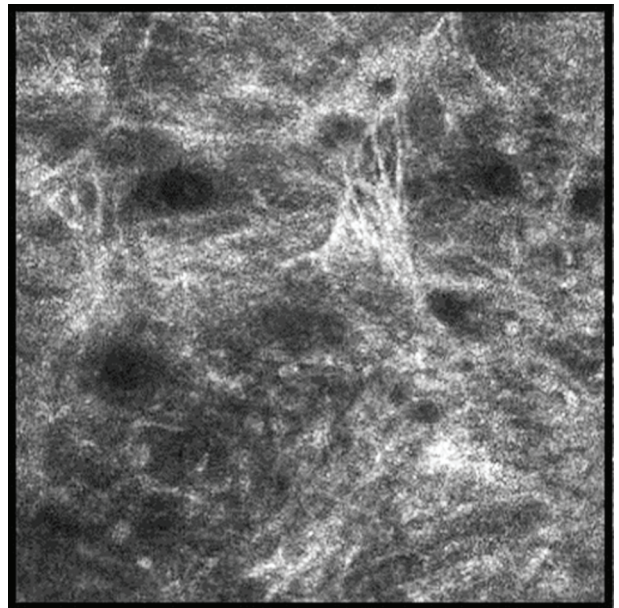
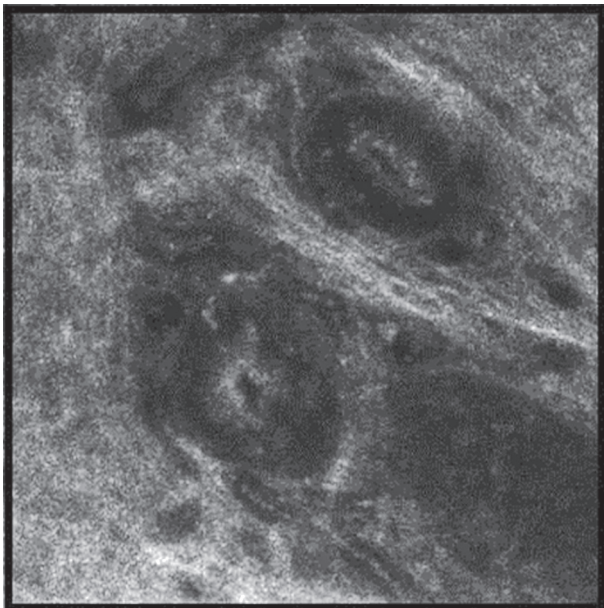


Figure 2 (A and B): The images demonstrated normal structure of periglandular tissue (A) and the picture of Meibomian gland atrophy with extensive periglandular fibrosis (B).

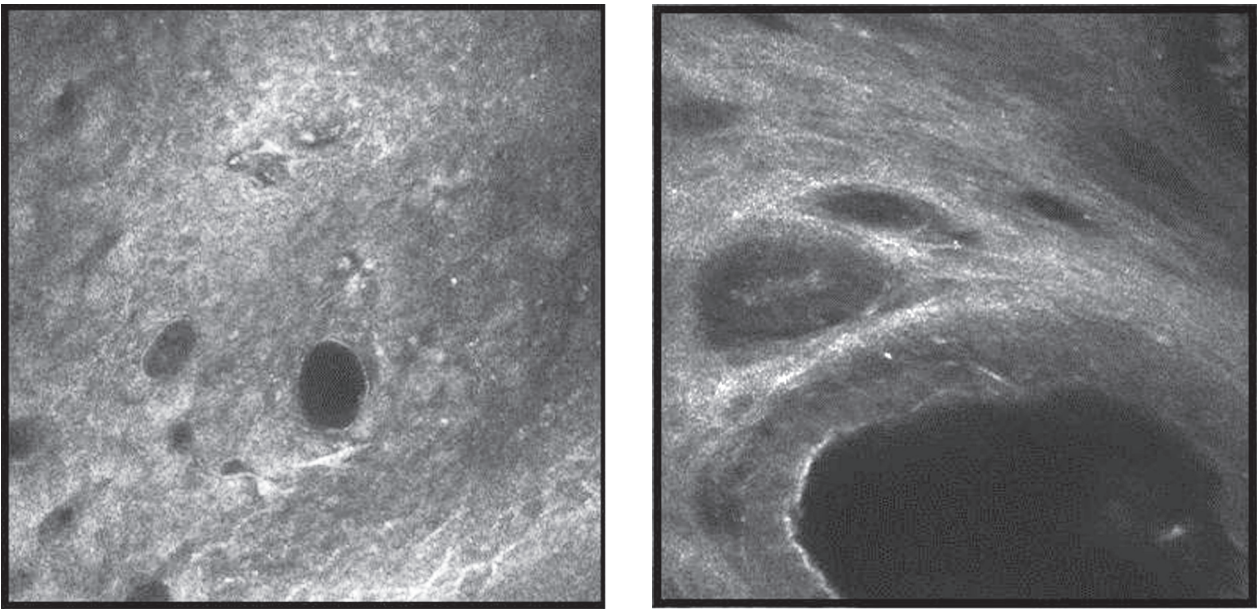


Figure 3 (A and B): On picture A it is demonstrated the normal structure of Meibomian gland with normal diameter of the ductus. On picture B it is seen “dry eye” Meibomian gland lumen with enlarged diameter.

as follows: occlusion of the lumen, impaired morphology of the acines, lack of normal structure and infiltration with inflammatory cells (fig. 4 A and B). This dysfunction may result in alteration of the tear film, and following symptoms: eye irritation, clinically apparent inflammation, ocular surface disease.

In vivo laser scanning confocal microscopy images presented the picture of inflammatory infiltration of the Meibomian glands. The mean inflammatory cell density in “dry eye” patients was 202 ± 24 cells/mm². The quantity of inflammatory cells correlated to the clinical observations and tear dysfunctions.

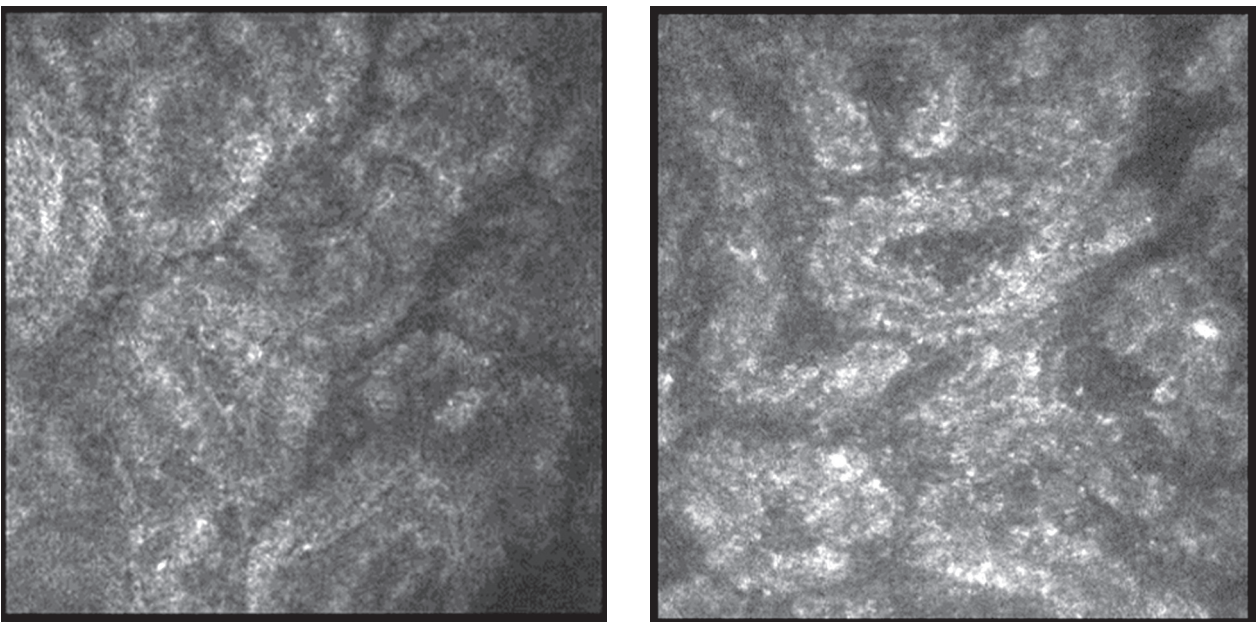


Figure 4 (A and B): It is demonstrated the normal structure of Meibomian glands (A) and inflammatory changes of the Meibomian glands (B).

In this study, *in vivo* laser scanning confocal microscopy of Meibomian glands was performed on all subjects with “dry eye” symptoms and on all controls. The mean acinar unit density was 116 ± 2 glands/mm² in the group of the controls. In patients with evaporative “dry eye” the mean acinar unit density was reduced to 47.6 ± 1.4 / mm². This value was significantly lower than the mean value measured in normal controls. The mean surface of a gland was measured to 0.0159/mm², and compared to normal controls (0.0086/mm²) was significantly lower.

The BUT values were significantly lower in patients with “dry eye” (5.1 ± 1.1 s) when compared to normal controls (12.5 ± 1.5 s). In all cases the lid hygiene and anti-inflammatory treatment demonstrated tendency to restoration the BUT and increased the value with 2mm.

DISCUSSION

Meibomian gland dysfunction (MGD) is a term that is mainly used to describe obstructive Meibomian glands disease. MGD is a major cause of “dry eyes” and has been reported to result in the alteration and/or reduction of lipid secretions, leading to increased tear evaporation, decreased tear stability, loss of lubrication, and damage to the ocular surface epithelium (15). This dysfunction may result in alteration of the tear film, and following symptoms: eye irritation, clinically apparent inflammation, ocular surface disease. MGD is one of the most common disorders encountered in ophthalmic practices. Hom et al. reported the prevalence of MGD to be 38.9% (16). Molinari and Stanek found the prevalence to be 33% in patients younger than 30 years and 71.7% in individuals 60 years or older (17).

The direct method allows visualization of the Meibomian glands by microscopy, or by meibography, via transillumination through the tarsus. Meiboscopy is the quantification of Meibomian gland dropout by using lid transillumination, while meibography is the same technique, but using photodocumentation, usually infrared camera, and long term record. Both methods, still have limited clinical and wider research applications (18).

Using *in vivo* laser scanning confocal microscopy, Ibrahim et al. made classification of the Meibomian gland presentation in 3 stages:

1. Typical acinar unit: the mean acinar unit density was 139 ± 8 glands/mm², the mean unit diameters were 45.3 ± 15.0 and 24.9 ± 7.3 μm, respectively, the mean inflammatory cell density (ICD) was 13 ± 1 cells/mm²
2. Meibomian gland dysfunction: the Meibomian gland dropout grade 2, and the Meibomian glands expressibility grade 2, atrophy in the glands with extensive perigranular inflammatory cells, the mean acinar unit density was 26 ± 3 glands/mm², the longest and shortest acinar unit diameters were 67.3 ± 27.4 and 37.9 ± 7.1 μm, respectively, the mean inflammatory cell density (ICD) was 1167 ± 10 cells/mm²
3. Meibomian gland dysfunction (enlargement of acinar unit): The Meibomian drop out grade was 2 and expressibility grade was 3, the mean acinar unit density was 40 ± 5 glands/mm², the longest and shortest acinar unit diameters were 133.5 ± 62.3 and 75.0 ± 8.1 μm, respectively, the mean inflammatory cell density (ICD) was 232 ± 9 cells/mm² (19).

To further investigate the morphological alterations in the MGs, Yakihiro Matsumoto et al. employed *in vivo* laser confocal microscopy and devised two new confocal microscopy based diagnostic parameters, glandular acinar unit density and acinar unit diameter, for the first time in the literature (20). They believed it might have had a relation with the morphological observations of the MGs such as the MG dropout and MG expressibility grades. Both the mean acinar unit density and diameter showed significant relations with MG dropout grades (20).

In vivo laser scanning confocal microscopy is a new emerging non-invasive technology, which is useful as a supplementary diagnostic tool for *in vivo* assessment of the histopathology of many ocular surface diseases. In this study, *in vivo* laser confocal microscopy of Meibomian glands was performed on all subjects with “dry eye” symptoms and on all controls.

The mean acinar unit density that we found in our study was 116 ± 2 glands/mm² in the group of the controls. In patients with evaporative “dry eye” the mean acinar unit density was reduced to 47.6 ± 1.4 /mm².

This value was significantly lower than the mean value measured in normal controls. The mean surface of a gland was measured to 0.0159/mm², and compared to normal controls (0.0086/mm²) was significantly lower.

In vivo laser scanning confocal microscopy images also disclosed morphological alterations in patients with MGD including the enlargement of glandular acinar units due to inspissation of meibum secretions. We noted the atrophy in the glands with extensive periglandular fibrosis. The atrophic acinar units appeared as small irregular structures with periglandular fibrosis compared to normal and round acini observed in healthy control subjects. The mean inflammatory cell density in “dry eye” patients was 202±24 cells/mm². The quantity of inflammatory cells correlated to the clinical observations and tear dysfunctions.

Meibomian glands function is judged on the basis of indirect and direct methods. The principle indirect method is tear break up time (TBUT). In accordance with previous published reports, we confirmed the presence of an ocular surface disease state, which was characterized by shorter BUT. Yakihiro Matsumoto et col. reported the BUT values significantly lower in MGD patients (4.9±2.4 s) when compared to normal controls (8.5±3.7s; <0.05)[20].

In our study we found the BUT values in patients with “dry eye” and in healthy patients. In all cases the BUT values were significantly lower in patients with “dry eye” when compared to normal controls.

CONCLUSION

Meibomian gland disease is a serious clinical problem with increasing incidence and prevalence. *In vivo* laser scanning confocal microscopy can effectively demonstrate the morphological changes of the Meibomian glands in patients with evaporative dry eye symptoms. This new non-invasive technology offers new opportunities for *in vivo* non-invasive histopathological evaluations of the ocular surface. It is useful as a supplementary diagnostic tool for *in vivo* assessment of many ocular surface disorders and monitoring of the therapeutic effect in patients with Meibomian glands dysfunction. Further studies regarding confocal features of Meibomian glands in various types of ocular surface diseases could lead to a new morpho-functional classification of Meibo-

mian glands alterations and to additional therapeutic modalities.

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