In Vivo Confocal Microscopy of Meibomian Glands in Contact Lens Wearing

Edoardo Villani, Gaia Ceresara, Silvia Beretta, Fabrizio Magnani, Francesco Viola, and Roberto Ratiglia

PURPOSE. To evaluate by in vivo laser scanning confocal microscopy (LSCM) the morphologic changes in the meibomian glands (MGs) and the status of periglandular inflammation in contact lens wearers (CLWs) and to investigate the correlations between clinical and confocal findings.

METHODS. Twenty CLWs and 20 age- and sex-matched control subjects were consecutively enrolled. Each participant completed an Ocular Surface Disease Index questionnaire and underwent a full eye examination, including tear film break-up time, fluorescein and lissamine green staining, and Schirmer test. LSCM of the MGs were performed to determine the cell density of the mucocutaneous junction epithelium, acinar unit density and diameter, glandular orifice diameters, meibum secretion reflectivity, and inhomogeneous appearance of the glandular interstice and acinar wall.

RESULTS. All clinical parameters showed statistically significant differences between groups (P < 0.01, Mann-Whitney U test) except the Schirmer test. Confocal data (Mann-Whitney U test) showed significantly decreased basal epithelial cell density (P < 0.01), lower acinar unit diameters (P < 0.05), higher glandular orifice diameters (P < 0.05), greater secretion reflectivity (P < 0.01), and greater inhomogeneity of the periglandular interstices (P < 0.05) in CLWs compared with controls. The duration of contact lens wear was correlated with the acinar unit diameters (P < 0.05, Spearman).

CONCLUSIONS. Morphologic changes in the MGs shown by LSCM were interpreted as signs of MG dropout, duct obstruction, and glandular inflammation. A comprehensive LSCM evaluation of the ocular surface in CLWs could better clarify the role of MG dropout and eyelid margin inflammation on the pathogenesis of CL-induced dry eye. (Invest Ophthalmol Vis Sci. 2011;52:5215–5219) DOI:10.1167/iovs.11-7427

There are more than 140 million contact lens wearers (CLWs) in the world.1 Although contact lenses (CLSs) are useful for correcting refractive errors without affecting the appearance of the wearer, CL use can induce various complications, including infection, allergic conjunctivitis, corneal disorders, and dry eye. Among these complications, dry eye is particularly troubling, because 30% to 50% of CLWs report symptoms, and the discomfort associated with dry eye may lead to intolerance of CL wear.2-5 Therefore, the notion that CL wear can interfere with the ocular surface is of immediate relevance, and careful clinical examination of the conjunctiva, cornea, and eyelids remains a key aspect of the ongoing management of CLWs.

Soft CLs completely cover the cornea and extend approximately 2 mm onto the bulbar conjunctiva. In the course of eye movement and blinking, the lens can momentarily become displaced and impinge farther onto the bulbar conjunctiva, perhaps up to 4 to 5 mm from the limbus. In addition, the palpebral conjunctiva comes into contact with the anterior surface of the lens during blinking and closed-eye lens wear.6 The effects of CLs on the cornea and conjunctiva are likely to be different, not only because different portions of the lens come into contact with these tissues, but also because of the different anatomic and physiological constructs of them.

Several causative mechanisms have been proposed for dry eye in CLWs including inflammation,8-10 increased evaporation and osmolarity of the tear film,11-13 and dewetting of the CL surface.14,15 The meibomian glands (MGs) are specialized sebaceous glands that secrete the oily layer of the tear film and prevent evaporation. Dysfunction of these glands leads to alterations in the lipid layer thickness and tear film stability. Therefore, abnormally functioning MGs have been investigated as a possible cause of dry eye in CLWs. A new meibographic technique using an infrared filter and an infrared charge-coupled device camera was recently introduced for this purpose.16 An alternative method of assessing ocular anterior segment tissues is in vivo laser scanning confocal microscopy (LSCM). It enables microstructural analysis of the cornea, allowing fresh insight into its structure in health and in inherited and acquired disease.17 The purpose of this study was to evaluate by in vivo LSCM the morphologic changes of conjunctival MGs and the status of periglandular inflammation in CLWs. We also investigated the correlations between clinical and confocal findings.

METHODS

Subjects

This study adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each subject before the examination. For the purposes of this pilot study, 20 asymptomatic CLWs (9 male and 11 female; mean age ± SD, 25 ± 3.2 years) and 20 age- and sex-matched non-CLW volunteer control subjects (10 male and 10 female, 25 ± 3.5 years) were consecutively enrolled.

For the CLWs, inclusion criteria were daily (5–7 d/wk, 5–12 h/d) soft hydrogel CLs worn for at least 1 year. For both groups, the Ocular Surface Disease Index (OSDI) score was <25, indicating no
or mild ocular surface symptoms.\textsuperscript{17} Exclusion criteria for both groups were dry eye, dystrophies, or inflammations of the ocular surface, signs of lymphomas, AIDS, sarcoidosis, diabetes mellitus, systemic treatments with drugs with known ocular surface toxicity, or use of topical antiglaucoma and/or anti-inflammatory agents within 6 months from inclusion or previous ophthalmic surgery.

**Clinical Evaluation**

An accurate medical history was prepared for each participant in the study, and each completed the OSDI questionnaire for a standardized evaluation of dry eye-related symptoms. All subjects underwent a thorough ophthalmic evaluation, including biomicroscopic examination of ocular adnexa and anterior segment.

Tear film break-up time (BUT), corneal staining with fluorescein, and bulbar conjunctival staining with lissamine green were also performed. The ocular surface staining was scored according to the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) scheme.\textsuperscript{18} Tear secretion was evaluated by the Schirmer test with oxybuprocaine hydrochloride 0.4%.

To evaluate the MGs, transilluminated obserivation observation (imibography) of the lower eyelid using a fiberoptic device was performed. The degree of MG dropout\textsuperscript{19,20} was scored as follows: grade 0, no dropout; grade 1, dropout in less than half of the inferior tarsus; and grade 2, dropout in more than half of the inferior tarsus. Assessment of MG orifice obstruction was conducted applying digital pressure on the lower tarsus, and the degree of ease in expressing meibomian secretion (meibum) was evaluated semiquantitatively as follows: grade 0, no dropout; grade 1, dropout in less than half of the inferior tarsus; and grade 2, dropout in more than half of the inferior tarsus. The ocular surface staining was scored according to the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) scheme.\textsuperscript{18} Tear secretion was evaluated by the Schirmer test with oxybuprocaine hydrochloride 0.4%.

Both eyes were examined in all subjects. For statistical analysis, the eye with the lower BUT was selected. In case of equal scores for the two eyes, the discriminant criteria considered were, by order of relevance, the fluorescein staining and the conjunctival staining scores.

**Confocal Microscopy**

**Image Acquisition.** LSCM was performed on all subjects with a new-generation confocal microscope (HRT II Corneal Rostock Module; Heidelberg Engineering GmbH, Dossenheim, Germany) using a scanning wavelength of 670 nm. The objective lens (65× immersion; Carl Zeiss Meditec, Inc., Dublin, CA) was covered by a polymethylacrylate sterile cap (Tomo-Cap; Heidelberg Engineering GmbH) and had a working distance of 0.0 to 2.0 mm. Before each examination, a drop of oxybuprocaine chloride 0.4% and an ophthalmic gel (polyacrylic gel 0.2%) were separately instilled into the conjunctival fornix. After the lower eyelid was partly everted, the center of the sterile cap was applanated onto the center of the eyelid margin, horizontally halfway between the inner and outer canthi. The instrument focus was manually adjusted while the microscope was in the sector mode acquisition modality. Scanning was begun at the most superficial tissues and progressed down to the deepest ones visualized with a satisfactory resolution. Ten images for every 10 μm of depth were taken, as well as other images in mid-depth while we attempted to manually assess the quality of the different structures in the examination. This procedure was repeated on the nasal and temporal eyelid margins. The two-dimensional image sizes were 584 × 584 pixels with 400 × 400-μm field of view. The duration of a single confocal microscopy examination session was approximately 3 to 5 minutes.

**Image Analysis.** For each variable examined, we analyzed three randomly chosen, nonoverlapping, high-quality digital images of the nasal, middle, and temporal lower eyelid margins. We quantified the following variables: (1) Cell densities of the eyelid superficial and basal epithelia within the manually identified most available region of interest were calculated automatically (Cell Count software; Heidelberg Engineering GmbH); (2) the acinar unit diameter was manually measured as the longest axis of the acinar unit; (3) the density of manually identified MGs inside each 400 × 400-μm frame was calculated automatically with the cell count software; (4) the diameter of each glandular orifice was manually measured along the longest axis of the orifice; (5) and the meibum reflectivity and the inhomogeneous appearance of the (6) interstices and (7) walls of the acinar units were scored. For meibum reflectivity and inhomogeneity of the MG interstices and walls, we adopted a grading scale of 1 to 4 for each by comparison with reference images reported in a previous study by our group.\textsuperscript{21}

**Statistical Analysis**

The statistical analysis was conducted with commercial software (SPSS for Windows, ver. 12.0; SPSS Sciences, Chicago, IL). All data were calculated as the means ± SD. For each variable, the Mann-Whitney U test was applied to determine the statistically significant

**TABLE 1. Clinical Data for CLWs and Controls**

<table>
<thead>
<tr>
<th></th>
<th>OSDI</th>
<th>BUT</th>
<th>Corneal Staining</th>
<th>Conjunctival Staining</th>
<th>Schirmer Test</th>
<th>MG Dropout</th>
<th>MG Expressibility</th>
</tr>
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<tbody>
<tr>
<td>CLW</td>
<td>12.2 ± 7.6</td>
<td>8.5 ± 2.3</td>
<td>0.9 ± 2.1</td>
<td>2.2 ± 1.8</td>
<td>11.8 ± 3.4</td>
<td>1.5 ± 0.7</td>
<td>1.9 ± 0.9</td>
</tr>
<tr>
<td>C</td>
<td>4.5 ± 3.2</td>
<td>10.5 ± 1.2</td>
<td>0.3 ± 3.6</td>
<td>—</td>
<td>13.2 ± 4.5</td>
<td>0.85 ± 0.8</td>
<td>0.4 ± 0.6</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*P* values obtained by Mann-Whitney U test. CLW, contact lens wearer group; C, non-contact lens-wearing control group.

**TABLE 2. Cell Density of Superficial and Basal Epithelium of the Eyelid Margin**

<table>
<thead>
<tr>
<th></th>
<th>Superficial Epithelium</th>
<th>Basal Epithelium</th>
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<tbody>
<tr>
<td>CLW</td>
<td>1735 ± 456</td>
<td>3565 ± 879</td>
</tr>
<tr>
<td>C</td>
<td>1775 ± 527</td>
<td>4440 ± 845</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are the cell density (in cells per millimeter square). *P* values obtained by Mann-Whitney U test. CLW, contact lens wearer group; C, non-contact lens-wearing control group.
differences between the two groups. Correlations among variables were analyzed with Spearman’s index of linear correlation. The minimum criterion for tests of significance was $P < 0.05$.

RESULTS

Clinical Data

All CLWs wore hydrogel contact lenses, primarily during the day. Two of the 20 subjects reported occasional overnight wear. The average longevity of wear was $9 \pm 6$ years.

All the measured clinical parameters showed statistically significant differences between the two groups ($P < 0.01$, Mann-Whitney U test; Table 1), except for the Schirmer test.

Cell Density Data

There were no significant differences between groups in superficial epithelial cell density. The basal epithelial cell density (Fig. 1) was significantly lower in the CLW group than in the control group ($P < 0.01$, Mann-Whitney U test, Table 2).

MG Confocal Data

LSCM of the MGs showed that CLWs had significantly smaller acinar unit diameters (Fig. 2) than did the control group ($P < 0.05$, Mann-Whitney U test, Table 3); however, there were no significant differences in acinar density. The glandular orifice diameters were significantly larger in the CLWs than in the controls ($P < 0.05$, Mann-Whitney U test; Table 3).

CLWs had significantly increased MG secretion reflectivity (Fig. 3) and significantly higher inhomogeneous appearance of the interstices (Fig. 4) compared with the controls ($P < 0.001$ and $P < 0.05$, respectively, Mann-Whitney U test, Table 4). There was no difference in the appearance of acinar wall between groups.

In CLW subjects, the grade of inhomogeneous appearance of periglandular interstices and secretion reflectivity correlated significantly ($P < 0.05$, Spearman). In the same group, glandular orifice diameters and inhomogeneous appearances of the acinar wall also correlated significantly ($P < 0.01$, Spearman). Moreover, the duration of CL wear was significantly correlated to the acinar unit diameters ($P < 0.05$, Spearman).

DISCUSSION

Several causative mechanisms have been proposed to initiate the onset of dry eye in CLWs, including the abnormal functioning of MGs. Ong and Larke$^{22}$ suggested that mechanical trauma from CLs cause duct blockage in the MGs. This hypothesis was supported in a recent study by Arita et al.,$^{16}$ who observed that in most of the CLWs with MG changes, the clusters of MGs were shortened. Moreover, they reported that the shortening of the MGs in CLWs began from the distal side. These results suggest that chronic irritation of conjunctival MGs by CLs is a major causative mechanism for MG changes in CLWs. This hypothesis seems to be consistent with the observation that damage to the MGs depended on the duration of CL wear but not on the CL materials.$^{16}$

LSCM was used by Efron et al.$^{6}$ to investigate the effects of CL wear on the human bulbar conjunctiva at a cellular level. Their observations suggested that CL wear induces changes in the bulbar conjunctiva such as epithelial thinning, increased epithelial cell density, and accelerated formation and enlargement of microcysts. LSCM has been recently applied to the armament of modalities used in the examination of MGs, providing a new noninvasive tool with which to study morphologic changes.$^{19-21}$ This technology allows description and testing of the diagnostic values of acinar density and diameter, secretion reflectivity, and perig-
landular inflammation in MG disease subjects. Previous studies\textsuperscript{19,20} described the appearance of MGs during in vivo confocal examinations performed through the tarsal conjunctiva of the everted superior eyelid. Confocal examination requires a few minutes of contact between the instrument and examined tissue, and this can be uncomfortable for the subject. Moreover the instrument has a space-fixed orientation, and so it requires the examined tissue to be positioned parallel to the sterile cap’s face. To better visualize the glandular structures in a less invasive way, we performed confocal microscopy of the inferior eyelid margin, as described and discussed in an our previous MG confocal study.\textsuperscript{21}

We found CLW subjects to be characterized by higher MG dropout, tear film instability, and ocular surface epithelial damage, all of which are consistent with the recent literature.\textsuperscript{16,23,24} However, other studies found no significant difference in MG secretion of CLWs\textsuperscript{25} and no significant structural changes in the MGs of CLWs affected by dry eye compared with those who were unaffected.\textsuperscript{26}

In the study reported here, the eyelid margins of CLWs were evaluated by LSCM. We found that the basal eyelid margin epithelium, MG acini, glandular orifices, and periglandular interstices exhibited peculiar changes compared with controls. The basal marginal epithelium had a lower cell density in CLWs compared with the control group, suggesting that mechanical and inflammatory damage cannot be compensated by proliferative changes of the basal epithelium, as happens in the cornea.\textsuperscript{21,27,28}

LSCM also showed that the MGs in CLWs had decreased acinar unit diameters, increased glandular orifice diameters, highly reflective secretion, and great inhomogeneity of periglandular interstices. We hypothesize that the increased diameter of glandular orifices and inhomogeneous appearances of the acinar wall and the changes in MG secretion are the result of duct blockage caused by mechanical trauma from the CL, as suggested by Arita et al.\textsuperscript{16} and Korb and Henriquez.\textsuperscript{24} The hypothesis of a common pathogenetic mechanism is supported by the strong correlation found between the glandular orifice diameter and acinar wall appearance. The decreased diameter of acinar units and the periglandular interstitial inhomogeneity could be, respectively, signs of MG dropout and eyelid margin inflammation\textsuperscript{25} caused by chronic CL irritation.

We found that the acinar unit diameter decreased as a function of the number of years of CL wear. This is consistent with the report by Arita et al.\textsuperscript{16} that the loss of MGs depended on the duration of CL wear. They also reported that CL materials do not play a significant role in CL-related dry eye. We deduced that our results could be generalized to all CL types. Further LSCM studies of the effects of specifically selected CL shapes and physical characteristics, including the materials are needed to test this hypothesis.

In conclusion, LSCM offers new opportunities for in vivo noninvasive histopathologic studies of the ocular anterior segment. This modality showed morphologic changes in conjunctival MGs associated with mechanical irritation by CL wear. These alterations were interpreted as signs of MG dropout, duct obstruction, and glandular inflammation. A comprehensive LSCM evaluation of the ocular surface in CLWs, including MG and mucocutaneous junction analysis, could better clarify the role of CL-related MG dropout and eyelid margin inflammation on the pathogenesis of CL-related dry eye.

### References


