Cornea

Time Course of Changes in Goblet Cell Density in Symptomatic and Asymptomatic Contact Lens Wearers

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PURPOSE. To investigate longitudinal changes in goblet cell density (GCD) in contact lens (CL) wearers who do and do not develop symptoms of dry eye (DE).

METHODS. Sixty healthy individuals fitted with daily disposable hydrogel CLs and 23 age-balanced non-CL-wearing controls underwent assessment using the 5-item dry eye questionnaire, noninvasive tear film break-up time measurement, ocular surface assessment, and phenol red thread test evaluation. Laser scanning confocal microscopy (LSCM) and conjunctival impression cytology (CIC) were used to assess GCD at baseline and follow-up visits at 1 week and 1 and 6 months. After 1 week, all CL wearers were categorized as those who were and were not symptomatic based on responses to the CL dry eye questionnaire-8 (CLDEQ-8). A linear mixed-model was used to examine changes in GCD over time.

RESULTS. The global mean GCD of the 83 participants at baseline (before CL wear) was 476 ± 41 and 467 ± 52 cells/mm² using LSCM and CIC, respectively. After 6 months of CL wear, GCD was reduced by approximately 13% and 29% in asymptomatic (N = 29) and symptomatic (N = 17) CL wearers (all P < 0.001), respectively, observed with both LSCM and CIC.

CONCLUSIONS. Contact lens wear induces a reduction of GCD over 6 months, which is exacerbated in those with DE symptoms. Either LSCM or CIC can be used to assess GCD in the conjunctiva.

Keywords: contact lens wear, symptoms, dry eye, conjunctival goblet cells

Conjunctival goblet cells (GCs) are secretory epithelial cells responsible for the production of the mucinous component of the tear film to support the integrity of the ocular surface. A decreased number of GCs is common in conditions of dry eye (DE), which is described as a multifactorial disorder causing damage to the ocular surface.3 Goblet cells may also contribute to the ocular immune response, as they have been shown to secrete cytokines and modulate dendritic cell phenotype.3 The presence of mucin during contact lens wear (CLW) promotes surface wettability and tolerance to CLW. However, a lack of mucin discharge onto the ocular surface can cause the patient to experience symptoms such as itching, burning, reduction of visual acuity, and dryness.5

Contact lens (CL) wearers frequently report symptoms of dryness but the reason for this is not completely understood. The resulting discomfort may cause CL wearers to reduce wearing time, or cease lens wear altogether. Indeed, up to 50% of wearers will discontinue CLW permanently due to DE symptoms.6 It is therefore important to understand whether CLW causes a reduction in GCD in those who are introduced to CLW and to determine the link between GCD and DE symptoms related to CLW.

Reports of the impact of CLW on conjunctival GCD are equivocal, perhaps as a result of methodologic differences in using conjunctival impression cytology (CIC) to determine GCD. The time-course of changes to GCD as a result of CLW is also unclear.

Conjunctival samples obtained using CIC can be assessed using flow cytometry and polymerase chain reaction, allowing mucin-type material to be analyzed, and mRNA expression of specific MUC genes to be quantified. Due to the invasive, time-intensive, and laboratory-based nature of CIC, in vivo laser scanning confocal microscopy (LSCM) may represent an alternative and fundamentally advantageous approach for morphologic assessment of cells at the ocular surface.

The aim of this study is to determine longitudinal changes in GCD in those with and without CL-induced DE symptoms, using both CIC and in vivo LSCM. A secondary aim of this study is to determine whether in vivo LSCM could essentially replace CIC for assessing GCD. This work could help researchers and practitioners better understand the importance of GCs and the role they play in CL comfort.

METHODS

Study Design and Participants

This was a prospective, longitudinal, observational, case-controlled clinical study. Ethical clearance was provided by the Queensland University of Technology Research Ethics Committee (Kelvin Grove, Australia) and written informed consent was obtained from all participants prior to enrollment. The research was conducted in accordance with the principles of the Declaration of Helsinki.

Potential participants interested in wearing daily disposable CLs (details below) were recruited from the staff and student population of the Queensland University of Technology, Brisbane, Australia. Individuals were not eligible if they had
TABLE 1. Dry Eye and Ocular Surface Assessment Pass/Fail Scores for Exclusion Due to Dry Eye and Identification of Symptomatic Contact Lens Wearers

<table>
<thead>
<tr>
<th>Test</th>
<th>Measurement</th>
<th>Instrument</th>
<th>Method</th>
<th>Pass</th>
<th>Fail</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIBUT,8 s</td>
<td>Tear fluid</td>
<td>Keratometer</td>
<td>Time from blink to first mire distortion</td>
<td>≥10</td>
<td>1–9</td>
</tr>
<tr>
<td>OSA,9 0–4</td>
<td>Ocular surface integrity</td>
<td>Slit-lamp biomicroscope, yellow observation filter; fluorescein-impregnated strip; Elfon grading scale for corneal staining</td>
<td>Estimate corneal staining to nearest 0.1 grading increment</td>
<td>0–2</td>
<td>3–4</td>
</tr>
<tr>
<td>PRT,10 mm/20s</td>
<td>Tear volume</td>
<td>Hamano cotton thread tear test</td>
<td>Thread in lower lacrimal river; wetting length after 20s</td>
<td>≥11</td>
<td>1–10</td>
</tr>
<tr>
<td>DEQ-5,7 0–22</td>
<td>Frequency and intensity of DE symptoms</td>
<td>DEQ-5 questionnaire</td>
<td>Score on validated shorter version of DEQ11</td>
<td>0–6</td>
<td>7–22</td>
</tr>
<tr>
<td>CLDEQ-8,12 0–37</td>
<td>DE symptoms in CL wear</td>
<td>CLDEQ-8 questionnaire</td>
<td>Score on validated shorter version of CLDEQ13</td>
<td>0–16</td>
<td>17–37</td>
</tr>
</tbody>
</table>

worn CLs during the 6 months prior to enrollment, current pregnancy, ocular trauma or surgery, ocular surface dysfunction, current classification as symptomatic for DE based on answers to the DE questionnaire 5 (DEQ-5),7 current or long-term use of topical ocular medication, or ocular or systemic disease that may affect the conjunctiva. Additional exclusion criteria for the CL-wearing group were astigmatism of more than 1.50 diopters (D), myopia more than −7.00 D, and hyperopia more than +2.00 D.

Participants assigned to wear CLs were fitted with ‘Biomedics 1 day Extra’ daily disposable CLs (CooperVision, Pleasanton, CA, USA). These lenses are made from the hydrogel material ‘ocufilcon D’ and had the following parameters and characteristics: water content 55%, diameter 14.2 mm, base curve 8.6 or 8.8 mm, center thickness (at −3.00 D) 0.07 mm, power range −10.00 D to +6.00 D, oxygen permeability (Dk) 19 × 10−11 cm2 mL O2/s mL mm Hg, oxygen transmissibility (Dk/t) at −3.00 D 27 × 10−9 cm mL O2/s mL mm Hg, and light blue handling tint.

Lenses were worn on a daily wear basis and all lens wearing participants as well as a group of age-balanced controls underwent detailed assessment of DE signs and symptoms over a 6-month period. Validated DE questionnaires7–8 (DEQ-5 for controls and CLDEQ-8 for CL wearers) were applied and all participants also underwent DE tests and GCD assessment using LSCM and CIC at baseline, 1 week, and 1 and 6 months. The testing procedures using both techniques were conducted by two experienced examiners (L.H.C. doing CIC and Y.A. doing LSCM) who were masked as to the group assignment of participants. All the tests were performed between 7 AM and 12 PM.

Dry Eye and Ocular Surface Assessment

Symptoms and signs of DE were assessed during recruitment in order to exclude individuals with this condition. Symptoms were determined using the DEQ-5 questionnaire and DE signs assessed by noninvasive break-up time (NIBUT),9 phenol red thread (PRT),10 and ocular surface assessment (OSA).11 To be considered eligible for this study, participants were required to pass the DEQ-5 and at least one of the objective DE tests, according to the pass/fail scores shown in Table 1. The methods used during this study to assess DE and ocular surface staining have been reported in detail elsewhere14 and are briefly described in Table 1.

After 1 week of CLW, the CLDEQ-8 was used to differentiate those in the CL-wearing group as either symptomatic or asymptomatic for CL-induced DE. The subjective (CLDEQ-8) and objective (NIBUT, PRT, or OSA) DE tests were repeated at 1 and 6 months. All tests were performed a few minutes after CL removal.

Goblet Cell Density

In Vivo Laser Scanning Confocal Microscopy. Conjunctival LSCM was performed using the Heidelberg Retinal Tomograph (HRT3) equipped with a Rostock Corneal Module (Heidelberg Engineering GmbH, Heidelberg, Germany). One eye was anaesthetized with 0.4% oxybuprocaine hydrochloride (Chauvin Pharmaceuticals Ltd., Surrey, UK). The participant was instructed to direct their gaze opposite to the region of measurement. The center of the surface of the TomoCap was positioned on the conjunctiva approximately 2 to 4 mm from the limbus.

Goblet cells at the nasal bulbar conjunctiva were scanned while focusing the applanating lens at approximately three different depths, using a 400 × 400 μm2 sampling area. As reported previously, a minimum of 11 images are necessary to determine the average GCD with an acceptably low variance15; thus, 11 images were selected from the 30 captured images on each measurement occasion.

Images were selected from LSCM scans that included GCs identified according to the following features: cell size of 25 to 30 μm in diameter,16,17 hyperreflective,18 bigger than surrounding cells,19 round to oval in shape,20 and sometimes with a visible nucleus.2

Conjunctival Impression Cytology. A few minutes after performing LSCM, the same eye was anaesthetized again and the center of a Biopore membrane (Millicell cell culture inserts; Millipore Corp, Cork, Ireland) was gently applied to the nasal bulbar conjunctival surface at approximately 2 to 4 mm from the limbus. The sample was allowed to air dry and then immersed in 95% methanol for fixation using a well culture plate sample holder. The sample was then refrigerated at 4°C for no more than 24 hours.

Giemsa stain was applied according to the following guidelines from the manufacturer (Sigma-Aldrich, Dorset, UK). The specimen was allowed to air dry at room temperature, the Giemsa stain was diluted 1:20 with deionized water and the specimen was immersed in the diluted Giemsa solution for 30 minutes. The sample was rinsed with tap water prior to examination.

A Leica DM2500 microscope (Leica Microsystems, Milton Keynes, UK) was used to visualize the specimen; this system...
has a magnification of ×200 and field of view of 640 × 480 μm². Approximately 10 images were captured from each sample by scanning in X and Y directions.

Acceptable images from CIC were those with nondisrupted cell material that contained GCs approximately 25 to 30 μm in diameter. The cells had a pale membrane with defined borders and a visible nucleus localized centrally, although sometimes eccentrically in bigger cells (approximately 30 μm). Goblet cells were easily differentiated from surrounding cells because of their balloon-like appearance and size. The mean GCD for each specimen was determined by averaging cell counts obtained from five of the 10 captured images with non-disrupted cell material.

The processing of CIC samples and selection of CIC and LSCM images were carried out by two experienced operators who were masked as to the group assignment of participants.

Statistical Analysis

The number of participants enrolled into this study was determined by a sample size calculation based on two previous investigations that used a similar design to the present work. We calculated that a minimum sample size of 23 participants was required per group, allowing for 20% attrition. This analysis gave 90% power, with a type I error of 5% to detect a difference in GCD between the two groups.

Normality of data was examined using the Shapiro-Wilk test. Differences in the demographic and clinical characteristics between the controls and the asymptomatic and symptomatic CL-induced DE groups were determined for the baseline and final visits. Dry eye and ocular surface assessments, including questionnaires, were compared among the controls and the asymptomatic and symptomatic CL-induced DE groups using paired and independent sample t-tests. Nonparametric data were analyzed using χ², Wilcoxon and Mann-Whitney U tests. All data are presented as mean ± SD unless otherwise indicated.

A linear mixed-model (LMM) was applied to examine changes in GCD of the nasal bulbar conjunctiva over time. Because changes of GCD in CLW over time was the main parameter of interest of this study, GCD was considered the response variable and time was added to the model to test the linear effect of CLW on GCD. The model contained GCD as the response variable. The primary fixed effects of interest were: group (i.e., controls, asymptomatic and symptomatic); test (i.e., LSCM and CIC); visit (i.e., baseline, 1 and 6 months); group*visit; and test*visit interactions. Type III sum of square test of fixed effects shows overall significance for the predictor. A significant reduction of GCD in the symptomatic and asymptomatic groups compared with the control group (P < 0.001) was observed. After 6 months of CLW, GCD was reduced by approximately 13% and 29% in asymptomatic (N = 29) and symptomatic (N = 17) CL wearers, respectively, observed with both LSCM and CIC.

The clinical characteristics and demographic data of participants who did and did not develop CL-induced DE symptoms after 1 week of CL wear and the controls, at the baseline and final visits, are presented in Table 2. The baseline measurements of the questionnaires were considered at 1 week after characterization of the symptomatic and asymptomatic groups among CL wearers. At baseline, before characterization, the 83 participants (36 males and 47 females) in the study had a mean age of 30 ± 8 years and were age- and sex-balanced (P = 0.89 and P = 0.22, respectively).

At baseline, the mean DEQ-5 score of the control group was significantly lower than that for the groups with and without symptoms of CL-induced DE (P < 0.01). At both the 1-week and 6-month visits, the CLDEQ-8 score was significantly higher (~2 times worse) in participants with symptoms compared with those without symptoms of CL-induced DE (P < 0.001). No significant difference existed between the three groups in regard to the NIBUT, OSA, and PRT test (P > 0.17) at baseline. Sixteen participants discontinued from the study, meaning that 83% of the enrolled participants completed the study. All CL wearers had refractive errors between −6.75 and +1.75 D and astigmatism from −0.25 to −1.25 D.

Contact lens wearing time, as shown in Table 2, after 6-month wear was not significantly different between symptomatic and asymptomatic groups (mean difference 1.5 hours, P = 0.38). The symptom score (DEQ-5) in the control group was the same at the baseline and 6-month visit (P = 0.64), whereas in both the symptomatic and asymptomatic groups the CLDEQ-8 symptom score was lower at the final visit than the baseline visit (P < 0.001). Noninvasive break-up time was not different at the final visit compared with baseline for the three groups (P > 0.10). Ocular surface assessment scores at baseline were greater at the final visit than at the baseline visit for both the symptomatic and asymptomatic groups (P < 0.01). A significant decrease was noted in PRT test values at the final visit, compared with the baseline visit, for the symptomatic group versus the control and asymptomatic groups (P < 0.003).

There was more than a 2-fold decrease of GCD at the 1-week visit in all study participants, observed using both LSCM and CIC. The reduction of GCD in non-CL wearers is thought to be an artefact caused by the removal of superficial cell layers in the nasal bulbar conjunctiva using the CIC technique at baseline. Therefore, to analyze the longitudinal effect of CLW on conjunctival GCD, the 1-week visit was removed from the analysis and LMM was applied. Figure 2 shows the longitudinal course of GCD over a 6-month period, excluding the 1-week visit data, assessed with LSCM (Fig. 2A) and CIC (Fig. 2B). To understand the effect of symptom grouping and time on the outcome variable, the LMM was applied. The Type III test of fixed effects shows overall significance for the predictor variables. There was a significant effect of group and visit; however, the effect of test (LSCM versus CIC) was not
TABLE 2. Demographic and Clinical Characteristics of Participants at Baseline and After 6 Months of Contact Lens Wear

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Month 6 Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Asymptomatic*</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Asymptomatic*</td>
</tr>
<tr>
<td>n (male/female)</td>
<td>23↑†‖ (19/4)</td>
<td>35† (10/25)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>30.0 ± 8.0</td>
<td>32.1 ± 9.8</td>
</tr>
<tr>
<td></td>
<td>30.5 ± 8.0</td>
<td>31.2 ± 9.3</td>
</tr>
<tr>
<td>CLW, h</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>9.8 ± 3.9</td>
</tr>
<tr>
<td>DEQ-5, 0–22</td>
<td>2 ± 2†‡</td>
<td>4 ± 2†‡</td>
</tr>
<tr>
<td></td>
<td>2 ± 2</td>
<td>9.8 ± 3.9</td>
</tr>
<tr>
<td>CLDEQ-8, 0–37</td>
<td>-</td>
<td>12 ± 3†§</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>11 ± 6*</td>
</tr>
<tr>
<td>NIBUT, s</td>
<td>12.7 ± 6.1</td>
<td>11.9 ± 5.6</td>
</tr>
<tr>
<td></td>
<td>10.0 ± 4.3</td>
<td>11.0 ± 4.7</td>
</tr>
<tr>
<td>OSA, 0–4</td>
<td>0.5 ± 0.6</td>
<td>0.5 ± 0.5**</td>
</tr>
<tr>
<td></td>
<td>0.3 ± 0.4#</td>
<td>0.7 ± 0.6**</td>
</tr>
<tr>
<td>PBT, mm/20s</td>
<td>22.7 ± 8.4</td>
<td>19.9 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>18.8 ± 7.7**</td>
<td>16.8 ± 8.4#</td>
</tr>
<tr>
<td>GCD–LSCM, cell/mm²</td>
<td>491 ± 43†</td>
<td>474 ± 40**</td>
</tr>
<tr>
<td></td>
<td>482 ± 33††</td>
<td>408 ± 50</td>
</tr>
<tr>
<td>GCD–CIC, cell/mm²</td>
<td>489 ± 47†</td>
<td>458 ± 55†‡‡</td>
</tr>
<tr>
<td></td>
<td>457 ± 41††</td>
<td>406 ± 50</td>
</tr>
</tbody>
</table>

* Groups were assigned at 1-week visit.
† P < 0.05, baseline comparison of controls vs asymptomatic.
‡ P < 0.05, controls vs. symptomatic.
§ P < 0.05, asymptomatic vs. symptomatic.
|| P < 0.05, month 6 follow-up comparison of controls vs. asymptomatic.
¶ P < 0.05, month 6 follow-up comparison of controls vs symptomatic.
# P < 0.05, month 6 follow-up comparison of asymptomatic vs. symptomatic.
** P < 0.05, baseline vs month 6 follow-up comparison of asymptomatic.
†† P < 0.05, baseline vs month 6 follow-up comparison of symptomatic.
significant. The interaction between groups and visits was significant. No significant interaction existed between test and visit. Figure 3 shows representative images of the conjunctiva, using LSCM and CIC, captured from a participant from each group at the 6-month visit.

A second subset, with restricted maximum likelihood estimation of fixed-effect parameters for GCD as the continuous response variable, was included in the LMM. In the model, there was no effect of test (LSCM versus CIC) on GCD ($P = 0.36$); however, there was a significant effect of group ($P < 0.001$). At the 1-month visit, the interaction between group and visit was significant for CL wearers compared with controls ($P < 0.05$). The LMM also showed a differential effect of time on GCD, with a decrease of 128 cells/mm$^2$ in individuals who developed symptoms versus a decrease of 84 cells/mm$^2$ in the asymptomatic group, compared with controls. There was no effect of test, and the interaction between test and visit was not significant ($P = 0.30$).

**DISCUSSION**

In vivo assessment of conjunctival GCD using LSCM has emerged as a valuable noninvasive technique that enhances the investigation of many CL-induced changes in the anterior eye. Goblet cell density assessment using LSCM has been used to assess ocular surface changes related to age, Sjögren’s syndrome DE, pterygium, chemical burns, glaucoma treatment with preserved and unpreserved levobunolol, and tafluprost therapy. To date, only one cross-sectional report of GCD assessed by LSCM in CLW has been published, which showed no significant difference between CL wearers and non-CL wearers; however, that study was limited by small sample size.

Previous longitudinal assessments of GCD in CLW have been undertaken using in vitro analysis with CIC. The present study examines, for the first time, longitudinal changes of GCD in CL wearers using both LSCM and the well-established CIC technique.

Over a 6-months period, GCD was reduced by approximately 13% in the asymptomatic and 29% in the symptomatic group. There was no significant difference between GCD assessed using LSCM versus CIC over time ($P = 0.30$). The control group remained relatively constant over time with a coefficient of variation of 4% and 7% using LSCM and CIC, respectively.

The LMM revealed that, regardless of group, the test had no significant effect on GCD. A test*time interaction term was not significant ($P = 0.30$), indicating that the assessment of GCD using LSCM and CIC did not differ over time regardless of the changes in GCD.

An additional model was developed in order to determine longitudinal change only for the control group. The Type III

**FIGURE 2.** Time course of GCD over a 6-month period, excluding the 1-week visit, in CLW and control groups assessed with (A) LSCM and (B) CIC. Solid line, control participants; dotted line, asymptomatic CL wearers; dashed line, symptomatic CL wearers. Error bars, mean ± SD.

**FIGURE 3.** Representative (A, C, E) CIC and (B, D, F) LSCM images captured at the 6-month visit. (A) and (B) are from a symptomatic CL wearer; (C) and (D) are from an asymptomatic CL wearer; and (E) and (F) are from a non-lens–wearing control participant.
fixed-effects showed no significant changes from the baseline to final visit ($P = 0.14$), suggesting that GCD decreased in the symptomatic and asymptomatic groups due to the effect of CLW.

The results of this study disagree with the results from Connor$^{32,33}$ Lievens,$^{34}$ and Corrales,$^{35}$ who reported a statistically significant increase or no changes to GCD assessed with CIC over a 6-month and 1-year period, in participants fitted with CLs. This disagreement may be attributed to variances in the following factors relating to CIC technique: sample inconsistency across the filter; number of images sampled; criteria for identifying GCs; quality of acetate filter used for cell attachment; units used to report GCD; and conjunctival region assessed. Also, differences in lens-related factors such as material (e.g., conventional hydrogels versus silicone hydrogels) and replacement frequency (e.g., daily/monthly replacement) make it difficult to compare results from various studies. Few of the previous studies$^{32–34}$ incorporated a non-CL wearing control group.

Connor$^{32}$ reported a 2-fold increase in GCD of 18 participants fitted with 38% water content CLs used on a daily basis, without CL replacement, initially for 6 months. The same authors repeated their study 3 years later using a larger sample size ($n = 28$) and replaced lenses every 2 weeks (instead of 6 months). The authors found no significant changes from the baseline to final visit. Lievens et al.$^{34}$ repeated the study 6 years later comparing hydrogel and silicone hydrogel lenses worn on an extended wear basis (6 consecutive days) with weekly and monthly lens replacement. This study again found a significant increase in GCD, but no difference between the two groups.

There are further methodologic problems with the three above-mentioned studies. Only one image per sample was used to determine GCD. Goblet cell density was expressed as the percentage of GCs of the total number of cells counted (including non-GCs) per field of view. In order to have GCs attach to an acetate filter, the sample must be multilayered.$^{15,21,22}$ If a multilayered sample is stained with PAS for GC detection and counter-stained for non-GCs, it is not possible to undertake a total cell count because there would be two to three non-GC layers obscuring the view. In contrast to the above studies, the present findings agree with the longitudinal findings of Simon et al.$^{24}$ who reported a decrease of GCD after fitting participants with soft and rigid CLs for 6 months. These authors used CIC and correlated the severity of symptoms with cytological alterations using a grading system for squamous metaplasia.$^{24}$

The findings of this study demonstrate a longitudinal equivalence between LSCM and CIC. The strengths of this study are the recruitment of sufficient participants as guided by power analysis; use of both a noninvasive, reiterative technique (LSCM) and a gold standard invasive technique (CIC) to assess GCD; careful phenotyping of symptomatic and asymptomatic lens wearers; incorporation of a non-CLW control group; appropriate masking of observers; use of validated image capture (LSCM) and sample collection (CIC) protocols; masked image selection and analysis methodology, and the use of robust statistical modelling.

A general limitation of all studies using CIC is the lack of evidence relating to the validity of undertaking repeated measures. We have demonstrated that it may take up to 4 weeks for GCD to recover post-CIC using Biopore Millicell inserts. The reduction of GCD in non-CL wearers at 1 week is attributed to an artefact caused by the removal of superficial cell layers of the epithelium. The conjunctival epithelium is known to have a rapid healing response, effected by cell migration and mitosis, whereby normal thickness is restored within 48 to 72 hours.$^{36}$ However, the time-course of GC differentiation, regeneration or migration from inner layers is unknown. The recovery time for the repopulation of GCs after having been removed from the conjunctival surface with different types of acetate filters is also unknown.

The 6-month time frame of this study may have been insufficient to determine the full extent of changes to GCD in symptomatic patients. The sex imbalance between groups limited our ability to draw conclusions regarding gender differences in CL-induced DE and GCD.

Further studies are required to determine mechanisms of mucin production and secretion by GCs in relation to DE symptoms during CL wear. It has recently been demonstrated that GCs play an important role in the mediation of dendritic cell phenotype by secreting cytokines.$^{3}$ New technologies are needed to fully elucidate functional aspects of conjunctival GCs and to determine the role of GCs in modulating the ocular environment in response to external stress such as that imposed by CLW.

In summary, the results of this study demonstrate that LSCM has the capability to detect changes of GCD in patients with CL-induced symptoms of DE. Furthermore, GCD is reduced in CLW, with the reductions being greater in those who develop symptoms.

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