Identification of Leukocytes Associated With Midday Fogging in the Post-Lens Tear Film of Scleral Contact Lens Wearers

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Submitted: June 19, 2018
Accepted: December 7, 2018

Citation: Postnikoff CK, Pucker AD, Laurent J, Huisingh C, McGwin G, Nichols JJ. Identification of leukocytes associated with midday fogging in the post-lens tear film of scleral contact lens wearers. *Invest Ophthalmol Vis Sci*. 2019;60:226–233. https://doi.org/10.1167/iovs.18-24664

Purpose. Midday fogging is a frequent complaint among scleral contact lens (ScCL) wearers, and the mechanism and cause of this is unknown. The purpose of this investigation was to understand the relation between midday fogging, ocular surface leukocytes, and ScCL fitting characteristics.

Methods. Subjects arrived at a clinical exam having worn ScCLs for at least 4 hours. ScCL were removed, and 150 μL of phosphate-buffered saline (PBS) was used to wash the bowl of the ScCL. Eyes were washed post-ScCL removal with 5 mL PBS per eye. Wash solutions were collected and leukocytes were then isolated and counted, followed by assessment with flow cytometry. Samples from the post-lens tear fluid were stained with fluorescently labeled antibodies to detect leukocyte distributions.

Results. Thirty-nine eyes from 19 adapted, full-time, ScCL wearers were included, and 46% presented with midday fogging. ScCL corneal clearance was 246 ± 61 μm for nonfoggers, while it was 308 ± 98 μm for those with fogging ($P < 0.05$). On average, the number of leukocytes collected from the ScCL bowl (9551 ± 18,926) was greater than the number of leukocytes recovered from the eye wash (2195 ± 4384, $P < 0.02$). ScCL corneal clearance was associated with the presence of fogging, with an odds ratio of 2.24 (95% confidence interval = 1.48–3.38, $P < 0.001$).

Conclusions. Leukocytes, predominated by neutrophils, are present in the post-lens tear film of ScCL wearers, and in particular wearers with greater ScCL corneal clearance have greater odds of having midday fogging.

Keywords: scleral contact lens, leukocytes, midday fogging, contact lens clearance, tear film

Scleral contact lenses (ScCLs) are large diameter, gas permeable contact lenses (CLs) that are designed to vault the cornea, rest on the conjunctiva, and envelop a layer of saline and tears between the ocular surface and the posterior lens itself.[1] Although the first ScCL was developed over 100 years ago, more recent improvements in CL materials (primarily oxygen permeability) and designs have reduced historical concerns about corneal hypoxia, which has now made ScCLs a viable option and often treatment of choice for correcting irregular corneas.[2–7] Improvements in ScCL have likewise resulted in their use for treatment of conditions like CL intolerance, ocular surface disease, and even uncomplicated refractive errors.[8,9]

While ScCL have gained momentum in the market, there is still a dearth of information related to uncomplicated ScCL wear.[7,10] Currently, evidence in the peer-reviewed literature suggests that ScCL are safe, though their use is not without risk of complication. For example, case reports have noted microbial keratitis in ScCL wearers who fail to comply with their care regimes.[11,12] Even though infectious keratitis is of most significant concern for practitioners and contact lens wearers since it can result in permanent vision loss,[13] other complications associated with hypoxia, inflammation, mechanical influence, deposition, and visual blur occur frequently and should be better understood in ScCL wearers.[14–21]

A ScCL specific type of visual blur, midday fogging, is thought to occur in 20% to 33% of all patients who wear ScCLs.[1,21] Midday fogging results from particulate matter that is trapped between the ocular surface and contact lens in the post-lens tear film.[22] Patients who experience midday fogging often describe blurred vision that worsens throughout the wear day, a burdensome symptom that can only be relieved by removing the ScCL, cleaning it, and reapplying it with fresh nonpreserved saline solution.[21,23] Midday fogging is visible to the trained observer upon slit-lamp biomicroscope examination, and the cloudy particulates can be digitally imaged with optical coherence tomography (OCT).[21] The composition of midday fogging particulate is unknown, yet prevailing theories include that it is a response to ScCL seal off (reduced tear exchange, increased hypoxia), mechanical irritation, dry eye, or the pathology that is being treated by the ScCLs.[22] As midday fogging may be different whether it is observed by the patient, or

by the clinician, hereafter midday fogging is referred to as either "subject-reported fogging" or "post-lens tear film fogging" to distinguish the different perspectives, where necessary.

Identification of the composition of ScCL post-lens tear film fogging is of significant clinical importance because this knowledge would point toward the condition's underlying mechanism, and it may lead to the development of a new treatment or prevention strategy. Our prior work showed a significant accumulation of leukocytes in the tear film associated with the closed eye environment (during sleep, with associated hypoxia induced with prolonged eye closure).[24–26] Hypoxia is associated with ScCL wear through a combination of reduced tear exchange, excessive corneal vault, increased ScCL center thicknesses, and material oxygen permeability.[3–7] Thus, it is hypothesized that ScCL wear may be related to the release of leukocytes into the post-lens tear film during ScCL wear, particularly in those with higher degrees of ScCL vault and patients with either clinically observed or patient-reported midday fogging. The purpose of this study was to determine if an enrichment of leukocytes in the post-lens tear film in ScCL wear is associated with clinical fitting characteristics of ScCL wear.

Materials and Methods

Subjects

The study was conducted in accordance with the tenets of the Declaration of Helsinki, was approved by the University of Alabama at Birmingham Institutional Review Board, and all subjects provided informed consent. Nineteen habitual ScCL wearers were enrolled and 39 eyes were measured. All but one subject wore a ScCL on both eyes. One subject was measured twice, in two different sets of ScCLs on two separate days. All subjects wore their ScCLs for at least 4 hours before their appointment.

Subjects underwent a clinical exam to assess their overall ocular comfort, using both the Ocular Surface Disease Index (OSDI)[27] and the Contact Lens Dry Eye Questionnaire (CLDEQ).[28] The ScCL fit was assessed using a G5 Ultra Slit Lamp Biomicroscope (Marco Ophthalmic, Jacksonville, FL, USA). A fully integrated digital ophthalmic camera (Marco Ophthalmic) was used to take pictures and videos of corneal cross-sections to evaluate ScCL clearance and post-lens tear film fogging. Post-lens tear film fogging was graded by consensus amongst three co-authors, and ScCL clearance was measured in Microsoft PowerPoint by comparing the area of clearance to a reference ScCL thickness of 350 μm. Subjects were also asked for the frequency of any visual disturbances such as blur or fog related to fogging during their ScCL wear. A multifunctional corneal topographer (Keratograph 5M; Oculus, Arlington, WA, USA) was used to capture bulbar conjunctival redness images, which were graded with the Brian Holden Vision Institute (BHVI) scale for bulbar redness.[29,30] Additional pictures were taken with the topographer (white light at 1× magnification) to assess overall lens fit. Specifically, areas of ScCL liftoff and blanching were quantified by number of clock-hours observed. ScCL fit and bulbar redness were graded from these images by consensus of three co-authors.

Cell Collection

Following the clinical exam, subjects removed their ScCLs, while standing and bent forward, with their head down, such that the concave side of the lens was oriented up (toward the ceiling) to ensure that the post-lens fluid was maintained inside the ScCL. One hundred fifty microliters of sterile phosphate-buffered saline (PBS) was added to the inside of the ScCL, and the total solution was pipetted three to four times to wash the inside of the lens. The total volume from within the ScCL was removed via micropipette and reserved by transferring it to a 1.5-mL microcentrifuge tube. The resulting cell population is hereafter referred to as the "in lens" cell population.

Before re-inserting their ScCLs, subjects had their eyes washed to remove residual leukocytes on the ocular surface. Similar to a previously established method for tear leukocyte collection, a polyethylene pipette containing sterile PBS was used to wash each eye individually with 5 mL PBS.[25] Runoff was collected in a sterile polypropylene tube. Between lens removal and eyewash, subjects were instructed not to rub their eyes to avoid excess tearing. The resulting cell population is hereafter referred to as the "eye wash" cell population. Collected samples were processed immediately. The cell collections were centrifuged at 270*g* and the supernatant was removed. Cells were counted and average cell size was obtained using a Moxi Z automated cell counter (ORFLO, Hailey, ID, USA).

Reagents and Monoclonal Antibodies

PBS (pH 7.4) was acquired from Lonza (Allendale, NJ, USA). All other chemicals were of analytical reagent grade and were purchased from Fisher Scientific (Pittsburgh, PA, USA). Brilliant-ultraviolet 395 (BUV395)-conjugated anti-CD4, PerCP-Cy5.5-conjugated anti-CD8, allophycocyanin (APC)-conjugated anti-CD3, brilliant blue 515 (BB515)-conjugated anti-CD66b, R-phycoerythrin (PE)-conjugated anti-CD19, allophycocyanin-H7 (APC-H7)-conjugated anti-CD45, and BV510-conjugated fixable viability stain (FVS) were all purchased from Becton Dickinson (BD) Biosciences (San Jose, CA, USA).

Flow Cytometry

After cell counting with the Moxi Z cell counter, tear samples were transferred into tubes containing fluorescently labeled antibodies against CD4 (helper T cell), CD8 (cytotoxic T cell), CD3 (T cell), CD66b (neutrophil), CD19 (B cell), CD45 (pan-leukocyte), as well as a FVS. Cells were incubated for 30 minutes at room temperature in the dark, and they were then washed twice by spinning down and resuspending them in 700 μL PBS. Finally, cells were filtered using a 35-μm cell-strainer cap (Corning, Corning, NY, USA), and were then fixed with 2% paraformaldehyde.

All samples were acquired on a BD LSR II flow cytometer within 8 hours of fixation using BD FACS Diva software, version 8.0.1. Viable leukocytes were defined by stepwise exclusion of doublets and cell clumps, CD45 negative cells, and dead cells by using flow cytometric gating strategies. Granulocytes and lymphocytes were specified by their appropriate side scatter characteristics; namely, granulocytes are distinguished as having a higher side scatter versus lymphocytes with a lower side scatter. Neutrophils (CD66b+), B cells (CD19+), and T cells (CD3+) were identified. A further analysis of T cells into CD4+ and CD8+ populations was performed. Single color compensation controls were employed, using compensation beads (BD Biosciences), to set appropriate voltages. A compensation matrix was initially calculated after acquisition of single-color controls using BD FACS Diva software, before post-acquisition adjustments in FlowJo V10 (Ashland, OR, USA), where necessary. Fluorescence-minus one controls were used to appropriately determine gating strategies. Interdaily variations in flow cytometry acquisition were controlled for using the Application Settings feature in BD FACS Diva software. All data were analyzed post-acquisition using FlowJo V10.

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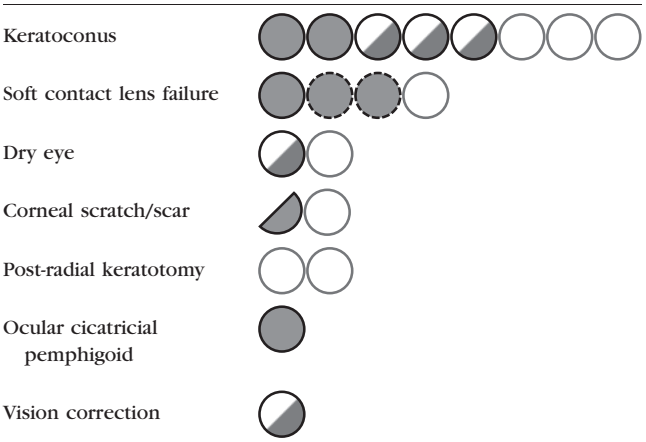
Table 1. Breakdown of Observed Post-Lens Tear Film Fogging by ScCL Indication With 39 Total Eyes



Each half circle represents one eye. Shaded half-circles indicate presence of fogging. Note that one subject was prescribed a ScCL in only one eye. Another subject was measured twice, in two different sets of ScCLs. Symbols legend: ○ = no eyes with fogging, ◔ = one eye with fogging, ● = both eyes with fogging, ◢ = only one eye with ScCL, ◌ = same subject, different ScCL.

Statistical Analyses

Reported values include means ± SD. Demographic, clinical, and ocular characteristics were compared between eyes with and without post-lens tear film fogging using logistic regression with generalized estimating equations (GEEs) to account for within-subject correlation that exists when using two eyes from the same subject. Factors associated with post-lens tear film fogging or those identified in the literature were considered potential confounders. To address the primary objective of the study, logistic regression using GEE was used to estimate crude and adjusted odds ratios, 95% confidence intervals (95% CIs), and *P* values between the number of leukocytes and each specific leukocyte type and presence or absence of post-lens tear film fogging. Leukocytes were examined as a continuous variable and as a dichotomous variable (high versus low numbers), with cut-offs guided by visual inspection and informed by median values and normative ratios of closed eye leukocyte populations.[25]

A paired *t*-test was used to compare the numbers of leukocytes recovered from inside the lens versus the post-lens removal eye wash. A linear regression using GEE adjusted for potential confounders was used to examine the relation between the number of leukocytes recovered from inside the lens, transformed via natural logarithm, versus the amount of central scleral lens clearance. All data were analyzed using SAS version 9.4 (SAS, Cary, NC, USA).

Results

Subject Characteristics

Of the 19 subjects, nine were male and 10 were female and had an overall average age of 57.2 ± 15.7 years (range, 28–81 years). Subjects were prescribed ScCLs for various reasons, as outlined in Table 1, with the majority of indications being keratoconus, followed by soft contact lens failure.

Subjects were characterized by the presence of post-lens tear film fogging, on a per eye basis. Descriptive statistics for nonfogging eyes ($n = 21$) versus fogging eyes ($n = 18$) are presented in Table 2. As shown, 46% of eyes presented with post-lens tear film fogging at the time of the visit. Compared to those without fogging, eyes with fogging had higher levels of ScCL central clearance ($P = 0.047$). Otherwise, those with post-lens tear film fogging shared similar characteristics to those without fogging.

Table 2. Comparison of Demographic and Clinical Characteristics Between Eyes With and Without Post-Lens Tear Film Fogging During the Time of the Visit

Demographic and Clinical Descriptors	Nonfogging Eyes	Fogging Eyes	*P**
N (%)	21 (54)	18 (46)	
Age, mean ± SD (y)	57.4 ± 12.2	55.8 ± 17.7	0.77
Hours of ScCl wear before removal, mean ± SD	6.1 ± 1.9	5.9 ± 1.3	0.70
Sex, *n* (%)			0.67
Males	10 (47.6)	7 (38.9)	
Females	11 (52.4)	11 (61.1)	
Prior keratoconus diagnosis, *n* (%)			0.51
Yes	11 (52.4)	7 (38.9)	
No	10 (47.6)	11 (61.1)	
CLDEQ positive, *n* (%)			0.41
Yes	9 (42.9)	5 (27.8)	
No	12 (57.1)	13 (72.2)	
Redness, mean ± SD	2.0 ± 0.7	2.3 ± 0.7	0.36
Total cell count (Moxi), mean ± SD	5909 ± 8786	13,801 ± 25,969	0.25
Prior dry eye diagnosis, *n* (%)			0.24
Yes	7 (33.3)	3 (16.7)	
No	14 (66.7)	15 (83.3)	
Visual disturbances related to fogging, *n* (%)			0.16
0 days per week	8 (42.9)	3 (16.7)	
>0 days per week	13 (57.1)	15 (83.3)	
OSDI score, mean ± SD	16.6 ± 17.0	24.1 ± 14.3	0.15
ScCl central clearance, mean ± SD (μm)	246 ± 61	308 ± 98	0.047

* Estimated using logistic regression with GEEs to compare eyes with and without fogging. All *P* values are unadjusted.

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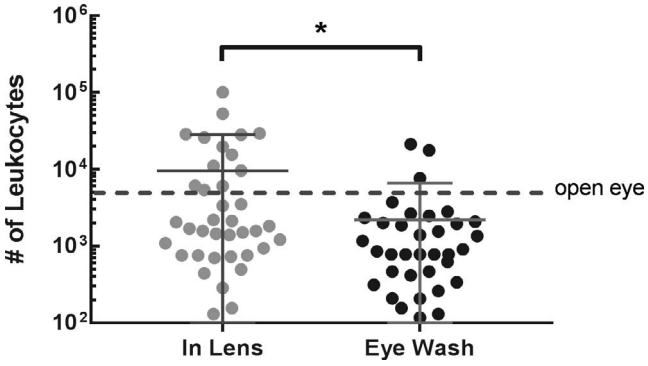

FIGURE 1. Comparison of leukocyte recovery from inside ScCL worn for at least 4 hours versus an eye wash to recover all cells remaining on the eye immediately after ScCL removal. Reference value for a cellular recovery from an eye wash during the middle of the day in a nonlens wearer (open eye) is illustrated as a *dotted line* (~7000 cells[24]). *Solid lines* indicate the mean of the values, with error bars representing the SD (lower line not shown because it exceeds lower limit of *y*-axis). *$P = 0.007$.

Leukocytes, Tear Samples, and ScCL Fitting Characteristics

Overall, subjects wore their ScCLs for 6.0 ± 1.6 hours on average (range, 4.0–10.2 hours) prior to lens removal and subsequent sample collection at the study visit. Comparisons of total leukocyte counts are shown in Figure 1. On average, the number of leukocytes collected from the in-lens portion (9551 ± 18,926) was significantly greater than the number of leukocytes recovered from the eye wash after lens removal (2195 ± 4384, $P = 0.007$). Figure 1 also shows an average value for recovered leukocytes from an open eye wash from a noncontact lens wearer for reference.[24] Of the 5 mL that was instilled in the eye wash, the average volume recovered was 4.2 ± 0.9 mL per eye. Total volumetric recovery from the inside of the ScCL, with the 150 µL PBS rinse was not measured as it also contained post-lens tear fluid. Figure 2 also shows the nonsignificant, but trending relation between leukocytes counts (transformed by natural logarithm) and ScCL corneal clearance. A linear regression analysis was performed using GEEs, and after adjusting for age, sex, inter and intrasubject variability, OSDI score, prior dry eye disease diagnosis, prior keratoconus diagnosis, and the presence of post-lens tear film fogging, there was a nonsignificant trend between recovered leukocytes and total clearance ($P = 0.07$).

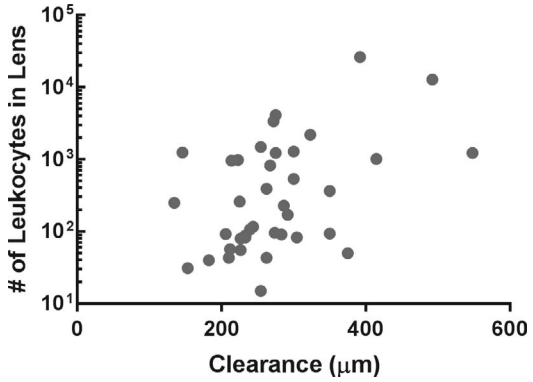

FIGURE 2. Relation between the number of leukocytes recovered from inside a ScCL following at least 4 hours of wear with measured ScCL corneal clearance. Controlled for age, sex, OSDI score, dry eye, keratoconus, and bulbar redness. $P = 0.07$, $n = 39$.

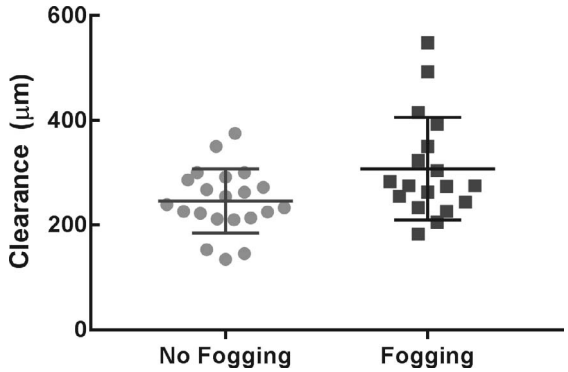

FIGURE 3. Relation between central scleral lens clearance with the presence or absence of observed post-lens tear film fogging at time of lens removal. *Solid lines* indicate the mean of the values, with error bars representing the SD.

Factors Associated With ScCL Post-Lens Tear Film Fogging

As shown in Table 2, the only factor associated with post-lens tear film fogging was ScCL clearance over the cornea, which is illustrated in Figure 3. The crude odds ratio for observed fogging was 1.72 (95% CI = 1.13–2.62, $P = 0.01$) for each 50-µm increase in clearance. After adjusting for OSDI score, bulbar redness, presence of blanching, age, sex, prior dry eye diagnosis, prior keratoconus diagnosis, and total number of leukocytes recovered from inside the worn ScCLs (natural logarithm transformed), the odds ratio for observed post-lens tear film fogging was 2.24 (95% CI = 1.48–3.38, $P < 0.001$) for each 50-µm increase in ScCL clearance.

It was hypothesized that there would be an observed relation between the number of recovered leukocytes and the presence of post-lens tear film fogging. The average number of CD45+ leukocytes recovered from inside a worn ScCL that had fogging was 2768 ± 6574 versus 597 ± 793 from a ScCL without fogging (Fig. 4; $P = 0.18$). In a crude model, the odds ratio for the presence of fogging was 1.07 for every log unit increase in the number of recovered leukocytes from within a ScCL (95% CI = 0.68–1.68, $P = 0.77$). After adjusting for OSDI score, bulbar redness, presence of blanching, age, sex, prior dry eye disease diagnosis, prior keratoconus diagnosis, and presence of fogging, the odds ratio for the presence of fogging was not significantly different from 1 for every log unit increase

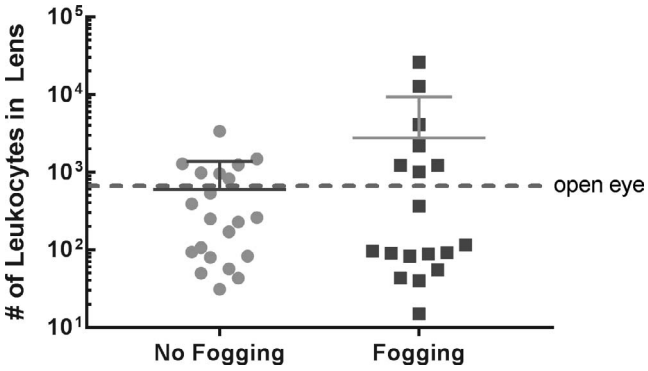

FIGURE 4. Relation between the number of leukocytes recovered from inside a ScCL following at least 4 hours of wear with the presence or absence of observed post-lens tear film fogging at time of lens removal. *Solid lines* indicate the mean of the values, with error bars representing the SD (lower lines not shown due to limits of *y*-axis).

Table 3. Comparison of the Distribution of Leukocytes Recovered From Worn ScCL Between Eyes With and Without Fogging

Leukocyte Type	Nonfogging Eyes	Fogging Eyes	P*
Total # CD45+ leukocytes			
mean ± SD	597 ± 793	2768 ± 6574	0.18
# with ≥250 cells, *n* (%)	11 (52.4)	8 (44.4)	0.70
# with <250 cells, *n* (%)	10 (47.6)	10 (55.6)	
Total # CD66b+ neutrophils			
mean ± SD	473 ± 725	2579 ± 6146	0.22
# with ≥250 cells, *n* (%)	7 (33.3)	8 (44.4)	0.58
# with <250 cells, *n* (%)	14 (66.6)	10 (55.6)	
Total # CD19+ B cells			
mean ± SD	19 ± 35	7 ± 21	0.28
# with ≥10 cells, *n* (%)	7 (33.3)	2 (11.1)	0.14
# with <10 cells, *n* (%)	14 (66.6)	16 (88.9)	
Total # CD3+ T cells			
mean ± SD	18 ± 19	19 ± 34	0.93
# with ≥10 cells, *n* (%)	11 (52.4)	7 (38.9)	0.45
# with <10 cells, *n* (%)	10 (47.6)	11 (61.1)	
# CD4+ cells, mean ± SD	11 ± 12	10 ± 23	0.89
# CD8+ cells, mean ± SD	4 ± 6	5 ± 7	0.43
% CD66b+ neutrophils			
mean ± SD	68 ± 20	68 ± 29	0.95
# with ≥80%, *n* (%)	8 (38.1)	9 (50)	0.54
# with <80%, *n* (%)	13 (61.9)	9 (50)	
% CD3+ T cells			
mean ± SD	4.4 ± 3.7	5.7 ± 7.4	0.56
% with ≥4%, *n* (%)	9 (42.9)	8 (44.4)	0.94
% with <4%, *n* (%)	12 (57.1)	10 (55.6)	
% CD19+ B cells			
mean ± SD	4.3 ± 6.2	0.5 ± 1.1	0.04
% with ≥4%, *n* (%)	8 (38.1)	1 (5.6)	0.03
% with <4%, *n* (%)	13 (61.9)	17 (94.4)	

* Estimated using logistic regression with GEEs to compare eyes with and without fogging. All *P* values are unadjusted.

in recovered leukocytes (odds ratio = 1.11, 95% CI = 0.53–2.31, $P = 0.79$).

Leukocyte Composition Distribution

Across all participants, there were 1445 ± 4278 neutrophils, 13.6 ± 29.9 B cells, and 18.5 ± 26.6 T cells recovered from inside worn ScCLs, which represented 67.9% ± 24.4%, 2.5% ± 4.9%, and 5.0% ± 5.6% of the total leukocyte population. T cells were further categorized as CD4+ or CD8+, which represented 55.8% ± 32.8% and 27.4% ± 30.6% of the total T-cell population, respectively. Importantly, CD4+ and CD8+ counts were very low, with an average of 10.6 ± 17.5 CD4+ T cells and 4.4 ± 6.4 CD8+ T cells per inside worn ScCLs.

Table 3 shows leukocyte composition observed between ScCL wearers with and without post-lens tear film fogging. Overall, there was a similar percentage of CD66b+ neutrophils observed without fogging compared to those with fogging (68.2% ± 19.9% vs. 67.6% ± 29.4%, $P = 0.95$). Notably, there was a significant reduction in CD19+ B cells recovered from worn ScCL in subjects with fogging, with an overall percentage of 4.3% ± 6.2% B cells without fogging compared to 0.5% ± 1.1% B cells with fogging ($P = 0.04$). Lastly, the overall percentage of CD3+ T cells was also similar between those that did not have fogging (4.4% ± 3.7%) compared to those that did have fogging (5.7% ± 7.4%, $P = 0.56$). Given the very low counts for CD4+ and CD8+ T cells, no comparison was performed for changes in their distribution.

To determine whether changes in the recovered leukocyte population, in terms of absolute counts or relative breakdown, was associated with ScCL post-lens tear film fogging, a logistic regression was performed reducing all independent variables to dichotomous outcomes. Results of this regression are presented in Table 4. There was a trend for fewer B cells associated with ScCL fogging eyes with an adjusted odds ratio of 0.18 (95% CI = 0.29–1.09, $P = 0.06$); however, this association was not statistically significant. No other associated trends in the types of leukocytes between ScCL wearing eyes with and without fogging were observed.

Discussion

Scleral lens wear has become an increasingly useful modality for assisting patients with many different ocular disorders broadly including corneal irregularities, ocular surface disease, and common refractive errors. This is largely because modern contact lens materials and advanced, customized designs have helped reduce common complications (e.g., hypoxia) historically associated with ScCL.[21] While ScCLs are beneficial for restoring sight and improving comfort in these conditions, they can still be associated with several complications due to their size, shape, and fit on the ocular surface. These complications can have associated etiologies relating to hypoxia, infection, inflammation, mechanics, or any combination of the aforementioned.

One more frequent complication associated with ScCL wear is subjective and/or objective midday fogging that occurs within the first few hours of ScCL wear.[17] There are several potential etiological factors that might be considered. For instance, even with modern designs and materials associated with improved oxygenation to the cornea, it is well known that ScCL wear continues to be associated with reductions in oxygenation to the cornea.[3] Specifically, ScCLs are associated with large reductions in post-lens tear exchange compared with soft contact lens wear (sometimes referred to as tear

Table 4. Crude and Adjusted Association Between Leukocyte Types and Presence of Post-Lens Tear Film Fogging

	Crude†	Adjusted*†		
Leukocyte Type	OR	OR	95% CI	P
Total # CD66b+ neutrophils (≥250 vs. <250 cells)	1.07	0.99	0.16–6.01	0.99
% CD66b+ neutrophils (≥80% vs. <80% total leukocytes)	1.63	0.72	0.12–4.50	0.73
Total # CD3+ T cells (≥ 10 vs. <10 cells)	1.01	0.71	0.17–2.93	0.64
% CD3+ T cells (≥4% vs. <4% total leukocytes)	1.07	1.96	0.26–14.74	0.51
Total # CD45+ leukocytes (≥250 vs. <250 cells)	1.06	0.37	0.06–2.37	0.29
% CD19+ B cells (≥4% vs. <4% total leukocytes)	0.10	0.07	0.002–2.49	0.15
Total # CD19+ B cells (≥10 vs. <10 cells)	0.84	0.18	0.29–1.09	0.06

* Adjusted for central scleral lens clearance, bulbar redness, prior dry eye disease diagnosis, and OSDI score.
† For all models, the reference group was the group with lower cell counts.

Table 5. Comparison of Leukocyte Breakdown Between Blood,[42–44] Closed Eye Tears,[25] and the Post-Lens Tear Film of Scleral Lens Wear*

				Post-Lens Tear Film ScCL		
Leukocyte Type	**Blood**	**Open Eye Tears**	**Closed Eye Tears**	**Overall**	**No Fogging**	**Fogging**
Neutrophils	60%	82%	65%	68%	68%	68%
B cells	4%	No data	No data	5%	4.3%	0.5%
T cells	26%	No data	3%	3%	4.4%	5.7%
CD4	47%	No data	53%	56%	–	–
CD8	27%	No data	22%	27%	–	–

The exact distribution of leukocyte types in the open eye condition is not yet known, given low cell counts measured on estimates to date.[24]
* Estimates from blood, open eye tears, and closed eye tears are referenced from literature sources.

elimination).[15,31] This reduction effectively seals the post-lens tear fluid behind the lens, reducing oxygenation to the cornea, allowing for the buildup of debris and mediators behind the ScCL. Likewise, ScCLs are thick centrally (~150 to 500 μm, dependent on ScCL power), and also vault the cornea with a substantial post-lens tear thickness, which impacts its transmissibility (typically greater than 150 μm, compared to approximately 20–30 μm for a corneal gas permeable lens, and 2–3 μm for a soft contact lens); this ScCL center thickness and post-lens tear film both contribute to further increasing the hypoxic state of the cornea.[3,4,7,16,32–35]

This study showed that 46% of the ScCL wearing subjects had clinically confirmed post-lens tear film fogging, and 83% of the subjects with post-lens tear film fogging had concurrent self-reported complaints of blur or vision disruption associated with fogging (subject-reported fogging). With that said, there were no differences found in either the CLDEQ or OSDI relative to subjective perceptions of ocular comfort and/or symptomatology when comparing the post-lens tear film foggers and nonfoggers, albeit neither survey was designed or validated for use in ScCL wearers. While there were no statistical differences in these subjective outcomes, there was a 45% increase in the OSDI score in the ScCL wearers with post-lens tear film fogging compared to those without fogging. This likely reflects the fact that the OSDI is broader than assessing only comfort-related symptoms, like the CLDEQ; in particular, the OSDI asks questions relating to quality blurred vision, poor vision, reading, driving, and working environments that are all more relevant to ScCL wearers with fogging than comfort-related questions.

This study also showed that central ScCL clearance was also significantly associated with the presence of post-lens tear film fogging. In particular, for every 50-μm increase in ScCL central clearance, there was a 2.24 times higher odds of presenting with post-lens tear film fogging. This finding supports the recommendation of minimizing corneal clearance, sometimes recommended to be less than 200 μm.[3]

Leukocytes are the effector cells of the immune system, and their presence at the ocular surface has only recently been elucidated.[24,36,37] During sleep, there is an influx of approximately 750,000 leukocytes into the conjunctival sac of the closed eye.[36] These cells are rapidly depleted upon awakening, but there is a constitutive expression of roughly 7000 leukocytes that are maintained in the open eye.[24] One question that remains is what drives the large recruitment of leukocytes into the tear film during sleep, and one hypothesis is that the hypoxic environment can induce leukocyte infiltration. It has been documented that the oxygen tension at the cornea in the closed eye is approximately 2% to 4% (unlike in the open eye state, when it is up to 21%), which is not much different than the oxygen tension at the cornea during ScCL wear, even under optimal fitting conditions (e.g., thin central lens, highly permeable material, reduced tear clearance).[38] Therefore, the hypoxic environment under a ScCL (albeit in an open eye state) somewhat mimics the closed eye environment in terms of corneal oxygen tension, which could possibly lead to a leukocyte influx. In this study, there were 2768 ± 6574 leukocytes associated with ScCL with post-lens tear film fogging and 597 ± 793 leukocytes associated with ScCL without fogging, but this was not statistically significant. Variability in the number of recovered leukocytes could also be derived from the presence of inflammatory etiologies present in some subjects (such as dry eye disease) that could promote or inhibit leukocyte recruitment, but this was not reflected in our small number of subjects.

In collecting the leukocytes from the post-lens tear film, a wash of subjects' ocular surfaces following lens removal was also conducted to understand whether there was a difference in leukocytes between the pre- and post-lens tear film of the ScCL wearer. Indeed, there were significantly more leukocytes recovered from within the ScCL compared to those washed from the ocular surface following lens removal (Fig. 1), there were significantly more leukocytes associated with greater levels of ScCL clearance (Fig. 2), and greater levels of ScCL clearance was strongly associated with post-lens tear film fogging (Fig. 3). Taken together, these findings suggest that greater levels of hypoxia are associated with greater accumulations of leukocytes, and leukocytes are associated with the fogging phenomenon associated with ScCL wear. However, the total number of recovered cells from inside a worn ScCL was still orders of magnitude less than from an eye at awakening.

These data might also suggest that there could be different etiologies for ScCL fogging. Among those that presented with post-lens tear film fogging, there appeared to be a group of subjects with a high number of leukocytes recovered, and a separate group with a low number of leukocytes recovered. For example, it could be that the ScCL foggers with higher leukocytes were those that had greater central vault, but better tear exchange (something that was not measured in this study); conversely, ScCL wearers with fogging, but lower leukocyte counts, could be those with greater central vault, but good tear exchange. Further, there were a few trends in the leukocyte composition in the post-lens tear film of ScCL wear worth noting when comparing to that found in the closed eye state,[36] in addition to human blood samples from the literature (Table 5).[39–41] It can be observed in the Table that there are smaller frequencies of T cells in tear film derived samples (both noncontact lens and ScCL) compared to the higher frequencies found in blood. The current study also showed that that B cells were largely absent from ScCL wearers with fogging ($P = 0.04$) and the implications of this finding are deserving of further investigation.

There appears to be a relative paucity of biological information in the literature relating ScCL wear to inflammation and hypoxia, and this initial study helps provide some insight into these issues. While informative, the study has several limitations worth noting. With the current sample size and evaluation techniques, the study was limited in extent of

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understanding the exact mechanisms between leukocyte migration and ScCL wear, but more targeted and prospective studies with larger sample sizes could better determine hypoxia-driven mechanisms, or whether interventions could improve overall health and safety associated with ScCL wear related to these outcomes (e.g., effects of change peripheral curves in flanges on leukocyte migration). In summary, these results relate increased ScCL clearance and leukocytes in the post-lens tear film with the presence of post-lens tear film fogging, suggesting that clinicians should be mindful of clearance as a fitting parameter and adjust to smaller vaults, as appropriate.

Acknowledgments

The authors thank Kelly Nichols for her productive discussions related to this project.

Supported by the core grant NEI P30 EY003039 for its support of the Ophthalmology Clinical Research Unit for statistical support. The BD LSR II flow cytometer is part of the UAB Comprehensive Flow Cytometry Core, supported by NIH P30 AR048311 and NIH P30 AI27667. CP is supported by the Natural Sciences and Engineering Research Council of Canada Alexander Graham Bell Doctoral Scholarship and the American Academy of Optometry Section on Cornea, Contact Lenses, and Refractive Technologies William C. Ezell Fellowship.

Disclosure: **C.K. Postnikoff**, None; **A.D. Pucker**, None; **J. Laurent**, None; **C. Huisingh**, None; **G. McGwin**, None; **J.J. Nichols**, None

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