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. 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 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 one of the most significant concerns 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 the post-lens tear film.22,23 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).23 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

**Correspondence:** Jason J. Nichols, School of Optometry, University of Alabama at Birmingham, 1716 University Boulevard, Birmingham, AL 35223, USA; jjn@uab.edu.

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**PURPOSE.** Midday fogging is a frequent complaint among 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
Leukocytes in Scleral Contact Lens Wear

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 thickness, and material oxygen permeability.2–7 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 Slt 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 1X magnification) to assess overall lens fit. Specifically, areas of ScCL liftoff and blanching were quantified by number of clock-pictures were taken with the topographer (white light at 1

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 270g 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-phycocerythrin (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. Interdially 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.
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.

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>
<thead>
<tr>
<th>TABLE 1. Breakdown of Observed Post-Lens Tear Film Fogging by ScCL Indication With 39 Total Eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratoconus</td>
</tr>
<tr>
<td>[Image]</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>TABLE 2. Comparison of Demographic and Clinical Characteristics Between Eyes With and Without Post-Lens Tear Film Fogging During the Time of the Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic and Clinical Descriptors</td>
</tr>
<tr>
<td>N (%)</td>
</tr>
<tr>
<td>Age, mean ± SD (y)</td>
</tr>
<tr>
<td>Hours of ScCl wear before removal, mean ± SD</td>
</tr>
<tr>
<td>Sex, n (%)</td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>Females</td>
</tr>
<tr>
<td>Prior keratoconus diagnosis, n (%)</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>CLDEQ positive, n (%)</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Redness, mean ± SD</td>
</tr>
<tr>
<td>Total cell count (Moxi), mean ± SD</td>
</tr>
<tr>
<td>Prior dry eye diagnosis, n (%)</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Visual disturbances related to fogging, n (%)</td>
</tr>
<tr>
<td>0 days per week</td>
</tr>
<tr>
<td>&gt;0 days per week</td>
</tr>
<tr>
<td>OSDI score, mean ± SD</td>
</tr>
<tr>
<td>ScCl central clearance, mean ± SD (µm)</td>
</tr>
</tbody>
</table>

* Estimated using logistic regression with GEEs to compare eyes with and without fogging. All P values are unadjusted.
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, 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 (\( P = 0.07 \)).

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 (\( P = 0.18 \)). 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.
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. 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.1 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 post-lens tear exchange compared to soft contact lens wear (sometimes referred to as tear...
Leukocytes in Scleral Contact Lens Wear

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

<table>
<thead>
<tr>
<th>Leukocyte Type</th>
<th>Blood</th>
<th>Open Eye Tears</th>
<th>Closed Eye Tears</th>
<th>Overall</th>
<th>No Fogging</th>
<th>Fogging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>60%</td>
<td>82%</td>
<td>65%</td>
<td>68%</td>
<td>68%</td>
<td>68%</td>
</tr>
<tr>
<td>B cells</td>
<td>4%</td>
<td>No data</td>
<td>No data</td>
<td>5%</td>
<td>4.3%</td>
<td>0.5%</td>
</tr>
<tr>
<td>T cells</td>
<td>26%</td>
<td>No data</td>
<td>3%</td>
<td>5%</td>
<td>4.4%</td>
<td>5.7%</td>
</tr>
<tr>
<td>CD4</td>
<td>47%</td>
<td>No data</td>
<td>53%</td>
<td>56%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD8</td>
<td>27%</td>
<td>No data</td>
<td>22%</td>
<td>27%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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.56 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...
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.

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