Do Endothelial Blebs Better Predict Corneal Edema than Pachometry after the Wear of Scleral Lenses with different clearances?

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Background

Endothelial blebs: transient disappearance of endothelial cells from the mosaic observed in specular reflection (Fig 1). Occurence:15-25 minutes after lens insertion in unadapted lens wearers. Blebs disappear shortly after their removal or once the cornea adapts. (Zantos, 1977) Blebs also occur after exposure of the cornea to 100% N_2 or to 9.8% CO_2-20.5% O_2. Endothelial acidosis, common to exposure of N_2 and CO_2 was suggested as the cause. (Holden, 1985)

The bleb response occurs after the wear of oxygen impermeable PMMA (Barr, 1980), Rigid Gas Permeable (RGP) (Inagaki, 2003) or hydrophilic contact lenses (Schoessler, 1982) The magnitude of the bleb effect (relative area of blebs or the number of blebs or the time for blebs to disappear) is inversely related to oxygen transmissibility. (Inagaki, 2003;Ohya, 1996). The effect seems larger in Asian subjects.(Hamano, 2002; Brennan, 2008) The diagram of Fig. 2 illustrates the phenomenon that produce blebs. The bleb is an edematous endothelial cell. (Vannas, 1984; Kaufman, 1996) Its warped apical surface causes the loss of the light ray reflecting on this surface, makin the cell appears black. The transitory appearance of bleb could represent an attempt of the cell to regulate its volume. (Bonanno, 2003)

There are concerns over the oxygenation of the cornea with scleral lenses with high clearance: the thickness of the liquid reservoir (clearance) with that of the material required for dimensional stability contribute to limit the passage of oxygen across this system. (Michaud, 2012) Recently, we concluded that the corneal surface pO_2 under SL200 and SL400 was lower than the theoretical values needed to prevent hypoxia during lens wear (9.9% and above). We obtained pO_2s measured at the corneal surface of 9.07 ± 0.86 and 6.19 ± 0.87% for similar scleral lenses fitted with a post-lens fluid thickness (PLFT) of 200 and 400 µm, respectively. (Giasson, 2017)

In the current study, we examine whether or not the corneal endothelium develops blebs during the wear of scleral lenses. The purpose of this study is evaluate if 1) blebs appear after wearing scleral lenses fitted with different PLFT, 2) blebs better indicate corneal edema than pachymetry and 3) the number of blebs is modified by the clearance heights of the scleral lenses.

Methods

SUBJECTS AND EXPERIMENTAL PROCEDURE

This study was approved by the institutional review board for experimentation on humans. 21 participants (mean age 25.0 ± 2.0 years old; 6 men and 15 women) free of any systemic or ocular disease, without previous eye surgery were recruited at the Clinique Universitaire de la Vision, Université de Montréal. Subjects had never worn contact lenses regularly except for 4 subjects with a previous history of silicone-hydrogel corneal lens wear. These last subjects were asked to stop lens wear 72 hours prior to the beginning of the study.

During each of the 2 sessions, participants were fitted with MSD (mini scleral design) scleral lenses (Laboratoires Blanchard, Sherbrooke, Qc, Canada), (Giasson, 2017) in order to obtain a clearance of 200 or 400 µm (SL200 and SL400). SL were worn on one eye for 25 minutes, the other eye was a control. Central corneal thickness (CT), endothelial images of both eyes, before (t_0) and after lens wear (t_1) were obtained with a non-contact specular microscope (NIDEK model CEM-530), shown in figure 3. Clearance or post-lens fluid thickness (PLFT) was measured just before lens removal with the anterior segment OCT RT-VUE (Optovue, Clarion, Cambridge, ON, Canada). The OCT was positioned to get a bright reflection in the geometric center of the scleral lens to estimate PLFT at the center of the scleral lens. OCT analysis software was then used to measure the exact distance, in µm, between the rear lens surface and the front surface of the cornea (Fig. 4). Two observers blind to the specific experimental condition checked for the number of blebs (NB) in the images before further processing of these images (see companion poster).

We observed 168 endothelial pictures (21 subjects x 2 eyes x 2 times x 2 lenses), 42 of which had been taken after scleral lens wear whereas 126 (either from the control eye at t_0 and t_1 and from the experimental eye at t_0) were taken without any scleral lens wear. Each of the two observers who collected data (JR, JR) took half of the measurements and masked the details of the image for the other observer to analyze the images in a blind way. As our routines defined in Image J software did not allow to include in the analysis (see companion poster) the peripheral blebs, the analysis consisted in counting the blebs in the endothelial field. Statistical analysis: all results were considered significant when $p < 0.05$. We compared the differences 1) in the number of blebs (NB) in the images between both lenses with a Wilcoxon signed-rank test; 2) in PLFT between the subgroup of subjects with and without blebs by independent t tests done separately for SL200 and SL400 and 3) in CT before and after wearing SL200 and SL400 with a 3-factor (time x lens x experimental/control side) repeated measure ANOVA.

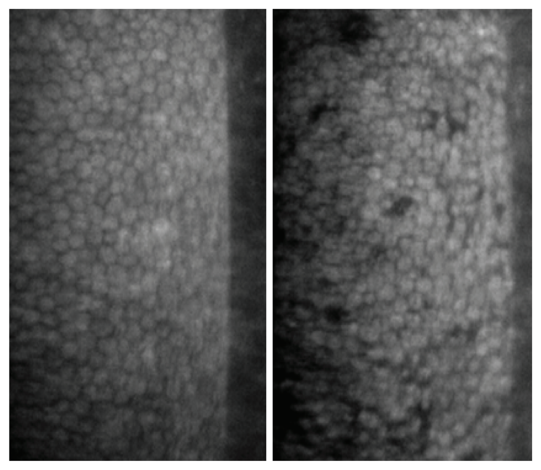

Figure 1. Image of a subject before and after 25 minutes of wear of a scleral lens with a targeted clearance of 400 µm.

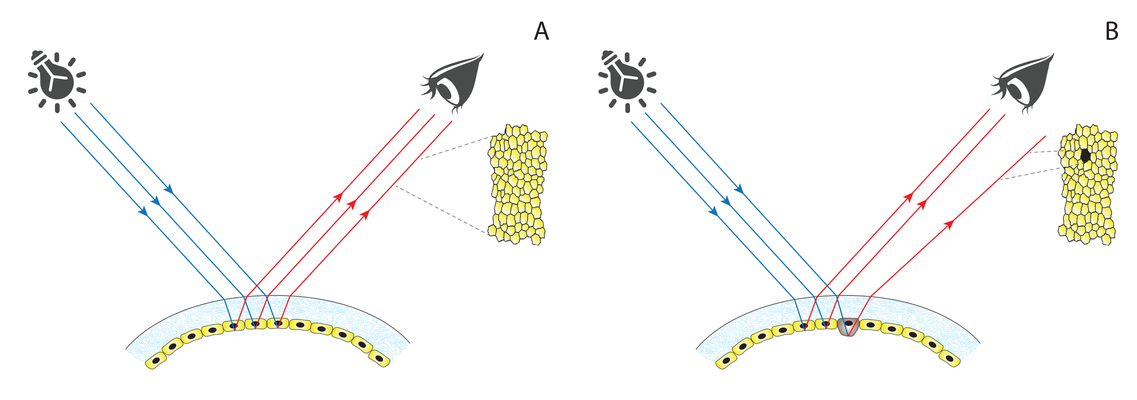

Figure 2. Drawing of the specular observation of the endothelium with a microscope. The light source is obliquely directed towards the cornea and the observer views endothelial cells reflected at the endothelium-aqueous humor interface. **A.** The apical side (aqueous-facing) of the endothelial cell is normal, providing an uniform view of endothelial cells. **B.** A swollen cell alters the regular interface for reflection and shift the reflected ray in a different direction so that the cell appears black for the observer.

Figure 3. One observer and her subject at the endothelial specular microscope NIDEK model CEM-530.

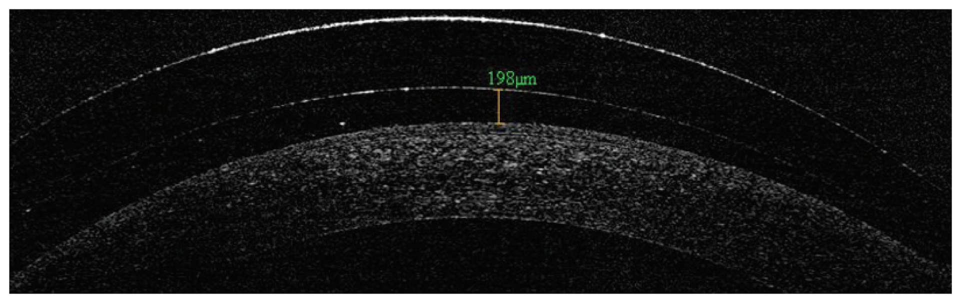

Figure 4. Picture showing the procedure to measure fluid thickness with the OCT.

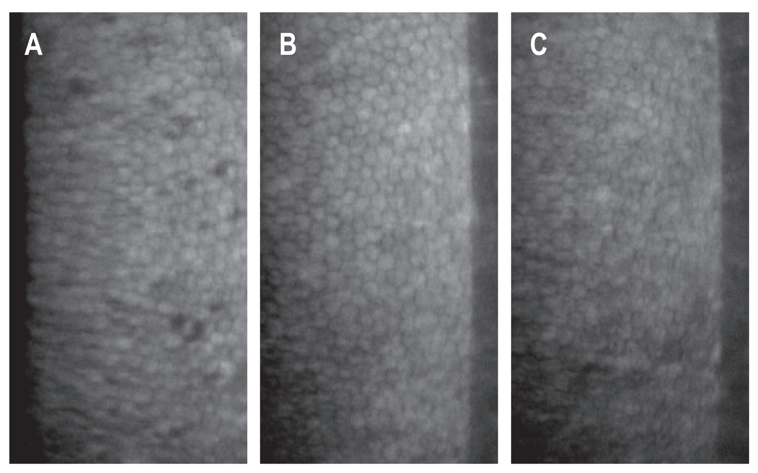

Figure 6. The eye of one subject having worn the lens is photographed at the opening of the eyes (A). The control eye was photographed thereafter (B). Immediately after taking these two images, the first eye as in fig 5A was photographed again (C). This small delay between the two measurements during which the eyes were open was sufficient for several blebs to disappear.

Results

One observer reported the occurrence of bleb in only one of the 126 endothelial pictures without any lens wear (either control eyes at t_0 and t_1, or experimental eyes at t_0). In retrospect, this individual had areas of darker zone over the surface of some cells. No other blebs were reported for this non-contact lens wearer in this eye or in the fellow one after contact lens wear. Because of the inexistence of variance for blebs for the control eye at t_0 and t_1, and for the experimental eye at t_0, it is not possible to include in a statistical analysis these 126 endothelial pictures. It is only possible to analyze the 42 endothelial pictures taken after lens wear. In these 42 remaining endothelial pictures taken after scleral lens wear, 9 and 14 subjects had at least one bleb after wearing SL200 and SL400, respectively. The maximal number of blebs observed in one subject was 5 and 15 for SL200 and SL400, respectively. Table 1 shows the mean, median, maximal and minimal number of blebs observed after the wear of SL 200 and SL400. Median NB after wearing SL200 and SL400 were significantly different (SL200: 0.00; SL400: 1.00; p = 0.02) when tested with the Wilcoxon signed rank test.

Figure 5 illustrates measurements of corneal thickness observed for control and experimental eyes during each visit for SL200 and 400. As may be observed, corneal thickness measured after the wear (t_1) of SL200 or of SL400 are the largest ones. The lower bound of their 95% confidence interval is larger than each other measured corneal thickness. Corneal thickness respectively increased from 561 ± 26 and 563 ± 26 to 577 ± 26 and 575 ± 24 µm in experimental eyes after the wear of the SL200 and SL400, but did not change much in control eyes. There is a significant triple interaction between time, type of lens and side (control/experimental) $F(1,20) = 4.96$ ($p = 0.038$), and a significant double interaction between time and side $F(1,20) = 92.10$ ($p = 0.000$). This indicates that time had different effects on corneal thickness depending on which side (control or experimental) the lens was applied. There was a significant main effect of time on corneal thickness, $F(1, 20) = 146.13$ ($p=0.000$), and of side on corneal thickness, $F(1, 20) = 39.28$ ($p=0.000$), but no effect of lens clearance on corneal thickness.

The PLFT of SL200 and SL400, measured by OCT, were closed to the expected values, suggesting a correct adjustment in terms of clearance. The PLFT of the LS200 and LS400 were respectively of 200.4 ± 41.8 µm (Median: 198.0) and 417.5 ± 58.6 µm (Median: 424.0) under SL200 and SL400, respectively. However, despite that there was more than twice the number of subjects in the current project, the standard deviation of the PLFT of the current sample was larger than the one (≈ 30) observed in a recent study on 8 subjects with the same lenses (Giasson, 2017). Therefore, we wondered if there could be differences in PLFT between those who developed blebs and those who did not. The difference in the PLFT between the 12 subjects who did not develop bleb after wearing the SL200 (197.3 ± 36.2; 12) and the mean PLFT of those 9 participants who had blebs (204.4 ± 50.4) was not significant. However, 7 subjects who did not develop any bleb with the SL400 had a mean PLFT of 382.4 ± 46.4 whereas the 14 participants who had blebs had a mean PLFT of 435.0 ± 57.4. This last difference was at the limit of significance (p= 0.05; t=-2.097, df19) (Table 1)..

TABLE 1 - Blebs observed after wear of SL 200 and SL400

N blebs observed after scleral lens wear	SL 200	SL 400
Mean ± Standard error	0.86* ± 1.42	2.29* ± 3.48
Median	.00*	1.00*
Minimum	.00	.00
Maximum	5.00	15.00
n	21	21
n subjects with blebs (PLFT)	9 (204.4 ± 50.4)	14 (435.0† ± 57.4)
n subjects wo blebs (PLFT)	12 (197.3 ± 36.2)	7 (382.4† ± 46.4)

* p <0.05 † p = 0.05

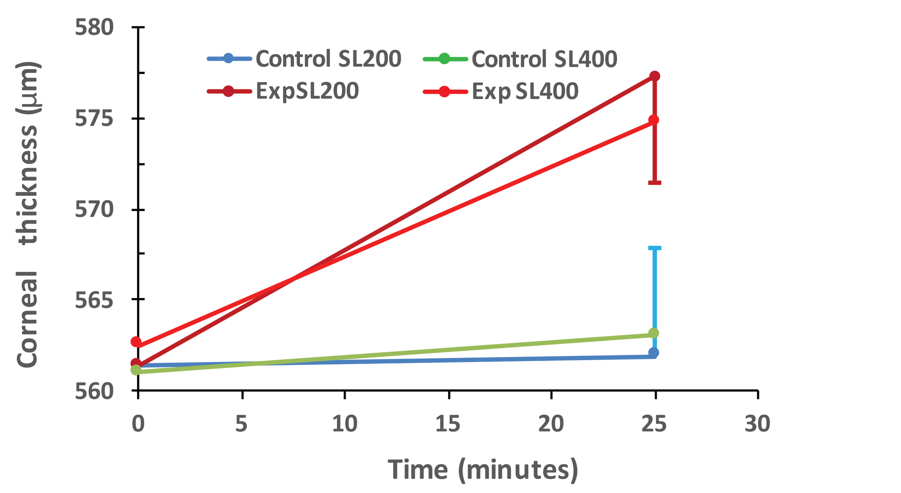

FIGURE 5. Evolution of corneal thickness before and after the wear of SL200 and SL400 for the experimental and control eyes.

Discussion

Each observer was masked in order to avoid bias during analysis, considering a potential subjectivity in counting blebs. Blebs are transitory structures which represent a darkened cell surface. In the current study, an image with a slightly darker zone was considered as a bleb by a masked observer. As this occurred for only 1 case out of the 126 situations without lenses revealed blebs, we consider the blind analysis very effective.

Corneal thickness increased significantly on the eye wearing scleral lenses, both SL200 and SL400. Whereas there were more blebs observed under SL400, corneal thickness increases observed under SL200 and SL400 were not significantly different. In a previous work, we measured a relative oxygen tension (pO_2) of 9.07 and 6.19 mm Hg after 5 minutes of scleral lens wear of SL200 and SL400, respectively.(Giasson, 2017) Both pO_2 values were lower than the range of thresholds for minimal pO_2 which has been evaluated at 9.9,(Holden, 1984) 10.4(Harvitt, 1999) or 10.9%.(Brennan, 1988) Here, we show that for similarly fitted lenses, a larger number of blebs are noticed after 25 minutes of wear of SL400 lenses, compared to SL200 lenses, despite a similar increase in corneal thickness.

Despite different pO_2s under both scleral lenses and different levels of endothelial edema (number of blebs), there is no difference in corneal thickness. Why would corneal thickness, a valuable index of corneal edema, would not reflect the endothelial edema observed as blebs? Which between corneal thickness and the number of blebs is the best indicator of edema. Whereas only the latter may distinguish overall between SL200 and SL400, corneal thickness increases occurs in everyone, not in the third or the half on the sample. The period of wear is different between both studies and the kinetics for each type of edema must be different. Hypoxic edema is related to lactate production in the corneal epithelium. Lactate migrates out of the epithelium via a lactate proton co-transporter. (Bonanno, 1990) Lactate diffuses across the corneal stroma down its gradient. The corneal endothelium also contains mechanisms of lactate transport,(Giasson, 1994) but these mechanisms are insufficient to evacuate lactate during hypoxic contact lens wear: under these conditions, the presence of lactate increases stromal osmotic pressure and causes edema.(Klyce, 1981) Bicarbonate transporters and carbonic anhydrase help to remove lactate from the corneal stroma into the aqueous humor. The lactate-H^+ co-transporter is an important component of the endothelial fluid pump.(Nguyen, 2012)

The measurement with the specular microscope after 25 minutes of wear had to be done quickly. Indeed, just after lens removal, participants were instructed to keep their eyes closed and be guided to the specular microscope until it was ready to take a picture of the eye which had worn the lenses. This approach is very relevant to maximize the transitory observation of blebs. Figure 6A shows blebs in the endothelial picture after the wear of a scleral lens and eye closure. The time taken by the endothelial camera to photograph the control eye (Fig. 6B) and to photograph again the lens-wearing eye, blebs had almost disappear from the endothelial field (Fig. 6C). It is quite obvious that this small delay between taking measurements and having eyes closed or open makes a difference in the presence or absence of blebs.

Despite that the differences in PLFT between the subjects who develop and those who did not develop any bleb with the SL400 was barely significant, it is clear from these results that increases in the PLFT increases the likelihood of developing transitory endothelial edema, or blebs. Since an increased thickness of the PLFT increases the resistance for the diffusion of oxygen, it is not surprising to observe a thicker PLFT of wearers of SL400 with blebs compared with the PLFT of these participants who did not develop blebs. Such a difference was not observed with SL200 as the range of PLFT thickness was smaller than PLFT with SL400. We believe that the fitting of scleral lenses with reduced central thicknesses (maximum of 250 µm) combined with a limited clearance (<200 µm, once stabilized) minimizes endothelial reaction and is therefore beneficial for the endothelium of patients.

Conclusion

Observation of the physiological changes, called blebs in the corneal endothelium after the wear of Boston XO_2 scleral lenses fitted with a clearance of 200 and 400 µm comes to the same conclusions as previous measurements of corneal pO_2: clearances of 400 µm should be avoided. We knew already that they penalize oxygen delivery to the cornea by 30% compared to those fitted with a clearance of 200 µm.(Giasson, 2017) Therefore, the principle of non-maleficence requires to avoid clearances as high as 400 µm whenever possible.(Gillon, 1994)

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