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Assessment of meibomian gland drop-out and visibility through a new quantitative method in scleral lens wearers: A one-year follow-up study

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ABSTRACT

Objectives: To validate a previously developed algorithm based on the visibility of meibomian gland images obtained with Cobra fundus camera and to assess the changes in meibomian glands in scleral lens wearers over one year of lens wear.

Methods: Infrared meibography was obtained from the upper eyelid using the Cobra fundus camera in forty-three volunteers (34.2 ± 10.1 years). Meibographies were classified into 3 groups: Group 1 = good subjective gland visibility and gland drop-out < 1/3 of the total area; Group 2 = low visibility and gland drop-out < 1/3; and Group 3 = low visibility and gland drop-out > 1/3. Meibomian gland visibility metrics were then calculated using the developed algorithm from the pixel intensity values of meibographies. Repeatability of new metrics and their correlations with gland drop-out were assessed. Meibographies and ocular symptoms were also assessed after 1 year of scleral lens wear in 29 subjects.

Results: Gland drop-out percentage was not statistically different between groups 1 and 2 ($p = 0.464$). Nevertheless, group 1 showed higher grey pixel intensity values than the other groups. Statistically significant correlations were found between gland visibility metrics and gland drop-out percentage. Repeatability was acceptable for all metrics, coefficient of variation achieving values between 0.52 and 3.18. While ocular symptoms decreased with scleral lens wear ($p < 0.001$), no statistically significant differences were found in gland drop-out percentage ($p = 0.157$) and gland visibility metrics ($p > 0.217$).

Conclusions: The proposed method can assess meibomian gland visibility in an objective and repeatable way. Scleral lens wear appears to not adversely affect meibomian gland drop-out and visibility while might improve dry eye symptoms after one year of lens wear. These preliminary results should be confirmed with a control group.

1. Introduction

Scleral lenses are large-diameter contact lenses that rest on the bulbar conjunctiva overlying the sclera. As scleral lenses are inserted with liquid (preservative-free saline solution), they create a tear reservoir that keeps the cornea moistened. This characteristic along with correcting anterior corneal aberrations allows these devices to often deliver clear as well as minimize dry eye-related symptoms in patients with irregular corneas and/or severe ocular surface disease [1–10].

Nevertheless, despite the use of scleral lenses is increasing Agreed and amended. Thank you., little is known about their effect on meibomian gland structure and function [11]. Meibomian glands are located

in the tarsal plate and are sebaceous glands that secrete meibum, which reduces the evaporation of the tear film and enhances its stability and spreading [12–18]. Meibomian Gland Dysfunction (MGD) occurs when meibomian glands produce an abnormal secretion, are obstructed or atrophied. It is a chronic disorder and is the leading cause of DED [12,15,17,19–21]. This disease was defined by the 2011 International Workshop on MGD as “a chronic, diffuse abnormality of the Meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion” [15]. Some studies concluded that soft and rigid contact lens wear may potentiate meibomian gland drop-out, alter gland morphology such as length and width, alter meibum expressibility and quality, and induce dry eye

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symptoms, ultimately classified as MGD [22–28].

The diagnosis of MGD and DED are challenging due to their multifactorial aetiology and the lack of agreement between signs and symptoms [29,30]. Therefore, the Tear Film and Ocular Surface Society Dry Eye Workshop II (TFOS DEWS II) Diagnostic Methodology Report acknowledged the need of developing new clinical tests as objective as possible to assess the ocular surface [29]. Some algorithms [13,14,31–38] have been recently developed to assess the meibomian glands objectively, avoiding subjective grading scales since they depend on the ability of the examiner to detect gland alterations, decreasing repeatability and agreement between clinicians [39].

A new algorithm based on the visibility of meibomian glands has been recently validated for Keratograph 5 M [35]. This algorithm is similar to the meibomian gland contrast measurement [38]. It was proven that, as meibomian glands are lighter than the background in a meibography, the visibility of meibomian glands could be used as a feature to aid in the assessment of those secretory structures. As previously defined, [35,40] visibility is defined as the grade in which meibomian glands are seen. Thus, in a subject with high gland drop-out, the glands have totally lost their visibility. With this premise, a new algorithm was developed, based on image analysis, to objectively assess the visibility of meibomian glands. Gland visibility is defined as the grade in which meibomian glands are seen. It was also demonstrated that gland visibility metrics are different from gland drop-out since gland visibility was different in subjects with similar gland drop-out percentages [35]. Thus, these metrics were useful to grade the level of visibility of the glands. Moreover, García-Marqués et al. [40] found that meibomian gland visibility metrics have good sensitivity and specificity to diagnose MGD, having higher diagnostic capability than current diagnostic metrics such as meibomian gland drop-out. Moreover, gland visibility metrics are correlated with gland expressibility, tear film stability, tear meniscus height and bulbar redness.

There is no previous study assessing the effect of scleral lenses on meibomian gland drop-out. Moreover, it has been recognized the need for assessing meibomian glands in subjects fitted with these lenses [41]. Since scleral lenses are usually fitted in subjects with irregular corneas, namely patients with keratoconus who are more likely to suffer from DED and MGD [42–44], understanding their effect on meibomian glands is clinically relevant. The present study has two main aims: The first one is to validate a previously developed algorithm based on the visibility of meibomian glands with a commercial fundus camera, which might help to further validate this algorithm, suggesting that it is not instrument-specific. The second aim is to assess dry eye symptoms and meibomian gland alterations in scleral lens wearers after one year of lens wear. The hypothesis is that as both eyelids and the tear film interact directly with scleral lenses, they might be affected by scleral lens wear [11].

2. Material and methods

2.1. Validation of an objective method to quantify meibomian gland visibility

The data from forty-three subjects participating in a prospective clinical study involving scleral lens fitting [1,9,45] was also used in the present study. The mean age of the participants was 34.2 ± 10.1 years old (ranging between 18 and 65 years). Subjects were not required to be previous soft or rigid contact lens wearers. No exclusion based on the state of the meibomian glands of subjects was made. A random eye was chosen for the analysis. Written consent of each subject was obtained after the explanation of the study purpose and clinical protocol. The study procedures were approved by the Ethics Subcommittee for Life and Health Sciences (SECVS) of the University of Minho and following the tenets of the Declaration of Helsinki.

2.2. Measurements

Non-contact infrared meibography of the upper eyelid was obtained using the Cobra fundus camera (CSO, Scandicci, Firenze, Italy) by the same experienced and masked researcher. Meibographies were obtained in the same laboratory under constant conditions of illumination. Illuminance was measured using an illuminance meter (T-10, Minolta - Sensing Inc, Tokyo, Japan). The varying daylight was removed using black-out curtains. The illuminance was 254.65 ± 8.19 lx. Gland drop-out percentage was calculated by using the Image J tool (Wayne Rasband, National Institutes of Health, Bethesda, MD) as the ratio between the eyelid area and gland loss area, as previously described [46].

2.3. Algorithm validation for the measurement of meibomian gland visibility

Meibographies were subjectively classified into 3 groups (Fig. 1), by a masked researcher, as follows: Group 1 = Patients with good subjective glands visibility and an Image J drop-out percentage less than one-third of the total area of meibomian glands; Group 2 = Patients with low subjective glands visibility and an Image J drop-out percentage lower than one-third of the total area; and Group 3 = Patients with low subjective glands visibility and an Image J drop-out percentage higher than one-third of the total area. This classification into groups is not based on any previous criterion, but subjects were classified into groups to validate the algorithm. However, the cut-off value of 1/3 of gland drop-out is similar to Arita's scale [28].

Once meibographies were classified into these 3 groups, they were analysed using the developed algorithm based on the grey level intensity of pixels. Examiner was masked to the study group allocation of meibographies during image processing analysis. The present algorithm was developed using Matlab® R2018a software (MathWorks, Natick, MA) as explained by García-Marqués et al. [35] for Keratograph 5 M. Meibographies obtained with Cobra fundus camera were lighter than ones obtained with Keratograph 5 M. Therefore, in this case, the algorithm included a reduction of 50% in the brightness of the meibography to avoid pixels saturation in the image processing.

2.4. Effect of scleral lens wear in ocular surface symptoms, gland drop-out and gland visibility

Out of 43 subjects, data from 29 of them (25.6 ± 11.03 years old, ranging from 18 until 65 years) was available to assess the effect of scleral lens wear after one year. Thus, meibographies were again obtained and analysed with Image J and the developed algorithm to assess differences in meibomian gland drop-out and visibility after one year of scleral lens wear. Ocular surface symptoms were also assessed at baseline and after one year of scleral lens wear using the Ocular Surface Disease Index (OSDI) questionnaire.

All volunteers were fitted with Senso MiniScleral lenses from Procornea (Eerbeek, The Netherlands) made of Boston XO material (DK 100 ISO/Fatt). Lenses were fitted by an experienced practitioner using a diagnostic fitting set. Other technical characteristics of the lenses and fitting procedure were previously described [1,9,45,47]. The lens should align evenly with the conjunctival surface and vault the entire corneal surface and limbus. If the fit was not satisfactory, another diagnostic lens was applied. When the best diagnostic lens fitting was achieved, participants were asked to continue with the diagnostic lens for another 90 min and then to return for a new assessment and to perform over-refraction. After the final assessment, minor adjustments were made and the final lenses were ordered [48].

2.5. Statistical analysis

Statistical analysis was performed using SPSS v26.0 for Windows (IBM Corp, Armonk, New York, USA). Results were displayed as the



Fig. 1. Examples of the 3 meibography groups. From left to right: Group 1, Group 2 and Group 3.

mean \pm SD and Shapiro-Wilk test was used to check the normality distribution for each group.

Differences between meibography groups for each metric were assessed using the Kruskal-Wallis test, while the Bonferroni test was used to evaluate the post-hoc differences between paired groups and p-values were shown according to the Bonferroni correction. A p-value < 0.05 was defined as statistically significant.

Correlations between each gland visibility metric and meibomian gland drop-out percentage were assessed by means of the Rho Spearman test; with the entire sample and after excluding group 2 from the analysis to not take into account the cases in which low visibility was not related to high gland drop-out. Thus, group 2 was excluded to further prove that not only was the algorithm measuring gland drop-out, but it also measured the visibility of the glands.

Since the algorithm was semiautomatic, the repeatability of the algorithm to calculate each metric was obtained by analyzing each meibography three times with the developed algorithm. Within-subject standard deviation (S_w), coefficient of variation (CoV) and the repeatability coefficient (CoR) were calculated to assess the repeatability of each new metric. S_w was defined as the square root of the mean within-subject variance [49–51], CoV was calculated as the ratio between S_w and the average value, and CoR as $1.96 \sqrt{2} \times S_w$ in accordance with the British Standard Institution and the International Organization of Standardization [52–54]. Finally, either paired t -test or Wilcoxon test were used to assess the effect of scleral lenses in meibomian glands and

ocular surface symptoms after one year of wear.

3. Results

3.1. Validation of the algorithm in Cobra fundus camera (CSO)

The new algorithm was applied to forty-three meibographies from the eyes of 43 subjects. Twenty were female (46.5%) and 23 male (53.5%), while the mean age was 34.2 ± 10.1 years (ranging from 18 to 65 years). From the total sample, 24 volunteers were classified in group 1 (34.3 ± 12.0 years), 12 in group 2 (34.2 ± 7.1 years) and 7 in group 3 (34.0 ± 6.7 years). No statistically significant differences in age were found between groups ($p = 0.998$). Table 1 shows the main results obtained for each metric per group and the statistical comparison between them. The algorithm performed properly in all meibographies.

Meibomian gland visibility metrics did not reveal statistically significant differences in gland drop-out percentage between groups 1 and 2. Nevertheless, meibographies of group 1 showed higher grey pixel intensity values than the other groups, which is reflected in relative energy, energy, standard deviation irregularity, mean, standard deviation, median, kurtosis and skewness. This suggests that the algorithm can distinguish between different types of meibographies.

Statistically significant correlations were found between the meibomian gland visibility metrics, based on grey level intensity pixels of meibographies, and gland drop-out percentage with the entire sample and after excluding group 2 (Table 2). Moreover, correlations were

Table 1

Mean values for each group and comparison between them.

METRIC	GROUP	MEAN \pm SD	SIGNIFICANCE LEVEL (p-value)	COMPARISON BETWEEN GROUPS (p-value)
DROP-OUT PERCENTAGE (%)	Group 1	25.51 \pm 7.09	$< 0.001 \dagger^*$	1–2 = 0.464
	Group 2	27.03 \pm 5.80		1–3 = $< 0.001^*$
	Group 3	60.19 \pm 20.57		2–3 = 0.004*
RELATIVE ENERGY	Group 1	0.34 \pm 0.07	$< 0.001 \dagger^*$	1–2 = 0.008*
	Group 2	0.24 \pm 0.05		1–3 = $< 0.001^*$
	Group 3	0.12 \pm 0.10		2–3 = 0.522
ENERGY	Group 1	226.79 \pm 9.93	$< 0.001 \dagger^*$	1–2 = 0.047*
	Group 2	210.57 \pm 7.92		1–3 = $< 0.001^*$
	Group 3	187.22 \pm 12.89		2–3 = 0.296
SD IRREGULARITY	Group 1	0.28 \pm 0.08	$< 0.001 \dagger^*$	1–2 = 0.006*
	Group 2	0.17 \pm 0.05		1–3 = $< 0.001^*$
	Group 3	0.09 \pm 0.10		2–3 = 0.573
MEAN ROI PIXELS INTENSITY	Group 1	95.77 \pm 14.30	$< 0.001 \dagger^*$	1–2 = 0.006*
	Group 2	77.03 \pm 9.62		1–3 = $< 0.001^*$
	Group 3	49.07 \pm 21.85		2–3 = 0.573
SD ROI PIXELS INTENSITY	Group 1	81.67 \pm 5.99	$< 0.001 \dagger^*$	1–2 = 0.012*
	Group 2	72.74 \pm 6.95		1–3 = $< 0.001^*$
	Group 3	53.69 \pm 15.62		2–3 = 0.600
MEDIAN ROI PIXELS INTENSITY	Group 1	72.08 \pm 20.72	$< 0.001 \dagger^*$	1–2 = 0.013*
	Group 2	49.50 \pm 10.13		1–3 = $< 0.001^*$
	Group 3	28.43 \pm 16.72		2–3 = 0.339
KURTOSIS	Group 1	0.0107 \pm 0.0010	$< 0.001 \dagger^*$	1–2 = 0.015*
	Group 2	0.0120 \pm 0.0009		1–3 = $< 0.001^*$
	Group 3	0.0167 \pm 0.0029		2–3 = 0.455
SKEWNESS	Group 1	0.105 \pm 0.006	$< 0.001 \dagger^*$	1–2 = 0.016*
	Group 2	0.112 \pm 0.004		1–3 = $< 0.001^*$
	Group 3	0.133 \pm 0.014		2–3 = 0.443

Where: ROI = Region of Interest; SD = Standard Deviation; Group 1 = Patients with good subjective gland visibility and gland drop-out less than one-third of the total meibomian gland area; Group 2 = Patients with low subjective gland visibility and gland drop-out less than one-third of the total meibomian gland area; Group 3 = patients with low subjective gland visibility and gland drop-out higher than one-third of the total meibomian gland area; * = Statistically significant; \dagger Kruskal-Wallis Test.

Table 2

Rho Spearman correlations between the developed metrics based on grey intensity pixels of meibographies and gland drop-out percentage with the entire sample and after excluding group 2.

METRIC	GROUPS	SPEARMAN'S CORRELATION COEFFICIENT (r)	SIGNIFICANCE LEVEL(p-value)
RELATIVE ENERGY	ALL THE SAMPLE	-0.606	<0.001*
	WITHOUT GROUP 2	-0.682	<0.001*
ENERGY	ALL THE SAMPLE	-0.564	<0.001*
	WITHOUT GROUP 2	-0.602	<0.001*
STANDARD DEVIATION IRREGULARITY	ALL THE SAMPLE	-0.589	<0.001*
	WITHOUT GROUP 2	-0.663	<0.001*
MEAN ROI PIXELS INTENSITY	ALL THE SAMPLE	-0.594	<0.001*
	WITHOUT GROUP 2	-0.674	<0.001*
STANDARD DEVIATION ROI PIXELS INTENSITY	ALL THE SAMPLE	-0.502	0.001*
	WITHOUT GROUP 2	-0.538	0.002*
MEDIAN ROI PIXELS INTENSITY	ALL THE SAMPLE	-0.620	<0.001*
	WITHOUT GROUP 2	-0.714	<0.001*
KURTOSIS	ALL THE SAMPLE	0.605	<0.001*
	WITHOUT GROUP 2	0.674	<0.001*
SKEWNESS	ALL THE SAMPLE	0.603	<0.001*
	WITHOUT GROUP 2	0.668	<0.001*

Where: ROI = Region of Interest; Group 2 = Patients with low subjective gland visibility and gland drop-out less than one-third of the total meibomian gland area; * = Statistically significant.

stronger after excluding group 2. Group 2 was excluded from the analysis to further prove that the software was not only measuring gland drop-out, but also able to detect differences in gland visibility.

All metrics showed moderate-acceptable repeatability since S_w and CoR values were low and the variability between the three measurements was not high, CoV achieving values between 0.52 and 3.18 (Table 3).

3.2. Differences in ocular surface symptoms and meibomian glands after 1 year of scleral lens wear

Twenty-nine subjects were fitted with scleral lenses and meibographies were assessed at baseline and after one year of scleral lens wear.

Table 3

Repeatability of each metric.

METRIC	Sw	CoR	CoV (%)
RELATIVE ENERGY	0.006	0.017	2.04
ENERGY	1.35	3.74	0.63
STANDARD DEVIATION IRREGULARITY	0.006	0.017	2.63
MEAN ROI PIXELS INTENSITY	1.29	3.56	1.46
STANDARD DEVIATION ROI PIXELS INTENSITY	0.54	1.5	0.70
MEDIAN ROI PIXELS INTENSITY	2.04	5.65	3.18
KURTOSIS	0.00012	0.0003	1.02
SKEWNESS	0.0006	0.002	0.52

Where: CoR = Repeatability coefficient; CoV = Coefficient of variation; ROI = Region of Interest; Sw = Within-subject standard deviation.

The mean age was 25.6 ± 11.03 , ranging from 18 to 65 years old. Fifteen were female (51.7 %) and fourteen were male (48.3 %). One eye of each patient was randomly chosen for the meibomian gland evaluation before fitting and 1 year after lens wear: Eighteen eyes had keratoconus (62.1%), four eyes with healthy cornea but high refractive error (13.8 %), four eyes had undergone penetrating keratoplasty (13.8 %) and three eyes had post-LASIK (Laser Assisted In-Situ Keratomileusis) ectasia (10.3 %).

No statistically significant differences were found in meibomian gland drop-out percentage and gland visibility metrics between baseline and after one year of scleral lens wear (Table 4). Nevertheless, an average improvement in OSDI of 24.5 was found after one year of scleral lens wear. Thus, this might suggest that scleral lens wear appears to not adversely affect the meibomian gland drop-out and visibility while promoting comfortable wear.

4. Discussion

4.1. Validation of the algorithm in Cobra fundus camera (CSO)

Image processing might be a suitable way to develop metrics with improved accuracy and repeatability against subjective scales [29,31–33,55–60]. In the present study, an objective, semiautomatic algorithm based on the analysis of the visibility of meibomian glands,

Table 4

Comparison of OSDI, gland drop-out and gland visibility in baseline and after one year of scleral lens fitting.

METRIC	GROUPS	Mean \pm SD	SIGNIFICANCE LEVEL(p-value)
DROP-OUT PERCENTAGE (%)	Pre	29.29 \pm 12.10	0.157 [‡]
	Post	30.44 \pm 11.84	
RELATIVE ENERGY	Pre	0.30 \pm 0.10	0.525 [‡]
	Post	0.29 \pm 0.10	
ENERGY	Pre	213.88 \pm 12.73	0.217 [‡]
	Post	211.05 \pm 12.59	
STANDARD DEVIATION IRREGULARITY	Pre	0.25 \pm 0.10	0.411 [‡]
	Post	0.23 \pm 0.09	
MEAN ROI PIXELS INTENSITY	Pre	89.09 \pm 19.82	0.369 [‡]
	Post	85.12 \pm 20.34	
STANDARD DEVIATION ROI PIXELS INTENSITY	Pre	78.13 \pm 10.54	0.539 [‡]
	Post	76.57 \pm 9.70	
MEDIAN ROI PIXELS INTENSITY	Pre	64.62 \pm 23.93	0.539 [‡]
	Post	60.97 \pm 25.84	
KURTOSIS	Pre	0.0114 \pm 0.0020	0.539 [‡]
	Post	0.0117 \pm 0.0022	
SKEWNESS	Pre	0.108 \pm 0.010	0.569 [‡]
	Post	0.109 \pm 0.010	
OSDI	Pre	50.17 \pm 22.40	<0.001 ^{†*}
	Post	25.69 \pm 14.34	

Where: OSDI = Ocular Surface Disease Index; ROI = Region of Interest; SD = Standard Deviation; * = Statistically significant; †Paired T-test; ‡Wilcoxon Test.

which was previously developed for Keratograph 5 M, was validated for a commercial fundus camera. Meibomian gland visibility assessment could be relevant because the link between gland drop-out and gland function is not clear [60].

The results in this study were similar to those found by García-Marqués et al. [35] in Keratograph 5 M, suggesting that the algorithm is not instrument-dependent. The only modification performed was a reduction of 50% in the brightness of the meibography to avoid pixels saturation since meibographies obtained with the Cobra fundus camera were lighter. As in the previous study [35], results showed lower pixel intensity values for group 2, which had lower gland visibility than group 1 but similar gland drop-out. This evidences that the algorithm was not only able to detect gland drop-out, but it can also objectively assess meibomian gland visibility. Moreover, this was even more evident when correlations between gland visibility metrics and gland drop-out percentage were stronger after excluding group 2 from the analysis. As previously reported [40], gland visibility metrics had higher diagnostic ability than current metrics such as meibomian gland drop-out and were correlated with different ocular surface metrics.

Despite the algorithm being semiautomatic - since the region of interest of glands needs to be manually delimited -, repeatability of gland visibility metrics showed to be moderate-acceptable because S_w , CoR and CoV indicate good repeatability when its values are near zero [49–52].

4.2. Effect of scleral lens wear in ocular surface symptoms, gland drop-out and gland visibility

Some studies have found that subjects with irregular corneas are more likely to suffer from DED and MGD [42–44]. Moreover, McMonnies [61] and Lema et al. [62] also suggested that a possible inflammatory role might be involved in the pathogenesis of keratoconus since inflammatory molecules were overexpressed in the tears of subjects with keratoconus.

Visual rehabilitation of subjects with irregular corneas is usually achieved with scleral lenses. Both eyelids and the tear film interact directly with scleral lenses and they might be affected by scleral lens wear [11]. For instance, changes in meibomian gland secretion could increase deposits on the anterior surface of scleral lenses [11]. Also, scleral lenses might increase the mechanical interaction with the tarsal structures on repeated blinking due to the thickness and space that occupy. However, few studies assessed the effect of scleral lenses on the tear film and meibomian glands [11,63,64]. The sample of this study represented irregular corneas, except for four of them. Since scleral lenses are usually fitted in subjects with irregular corneas, who are more likely to suffer from DED [42–44], assessing the effect of these lenses on meibomian glands is vital. Thus, a recent review confirmed that there is a need for prospective evaluation of meibomian gland appearance and function during scleral lens wear [11].

To the authors' knowledge, only one study [63] attempted to measure some features related to meibomian glands after scleral lens wearing. The authors did not find statistically significant differences in meibum expression after 1 year of scleral lens wear in moderate-severe DED subjects. These results are aligned with ones found in the present study since no statistically significant differences were found in meibomian gland drop-out and meibomian gland visibility after one year of scleral lens wearing. This is the first study evaluating the effect of scleral lenses on the structural aspects of Meibomian gland dysfunction evaluated objectively.

Regarding ocular surface symptoms, it was already shown that scleral lenses can improve OSDI scores compared to other treatment options and that this improvement was maintained over 12 months of lens wear [9]. In the present study a clinically and statistically significant reduction from 50.17 ± 22.40 to 25.69 ± 14.34 was found between Baseline (prior scleral lens fitting) and after 1 year of scleral lens wear ($p < 0.001$). Furthermore, these results are also in agreement with previous

studies that claimed that tear film stability, tear film volume, tear film osmolarity, dry eye symptoms, tear film temperature and volume are not altered or are even improved after scleral lens fitting [11,63,64].

The present study had some limitations that must be taken into accounts such as the sample size and the lack of a control group. No control group was included in the study and results were only compared with the baseline. Three cases could have happened if a control group was included. First, no differences in the control and scleral lens group would mean that scleral lens did not affect positively and negatively meibomian glands. Second, the control group showed a negative impact on meibomian glands whilst the scleral group did not show changes would mean that scleral lenses prevent the alteration of meibomian glands. Third, the control group improved meibomian glands while the scleral lens group did not show changes would mean that scleral lenses negatively impacted meibomian glands. Nonetheless, this last case might be improbable since it is not expected that meibomian glands improved in the control group without any treatment.

As there is no control group, we cannot conclude whether the meibomian glands improved after scleral lens wear. Nevertheless, what we can claim is that scleral lenses did not affect negatively the structure and visibility of meibomian glands. Future research with a larger sample, with a longer follow-up and with a control group would be required in aged subjects in order to confirm these findings. This work represents the first step in the study of meibomian glands linked to scleral lens wear and further studies with a control group should confirm these preliminary results. In any case, the results of the present study allow a hypothesis to be built for testing in future studies.

Another limitation was that this study focused on the anatomical characteristics of meibomian glands rather than their function. Nevertheless, it has been previously reported that meibomian gland visibility is correlated with gland expressiveness, tear film stability, tear meniscus height and bulbar redness [40]. Moreover, in this study, the DED status was not taken into account. The procedure was semiautomatic since the clinician still has to manually eliminate the reflexes and delineate the region of interest of glands. However, despite it being semiautomatic, repeatability was moderate-acceptable. Besides, this study was only focused on meibographies of the upper eyelids because it was easier to capture a uniformly focused image of the tarsal plate [58].

Overall, the new algorithm based on grey level intensity pixels of meibographies is also able to assess meibomian gland visibility in an objective and repeatable way in the Cobra fundus camera. This might suggest that the algorithm is not instrument-specific. Moreover, gland visibility metrics might help to assess the follow-up of meibomian glands after scleral lens wear. The present work adds valuable information regarding the effect of scleral lenses on meibomian gland drop-out and visibility. Scleral lens wear appears to not adversely affect meibomian gland drop-out and visibility while promoting comfortable wear and might improve dry eye symptoms after one year of lens wear. All of this may suggest that scleral lenses are not only preferable for visual rehabilitation of subjects with irregular cornea (86.2 % of the sample) but might also benefit their ocular surface comfort as these patients may be predisposed to DED and MGD. Further studies are needed to confirm this preliminary results with a control group and to assess the effect of scleral lenses on meibomian glands depending on DED diagnosis, in different age groups and with longer follow-up periods.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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