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The effect of sodium fluorescein on anterior eye surface measurements

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ABSTRACT

Purpose: During image acquisition, certain topographers require the addition of sodium fluorescein (NaFl) dye to the tear film. This study investigates the effect of NaFl dye on corneal topography and tear surface quality.

Method: The E300 corneal topographer (Medmont International Pty Ltd., Victoria, Australia) was used to measure ocular surface topography and quality of 57 eyes of 57 healthy individuals without dry eye symptoms, age 35.1 ± 15.2 years (mean ± standard deviation) ranging between 19 and 65 years. The mean of three simulated keratometry values, a variety of corneal shape descriptors, and Tear Film Surface Quality (TFSQ) were measured under three different conditions; without NaFl (baseline), with the addition of a single dose NaFl, and using a double dose of NaFl.

Results: Compared to baseline, the Inferior-Superior (IS) index decreased significantly after a single dose ($P = 0.034$) or double dose of NaFl ($P = 0.030$). The corneal surface was significantly more regular without NaFl ($P = 0.003$) or one insertion of NaFl ($P = 0.024$) when compared to two doses of NaFl. There was no association with age, or dry eye signs or symptoms on the variance observed in any of the indices between baseline, intervention I, and intervention II ($P > 0.05$). Agreement between corneal surface indices reduced following the addition of NaFl.

Conclusion: In comparison to measurements taken without an ocular dye, one dose of NaFl resulted in increased reliability and consistency in corneal topography measurements using the E300 topographer, but 2 doses decreased reliability and consistency. Practitioners ought to be aware that tear film surface regularity and inferior-superior corneal power changed significantly following the addition of NaFl in those with healthy corneas. Its effect in diseased corneas is unknown.

1. Introduction

Most common corneal surface devices in an optometric practice measure the curvature of the cornea, a baseline measurement in soft contact lens fittings, over a relatively small central area. These include keratometers (2–4 mm) and small- or large-cone Placido disc videokeratographs (6 − 8 mm) [1]. Using Placido disc videokeratography, attempts have been made to extend the corneal coverage to approximately 11 mm using extrapolation techniques [2,3]. Following the recent increase in fittings of large diameter gas permeable contact lenses, such as contact lenses for orthokeratology (> 11 mm) and (semi-/ mini-) scleral (13 to 24 + mm) lenses, devices are needed to visualize and measure large geometric areas of the cornea and sclera.

Other approaches to corneal topography, including Optical Coherence Tomographers (OCT) and Scheimpflug-based imaging devices are able to generate a three-dimensional profile of the anterior segment and provide topography information about the anterior as well as posterior cornea including corneal thickness. These imaging techniques are able to measure a larger surface of the anterior eye up to 16 mm [4]. Scheimpflug camera systems have shown good agreement for anterior corneal geometry compared to Placido-based videokeratography [5], although this is significantly reduced for the posterior cornea [5–7]. Similar results have been reported for OCT devices when compared to Placido-based videokeratography [8–10], producing sub-optimal peripheral output predominantly associated with refractive and elevation data. It is possible that three-dimensional information obtained using a radial scan mode plays a role, causing oversampling in the central region while undersampling in the outer region, and misalignment of the OCT system caused by the patient or operator proving difficulties with acquiring perfectly centered radial scans [9].

Devices based on Fourier transform profilometry have been developed to measure the corneo-scleral region up to 16.5 mm [11,12]. Examples of this are the Eye Surface Profiler (ESP, Eaglet-Eye, The Netherlands) and sMap3D (Precision Ocular Metrology, LLC., Cedar

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Crest, New Mexico, United States). These devices directly measure the elevation of both the anterior and posterior cornea via time domain or light-based analysis, while converting elevation data into anterior and posterior curvatures (in diopters) as well as corneal thickness. To obtain a measurement, the profilometer uses the phase information of the projected images, which only exist by the mirror function of the tear film [11,12]. To obtain reflected light from the scleral epithelium, a significant amount of sodium fluorescein (NaFl) dye is required prior to image capture. NaFl dye is highly water soluble and is used as a diagnostic dye to detect the tear film stability as well as damage of the epithelial cells of the anterior surface of the eye. When artificially increasing tear volume by introducing an ocular dye like NaFl, it is important to understand the effect of this volume increase on the regularity of the corneal topography and quality of resulting images. This cross-sectional study aims to evaluate the effect of various doses of NaFl dye on the image quality of corneal topography when using a Placido disc videokeratograph. Adding NaFl was expected to increase the volume, smooth the tear film and improve optical regularity, and possibly increase reliability and consistency of the corneal curvature measurements. A particular interest was to investigate if topography and tear film quality measurements are affected by multiple doses of NaFl. To do this, variations in the ocular surface topography following instillation of different amounts of NaFl were observed, predominantly in participants with low tear film quality and/or signs of dry eyes.

2. Methods

The research conducted in this study complied with the requirements of the Declaration of Helsinki (2008) and the research protocol and documentation received approval from the School of Health Sciences Research Ethics Committee at City, University of London (United Kingdom) and University of Applied Sciences Utrecht (UAS Utrecht; the Netherlands). Written consent was obtained prior to participation. The study included 57 healthy participants from the Healthcare department at the UAS Utrecht Eyecare Clinic between February and June 2017. Health was determined by general and ocular health questionnaires and anterior eye examination using slit lamp biomicroscopy. Exclusion criteria included a history of ocular surgery, anterior eye trauma or corneal/ corneal-scleral disease resulting in reduced visual acuity. Volunteers were excluded if they were pregnant, diagnosed with amblyopia, rigid contact lenses wearers, or presented with any other corneal abnormalities including suspect keratoconus. Participants were either neophytes or were asked to discontinue soft contact lens wear for at least 48 h prior to the assessment.

All measurements were taken on both eyes during a single visit (Table 1), including a 20-minute break between the baseline measurements and interventions I and II. To rule out observer bias, all measurements were obtained by the same experienced investigator (JM). At the baseline visit, participants underwent a clinical anterior eye examination and symptom assessment. The prevalence of ocular surface disease was determined using the Ocular Surface Disease Index (OSDI) questionnaire culturally translated into Dutch (Oogoppervlak Beperkingen Vragenlijst, Alcon, 1995). OSDI results were considered normal (0–12 points), mild (13–22 points), moderate (23–32 points) or severe (33–100 points) diseased [13]. A subjective refraction was performed to determine the degree of ametropia and visual acuity. Snellen visual acuity was measured with full correction. A standard optometric slit lamp examination of the anterior eye including (palpebral) eyelids, lid margins, conjunctiva, limbus and cornea was performed and evaluated using CCLRU grading scales (0–4 grades) in 0.5 increments [14].

2.1. Corneal topography and surface assessment

Corneal topography and surface assessment were determined using the E300 corneal topographer (Medmont International Pty Ltd., Victoria, Australia. Software: Medmont Studio 6.0). Three high-quality measurements were obtained whereby the instrument‘s software automatically calculates the geometric shape of the measured area and provides information about the regularity of the surface using indices including Simulated keratometry values, Inferior Superior index (IS), Surface Asymmetry Index (SAI), Surface Regularity Index (SRI) and the Tear Film Surface Quality (TFSQ; see Table 2) [16,17]. For all indices, the average of three measurements were used for analysis.

2.2. Tear Break Up Time (TBUT)

To measure TBUT, a sterile BIO-GLO 100 fluorescein sodium 1 mg ophthalmic (HUB Pharmaceuticals, LLC Rancho Cucamonga CA, USA) was moistened with 1 drop of non-preserved saline from a minim (Oté Pharma, Uden, The Netherlands), and was gently shaken once after moistening to remove excess fluorescein solution from the strip. After application on the superior-temporal conjunctiva, the TBUT was observed with a SL-9900D LED slit lamp biomicroscope (CSO Srl, Firenze, Italy) with a cobalt blue filter using a Wratten no 12 (yellow) filter after two blinks. Time in seconds was recorded when the first dry spot was observed after blink using a full width beam at 10x magnification. The mean of three TBUT measurements was calculated for each eye with a time period of at least one minute between measurements to improve measurement accuracy [15].

2.3. Intervention I and II

After 20 min, corneal topography measurements were repeated after the insertion of NaFl as described above. During the first intervention, one lubricated strip of BIO-GLO 100 was used, while during the second intervention (20 min apart) this was immediately followed by a second strip of NaFl.

2.4. Statistical analysis

Statistical analyses were calculated using SPSS statistical package version 25 (SPSS Inc., Chicago, IL, USA). Mean spherical equivalent and corneal topography indices showed strong positive correlations between both eyes ($p < 0.0005$); therefore, only right eyes were included for analysis to alleviate any inter ocular dependency issues and statistical bias due to the mirror-image relations [20]. At baseline, age-related differences were calculated between mean values (Mann-Whitney test) and proportions (one-sample *t*-test between percents). Following violation of the assumptions of normality (Kolmogorov-Smirnov tests)

Table 1
Single visit study protocol.

Baseline	Intervention I *20 min after baseline*	Intervention II *20 min after intervention I*
Health questionnaire Subjective refraction including visual acuity Slit lamp examination of anterior eye Corneal topography and surface assessment (Medmont topographer) Tear Break Up Time (TBUT) [15]	Insertion of one application of NaFl Corneal topography and surface assessment	Insertion of two applications of NaFl Corneal topography and surface assessment

Table 2
Medmont E300 output explanation [16].

Abbreviation		Description
IS	Inferior Superior Index (Diopters)	The difference between the average inferior and superior power in the eye is called the IS value. Measured over a 4 − 5 mm area depending on the position of the eyelids.
SAI	Surface Asymmetry Index (Diopters)	Calculated from the centrally weighted summation of differences in corneal power between corresponding points at one of the 128 equidistant chords, 180 degrees apart on the eyes surface. A regular cornea shows a SAI value of < 1.0.
SRI	Surface Regularity Index (Diopters)	Description of the corneal shape in the central 4.5 mm zone. The power of each point is compared with contiguous points. The calculation is based on the determination of the most frequently occurring dioptric power and the comparative analysis of dioptric powers of adjacent points in 256 hemi-meridians in the 10 central rings [18]. A cornea with an SRI index of < 0.8D is considered regular.
TFSQ	Tear Film Surface Quality	Tear film quality indicated by the average organisation of the Placido disc image reflections over the entire cornea. A local TFSQ value of 0.30 or higher correspondents with a visual tear break up [19].

for indices SAI, SRI, and TFSQ, data was transformed on a logarithmic scale to achieve normality and for statistical analyses, whereas raw (sample) data is presented as summary statistics (mean ± SD, 95 % confidence intervals CI, etc) [21]. A one-way repeated measures ANOVA including Least Significant Difference post hoc tests determined the significance between measurements under different conditions (baseline, intervention I and II), while mixed between-within AVOVA tests were used to explore the effect of covariates such as age and dry eye. Intra class correlation (ICC) estimates and their 95 % confident intervals were calculated based on a mean-rating (k = 3), absolute-agreement, 2-way mixed-effects model. Coefficient of Repeatability (CoR) for each of the parameters measured at baseline and each intervention were calculated as 1.96 x Sw (within-subject standard deviation). Agreement between the different interventions was calculated using the mean differences and 95 % limits of agreement (LoA). Statistical significance was accepted at the 95 % CI ($p < 0.05$). The participants were grouped by age, and two-way ANOVA power statistics revealed that a sample size of 39, 19 subjects per group, was needed to detect a standardized difference between the groups using a partial eta squared of 0.033 and 80 % power at 5% significance level [22]. This calculation was based on an estimated mean of 3 repeated flat keratometry readings of 7.87 mm with group SDs of 0.29 mm, based on data collected from the first 15 subjects.

3. Results

Demographic and dry eye characteristics of the participants are summarized in Table 3. A total of 57 participants (34 females, 23 males), age range between 19 and 65 years, were divided in two age groups: group A < 40 years (n = 34) and group B ≥ 40 years (n = 23). All participants presented with healthy corneas, without significant ocular surface, eyelid diseases or corneal staining. Both groups were well-matched for gender, OSDI scores, mean spherical equivalent (MSE), and average TBUT measured at baseline.

3.1. Influence of NaFl on corneal topography and surface assessment

One-way, repeated-measures analysis of variance (ANOVA) was conducted to compare the average of 3 measurements of quantitative descriptors in corneal topography under three different conditions: baseline, intervention I (following application of single amount of NaFl) and intervention II (following two applications of NaFl). Except for the IS index ($F(2,55) = 3.288$, $p = 0.045$, partial eta squared 0.107) and SRI index ($F(2,55) = 4.603$, $p = 0.014$, partial eta squared 0.143), insertion of NaFl did not have a statistically significant effect on corneal topography measurements (Table 4). Posthoc analysis revealed that compared to baseline, the inferior part of the cornea became significantly flatter after a single dose ($p = 0.034$) or double dose of NaFl ($p = 0.030$). Additionally, the corneal surface (SRI) was significantly more regular without NaFl ($p = 0.003$) or one dose of NaFl ($p = 0.024$) when compared to two doses of NaFl.

Table 3
Demographics and dry eye characteristics. Parameters are shown in mean ± standard deviation (SD) and [95 % confidence intervals around the mean]. Statistical significance is highlighted in bold. *Abbreviations: OSDI Ocular Surface Disease Index; MSE Mean Spherical Equivalent; VA Visual Acuity*.

	All subjects n = 57	< 40 years n = 34	≥40 years n = 23	*p*
Gender (male: female)	23 : 34	14 : 20	9 : 14	0.88
Age (years)	35.1 ± 15.2 [31–39]	23.8 ± 4.5 [22–25]	51.7 ± 8.4 [48–55]	< **0.0005**
OSDI score	11.9 ± 9.9 [9.2–14.5]	12.0 ± 9.3 [8.8–15.3]	11.6 ± 10.9 [6.9–16.3]	0.83
% normal OSDI score (< 13)	68 %	62 %	78 %	0.21
MSE (Diopters)	−0.82 ± 2.38 [−1.45 to −0.19]	−1.11 ± 2.17 [−1.86 to −0.35]	−0.39 ± 2.65 [−1.54 to −0.76]	0.21
Best corrected VA (Snellen decimal)	1.23 ± 0.21 [1.18–1.29]	1.34 ± 0.17 [1.28–1.40]	1.08 ± 0.14 [1.02–1.14]	< **0.0005**
Mean of three TBUT (seconds)	8.1 ± 6.9 [6.3–10.0]	7.8 ± 7.2 [5.3–10.3]	8.6 ± 6.5 [5.8–11.4]	0.43

Table 4
Corneal topography and surface assessment at baseline, intervention I (1x NaFl) and intervention II (2x NaFl). Parameters are shown in mean ± standard deviation. P-values represent the one-way repeated measures analysis of variance (ANOVA).

	Baseline n = 57	Intervention I n = 57	Intervention II n = 57	*p*
K flat (mm)	7.87 ± 0.26	7.88 ± 0.26	7.88 ± 0.26	0.29
K steep (mm)	7.71 ± 0.25	7.72 ± 0.25	7.71 ± 0.25	0.68
IS index (Diopters)	0.03 ± 0.59	−0.06 ± 0.58	−0.06 ± 0.40	**0.045**
SAI index (Diopters)	0.75 ± 0.28	0.77 ± 0.33	0.81 ± 0.34	0.12
SRI index (Diopters)	0.52 ± 0.16	0.54 ± 0.17	0.60 ± 0.18	**0.014**
TFSQ index	0.96 ± 0.038	0.93 ± 0.049	0.94 ± 0.044	0.45

3.2. Effect of age, OSDI score and TBUT

The effects of the covariates age (< 40 versus ≥40 years of age), dry eye symptoms (OSDI score normal < 13 versus dry eye ≥13), and dry eye signs (TBUT normal ≥5 versus dry eye < 5 s) were explored. None of these covariates were significantly associated with any of the topography indices at baseline ($p > 0.05$), except for TFSQ which was significantly increased in the older group ($p = 0.023$). Additionally, which covariate had most effect on the significant changes observed following installation of NaFl was investigated. A mixed between-within ANOVA showed no significant impact on the IS, SRI, or TFSQ indices variance observed between baseline, intervention I, and intervention II due to age, or dry eye signs or symptoms ($p > 0.05$).

Table 5
Repeatability and reliability of 3 corneal topography measurements at baseline, intervention I and II (n = 57). Results show Coefficient of Repeatability (CoR) including [95 % confidence intervals CI], and Intraclass Correlation Coefficients (ICC) with [95 % CI around the mean].

	Baseline		Intervention I		Intervention II	
	N = 57		N = 57		N = 57	
	CoR	ICC	CoR	ICC	CoR	ICC
K flat	0.062 [−0.431 to 0.555]	0.991 [0.987–0.995]	0.003 [−0.097 to 0.103]	0.992 [0.987–0.995]	0.088 [−0.498 to 0.674]	0.988 [0.981–0.992]
K steep	0.062 [−0.431 to 0.555]	0.991 [0.986–0.994]	0.062 [−0.431 to 0.555]	0.993 [0.989–0.996]	0.062 [−0.431 to 0.555]	0.982 [0.972–0.989]
IS index	0.782 [−0.968 to 2.532]	0.852 [0.781–0.904]	0.894 [−0.978 to 2.766]	0.763 [0.661–0.843]	0.885 [−0.978 to 2.748]	0.825 [0.745–0.886]
SAI index	0.196 [−0.681 to 1.073]	0.771 [0.672–0.849]	0.476 [−0.890 to 1.842]	0.676 [0.551–0.780]	0.480 [−0.892 to 1.852]	0.587 [0.444–0.713]
SRI index	0.062 [−0.431 to 0.555]	0.557 [0.409–0.690]	0.438 [−0.873 to 1.749]	0.602 [0.462–0.725]	0.291 [−0.777 to 1.359]	0.371 [0.207–0.536]
TFSQ index	0.062 [−0.431 to 0.555]	0.619 [0.482–0.737]	0.014 [−0.224 to 0.252]	0.726 [0.614–0.817]	0.175 [−0.654 to 1.004]	0.099 [−0.050–0.274]

3.3. Comparison subjective and automated TBUT

To understand the effect of NaFl on the regularity and image quality of the tear film, the traditional subjective measure of fluorescein TBUT was compared to the automated objective measure of tear stability (TFSQ) with and without NaFl. No statistically significant differences between the 3 repeated measurements of TBUT ($p = 0.62$), and 2 repeated measures of TFSQ without NaFl ($p = 0.67$) or TFSQ with NaFl ($p = 0.96$) were observed. However, TBUT was found to be significantly shorter (8.1 ± 0.91 s) compared to the automated method, without (12.6 ± 1.71 s) or with NaFl (13.6 ± 1.66 s; $F(2,55) = 7.085$; $p = 0.002$). These results were irrespective of age ($p = 0.36$), gender ($p = 0.60$), or OSDI score ($p = 0.67$). Although not significant, those classified as having no dry eye symptoms (OSDI score < 13) showed increased TBUTs independent of the method used.

3.4. Repeatability, reliability and agreement

Repeatability of three measurements for each of the parameters measured at baseline and each intervention were reported as the Coefficient of Repeatability (CoR; Table 5). Compared to baseline, repeatability of all parameters except K flat and TFSQ decreased following one application of NaFl (intervention I), while all parameters showed reduced repeatability following two applications of NaFl (intervention II). The amount of NaFl (one or two applications) had little effect on the repeatability of any of the parameters. In addition, the reliability of repeated measurements of corneal topography was established by calculating the Intra Class Correlation (ICC) of quantitative descriptors determined by the instrument's computerized algorithm (Table 5). At baseline, indices show moderate (SRI and TFSQ), good (IS and SAI) and excellent (K flat and steep) reliability. Reliability improved following the instillation of NaFl compared to baseline for all indices except IS and SAI. Compared to measurements taken without NaFl (baseline), all indices showed reduced ICC after insertion of NaFl twice (intervention II). Agreement between the interventions are presented as mean differences of average (out of 3) quantitative descriptors including 95 % LoA (Table 6). Mean differences increased when interventions (I and II) were compared to baseline for the corneal curvature in the flatter meridian, IS and TFSQ. On the other hand, SAI and SRI indices agreed better between baseline and 1 application of NaFl, compared to two applications. Similar to age (cut off 40 years), there was no effect of dry eye on the reliability of corneal topography measurements under the three conditions, irrespective of whether this was measured by OSDI scores (cut off 13) or TBUT measurements (cut off 5 s; data not shown).

Table 6
Agreement between average corneal topography measurements at baseline, intervention I and II (n = 57). Results show mean differences between the interventions and [95 % LoA] [23].

	Baseline vs Intervention I	Baseline vs Intervention II	Intervention I vs Intervention II
K flat	0.008 [−0.074 to 0.090]	0.009 [−0.073 to 0.091]	0.001 [−0.047 to 0.049]
K steep	0.004 [−0.096 to 0.104]	0.000 [−0.095 to 0.095]	−0.004 [−0.069 to 0.062]
IS index	−0.095 [−0.740 to 0.549]	−0.090 [−0.696 to 0.515]	0.005 [−0.656 to 0.666]
SAI index	0.022 [−0.336 to 0.380]	0.066 [−0.288 to 0.420]	0.044 [−0.388 to 0.476]
SRI index	0.013 [−0.267 to 0.293]	0.073 [−0.283 to 0.429]	0.060 [−0.323 to 0.443]
TFSQ index	−0.003 [−0.085 to 0.078]	−0.002 [−0.091 to 0.086]	0.001 [−0.099 to 0.101]

4. Discussion

Understanding the geometry of the peripheral cornea and sclera may enhance the successful fitting of these lenses and improve designs by manufacturers, in return resulting in increased comfort for the contact lens wearer. The regularity of the cornea may be reflected by means of the tear film layer stability and the corneal surface. Corneal regularity can be measured with or without the addition of NaFl dye to the tear film, but using NaFl is expected to enhance the quality of the image [24]. The aim of this study was to investigate the effect of NaFl on corneal topography measurements, including simulated flat and steep meridians, IS, SAI, SRI indices and TFSQ, using the E300 topographer. These computer-assisted corneal topographic analyses have been essential for understanding pathological alterations of the shape of the anterior corneal surface [25]. It was hypothesised that NaFl instillation would induce measurable differences in the ocular surface topography. This could be caused by 1) NaFl reduces tear film stability [26] and affects the ring pattern reflection from the ocular surface, in the absence of absolute changes in corneal surface topography; 2) the added fluid increases tear film volume and therefore affects the topography measurements; or 3) NaFl itself interacts (temporarily) with the cornea and changes its topography [27]. In addition, ocular surface topography could be affected due to a combination of two, or all of the above.

Since NaFl dye is soluble in water (saline), only a small amount is needed to colour the tear film. The method of delivering NaFl to the eye was based on that used in current clinical practice. A saline wetted

NaFl-impregnated paper strip adds approximately 3 μl to the tear film [28,29]. According to the results, inducing a change in tear volume, by insertion of NaFl using a paper strip and saline, seems to have little effect on corneal shape measurements (simulated Keratometry readings, SAI, and TFSQ), except for IS and SRI. The results revealed a statistically significant decrease in IS index score, representing a flatter inferior segment, following the application of a single dose of NaFl to the tear film. Considering the addition of tear volume results in an increased tear volume specifically in the inferior tear meniscus, it is expected that the inferior part of the central cornea over a 6.0 mm chord flattened due to the collection of tears on the lower eye lid. The method of NaFl instillation has been shown to reduce tear film stability [30], possibly leading to reflex lacrimation and/or subsequently increased tear meniscus heights. Compared to a single dose of NaFl, no significant difference was observed after applying a double dose, possibly indicative of tear meniscus saturation reached within the exposed tear volume or the lower lacrimal lake [31]. In addition, the SRI increased significantly with the insertion of NaFl, representative of decreased tear film surface regularity. This index is considered a measure of central corneal optical quality within the pupil size [32], based on the determination of the most frequently occurring dioptric power and the comparative analysis of dioptric powers of adjacent points in 256 hemimeridians in the central 10 Placido disc rings representing the average virtual pupil size [18]. For a perfectly smooth surface, SRI would approach 0 [32], whereas a cornea presenting an SRI index < 0.8D has been considered regular [16]. Hence, the significant change in surface regularity following NaFl (from 0.52 to 0.60) observed in this study did not signify a clinically irregular corneal surface caused by NaFl.

Compared to baseline, the addition of 1 or 2 applications (approximately 3 or 6 μl), to the tear volume prior to image acquisition also had a negative effect on the repeatability of the dioptre difference between the average inferior and superior power in both corneal hemispheres (IS), and the surface asymmetry where the centrally weighted average of the difference in power between corresponding points at the same chord is calculated (SAI) [32]. However, increased reliability and consistency in corneal shape was observed after instilling a single dose of NaFl for all other indices. Theoretically, the SAI value would be 0 for a perfect sphere; a surface with perfectly spherocylindrical regular astigmatism, or for any surface with a power that is radially symmetrical [34]. After instilling a double dose of NaFl there was a decrease in Intra class correlation in all quantitative descriptors. Similar to the current study, ICC for corneal topography parameters without the addition of NaFl have been shown to be highly repeatable, with an ICC of > 0.95 for simulated keratometric values [33–35]. Besides TFSQ values [19], no previous studies have reported the reliability of the Medmont E300's automatic computer assisted analysis of corneal shape. Moderate to good agreement between 3 repeated measures of IS, SAI and SRI indices were observed at baseline, indicating no need for multiple measurements when used in clinical practice. In addition, the agreement between the different interventions and the baseline measurements highlighted that the addition of NaFl to the tear film has an impact on most indices. Simulated keratometry, IS and TFSQ indices show reduced repeatability, irrespective of the amount of NaFl. On the other hand, SAI and SRI indices are more in agreement with baseline measurements after the application of one dose compared to two doses of NaFl.

Similar to previous reports, TBUT measured at baseline was found to be significantly reduced compared to non-invasive tear film stability (TFSQ) (p = 0.002) [19]. In addition, although tear film stability may be negatively affected following the application of NaFl [26,27], tear film quality measured using the Medmont E300 allowed for improved Placido disc image reflections (TFSQ). This finding is supported by previous studies showing that tear film stability can be affected due to an artificially increased TBUT following the application of an amount of NaFl exceeding the average tear volume of approximately 6 − 7 μl [36,37]. Additionally, the repeatability of TBUT measurement is improved when only small amounts of NaFl solution are added into the tear film [38]. However, when comparing the TFSQ with and without NaFl, the current results with the E300 topographer, differed from those of Mengher et al. [39] who, using a grid xeroscope, showed that NaFl significantly decreased non-invasive BUT when measured within 2 min after instillation [40].

4.1. Limitations

It is recognised that the sample size was too small to confirm if the variance observed in IS and SRI subsequent to the application of NaFl was induced due to age or dry eye signs/ symptoms, and as a result further studies are warranted. Although randomisation of data collection is desired to minimize bias error, this was not applied due to its potential significant effect on the diurnal variation in tear production, dry eye, and room/ environmental conditions. It is possible that the additional variability observed with the larger volume of fluorescein could have occurred because this was always the last intervention. However, this approach had the advantage that, since the washout period for fluorescein in the tear film is known to be at least 30 min [41], any effects due to residual fluorescein would tend to be minimised by always having the larger volume occur after the smaller one.

Another limitation is the lack of controlled fluorescein volume used in this investigation. To mimic daily practice, NaFl from a sterile strip was applied using one drop of saline from a minim. Although a controlled volume may have resulted in less variability, the current method reflects a more realistic outcome similar to those observed in clinical practice. Additional detailed measurements of the quantity and physiology of the tear film such as tear meniscus height and lipid layer quality were not included, which could also be considered a limitation of this study. Previous reports of tear meniscus height measured with OCT following careful installation of NaFl [39] do not seem to support the current findings whereby differences in IS indices found between the single and double dosages. However, it is known that the instillation methods of NaFl vary significantly between studies depending on the volume of saline used to wet the strip. Lastly, it is difficult to draw conclusions about how NaFl will affect elevation data using the ESP device, considering that this instrument was not used in this study. However, it is expected that elevation data in the inferior hemisphere of the corneal surface will behave similarly as was observed in this study, particularly when measured in primary gaze.

4.2. Clinical relevance

Statistically significant average differences in IS and SAI indices found in this study ranged from 0.09 to 0.18D, which is too small to distinguish between a normal or abnormal cornea descriptor values [16]. However, this study only included healthy corneas and it is therefore not surprising these differences are clinically insignificant. Using a spherical 8 mm test object, Medmont E300 repeated measurements of corneal shape are in 100 % agreement and quantitative descriptors are assumed to be 0, representing a perfectly spherical shape. However, in addition to the fact that the human cornea is aspheric, factors such as image focus, palpebral aperture height and tear meniscus height are expected to affect the measurements, particularly towards the periphery. The effects of these anatomical factors vary widely within the general population and it is unknown how these influence the algorithms used for calculating the quantitative descriptors in unhealthy (for example keratoconus) corneas.

This study investigated the effect of fluorescein dye on the quantitative descriptors in corneal topography. In conclusion, when using the Medmont E300 topographer, an increased reliability and consistency in corneal topography after instilling one dose of NaFl in the eye in all corneal descriptors was found, except for IS and SAI indices. On the other hand, larger amounts of NaFl decrease reliability and consistency. Practitioners should be aware that tear film surface regularity and inferior-superior corneal power change significantly following the addition of NaFl, although this does not seem to be clinically significant in healthy corneas. More work is needed to understand the effect of NaFl on corneal shape particularly during scleral and ortho-K contact lens fittings.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.clae.2020.02.016.

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