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The effect of sodium fluorescein on anterior eye surface measurements

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Highlights

- Corneal topography measurements are affected by the insertion of fluorescein sodium (NaFl) ocular dye
- A single (not double) dose of NaFl resulted in increased reliability and consistency in corneal topography measurements
- Tear film surface regularity changes significantly with NaFl, although this is not clinically significant in healthy corneas

Abstract

Purpose: During image acquisition, certain topographers require the addition of sodium fluorescein (NaFI) dye to the tear film. This study investigates the effect of NaFI 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 \pm 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 NaFI (baseline), with the addition of a single dose NaFI, and using a double dose of NaFI.

Results: Compared to baseline, the Inferior-Superior (IS) index decreased significantly after a single dose ($P=0.034$) or double dose of NaFI ($P=0.030$). The corneal surface was significantly more regular without NaFI ($P=0.003$) or one insertion of NaFI ($P=0.024$) when compared to two doses of NaFI. 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 NaFI.

Conclusion: In comparison to measurements taken without an ocular dye, one dose of NaFI 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 NaFI in those with healthy corneas. Its effect in diseased corneas is unknown.

Keywords: corneal shape, corneal topography, Break Up Time, reliability, sodium fluorescein

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1 **Introduction**

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

12

13 Other approaches to corneal topography, including Optical Coherence Tomographers
14 (OCT) and Scheimpflug-based imaging devices are able to generate a three-
15 dimensional profile of the anterior segment and provide topography information about
16 the anterior as well as posterior cornea including corneal thickness. These imaging
17 techniques are able to measure a larger surface of the anterior eye up to 16 mm [4].
18 Scheimpflug camera systems have shown good agreement for anterior corneal
19 geometry compared to Placido-based videokeratography [5], although this is
20 significantly reduced for the posterior cornea [5-7]. Similar results have been reported
21 for OCT devices when compared to Placido-based videokeratography [8-10],
22 producing suboptimal peripheral output predominantly associated with refractive and
23 elevation data. It is possible that three-dimensional information obtained using a radial
24 scan mode plays a role, causing oversampling in the central region while
25 undersampling in the outer region, and misalignment of the OCT system caused by
26 the patient or operator proving difficulties with acquiring perfectly centered radial scans
27 [9].

28

29 Devices based on Fourier transform profilometry have been developed to measure
30 the corneo-scleral region up to 16.5 mm [11,12]. Examples of this are the Eye Surface
31 Profiler (ESP, Eaglet-Eye, The Netherlands) and sMap3D (Precision Ocular
32 Metrology, LLC., Cedar Crest, New Mexico, United States). These devices directly
33 measure the elevation of both the anterior and posterior cornea via time domain or
34 light-based analysis, while converting elevation data into anterior and posterior

35 curvatures (in diopters) as well as corneal thickness. To obtain a measurement, the
36 profilometer uses the phase information of the projected images, which only exist by
37 the mirror function of the tear film [11,12]. To obtain reflected light from the scleral
38 epithelium, a significant amount of sodium fluorescein (NaFI) dye is required prior to
39 image capture. NaFI dye is highly water soluble and is used as a diagnostic dye to
40 detect the tear film stability as well as damage of the epithelial cells of the anterior
41 surface of the eye. When artificially increasing tear volume by introducing an ocular
42 dye like NaFI, it is important to understand the effect of this volume increase on the
43 regularity of the corneal topography and quality of resulting images. This cross-
44 sectional study aims to evaluate the effect of various doses of NaFI dye on the image
45 quality of corneal topography when using a Placido disc videokeratograph. Adding
46 NaFI was expected to increase the volume, smooth the tear film and improve optical
47 regularity, and possibly increase reliability and consistency of the corneal curvature
48 measurements. A particular interest was to investigate if topography and tear film
49 quality measurements are affected by multiple doses of NaFI. To do this, variations in
50 the ocular surface topography following instillation of different amounts of NaFI were
51 observed, predominantly in participants with low tear film quality and/or signs of dry
52 eyes.

53

54

55 **Methods**

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

68 suspect keratoconus. Participants were either neophytes or were asked to discontinue
69 soft contact lens wear for at least 48 hours prior to the assessment.

70

71 All measurements were taken on both eyes during a single visit (Table 1), including a
72 20-minute break between the baseline measurements and interventions I and II. To
73 rule out observer bias, all measurements were obtained by the same experienced
74 investigator (JM). At the baseline visit, participants underwent a clinical anterior eye
75 examination and symptom assessment. The prevalence of ocular surface disease was
76 determined using the Ocular Surface Disease Index (OSDI) questionnaire culturally
77 translated into Dutch (Oogoppervlak Beperkingen Vragenlijst, Alcon, 1995). OSDI
78 results were considered normal (0-12 points), mild (13-22 points), moderate (23-32
79 points) or severe (33-100 points) diseased [13]. A subjective refraction was performed
80 to determine the degree of ametropia and visual acuity. Snellen visual acuity was
81 measured with full correction. A standard optometric slit lamp examination of the
82 anterior eye including (palpebral) eyelids, lid margins, conjunctiva, limbus and cornea
83 was performed and evaluated using CCLRU grading scales (0-4 grades) in 0.5
84 increments [14].

85

86 **Table 1. Single visit study protocol**

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

87

88 *Corneal topography and surface assessment*

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

98

99 **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 corresponds with a visual tear break up [19]. |

100

101

102 *Tear Break Up Time (TBUT)*

103 To measure TBUT, a sterile BIO-GLO 100 fluorescein sodium 1 mg ophthalmic (HUB
104 Pharmaceuticals, LLC Rancho Cucamonga CA, USA) was moistened with 1 drop of
105 non-preserved saline from a minim (Oté Pharma, Uden, The Netherlands), and was
106 gently shaken once after moistening to remove excess fluorescein solution from the
107 strip. After application on the superior-temporal conjunctiva, the TBUT was observed
108 with a SL-9900D LED slit lamp biomicroscope (CSO Srl, Firenze, Italy) with a cobalt
109 blue filter using a Wratten no 12 (yellow) filter after two blinks. Time in seconds was
110 recorded when the first dry spot was observed after blink using a full width beam at
111 10x magnification. The mean of three TBUT measurements was calculated for each
112 eye with a time period of at least one minute between measurements to improve
113 measurement accuracy [15].

114

115 *Intervention I and II*

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

120

121 *Statistical Analysis*

122 Statistical analyses were calculated using SPSS statistical package version 25 (SPSS
123 Inc., Chicago, IL, USA). Mean spherical equivalent and corneal topography indices
124 showed strong positive correlations between both eyes ($p < 0.0005$); therefore, only
125 right eyes were included for analysis to alleviate any inter ocular dependency issues
126 and statistical bias due to the mirror-image relations [20]. At baseline, age-related
127 differences were calculated between mean values (Mann-Whitney test) and
128 proportions (one-sample t-test between percents). Following violation of the
129 assumptions of normality (Kolmogorov-Smirnov tests) for indices SAI, SRI, and TFSQ,
130 data was transformed on a logarithmic scale to achieve normality and for statistical
131 analyses, whereas raw (sample) data is presented as summary statistics (mean \pm SD,
132 95% confidence intervals CI, etc) [21]. A one-way repeated measures ANOVA
133 including Least Significant Difference post hoc tests determined the significance
134 between measurements under different conditions (baseline, intervention I and II),
135 while mixed between-within ANOVA tests were used to explore the effect of covariates
136 such as age and dry eye. Intra class correlation (ICC) estimates and their 95%

137 confident intervals were calculated based on a mean-rating ($k = 3$), absolute-
138 agreement, 2-way mixed-effects model. Coefficient of Repeatability (CoR) for each of
139 the parameters measured at baseline and each intervention were calculated as 1.96
140 \times S_w (within-subject standard deviation). Agreement between the different
141 interventions was calculated using the mean differences and 95% limits of agreement
142 (LoA). Statistical significance was accepted at the 95% CI ($p < 0.05$). The participants
143 were grouped by age, and two-way ANOVA power statistics revealed that a sample
144 size of 39, 19 subjects per group, was needed to detect a standardized difference
145 between the groups using a partial eta squared of 0.033 and 80% power at 5%
146 significance level [22]. This calculation was based on an estimated mean of 3 repeated
147 flat keratometry readings of 7.87 mm with group SDs of 0.29 mm, based on data
148 collected from the first 15 subjects.

149

150

151 **Results**

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

159

160 **Table 3. Demographics and dry eye characteristics.** Parameters are shown in
 161 mean \pm standard deviation (SD) and [95% confidence intervals around the mean].
 162 *Abbreviations: OSDI Ocular Surface Disease Index; MSE Mean Spherical Equivalent;*
 163 *VA Visual Acuity*

| | All subjects n=57 | <40 years n=34 | \geq40 years n=23 | <i>p</i> |
|--|--------------------------------------|--------------------------------------|--|--------------------|
| Gender (male: female) | 23 : 34 | 14 : 20 | 9 : 14 | 0.88 |
| Age (years) | 35.1 \pm 15.2 [31 to 39] | 23.8 \pm 4.5 [22 to 25] | 51.7 \pm 8.4 [48 to 55] | <0.0005* |
| OSDI score | 11.9 \pm 9.9 [9.2 to 14.5] | 12.0 \pm 9.3 [8.8 to 15.3] | 11.6 \pm 10.9 [6.9 to 16.3] | 0.83 |
| % normal OSDI score (<13) | 68% | 62% | 78% | 0.21 |
| MSE (Diopters) | -0.82 \pm 2.38 [-1.45 to -0.19] | -1.11 \pm 2.17 [-1.86 to -0.35] | -0.39 \pm 2.65 [-1.54 to -0.76] | 0.21 |
| Best corrected VA (Snellen decimal) | 1.23 \pm 0.21 [1.18 to 1.29] | 1.34 \pm 0.17 [1.28 to 1.40] | 1.08 \pm 0.14 [1.02 to 1.14] | <0.0005* |
| Mean of three TBUT (seconds) | 8.1 \pm 6.9 [6.3 to 10.0] | 7.8 \pm 7.2 [5.3 to 10.3] | 8.6 \pm 6.5 [5.8 to 11.4] | 0.43 |

164
 165 *Influence of NaFI on corneal topography and surface assessment*
 166 One-way, repeated-measures analysis of variance (ANOVA) was conducted to
 167 compare the average of 3 measurements of quantitative descriptors in corneal
 168 topography under three different conditions: baseline, intervention I (following
 169 application of single amount of NaFI) and intervention II (following two applications of
 170 NaFI). Except for the IS index ($F(2,55) = 3.288$, $p=0.045$, partial eta squared 0.107)
 171 and SRI index ($F(2,55) = 4.603$, $p=0.014$, partial eta squared 0.143), insertion of NaFI
 172 did not have a statistically significant effect on corneal topography measurements
 173 (Table 4). Posthoc analysis revealed that compared to baseline, the inferior part of the
 174 cornea became significantly flatter after a single dose ($p=0.034$) or double dose of
 175 NaFI ($p=0.030$). Additionally, the corneal surface (SRI) was significantly more regular

176 without NaFI ($p=0.003$) or one dose of NaFI ($p=0.024$) when compared to two doses
 177 of NaFI.

178

179 **Table 4. Corneal topography and surface assessment at baseline, intervention I**
 180 **(1x NaFI) and intervention II (2x NaFI).** Parameters are shown in mean \pm standard
 181 deviation. P-values represent the one-way repeated measures analysis of variance
 182 (ANOVA).

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

183

184 *Effect of age, OSDI score and TBUT*

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

194

195 *Comparison subjective and automated TBUT*

196 To understand the effect of NaFI on the regularity and image quality of the tear film,
 197 the traditional subjective measure of fluorescein TBUT was compared to the
 198 automated objective measure of tear stability (TFSQ) with and without NaFI. No
 199 statistically significant differences between the 3 repeated measurements of TBUT
 200 ($p=0.62$), and 2 repeated measures of TFSQ without NaFI ($p=0.67$) or TFSQ with NaFI

201 ($p=0.96$) were observed. However, TBUT was found to be significantly shorter ($8.1 \pm$
202 0.91 sec) compared to the automated method, without (12.6 ± 1.71 sec) or with NaFI
203 (13.6 ± 1.66 sec; $F(2,55)=7.085$; $p=0.002$). These results were irrespective of age
204 ($p=0.36$), gender ($p=0.60$), or OSDI score ($p=0.67$). Although not significant, those
205 classified as having no dry eye symptoms (OSDI score <13) showed increased TBUTs
206 independent of the method used.

207

208 *Repeatability, reliability and agreement*

209 Repeatability of three measurements for each of the parameters measured at baseline
210 and each intervention were reported as the Coefficient of Repeatability (CoR; Table
211 5). Compared to baseline, repeatability of all parameters except K flat and TFSQ
212 decreased following one application of NaFI (intervention I), while all parameters
213 showed reduced repeatability following two applications of NaFI (intervention II). The
214 amount of NaFI (one or two applications) had little effect on the repeatability of any of
215 the parameters. In addition, the reliability of repeated measurements of corneal
216 topography was established by calculating the Intra Class Correlation (ICC) of
217 quantitative descriptors determined by the instrument's computerized algorithm (Table
218 5). At baseline, indices show moderate (SRI and TFSQ), good (IS and SAI) and
219 excellent (K flat and steep) reliability. Reliability improved following the instillation of
220 NaFI compared to baseline for all indices except IS and SAI. Compared to
221 measurements taken without NaFI (baseline), all indices showed reduced ICC after
222 insertion of NaFI twice (intervention II). Agreement between the interventions are
223 presented as mean differences of average (out of 3) quantitative descriptors including
224 95% LoA (Table 6). Mean differences increased when interventions (I and II) were
225 compared to baseline for the corneal curvature in the flatter meridian, IS and TFSQ.
226 On the other hand, SAI and SRI indices agreed better between baseline and 1
227 application of NaFI, compared to two applications.

228

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

| | Baseline N=57 | | Intervention I N=57 | | Intervention II 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] |

233

234

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

238

| | 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] |

239

240

241 Similar to age (cut off 40 years), there was no effect of dry eye on the reliability of
 242 corneal topography measurements under the three conditions, irrespective of whether
 243 this was measured by OSDI scores (cut off 13) or TBUT measurements (cut off 5
 244 seconds; data not shown).

245

246

247 **Discussion**

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

264 its topography [27]. In addition, ocular surface topography could be affected due to a
265 combination of two, or all of the above.

266

267 *Influence of NaFI on corneal topography and surface assessment*

268 Since NaFI dye is soluble in water (saline), only a small amount is needed to colour
269 the tear film. The method of delivering NaFI to the eye was based on that used in
270 current clinical practice. A saline wetted NaFI-impregnated paper strip adds
271 approximately 3 μ l to the tear film [28,29]. According to the results, inducing a change
272 in tear volume, by insertion of NaFI using a paper strip and saline, seems to have little
273 effect on corneal shape measurements (simulated Keratometry readings, SAI, and
274 TFSQ), except for IS and SRI. The results revealed a statistically significant decrease
275 in IS index score, representing a flatter inferior segment, following the application of a
276 single dose of NaFI to the tear film. Considering the addition of tear volume results in
277 an increased tear volume specifically in the inferior tear meniscus, it is expected that
278 the inferior part of the central cornea over a 6.0 mm chord flattened due to the
279 collection of tears on the lower eye lid. The method of NaFI instillation has been shown
280 to reduce tear film stability [30], possibly leading to reflex lacrimation and/or
281 subsequently increased tear meniscus heights. Compared to a single dose of NaFI,
282 no significant difference was observed after applying a double dose, possibly
283 indicative of tear meniscus saturation reached within the exposed tear volume or the
284 lower lacrimal lake [31]. In addition, the SRI increased significantly with the insertion
285 of NaFI, representative of decreased tear film surface regularity. This index is
286 considered a measure of central corneal optical quality within the pupil size [32], based
287 on the determination of the most frequently occurring dioptric power and the
288 comparative analysis of dioptric powers of adjacent points in 256 hemi-meridians in
289 the central 10 Placido disc rings representing the average virtual pupil size [18]. For a
290 perfectly smooth surface, SRI would approach 0 [32], whereas a cornea presenting
291 an SRI index <0.8 D has been considered regular [16]. Hence, the significant change
292 in surface regularity following NaFI (from 0.52 to 0.60) observed in this study did not
293 signify a clinically irregular corneal surface caused by NaFI.

294

295 Compared to baseline, the addition of 1 or 2 applications (approximately 3 or 6 μ l), to
296 the tear volume prior to image acquisition also had a negative effect on the
297 repeatability of the dioptre difference between the average inferior and superior power

298 in both corneal hemispheres (IS), and the surface asymmetry where the centrally
299 weighted average of the difference in power between corresponding points at the
300 same chord is calculated (SAI) [32]. However, increased reliability and consistency in
301 corneal shape was observed after instilling a single dose of NaFI for all other indices.
302 Theoretically, the SAI value would be 0 for a perfect sphere; a surface with perfectly
303 spherocylindrical regular astigmatism, or for any surface with a power that is radially
304 symmetrical [34]. After instilling a double dose of NaFI there was a decrease in Intra
305 class correlation in all quantitative descriptors. Similar to the current study, ICC for
306 corneal topography parameters without the addition of NaFI have been shown to be
307 highly repeatable, with an ICC of >0.95 for simulated keratometric values [33-35].
308 Besides TFSQ values [19], no previous studies have reported the reliability of the
309 Medmont E300's automatic computer assisted analysis of corneal shape. Moderate to
310 good agreement between 3 repeated measures of IS, SAI and SRI indices were
311 observed at baseline, indicating no need for multiple measurements when used in
312 clinical practice. In addition, the agreement between the different interventions and the
313 baseline measurements highlighted that the addition of NaFI to the tear film has an
314 impact on most indices. Simulated keratometry, IS and TFSQ indices show reduced
315 repeatability, irrespective of the amount of NaFI. On the other hand, SAI and SRI
316 indices are more in agreement with baseline measurements after the application of
317 one dose compared to two doses of NaFI.

318

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

332

333 *Limitations*

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

345

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

362

363 *Clinical relevance*

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

376

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

387

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