The Ocular Surface xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

The Ocular Surface

journal homepage: www.elsevier.com/locate/jtos

Research Paper

Randomised double-masked placebo-controlled trial of the cumulative treatment efficacy profile of intense pulsed light therapy for meibomian gland dysfunction

Ally L. Xue, Michael T.M. Wang, Susan E. Ormonde, Jennifer P. Craig*

Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, New Zealand

ARTICLE INFO

Keywords:
Intense pulsed light
Meibomian gland
Lipid layer
Ocular surface
Tear film
Dry eye

ABSTRACT

Purpose: To assess long-term cumulative treatment effects of intense pulsed light (IPL) therapy in meibomian gland dysfunction (MGD).

Methods: Eighty-seven symptomatic participants (58 female, mean ± SD age, 53 ± 16 years) with clinical signs of MGD were enrolled in a prospective, double-masked, parallel-group, randomised, placebo-controlled trial. Participants were randomised to receive either four or five homogeneously sequenced light pulses or placebo treatment to both eyes, (E-Eye Intense Regulated Pulsed Light, E-Swin, France). Visual acuity, dry eye symptomology, tear film parameters, and ocular surface characteristics were assessed immediately before treatment on days 0, 15, 45, 75, and four weeks after treatment course completion on day 105. Inflammatory and goblet cell function marker expression, and eyelid swab microbiology cultures were evaluated at baseline and day 105.

Results: Significant decreases in OSDI, SPEED, and SANDE symptomology scores, and meibomian gland capping, accompanied by increased tear film lipid layer thickness, and inhibited *Corynebacterium macginleyi* growth were observed in both treatment groups (all $p < 0.05$). Sustained clinical improvements occurred in both treatment groups from day 75, although significant changes from day 45, in lipid layer quality, meibomian gland capping, OSDI and SANDE symptomology, were limited to the five-flash group (all $p < 0.05$).

Conclusions: IPL therapy effected significant improvements in dry eye symptomology, tear film lipid layer thickness, and meibomian gland capping in MGD patients. Five-flash IPL treatment showed superior clinical efficacy to four-flash, and an initial course of at least four treatments is suggested to allow for establishment of sustained cumulative therapeutic benefits prior to evaluation of overall treatment efficacy.

Trial registration number: ACTRN12616000667415.

1. Introduction

Evaporative disease is recognised to be the most common dry eye etiological subtype [1], and can be associated with profound impacts on ocular comfort, visual function, quality of life, and work productivity [1–4]. The condition is frequently caused by underlying meibomian gland dysfunction (MGD), whereby the increased viscosity and melting points of gland secretions can predispose towards obstruction and inflammation of the ductal system [5,6]. The consequent reduction in the quality and quantity of meibomian lipids delivered to the tear film compromises the integrity of the surface lipid layer, triggering a self-perpetuating vicious circle of tear film hyper-evaporation, instability, hyperosmolarity, and inflammation [5,7].

A large number of treatments are currently available for MGD, including warm compress therapy, eyelid hygiene regimens, mechanical meibum expression, lipid-containing artificial tear supplements, and omega-3 fatty acid supplementation [8,9]. In addition, intraductal probing, tetracyclines, antibiotic, anti-inflammatory and immunomodulatory agents may also be considered judiciously in more severe and refractory cases [8,9]. Nevertheless, adequate symptomatic control is frequently difficult to achieve or sustain, highlighting the ongoing need for the development of alternative management options [8].

Intense pulsed light (IPL) therapy is frequently used in the cosmetic industry, and has demonstrated favourable clinical efficacy and tolerability for the treatment of various dermatological conditions [10]. IPL

* Corresponding author. Department of Ophthalmology, New Zealand National Eye Centre, The University of Auckland, Private Bag 92019, Auckland, 1142, New Zealand.
E-mail address: jp.craig@auckland.ac.nz (J.P. Craig).

https://doi.org/10.1016/j.jtos.2020.01.003
Received 17 October 2019; Received in revised form 6 January 2020; Accepted 28 January 2020

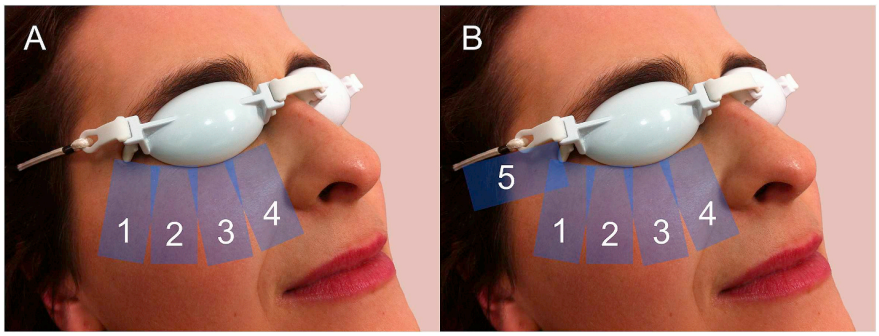

Fig. 1. Intense pulsed light therapy was delivered to four overlapping periocular zones inferior to each eye (panel A), and the fifth pulse was applied temporally adjacent to the lateral canthus in those randomised to the five pulse group (panel B).

devices emit high-intensity polychromatic light, ranging from the visible to infrared spectrum, and the wavelength, penetration depth, and targeted areas can be tailored for selective thermal delivery to specific structures [10]. In recent years, IPL therapy has garnered significant interest as a potential treatment for MGD [11–20], following serendipitous reports of improved ocular surface health with IPL treatment of cutaneous rosacea [21]. Although various clinical trials have demonstrated the reduction of dry eye signs and symptoms following IPL therapy [11–20], its mechanism of action and cumulative therapeutic profile remains poorly understood [21].

The purpose of the current double-masked randomised controlled trial was to further characterise the long-term cumulative treatment effects of IPL therapy in MGD patients, through the clinical assessment of dry eye signs and symptoms, and the laboratory analysis of ocular surface microbiological profiles and cytology markers.

2. Materials and methods

2.1. Subjects

This prospective, fifteen-week, double-masked, parallel-group, randomised, placebo-controlled trial, adhered to the tenets of the Declaration of Helsinki, and was approved by the University of Auckland Human Participants Ethics Committee (UAHPEC 017173), and prospectively registered as a clinical trial (ACTRN12616000667415). Participants were required to be 18 years or older, with symptoms of dry eye disease (McMonnies dry eye questionnaire score $\geq$10 and/or Ocular Surface Disease Index score $\geq$13) [22] and clinically significant signs of MGD (eyelid margin or mucocutaneous junction abnormalities, meibomian gland orifice capping, and/or decreased expressed meibum quality) [23,24], and no contact lens wear or use of systemic medications known to affect the eye two weeks prior to baseline assessment or during the treatment period. In addition, eligibility required participants to be non-pregnant; report no history of major systemic, dermatologic or ocular conditions; no ocular surgery or dermatologic treatments in the previous three months or during the treatment period; no implants, tattoos, semi-permanent makeup, or pigmented lesions in the treatment area; and no contraindications to IPL therapy, including the use of photosensitive medications. Eligible participants were enrolled after providing written consent.

A total of 87 eligible participants were recruited, exceeding the sample size requirements for the desired study power. Power calculations were conducted with non-invasive tear film breakup time as the designated outcome, and showed that a minimum of 25 participants was required in each of the three treatment groups (a total of 75 participants), to detect a clinically significant difference of 3–4 s in pairwise comparisons, at 80% power ($\beta = 0.2$) and a two-sided statistical significance level of 5% ($\alpha = 0.05$), with the SD of normal values being estimated to be approximately 4–6 s [25]. Sample size estimates were determined using a uniform non-parametric adjustment, with NCSS PASS 2002 (Utah, USA).

2.2. Treatments

Participants were randomly assigned to one of three treatment groups, and underwent IPL therapy with four or five homogeneously sequenced light pulses (E-Eye Intense Regulated Pulsed Light; E-Swin, Paris, France) or placebo treatment to both eyes, applied during in-office visits on days 0, 15, 45, and 75, by a trained unmasked clinician, who was not involved in study data collection. Randomisation was conducted by computer-generated random number allocation, and applied to sequentially enrolled participants. The randomisation schedule was pre-determined, prior to commencing participant recruitment, such that the investigator involved in baseline participant assessment had no involvement in treatment allocation. During each visit, participants were fitted with opaque, metal goggles covering both eyes to protect the globes, with clear conducting gel applied to the inferior, lateral, and medial aspects of the goggles, as per manufacturer recommendations. Light pulses were delivered to four overlapping periocular zones inferior to each eye, and the fifth pulse was applied temporally adjacent to the lateral canthus in those randomised to the five pulse group (Fig. 1). Pulse intensity ranged from 9 to 13 J/cm^2 and was inversely related to the Fitzpatrick skin phototype classification of the participant (Table 1) [20]. Participants allocated to the placebo group underwent sham treatment, and participant masking was achieved using an identical device with a non-illuminating handpiece applied to the periocular area, while an active piece was directed away from the participant towards the corner of the room to imitate the illumination and sounds of the IPL device in order to simulate treatment. For the purpose of characterising and isolating the cumulative treatment effects of IPL therapy, mechanical meibum expression was not conducted during the study period, and antibiotic or anti-inflammatory treatment was not prescribed.

2.3. Measurements

The investigator conducting clinical and laboratory measurements was masked to treatment randomisation. Participants were assessed at a single site, with a mean $\pm$ SD room temperature of 20.3 $\pm$ 0.5 °C and

Table 1
Intense pulsed light (IPL) therapy intensity applied according to Fitzpatrick skin phototype classification.

Fitzpatrick skin phototype	Skin appearance	IPL Treatment level	Fluence (J/cm^2)
Fitzpatrick type I	Pale white	6	13.0
Fitzpatrick type II	White	5	12.2
Fitzpatrick type III	Light brown	4	11.4
Fitzpatrick type IV	Medium brown	3	10.6
Fitzpatrick type V	Dark brown	2	9.8
Fitzpatrick type VI	Very dark brown	(Not suitable for IPL therapy)	

Table 2
Order of clinical and laboratory measurements conducted during the study period. Measurements were performed immediately before treatment application on days 0, 15, 45, and 75, and four weeks following the completion of the treatment course on day 105.

Assessments	Day 0	Day 15	Day 45	Day 75	Day 105
Fitzpatrick skin phototype classification	x				
McMonnies dry eye questionnaire	x				
OSDI dry eye questionnaire	x	x	x	x	x
SPEED dry eye questionnaire	x	x	x	x	x
SANDE dry eye questionnaire	x	x	x	x	x
Best corrected visual acuity	x				x
Conjunctival bulbar hyperaemia	x	x	x	x	x
Tear meniscus height	x	x	x	x	x
Non-invasive tear film breakup time	x	x	x	x	x
Tear film lipid layer grade	x	x	x	x	x
Tear osmolarity	x	x	x	x	x
Corneal and lid margin aethesiometry	x				x
Slit lamp biomicroscopy examination	x	x	x	x	x
Ocular surface staining	x	x	x	x	x
Meibomian gland expressibility	x		x		x
Infrared meibography	x		x		x
In vivo confocal microscopy evaluation	x				x
Eyelid margin swab for microbiology cultures	x				x
Eyelash epilation for ocular *Demodex* load	x				x
Conjunctival impression cytology for expression of ocular surface inflammation and goblet cell function markers	x				x

a mean ± SD relative humidity of 62.5 ± 6.8%, and ocular measurements were conducted on the right eye of each participant. Clinical and laboratory measurements were conducted in accordance with the recommendations of the TFOS DEWS II Diagnostic Methodology subcommittee [22], and performed immediately before treatment application on days 0, 15, 45, and 75, and four weeks following the completion of the treatment course, on day 105, in order to characterise the longer term cumulative treatment effects. To minimise the impact on ocular surface and tear film physiology for subsequent tests, clinical and laboratory measurements were performed in ascending order of invasiveness [22], as summarised in Table 2.

Six-metre best spectacle-corrected logMAR visual acuity was recorded as a safety measure. The McMonnies dry eye questionnaire was administered to screen for dry eye symptoms at baseline, while the Ocular Surface Disease Index (OSDI), Standard Patient Evaluation of Eye Dryness (SPEED), and Symptom Assessment in Dry Eye (SANDE) questionnaires were the instruments administered for the purpose of comparing symptomology across the treatment period. The overall SANDE score was calculated as the geometric mean of the frequency and severity scores [26]. Participants were advised to contact the study investigators during the study period to report adverse events at any time.

Bulbar conjunctival hyperemia, tear meniscus height, non-invasive tear film breakup time, and tear film lipid layer grade were assessed using the Keratograph 5M (Oculus Optikgeräte GmbH, Wetzlar, Germany). Bulbar conjunctival hyperemia was evaluated by automated objective evaluation of high magnification digital imaging, using the proprietary JENVIS grading scale from 0 to 4 [27]. The lower tear meniscus height was assessed using high magnification pre-calibrated digital imaging, and three measurements near the center of the lower meniscus were averaged. Non-invasive tear film breakup time was measured using automated detection of first break-up, while the subject maintained fixation and was requested to refrain from blinking. Three breakup time readings were averaged in each case [22]. Tear film lipid layer interferometry was graded according to the modified Guillon-Keeler system: grade 1, open meshwork; grade 2, closed meshwork; grade 3, wave or flow; grade 4, amorphous; grade 5, colored fringes; grade 0, non-continuous layer (non-visible or abnormal colored fringes) [28,29].

Tear film osmolarity measurements were performed, in-office, with a clinical osmometer (TearLab, California, USA), from 50 nL of tears sampled from the lower lateral canthal tear meniscus. A measurement was taken for each eye, and the higher reading and the inter-ocular difference recorded [22].

Central corneal and inferior eyelid margin sensitivity were assessed using non-contact air-jet aesthesiometry (NCCA, SDZ electronics, New Zealand) to evaluate potential functional changes in the peripheral nerve supply of regions local to IPL application [30]. An intermittent, barely susceptible flow of air was used to determine threshold sensitivity via a forced-choice double-staircase method [31]. Sensitivity thresholds were measured in a quiet room devoid of distractions, using a 0.9 s stimulus duration and a standardised 10 mm working distance from the ocular region assessed. Measurements were conducted at the geometric centre of the cornea, and at the lid wiper zone of the central inferior eyelid margin during slight lower eyelid eversion. Participants were instructed to blink frequently and the inferior eyelid margin was released to normal position between stimulus presentations, in order to avoid excessive ocular surface drying and subsequent dampening of the sensitivity threshold, and to minimise disruption to subsequent measurements [31,32].

Lid margin and eyelash abnormalities, including lid margin thickening, rounding, notching, foaming, telangiectasia, meibomian gland capping, staphylococcal lash crusting, seborrhoeic lash crusting, *Demodex* lash cylindrical dandruff, madarosis, poliosis, and trichiasis were assessed by slit lamp biomicroscopy examination. Grading of the clinical features was based on a four-point scale: grade 0, absent; grade 1, mild; grade 2, moderate; grade 3, severe [27].

Lid parallel conjunctival folds (LIPCOF) were graded, and sodium fluorescein and lissamine green dyes were applied using the recommended technique described in TFOS DEWS II Diagnostic Methodology report [22], in order to evaluate localised corneal and conjunctival areas of epithelial desiccation. Staining was recorded using the modified Oxford grading scheme [33], and lid wiper epitheliopathy (LWE) was evaluated relative to Korb's grading [34].

Expressibility of the inferior eyelid meibomian glands was assessed with the Meibomian Gland Evaluator (TearScience, North Carolina, USA), with a pressure of 1.2 g/mm ^{2} applied immediately inferior to the lash line, at the nasal, central, and temporal aspects of the eyelid margin. The number of meibomian gland orifices yielding lipid secretion was graded on a five-point scale: 0, more than 75%; 1, 50% to 75%; 2, 25% to 50%; 3, less than 25%; 4, none. The quality of expressed meibum was graded as: grade 0, clear fluid; grade 1, slightly turbid;

grade 2, thick opaque; grade 3, toothpaste like; grade 4, complete orifice blockage [29]. Infrared meibography was imaged with the Oculus Keratograph 5M, with the superior and inferior eyelids everted in turn. From the captured image, the proportion of meibomian glands visible within the tarsal area was graded according to the five-point Meiboscale [35].

In vivo confocal microscopy evaluation of the central cornea and inferior eyelid margin was performed using the Heidelberg Retinal Tomograph (HRT) III with Anterior Segment Module (Heidelberg Engineering GmbH, Germany), following instillation of a drop of 0.4% benoxinate hydrochloride into the conjunctival fornix. The objective lens was covered by a disposable polymethacrylate sterile cap (Tomo-Cap, Heidelberg Engineering GmbH, Germany), and Viscotears (Carbomer 980, 0.2%; Novartis, North Ryde, NSW, Australia) was applied as the coupling agent. Participants were requested to fix their gaze on a central target to allow for full thickness scanning of the central cornea in 2 μm increments using the Section Mode setting of the tomograph. Meibomian gland imaging was conducted with slight eversion of the inferior eyelid margin, with the Tomo-Cap positioned perpendicularly to the central, nasal, and temporal thirds of the eyelid margin, close to the mucocutaneous junction. For each measurement, three non-overlapping, high-resolution images (400 μm x 400 μm frame) were analysed using Image J software with the NeuronJ plug-in (National Institutes of Health, USA). Central cornea sub-basal nerve fibre density was assessed by measuring the total corneal nerve length per square millimetre [36], and dendritic cells were quantified as cellular density per unit area [37]. Inferior eyelid margin rete ridges per square millimetre were evaluated, and the diameter measured along the longest axis [38–40]. Meibum secretion reflectivity was graded according to a 4-point scale developed by Villani et al.: grade 1, black; grade 2, dark grey; grade 3, light grey; grade 4, white [41].

Microbiological swabs from the inferior eyelid margin were collected using a sterile cotton-tipped applicator moistened with buffered saline, and placed immediately into Amies transport medium (Fort Richard Laboratories, Auckland, NZ) and processed on the same day. Anaerobic and aerobic microbiological evaluation was performed by a dedicated, independent laboratory (LabPlus, Auckland, NZ), with the total number of colony forming units (CFUs) enumerated for each cultured sample, and the load of each of the identified microbial colonies was graded on an ordinal scale: grade 0, no growth; grade 1, single colony; grade 2, few colonies; grade 3, light growth; grade 4, moderate growth; grade 5 heavy growth.

Ocular *Demodex* load was assessed by epilating four eyelashes from the upper eyelids under slit lamp examination. Lashes were gently grasped with fine forceps close to the base and rotated for 20 s before epilation [42]. The epilated lashes were placed onto glass slides and examined under light microscopy at 200 times magnification [43]. Adult *Demodex* mites were identified morphologically, and the mite count from each of the four lashes was averaged.

Conjunctival impression cytology was conducted following topical anesthesia with one drop of 0.4% benoxinate hydrochloride. Bulbar conjunctival cells from the inferior temporal ocular surface were collected with the EYEPRIM™ conjunctival impression device (OPIA, France) [44]. Conjunctival cell sample RNA extraction and purification was performed with PureLink® RNA Mini Kit (Invitrogen™ by Life Technologies), and tested for the presence of inhibitors before undergoing cDNA synthesis using SuperScript™ IV VILO™ Master Mix (Invitrogen™ by Life Technologies). A standard β-actin PCR and gel electrophoresis was conducted on the synthesized samples to confirm successful cDNA synthesis [44,45]. Seven reference genes (Beta-Actin, HPRT1, B2M, PPIA, TBP, GUSB, RPLP0 and POLR2A) were tested amongst the sample population, with the combination of GUSB and RPLP0 producing the best stability value according to the Normfinder algorithm (MOMA, Aarhus, Denmark). The geometric mean of GUSB and RPLP0 data was subsequently used for normalisation and relative quantification of the target genes (MMP-9, IL-6 and MUC5AC) [44–46]. The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines were followed to ensure validity of the qPCR experiments [47], which were set up using QiAgility® PCR robot (Qiagen, Germany) with PrimeTime® Assays (Integrated DNA Technologies), and internal calibrators were used to enable the removal of inter-run variations [48].

2.4. Statistics

Statistical analysis was conducted with Graph Pad Prism version 8.01 (California, USA) and IBM SPSS version 24 (New York, USA). Two-way mixed model analysis of variance (ANOVA) testing was performed to test the significance of treatment, time and interaction (treatment-by-time) effects on measurements over the fifteen-week period, where continuous variables with a normal distribution had been confirmed (Kolmogorov-Smirnov test $p > 0.05$). Non-normally distributed continuous measures were logarithmically transformed prior to undergoing analysis. Post hoc analysis for the significance of treatment effects at each time point was conducted using the multiplicity-adjusted Tukey's test. Analysis of ordinal data was performed using multiple ordinal regression, with post hoc analysis of treatment effects at each time point conducted using the multiplicity-adjusted, non-parametric Dunn's test. Categorical data at baseline were analysed using chi-squared or Fisher's exact tests. All tests were two tailed, and $p < 0.05$ was considered significant. Data are presented as mean ± SD, or median (IQR) unless otherwise stated.

3. Results

The mean ± SD age of the 87 participants (58 females, 29 males) was 53 ± 16 years (range, 21–85 years). Baseline characteristics, clinical and laboratory measurements of participants during the fifteen-week study period are presented by treatment group in Table 3 to 6. Baseline measurements did not differ between treatment groups (all $p > 0.05$; Table 3).

3.1. Visual acuity and adverse events

There were no significant treatment, time, or treatment-by-time interaction effects for best-corrected visual acuity (all $p > 0.20$, Table 5). No adverse events were reported during the study period.

Table 3
Baseline characteristics of participants randomised to placebo treatment, or intense pulse light therapy with four or five homogeneously sequenced light pulses. Data are presented as mean ± SD, median (IQR), or number of subjects (% of subjects).

Characteristic	Treatment group			
	Placebo (n = 30)	4 Flashes (n = 28)	5 Flashes (n = 29)	p-value
Age (years)	55 ± 14	48 ± 15	56 ± 17	0.15
Female gender	21 (70%)	19 (68%)	18 (62%)	0.80
Ethnicity				
European ethnicity	25 (83%)	19 (68%)	23 (79%)	0.35
East Asian ethnicity	4 (13%)	8 (29%)	3 (10%)	0.15
Other ethnicity	1 (3%)	1 (4%)	3 (10%)	0.43
McMonnies score (out of 45)	20 ± 5	19 ± 7	18 ± 7	0.38
Fitzpatrick skin phototype classification				
Fitzpatrick type I	0 (0%)	3 (11%)	2 (7%)	0.20
Fitzpatrick type II	11 (37%)	5 (18%)	8 (28%)	0.28
Fitzpatrick type III	11 (37%)	8 (29%)	15 (52%)	0.19
Fitzpatrick type IV	8 (27%)	10 (36%)	4 (14%)	0.16
Fitzpatrick type V	0 (0%)	2 (7%)	0 (0%)	0.12
Fitzpatrick type VI	0 (0%)	0 (0%)	0 (0%)	> 0.99

Table 4
Clinical and laboratory measurements at days 0. 15, 45, 75 and 105, for participants randomised to placebo treatment, or intense pulse light therapy with four or five homogeneously sequenced light pulses. Data are presented as mean ± SD or median (IQR).

Measurement	Treatment	Day 0	Day 15	Day 45	Day 75	Day 105
Visual acuity						
Best corrected visual acuity (logMAR)	Placebo	0.1 ± 0.1	–	–	–	0.0 ± 0.1
	4 Flashes	0.0 ± 0.2	–	–	–	0.0 ± 0.2
	5 Flashes	0.1 ± 0.2	–	–	–	0.1 ± 0.1
Dry eye symptomology						
OSDI score (out of 100)	Placebo	34 ± 16	34 ± 21	32 ± 18	32 ± 23	31 ± 22
	4 Flashes	28 ± 16	23 ± 17	25 ± 18	18 ± 11	22 ± 18
	5 Flashes	28 ± 20	24 ± 20	22 ± 16	22 ± 17	21 ± 17
SPEED score (out of 28)	Placebo	14 ± 5	14 ± 5	12 ± 5	13 ± 6	13 ± 6
	4 Flashes	12 ± 5	10 ± 5	11 ± 5	9 ± 4	9 ± 5
	5 Flashes	14 ± 5	12 ± 5	10 ± 5	10 ± 5	10 ± 5
SANDE score (out of 100)	Placebo	60 ± 17	61 ± 20	57 ± 23	54 ± 24	51 ± 24
	4 Flashes	60 ± 20	54 ± 23	51 ± 22	41 ± 24	39 ± 22
	5 Flashes	56 ± 23	44 ± 24	41 ± 22	37 ± 23	36 ± 23
Tear film quality						
Tear meniscus height (mm)	Placebo	0.29 ± 0.14	0.33 ± 0.21	0.32 ± 0.18	0.32 ± 0.22	0.33 ± 0.19
	4 Flashes	0.28 ± 0.08	0.29 ± 0.08	0.30 ± 0.08	0.29 ± 0.08	0.31 ± 0.09
	5 Flashes	0.30 ± 0.10	0.30 ± 0.08	0.32 ± 0.11	0.31 ± 0.08	0.32 ± 0.07
Tear film lipid layer grade (out of 5)	Placebo	3 (2–4)	3 (3–4)	3 (2–4)	3 (2–4)	3 (2–4)
	4 Flashes	3 (2–4)	3 (3–4)	3 (2–4)	3 (3–4)	3 (3–4)
	5 Flashes	3 (2–4)	3 (3–4)	3 (3–4)	3 (3–4)	3 (3–4)
Non-invasive tear film breakup time (s)	Placebo	5.4 (4.8–9.0)	5.5 (5.2–6.6)	5.8 (4.1–7.8)	5.4 (4.2–7.4)	5.6 (4.1–7.3)
	4 Flashes	5.6 (4.2–7.3)	4.3 (3.8–5.9)	6.0 (4.9–7.7)	5.4 (4.2–6.7)	6.0 (3.8–7.8)
	5 Flashes	5.4 (4.2–7.8)	5.5 (4.4–7.2)	6.1 (4.9–8.3)	5.3 (4.1–7.7)	5.8 (4.1–8.2)
Tear osmolarity (mOsmol/L)	Placebo	315 ± 12	–	314 ± 11	–	311 ± 11
	4 Flashes	311 ± 13	–	309 ± 13	–	309 ± 14
	5 Flashes	310 ± 14	–	309 ± 12	–	308 ± 13
Inter-ocular difference in osmolarity (mOsmol/L)	Placebo	10 ± 8	–	11 ± 9	–	9 ± 5
	4 Flashes	9 ± 8	–	8 ± 7	–	9 ± 7
	5 Flashes	8 ± 6	–	9 ± 7	–	10 ± 8
Ocular surface characteristics						
Bulbar conjunctival hyperaemia (out of 4)	Placebo	1.0 ± 0.4	1.0 ± 0.5	1.1 ± 0.4	1.1 ± 0.5	1.1 ± 0.6
	4 Flashes	1.0 ± 0.4	1.0 ± 0.5	1.0 ± 0.4	1.1 ± 0.5	1.1 ± 0.4
	5 Flashes	1.2 ± 0.4	1.3 ± 0.5	1.2 ± 0.5	1.2 ± 0.4	1.2 ± 0.4
Lid margin thickening grade (out of 3)	Placebo	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
	4 Flashes	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
	5 Flashes	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Lid margin rounding grade (out of 3)	Placebo	0 (0–2)	0 (0–2)	0 (0–2)	0 (0–2)	0 (0–2)
	4 Flashes	0 (0–2)	0 (0–2)	0 (0–1)	0 (0–1)	0 (0–1)
	5 Flashes	1 (0–2)	0 (0–2)	0 (0–2)	0 (0–2)	0 (0–2)
Lid margin notching grade (out of 3)	Placebo	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
	4 Flashes	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
	5 Flashes	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
Lid margin foam grade (out of 3)	Placebo	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	4 Flashes	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	5 Flashes	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Lid margin telangiectasia grade (out of 3)	Placebo	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	4 Flashes	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	5 Flashes	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Meibomian gland capping grade (out of 3)	Placebo	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)
	4 Flashes	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (0–1)
	5 Flashes	1 (1–1)	1 (0–1)	0 (0–1)	1 (1–1)	0 (0–1)
Lid parallel conjunctival folds grade (out of 3)	Placebo	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
	4 Flashes	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
	5 Flashes	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–1)
Staphylococcal lash crusting grade (out of 3)	Placebo	0 (0–1)	0 (0–0)	0 (0–1)	0 (0–0)	0 (0–0)
	4 Flashes	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	5 Flashes	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Seborrhoeic lash crusting grade (out of 3)	Placebo	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	4 Flashes	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	5 Flashes	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Demodex lash cylindrical dandruff grade (out of 3)	Placebo	1 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)	1 (0–1)
	4 Flashes	1 (0–1)	1 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)
	5 Flashes	1 (0–1)	1 (0–1)	1 (0–1)	0 (0–0)	0 (0–0)
Madarosis grade (out of 3)	Placebo	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–1)
	4 Flashes	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	5 Flashes	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–0)
Poliosis grade (out of 3)	Placebo	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
	4 Flashes	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–1)
	5 Flashes	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–0)	0 (0–1)
Trichiasis grade (out of 3)	Placebo	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	4 Flashes	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
	5 Flashes	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)

(*continued on next page*)

Table 4 (*continued*)

Measurement	Treatment	Day 0	Day 15	Day 45	Day 75	Day 105
Ocular *Demodex* load (mites per lash)	Placebo	0 (0–0.3)	–	–	–	0 (0–0.3)
	4 Flashes	0 (0–0.2)	–	–	–	0 (0–0.2)
	5 Flashes	0 (0–0.2)	–	–	–	0 (0–0.3)
Sodium fluorescein staining score (out of 55)	Placebo	4 (1–7)	4 (2–6)	4 (2–5)	4 (2–7)	4 (2–7)
	4 Flashes	4 (2–8)	4 (2–10)	4 (2–8)	4 (2–9)	5 (2–8)
	5 Flashes	4 (3–5)	4 (2–7)	4 (3–7)	4 (4–7)	5 (2–9)
Lissamine green staining score (out of 55)	Placebo	2 (0–3)	2 (1–4)	2 (0–2)	2 (0–3)	2 (0–3)
	4 Flashes	2 (1–5)	3 (1–7)	2 (1–6)	2 (0–6)	2 (1–5)
	5 Flashes	2 (0–4)	2 (0–5)	3 (1–6)	2 (0–5)	2 (1–5)
Superior lid wiper epitheliopathy grade (out of 3)	Placebo	0 (0–1)	0 (0–1)	0 (0–2)	0 (0–1)	0 (0–1)
	4 Flashes	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–2)	0 (0–1)
	5 Flashes	0 (0–2)	0 (0–1)	0 (0–1)	0 (0–2)	0 (0–1)
Inferior lid wiper epitheliopathy grade (out of 3)	Placebo	1 (0–2)	0 (0–3)	0 (0–1)	1 (0–2)	0 (0–1)
	4 Flashes	0 (0–3)	1 (0–3)	1 (0–2)	1 (0–2)	0 (0–2)
	5 Flashes	0 (0–2)	0 (0–1)	1 (0–2)	0 (0–1)	0 (0–1)
Superior lid meibography grade (out of 4)	Placebo	1 (1–2)	–	2 (1–3)	–	2 (1–3)
	4 Flashes	2 (1–3)	–	1 (1–3)	–	1 (1–3)
	5 Flashes	1 (1–3)	–	1 (1–3)	–	1 (0–3)
Inferior lid meibography grade (out of 4)	Placebo	1 (1–1)	–	1 (0–2)	–	1 (0–1)
	4 Flashes	1 (0–1)	–	1 (0–1)	–	1 (0–1)
	5 Flashes	1 (0–2)	–	1 (0–2)	–	1 (0–2)
Meibum expressibility grade (out of 4)	Placebo	2 (1–4)	–	3 (2–5)	–	2 (1–5)
	4 Flashes	2 (1–4)	–	3 (2–5)	–	2 (1–5)
	5 Flashes	2 (1–4)	–	3 (2–5)	–	3 (1–5)
Expressed meibum quality grade (out of 4)	Placebo	2 (1–2)	–	2 (1–2)	–	1 (1–2)
	4 Flashes	1 (1–2)	–	1 (1–2)	–	1 (1–2)
	5 Flashes	2 (1–2)	–	2 (1–2)	–	2 (1–2)
Central corneal sensitivity	Placebo	0.8 ± 0.6	–	–	–	0.8 ± 0.6
	4 Flashes	0.7 ± 0.6	–	–	–	0.7 ± 0.6
	5 Flashes	1.0 ± 0.8	–	–	–	0.9 ± 0.7
Inferior lid margin sensitivity	Placebo	0.8 ± 0.6	–	–	–	0.7 ± 0.5
	4 Flashes	0.7 ± 0.6	–	–	–	0.7 ± 0.6
	5 Flashes	1.0 ± 0.9	–	–	–	0.9 ± 0.8
***In vivo* confocal microscopy evaluation**						
Corneal sub-basal nerve fibre density (μm/mm^2)	Placebo	17887 ± 3531	–	–	–	18623 ± 3890
	4 Flashes	19696 ± 4764	–	–	–	20908 ± 3835
	5 Flashes	20102 ± 5281	–	–	–	20156 ± 4850
Corneal sub-basal dendritic cell density (cells/mm^2)	Placebo	46 (15–130)	–	–	–	30 (16–71)
	4 Flashes	21 (10–31)	–	–	–	40 (24–58)
	5 Flashes	28 (17–86)	–	–	–	30 (17–72)
Inferior lid margin rete ridge diameter (μm)	Placebo	67.68 ± 24.30	–	–	–	63.57 ± 12.38
	4 Flashes	72.41 ± 21.19	–	–	–	61.81 ± 17.15
	5 Flashes	67.66 ± 22.00	–	–	–	62.97 ± 18.95
Inferior lid margin rete ridge density (units/mm^2)	Placebo	109 ± 52	–	–	–	110 ± 47
	4 Flashes	109 ± 40	–	–	–	113 ± 44
	5 Flashes	123 ± 72	–	–	–	127 ± 66
Inferior lid margin acinar secretion reflectivity (out of 4)	Placebo	3 (2–4)	–	–	–	3 (2–4)
	4 Flashes	2 (2–3)	–	–	–	3 (1–3)
	5 Flashes	3 (2–4)	–	–	–	3 (2–3)
Eyelid margin swab microbiology						
Total bacterial colony forming units	Placebo	45 (12–170)	–	–	–	22 (8–118)
	4 Flashes	28 (8–57)	–	–	–	49 (19–219)
	5 Flashes	12 (2–28)	–	–	–	19 (5–47)
Corynebacterium macginleyi growth	Placebo	0 (0–1)	–	–	–	0 (0–1)
	4 Flashes	0 (0–1)	–	–	–	0 (0–0)
	5 Flashes	0 (0–1)	–	–	–	0 (0–0)
Corynebacterium tuberculostericum growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Corynebacterium propinquum growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Corynebacterium pseudodiphtheriticum growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Enterobacter aerogenes growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)

(*continued on next page*)

Table 4 (*continued*)

Measurement	Treatment	Day 0	Day 15	Day 45	Day 75	Day 105
Enterococcus faecalis growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Micrococcus luteus growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Propionibacterium acnes growth	Placebo	2 (0–2)	–	–	–	2 (0–2)
	4 Flashes	2 (0–2)	–	–	–	2 (0–2)
	5 Flashes	1 (0–2)	–	–	–	1 (0–2)
Propionibacterium granulosum growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Proteus mirabilis growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Staphylococcus epidermidis growth	Placebo	2 (0–3)	–	–	–	2 (0–2)
	4 Flashes	2 (0–3)	–	–	–	2 (0–3)
	5 Flashes	1 (0–3)	–	–	–	1 (0–3)
Staphylococcus capitis growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Staphylococcus aureus growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Staphylococcus caprae growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Staphylococcus haemolyticus growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Staphylococcus hominis growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Staphylococcus lugdunensis growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Staphylococcus pasteuri growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Staphylococcus warneri growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Streptococcus viridans growth	Placebo	0 (0–0)	–	–	–	0 (0–0)
	4 Flashes	0 (0–0)	–	–	–	0 (0–0)
	5 Flashes	0 (0–0)	–	–	–	0 (0–0)
Conjunctival impression cytology markers						
MU5AC expression	Placebo	0.20 ± 0.16	–	–	–	0.25 ± 0.20
	4 Flashes	0.31 ± 0.28	–	–	–	0.24 ± 0.17
	5 Flashes	0.32 ± 0.19	–	–	–	0.25 ± 0.19
MMP-9 expression	Placebo	0.23 ± 0.57	–	–	–	0.24 ± 0.61
	4 Flashes	0.20 ± 0.38	–	–	–	0.14 ± 0.21
	5 Flashes	0.12 ± 0.21	–	–	–	0.12 ± 0.19
IL-6 expression	Placebo	0.16 ± 0.25	–	–	–	0.19 ± 0.31
	4 Flashes	0.18 ± 0.27	–	–	–	0.18 ± 0.29
	5 Flashes	0.12 ± 0.20	–	–	–	0.11 ± 0.17

3.2. Dry eye symptomology

Two-way ANOVA demonstrated significant treatment effects for OSDI, SPEED and SANDE dry eye symptomology scores (all $p \leq 0.001$, Table 5), and significant time effects for SPEED and SANDE scores (both $p < 0.001$, Table 5). Multiplicity-adjusted post-hoc testing showed that participants receiving four flashes of IPL demonstrated transient improvements in OSDI and SPEED scores relative to those in the placebo group on day 15 (both $p < 0.05$, Table 6), and then sustained improvements in OSDI, SPEED, and SANDE scores from day 75 onwards (all $p < 0.05$, Table 6 and Fig. 2). Participants in the five-flash IPL group exhibited significantly lower OSDI and SANDE scores from day 15 onwards, although sustained reductions in the SPEED score did not occur until day 75 onwards (all $p < 0.05$, Table 6).

3.3. Tear film quality

A significant treatment effect was detected for tear film lipid layer grade ($p = 0.01$, Table 5). Post-hoc multiplicity-adjusted analysis demonstrated enhanced tear film lipid layer quality in the four-flash IPL group from day 75 onwards, and improvements were observed in the five-flash IPL group from day 45 onwards (all $p < 0.05$, Table 6 and Fig. 3). Treatment, time, and interaction effects for tear meniscus height, non-invasive tear film stability, and tear osmolarity were not statistically significant (all $p > 0.05$, Table 5).

3.4. Ocular surface characteristics

Treatment and time effects were significant for meibomian gland

Table 5
Two-way analysis of variance of measurements for treatment, time and interaction (treatment-by-time) effects. Non-normally distributed continuous and ordinal data were converted to rank-values prior to non-parametric assessment. Data are presented as p-values. Asterisks denote statistically significant effects ($p < 0.05$).

Measurement	p-value		
	Treatment	Time	Interaction
Visual acuity			
Best corrected visual acuity	0.24	0.42	0.79
Dry eye symptomology			
OSDI score	< 0.001*	0.16	0.98
SPEED score	0.001*	< 0.001*	0.92
SANDE score	< 0.001*	< 0.001*	0.71
Tear film quality			
Tear meniscus height	0.59	0.08	0.52
Tear film lipid layer grade	0.01*	0.14	0.77
Non-invasive tear film breakup time	0.11	0.12	0.53
Tear osmolarity	0.12	0.32	0.68
Inter-ocular difference in osmolarity	0.14	0.98	0.21
Ocular surface characteristics			
Bulbar conjunctival hyperaemia	0.07	0.49	0.64
Lid margin thickening grade	0.16	0.12	0.19
Lid margin rounding grade	0.19	0.09	0.19
Lid margin notching grade	0.43	0.07	0.58
Lid margin foam grade	0.23	0.49	0.69
Lid margin telangiectasia grade	0.13	0.37	0.97
Meibomian gland capping grade	0.005*	0.02*	0.71
Lid parallel conjunctival folds grade	0.12	0.19	0.37
Staphylococcal lash crusting grade	0.56	0.25	0.47
Seborrhoeic lash crusting grade	0.11	0.21	0.27
Demodex lash cylindrical dandruff grade	0.27	0.12	0.59
Madarosis grade	0.19	0.16	0.61
Poliosis grade	0.93	0.08	0.62
Trichiasis grade	0.11	0.53	0.64
Ocular *Demodex* load	0.59	0.86	0.89
Sodium fluorescein staining score	0.13	0.40	0.19
Lissamine green staining score	0.63	0.36	0.19
Superior lid wiper epitheliopathy grade	0.08	0.48	0.97
Inferior lid wiper epitheliopathy grade	0.30	0.85	0.90
Superior lid meibography grade	0.97	0.65	0.98
Inferior lid meibography grade	0.29	0.62	0.94
Meibum expressibility grade	0.14	0.35	0.06
Expressed meibum quality grade	0.28	0.16	0.73
Central corneal sensitivity	0.31	0.37	0.44
Inferior lid margin sensitivity	0.12	0.32	0.19
***In vivo* confocal microscopy evaluation**			
Corneal sub-basal nerve fibre density	0.07	0.26	0.42
Corneal sub-basal dendritic cell density	0.24	0.74	0.50
Inferior lid margin rete ridge diameter	0.64	0.07	0.88
Inferior lid margin rete ridge density	0.24	0.69	0.98
Inferior lid margin acinar secretion reflectivity	0.06	0.09	0.29
Eyelid margin swab microbiology			
Total bacterial colony forming units	0.12	0.10	0.99
Corynebacterium macginleyi growth	0.003*	0.24	0.35
Corynebacterium tuberculostericum growth	0.16	0.45	0.82
Corynebacterium propinquum growth	0.18	0.67	0.83
Corynebacterium pseudodiphtheriticum growth	0.38	0.34	0.41
Enterobacter aerogenes growth	0.39	0.34	0.39
Enterococcus faecalis growth	0.59	0.46	0.59
Micrococcus luteus growth	0.52	0.41	0.52
Propionibacterium acnes growth	0.08	0.28	0.98
Propionibacterium granulosum growth	0.15	> 0.99	> 0.99
Proteus mirabilis growth	0.15	> 0.99	> 0.99
Staphylococcus epidermidis growth	0.40	0.58	0.48
Staphylococcus capitis growth	0.80	0.19	0.74
Staphylococcus aureus growth	0.61	0.62	0.74
Staphylococcus caprae growth	0.59	0.37	0.65
Staphylococcus haemolyticus growth	0.36	0.30	0.36
Staphylococcus hominis growth	0.36	0.31	0.36
Staphylococcus lugdunensis growth	0.61	0.80	0.25
Staphylococcus pasteuri growth	0.52	0.46	0.53
Staphylococcus warneri growth	0.83	0.12	0.83
Streptococcus viridans growth	0.16	> 0.99	> 0.99
Conjunctival impression cytology markers			
MU5AC expression	0.34	0.25	0.19
MMP-9 expression	0.39	0.82	0.91
IL-6 expression	0.47	0.92	0.90

capping grade (both $p < 0.05$, Table 5). Multiplicity-adjusted post-hoc analysis demonstrated significant reductions in meibomian gland capping severity in both IPL treatment groups on day 105, although improvements were limited to the five-flash IPL group on day 45 (all $p < 0.05$, Table 6. No significant treatment, time, or interaction effects were detected for conjunctival hyperaemia, eyelid margin and eyelash characteristics, ocular surface staining, meibomian gland dropout, meibum quality, and non-contact aesthesiometry (all $p > 0.05$, Table 5).

3.5. *In vivo* confocal microscopy evaluation

There were no significant treatment, time, and interaction effects for corneal sub-basal nerve fibre and dendritic cell densities, and inferior lid margin rete ridge diameter, density and secretion reflectivity (all $p > 0.05$, Table 5).

3.6. Eyelid margin swab microbiology

A significant treatment effect for *Corynebacterium macginleyi* growth was observed ($p = 0.003$, Table 5), with post-hoc multiplicity-adjusted analysis demonstrating inhibited growth in both IPL treatment groups on day 105 (both $p < 0.05$, Table 6). No significant treatment, time, or interaction effects were detected for total bacterial colony forming units, and the growth of all other bacterial species (all $p > 0.05$, Table 5).

3.7. Conjunctival impression cytology markers

Treatment, time, and interaction effects for ocular surface inflammation and goblet cell function markers were not statistically significant (all $p > 0.05$, Table 5).

4. Discussion

In agreement with the findings reported in earlier studies [11–20], the results of the current double-masked, randomised, placebo-controlled trial demonstrated clinical efficacy of intense pulsed light therapy in the treatment of patients with MGD. Clinical improvements in objective and subjective markers of ocular surface homeostasis were observed during the fifteen-week period in both IPL treatment groups, with significant decreases in OSDI, SPEED, and SANDE symptomology scores, and meibomian gland capping, which was accompanied by augmentation of tear film lipid layer thickness. In addition, *Corynebacterium macginleyi* growth appeared to be inhibited following treatment courses with both four and five-flash IPL therapy. It is, nevertheless, acknowledged that the treatment effects observed in the current study appear to be more modest than those previously reported in the literature [12–17,19,20]. Not unexpected, this is thought likely to be related to the intrinsic methodological design of the current trial, including the lack of mechanical meibum expression immediately following IPL therapy, and the evaluation of outcome measures either two weeks following the first treatment or four weeks following allother treatments, which was intentional, in order to isolate and provide better characterisation of long term the extended cumulative treatment effects of IPL therapy.

Table 6
Post-hoc multiplicity-adjusted Tukey's test for treatment effects at each individual time point. Ordinal data were converted to rank-values prior to non-parametric assessment. Data are presented as p-values. Asterisks denote statistically significant differences ($p < 0.05$).

Measurement	Treatment	p-value				
		Day 0	Day 15	Day 45	Day 75	Day 105
OSDI dry eye score	Placebo vs. 4 Flashes	0.12	0.02*	0.14	0.003*	0.04*
	Placebo vs. 5 Flashes	0.15	0.02*	0.03*	0.04*	0.03*
	4 Flashes vs. 5 Flashes	0.92	0.93	0.52	0.36	0.85
SPEED score	Placebo vs. 4 Flashes	0.11	0.005*	0.28	0.008*	0.006*
	Placebo vs. 5 Flashes	0.49	0.08	0.16	0.03*	0.04*
	4 Flashes vs. 5 Flashes	0.30	0.22	0.75	0.37	0.31
SANDE score	Placebo vs. 4 Flashes	> 0.99	0.23	0.31	0.03*	0.04*
	Placebo vs. 5 Flashes	0.49	0.003*	0.006*	0.004*	0.01*
	4 Flashes vs. 5 Flashes	0.50	0.09	0.10	0.48	0.61
Tear film lipid layer grade	Placebo vs. 4 Flashes	0.69	0.88	0.37	0.02*	0.03*
	Placebo vs. 5 Flashes	0.67	0.63	0.02*	0.01*	0.04*
	4 Flashes vs. 5 Flashes	0.42	0.53	0.46	0.89	0.69
Meibomian gland capping grade	Placebo vs. 4 Flashes	0.48	0.47	0.61	0.77	0.03*
	Placebo vs. 5 Flashes	0.50	0.19	0.03*	0.81	0.004*
	4 Flashes vs. 5 Flashes	0.96	0.55	0.11	0.95	0.28
Corynebacterium macginleyi growth	Placebo vs. 4 Flashes	0.33	–	–	–	0.02*
	Placebo vs. 5 Flashes	0.17	–	–	–	0.004*
	4 Flashes vs. 5 Flashes	0.72	–	–	–	0.59

The mechanisms by which IPL therapy effects clinical improvements in patients with MGD remains poorly understood, although a number of different hypotheses have been proposed [11,13,18,21,49]. Thermal energy transferred by IPL therapy is thought to liquefy the inspissated meibum observed in MGD, relieving ductal obstruction and promoting the release meibomian lipids into the tear film [8,9,11,21,49]. Restoration of the integrity and quality of the surface lipid layer can enhance tear film stability [8,9], and it has been recognised that a continuous lipid layer is necessary to retard excessive aqueous tear evaporation [50]. This hypothesis appears to be supported by the trends observed in the current study which demonstrate an improvement in tear film lipid layer thickness and meibomian gland capping following treatment with IPL therapy. Interestingly, despite a significant reduction of subjective dry eye symptomology scores being observed in association with improvements in markers of meibomian gland function, no significant changes in non-invasive tear film stability were detected in the current study, which contrasts with trends described in earlier reports [12–17,19,20]. It cannot be reliably determined whether this might be partially attributed to the more modest treatment effects in the absence of mechanical meibum expression immediately following IPL therapy in the current study. In addition, the measurement of outcome measures were conducted two or four weeks following each course of treatment, and may have failed to capture immediate or more transient treatment effects, especially in the context of the intrinsic variability of tear film stability measurements [22,51]. Finally, the possibility for the four IPL treatments during the fifteen-week study period to be insufficient to effect sustained cumulative improvements in tear film stability cannot be excluded, and would warrant investigation in future studies with a greater number of treatments.

It has also been previously suggested that IPL therapy might potentially decrease the bacterial load of the peri-ocular micro-environment and alter the composition of the ocular surface microbiota, thereby dampening triggers for host immune and inflammatory responses [21,49]. In the current study, inhibition of *Corynebacterium macginleyi* growth was observed with IPL treatment. Other mechanisms that have been previously raised include the therapeutic effects mediated by the thrombosis of abnormal blood vessels in the peri-ocular skin, reduction of epithelial turnover, fibroblast activation and promotion of collagen synthesis, reduction in ocular *Demodex* load, modulation of pro-inflammatory and anti-inflammatory cascades, and alteration in the levels of reactive oxidative species [13,18,49,52], although evidence supporting these hypotheses were either not directly investigated or observed in the findings of the current study.

The trends observed in the current study are generally supportive of the manufacturer recommendations of applying five flashes of IPL during each treatment. Sustained improvements in clinical signs and symptoms of MGD were detected earlier in the study period in participants randomised to receiving five flashes of IPL than those receiving four flashes. Indeed, on day 45, clinical improvements in tear film lipid layer thickness, meibomian gland capping, OSDI and SANDE symptomology scores were limited to those receiving five flashes. Although the mechanisms of improved treatment efficacy associated with the fifth flash applied temporally adjacent to the lateral canthus are not fully understood, it is possible that the enhanced transfer of thermal energy to the eyelids might have potentially contributed [21,49]. Possible neuromodulatory effects on the parasympathetic innervation of the meibomian glands originating from the pterygopalantine ganglion have also been hypothesised [53,54]. Interestingly, although significant reductions in OSDI scores were observed in both treatment groups on day 15, changes in SPEED scores were limited to those receiving four flashes, while improvements in SANDE scores were limited to those receiving five flashes. However, the initial changes in OSDI and SPEED scores were not sustained on day 45 in participants receiving four flashes, which contrasted with the continued improvements in OSDI and SANDE scores observed in participants receiving five flashes. These trends would suggest that the initial treatment effects of IPL therapy with four flashes might be more short-lived than those with five flashes. The contrasting trends observed in the three symptomology scores during the study period might also partially reflect the differing diagnostic sensitivity of these subjective measurements [55].

The methodological design of the current trial was performed to assess the cumulative profile of the treatment effects of IPL therapy. Although inconsistent changes in a number of subjective and objective ocular surface parameters were observed on days 15 and 45, sustained improvements in both IPL treatment groups for OSDI, SPEED, and SANDE symptomology scores, and tear film lipid layer thickness were consistently observed on days 75 and 105. Although an initial improvement in meibomian gland capping was observed on day 45 in participants receiving five flashes, a consistent improvement observed across both IPL treatment groups was not detected until day 105. Overall, these trends would suggest that an initial course of four treatments of IPL therapy is warranted in the clinical setting, to allow sufficient time for a sustained cumulative therapeutic effect to be established, before the evaluation of the treatment efficacy can be reliably

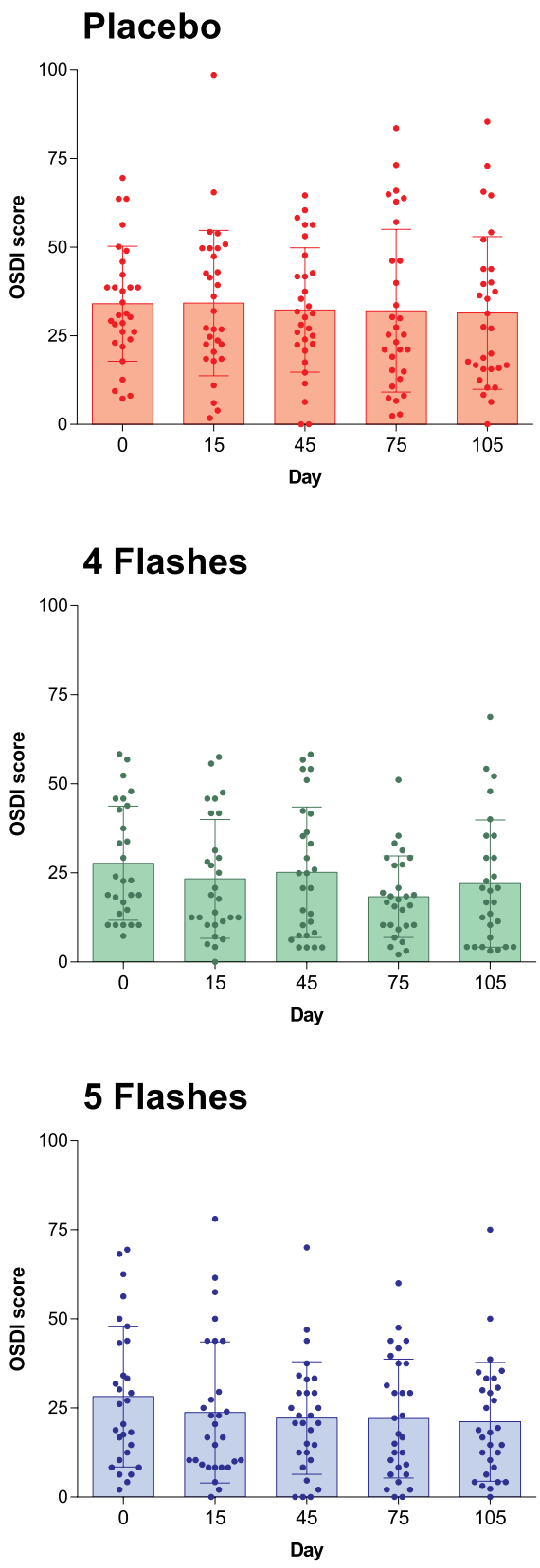

Fig. 2. OSDI scores at days 0, 15, 45, 75 and 105, for participants randomised to placebo treatment, or intense pulse light therapy with four or five homogeneously sequenced light pulses. Each point represents the OSDI score of an individual participant. Bars represent the mean OSDI scores. Error bars represent the standard deviation.

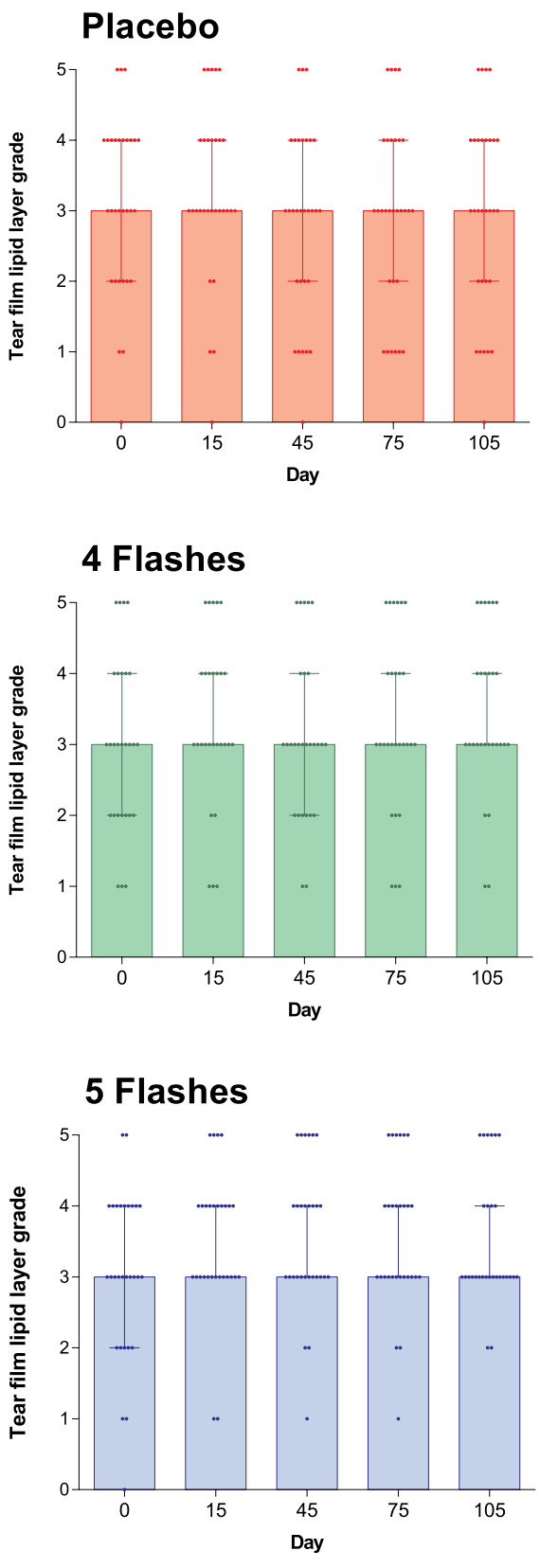

Fig. 3. Tear film lipid layer grades at days 0, 15, 45, 75 and 105, for participants randomised placebo treatment, or intense pulse light therapy with four or five homogeneously sequenced light pulses. Each point represents the lipid layer grade of an individual participant. Bars represent the median lipid layer grades. Error bars represent the interquartile range.

conducted in an individual patient. Future studies with longer treatment periods are required to confirm whether further extended treatment courses might confer an additional advantage through the clinical improvement of other signs of meibomian gland dysfunction.

5. Conclusions

In conclusion, IPL therapy effected improvements in dry eye symptomology, tear film lipid layer thickness, and meibomian gland capping in MGD patients in this double-masked, randomised, placebo-controlled trial. The findings also demonstrated superior clinical efficacy of applying five flashes of IPL during each treatment relative to four flashes, and would suggest that an initial course of four treatments would be required to allow for sustained cumulative therapeutic effects to be established, prior to the evaluation of overall treatment efficacy.

Funding

The authors are grateful to the New Zealand Optometric Vision Research Foundation, and to E-Swin, France, for grants-in-aid to support this investigator-initiated trial. AX was a recipient of a University of Auckland Doctoral Scholarship and a New Zealand Association of Optometrists Postgraduate Scholarship. The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Declaration of competing interest

The authors have no commercial or proprietary interest in any concept or product described in this article.

Acknowledgements

The authors are grateful for the unmasked clinical assistance of, Drs Joevy Lim, Ji Soo Kim, Isabella Cheung, Sanjay Marasini, and Mr Sang Hoo Lee in applying treatment and sham therapy according to the predetermined randomisation schedule, enabling study masking to be preserved.

References

[1] Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK, et al. TFOS DEWS II definition and classification Report. Ocul Surf 2017;15:276–83.
[2] Stapleton F, Alves M, Bunya VY, Jalbert I, Lekhanont K, Malet F, et al. TFOS DEWS II epidemiology Report. Ocul Surf 2017;15:334–65.
[3] Belmonte C, Nichols JJ, Cox SM, Brock JA, Begley CG, Bereiter DA, et al. TFOS DEWS II pain and sensation Report. Ocul Surf 2017;15:404–37.
[4] Mathews PM, Ramulu PY, Swenor BS, Utine CA, Rubin GS, Akpek EK. Functional impairment of reading in patients with dry eye. Br J Ophthalmol 2017;101:481–6.
[5] Bron AJ, de Paiva CS, Chauhan SK, Bonini S, Gabison EE, Jain S, et al. TFOS DEWS II pathophysiology Report. Ocul Surf 2017;15:438–510.
[6] Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international Workshop on meibomian gland dysfunction: Report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. Invest Ophthalmol Vis Sci 2011;52:1938–78.
[7] Baudouin C, Messmer EM, Aragona P. Revisiting the vicious circle of dry eye disease: a focus on the pathophysiology of meibomian gland dysfunction. Br J Ophthalmol 2016;100:300–6.
[8] Jones L, Downie LE, Korb D, Benitez-Del-Castillo JM, Dana R, Deng SX, et al. TFOS DEWS II management and therapy Report. Ocul Surf 2017;15:575–628.
[9] Geerling G, Tauber J, Baudouin C, Goto E, Matsumoto Y, O'Brien T, et al. The international Workshop on meibomian gland dysfunction: Report of the subcommittee on management and treatment of meibomian gland dysfunction. Invest Ophthalmol Vis Sci 2011;52:2050–64.
[10] Wat H, Wu DC, Rao J, Goldman MP. Application of intense pulsed light in the treatment of dermatologic disease: a systematic review. Dermatol Surg 2014;40:359–77.
[11] Ahmed SA, Taher IME, Ghoneim DF, Safwat AEM. Effect of intense pulsed light therapy on tear proteins and lipids in meibomian gland dysfunction. J Ophthalmic Vis Res 2019;14:3–10.
[12] Arita R, Fukuoka S, Morishige N. Therapeutic efficacy of intense pulsed light in patients with refractory meibomian gland dysfunction. Ocul Surf 2019;17:104–10.
[13] Choi M, Han SJ, Ji YW, Choi YJ, Jun I, Alotaibi MH, et al. Meibum expressibility improvement as a therapeutic target of intense pulsed light treatment in meibomian gland dysfunction and its association with tear inflammatory cytokines. Sci Rep 2019;9:7648.
[14] Li D, Lin SB, Cheng B. Intense pulsed light treatment for meibomian gland dysfunction in skin types III/IV. Photobiomodul Photomed Laser Surg 2019;37:70–6.
[15] Vigo L, Giannaccare G, Sebastiani S, Pellegrini M, Carones F. Intense pulsed light for the treatment of dry eye owing to meibomian gland dysfunction. J Vis Exp 2019;146:e57811https://doi.org/10.3791/57811.
[16] Albietz JM, Schmid KL. Intense pulsed light treatment and meibomian gland expression for moderate to advanced meibomian gland dysfunction. Clin Exp Optom 2018;101:23–33.
[17] Rong B, Tang Y, Liu R, Tu P, Qiao J, Song W, et al. Long-term effects of intense pulsed light combined with meibomian gland expression in the treatment of meibomian gland dysfunction. Photomed Laser Surg 2018;36:562–7.
[18] Liu R, Rong B, Tu P, Tang Y, Song W, Toyos R, et al. Analysis of cytokine levels in tears and clinical correlations after intense pulsed light treating meibomian gland dysfunction. Am J Ophthalmol 2017;183:81–90.
[19] Jiang X, Lv H, Song H, Zhang M, Liu Y, Hu X, et al. Evaluation of the safety and effectiveness of intense pulsed light in the treatment of meibomian gland dysfunction. J Ophthalmol 2016;2016. 1910694.
[20] Craig JP, Chen YH, Turnbull PR. Prospective trial of intense pulsed light for the treatment of meibomian gland dysfunction. Invest Ophthalmol Vis Sci 2015;56:1965–70.
[21] Vora GK, Gupta PK. Intense pulsed light therapy for the treatment of evaporative dry eye disease. Curr Opin Ophthalmol 2015;26:314–8.
[22] Wolffsohn JS, Arita R, Chalmers R, Djalilian A, Dogru M, Dumbleton K, et al. TFOS DEWS II diagnostic methodology report. Ocul Surf 2017;15:539–74.
[23] Tomlinson A, Bron AJ, Korb DR, Amano S, Paugh JR, Pearce EI, et al. The international Workshop on meibomian gland dysfunction: Report of the diagnosis subcommittee. Invest Ophthalmol Vis Sci 2011;52:2006–49.
[24] Foulks GN, Bron AJ. Meibomian gland dysfunction: a clinical scheme for description, diagnosis, classification, and grading. Ocul Surf 2003;1:107–26.
[25] Wang MT, Jaitley Z, Lord SM, Craig JP. Comparison of self-applied heat therapy for meibomian gland dysfunction. Optom Vis Sci 2015;92:e321–6.
[26] Gulati A, Sullivan R, Buring JE, Sullivan DA, Dana R, Schaumberg DA. Validation and repeatability of a short questionnaire for dry eye syndrome. Am J Ophthalmol 2006;142:125–31.
[27] Sung J, Wang MTM, Lee SH, Cheung IMY, Ismail S, Sherwin T, et al. Randomized double-masked trial of eyelid cleansing treatments for blepharitis. Ocul Surf 2018;16:77–83.
[28] Guillon JP. Use of the Tearscope Plus and attachments in the routine examination of the marginal dry eye contact lens patient. Adv Exp Med Biol 1998;438:859–67.
[29] Craig JP, Wang MT, Kim D, Lee JM. Exploring the predisposition of the Asian eye to development of dry eye. Ocul Surf 2016;14:385–92.
[30] Golebiowski B, Chim K, So J, Jalbert I. Lid margins: sensitivity, staining, meibomian gland dysfunction, and symptoms. Optom Vis Sci 2012;89:1443–9.
[31] Patel DV, Tavakoli M, Craig JP, Efron N, McGhee CN. Corneal sensitivity and slit scanning in vivo confocal microscopy of the subbasal nerve plexus of the normal central and peripheral human cornea. Cornea 2009;28:735–40.
[32] Cox SM, Nichols JJ. Association between meibomian gland testing and ocular surface sensitivity. Cornea 2015;34:1187–92.
[33] Bron AJ, Evans VE, Smith JA. Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea 2003;22:640–50.
[34] Korb DR, Herman JP, Greiner JV, Scaffidi RC, Finnemore VM, Exford JM, et al. Lid wiper epitheliopathy and dry eye symptoms. Eye Contact Lens 2005;31:2–8.
[35] Pult H, Riede-Pult B. Comparison of subjective grading and objective assessment in meibography. Contact Lens Anterior Eye 2013;36:22–7.
[36] Kheirkhah A, Satitpitakul V, Hamrah P, Dana R. Patients with dry eye disease and low subbasal nerve density are at high risk for accelerated corneal endothelial cell loss. Cornea 2017;36:196–201.
[37] Kheirkhah A, Rahimi Darabad R, Cruzat A, Hajrasouliha AR, Witkin D, Wong N, et al. Corneal epithelial immune dendritic cell alterations in subtypes of dry eye disease: a pilot in vivo confocal microscopic study. Invest Ophthalmol Vis Sci 2015;56:7179–85.
[38] Zhou S, Robertson DM. Wide-field in vivo confocal microscopy of meibomian gland acini and rete ridges in the eyelid margin. Invest Ophthalmol Vis Sci 2018;59:4249–57.
[39] Villani E, Magnani F, Viola F, Santaniello A, Scorza R, Nucci P, et al. In vivo confocal evaluation of the ocular surface morpho-functional unit in dry eye. Optom Vis Sci 2013;90:576–86.
[40] Villani E, Canton V, Magnani F, Viola F, Nucci P, Ratiglia R. The aging Meibomian gland: an in vivo confocal study. Invest Ophthalmol Vis Sci 2013;54:4735–40.
[41] Villani E, Beretta S, De Capitani M, Galimberti D, Viola F, Ratiglia R. In vivo confocal microscopy of meibomian glands in Sjogren's syndrome. Invest Ophthalmol Vis Sci 2011;52:933–9.
[42] Gao YY, Di Pascuale MA, Li W, Baradaran-Rafii A, Elizondo A, Kuo CL, et al. In vitro and in vivo killing of ocular Demodex by tea tree oil. Br J Ophthalmol 2005;89:1468–73.
[43] Cheung IMY, Xue AL, Kim A, Ammundsen K, Wang MTM, Craig JP. In vitro anti-demodectic effects and terpinen-4-ol content of commercial eyelid cleansers. Contact Lens Anterior Eye 2018;41:513–7.
[44] Ganesalingam K, Ismail S, Craig JP, Sherwin T. Use of a purpose-built impression cytology device for gene expression quantification at the ocular surface using

quantitative PCR and droplet digital PCR. Cornea 2019;38:127–33.
[45] Craig JP, Wang MTM, Ganesalingam K, Rupenthal ID, Swift S, Loh CS, et al. Randomised masked trial of the clinical safety and tolerability of MGO Manuka Honey eye cream for the management of blepharitis. BMJ Open Ophthalmol 2017;1. e000066.
[46] Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 2002;3. research0034.1.
[47] Andersen CL, Jensen JL, Orntoft TF. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. Canc Res 2004;64:5245–50.
[48] Ruijter JM, Ruiz Villalba A, Hellemans J, Untergasser A, van den Hoff MJ. Removal of between-run variation in a multi-plate qPCR experiment. Biomol Detect Quantif 2015;5:10–4.
[49] Dell SJ. Intense pulsed light for evaporative dry eye disease. Clin Ophthalmol 2017;11:1167–73.
[50] Craig JP, Tomlinson A. Importance of the lipid layer in human tear film stability and evaporation. Optom Vis Sci 1997;74:8–13.
[51] Sullivan BD, Crews LA, Sonmez B, de la Paz MF, Comert E, Charoenrook V, et al. Clinical utility of objective tests for dry eye disease: variability over time and implications for clinical trials and disease management. Cornea 2012;31:1000–8.
[52] Zhang X, Song N, Gong L. Therapeutic effect of intense pulsed light on ocular demodicosis. Curr Eye Res 2019;44:250–6.
[53] LeDoux MS, Zhou Q, Murphy RB, Greene ML, Ryan P. Parasympathetic innervation of the meibomian glands in rats. Invest Ophthalmol Vis Sci 2001;42:2434–41.
[54] Karaca EE, Evren Kemer O, Ozek D. Intense regulated pulse light for the meibomian gland dysfunction. Eur J Ophthalmol 2018 Dec 4. https://doi.org/10.1177/1120672118817687. [Epub ahead of print].
[55] Wang MTM, Xue AL, Craig JP. Comparative evaluation of 5 validated symptom questionnaires as screening instruments for dry eye disease. JAMA Ophthalmol 2019;137:228–9.