Mechanisms of action of light-based therapies in the management of dry eye disease and Meibomian gland dysfunction

Study Protocol

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Background and purpose

As the mechanisms of action underlying commonly prescribed light-based therapies are still not fully understood, our study aims to investigate clinical and physiological changes on the surface of the eyes and eyelids using clinically and readily available instruments. Two light-based therapies commonly prescribed are intense pulsed light (IPL) and low-level light therapy (LLLT), often in combination (IPL+LLLT). While IPL+LLLT is becoming a more common treatment regimen worldwide, recent evidence has also shown that LLLT alone is more effective at alleviating dry eye signs and symptoms better than IPL (Giannaccare et al, 2023). The proposed study aims to investigate the short- and long-term morphological, functional and physiological changes in the eyelid skin and ocular surface following IPL+LLLT versus LLLT alone (with prior sham IPL) for the treatment of Meibomian gland dysfunction in dry eye disease. The findings from this study will contribute knowledge to eyecare practitioners regarding the cellular and molecular changes these treatments and strengthen clinical guidelines associated with the prescription of light-based therapies. Understanding the short- and long-term implications of these treatments will also enable eyecare practitioners to treat patients with a more evidence-based approach.

Methods

Materials and Instruments

1. Light-based treatment protocol

The Eye-light unit (Espansione Group Ltd, Funo, Italy) was used to deliver the light-based therapies. Prior to the intense pulsed light therapy (IPL), the highest meiboscore of either eyelid and the Fitzpatrick skin grading was inputted into the unit system. This automatically calibrated the optical radiance output of the IPL. Table 1 details the exact output for each combination of settings.

Table 1. Optical radiance output of the intense pulsed light (IPL) therapy of the Eye-light unit (Espansione Group Ltd, Funo, Italy) according to inputted grading from the meiboscale and Fitzpatrick skin grading.

Pult Meiboscale	Fitzpatrick skin grading	Optical radiance output (J)
1	1	59
	2	59
	3	59
	4	59
2	1	60
	2	62
	3	62
	4	60
3	1	62
	2	66
	3	66
	4	64
4	1	65
	2	69
	3	69
	4	67

Figure 1 demonstrates the light-based treatment administration protocol used in this clinical trial for four treatment sessions.

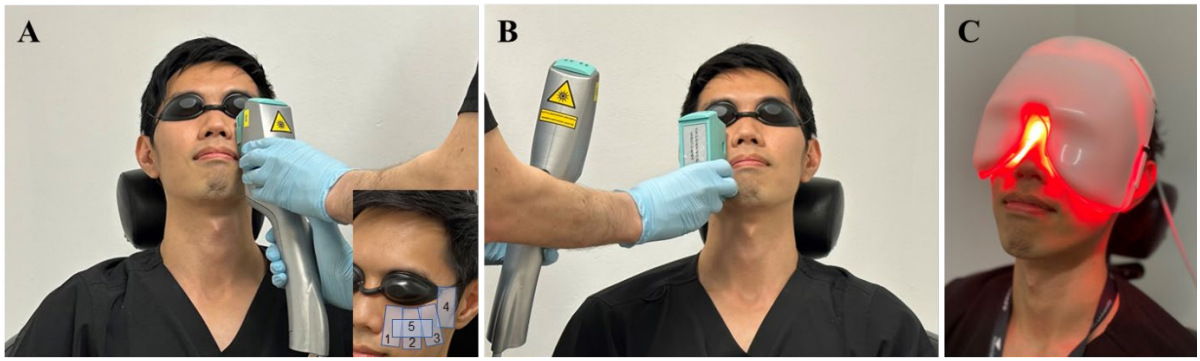

Figure 1. Intense pulsed light (IPL) protocol with (**A**) sequential placements of treatment cartridge for the five different regions involved, and (**B**) sham IPL placement of a disconnected empty cartridge along the same five different regions on the contralateral side but with the working IPL cartridge pointed away from the patient's face to simulate a pulse. Low-level light therapy (LLLT) was then administered for 15 minutes (**C**) with placement of the mask consisting of a series of red light-emitting diodes (LEDs). These were conducted with the Eye-light unit (Espansione Group Ltd, Funo, Italy).

2. Ocular assessment schedules and details

The study was structured into multiple visits and all conducted at the Aston Dry Eye Clinic in Aston University, Birmingham, United Kingdom. Each visit lasted for about 1.5 hours, with the sequence of assessments detailed in Table 2:

- Visit 1: Screening ± First treatment session
- Visit 2 (2 to 3 weeks after Visit 1): Second treatment session
- Visit 3 (2 to 3 weeks after Visit 2): Third treatment session
- Visit 4 (2 to 3 weeks after Visit 3): Fourth and final treatment session
- Visit 5 (2 weeks after visit 4): First follow-up
- Visit 6 (3 months after visit 4): Second and final follow-up

Table 2. The sequence of assessments for each visit

Procedures	Visits					
	1	2	3	4	5	6
Screening with inclusion and exclusion criteria	✓					
OSDI, DEQ5 and adapted SANDE questionnaires	✓	✓	✓	✓	✓	✓
General medical and ocular history, and lifestyle questions	✓	✓	✓	✓	✓	✓
Visual acuity assessment	✓	✓	✓	✓	✓	✓
Oculus Keratograph 5M, Part 1 - Number of total and partial blinks in 30 s - Tear meniscus height - Non-invasive tear break up time - Lipid layer pattern - Bulbar and limbal hyperaemia grading	✓	✓	✓	✓	✓	✓
Laser Doppler flowmetry and multimodal spectroscopy at central lower eyelids	✓				✓	✓
Slit lamp biomicroscopy - Telangiectasia grading	✓	✓	✓	✓	✓	✓

Procedures	Visits					
	1	2	3	4	5	6
- Number of blocked or capped Meibomian gland orifices - Demodex detection (2 lashes randomly chosen on either lid and assessed with lateral traction method) - Meibomian gland expression with Korb expressor						
Oculus Keratograph 5M, Part 2 - Fluorescein sodium and lissamine green staining grading on ocular surface and lid wiper epitheliopathy	✓	✓	✓	✓	✓	✓
Meibography	✓				✓	✓
In-vivo corneal confocal microscopy	✓				✓	✓

The following provides further details the instrumentation or procedures involved:

i. OSDI and DEQ5 questionnaires

These are validated questionnaires used to assess the severity and frequency of the dry eye symptoms experienced by the participants. OSDI consists of 12 questions regarding the frequency of symptoms and impact of daily activities over the past week. The scores range from 0 to 100 [1]. The DEQ5 assesses the severity and frequency of the discomfort, dryness and wateriness during a typical day in the past month. The scores range from 0 to 22 [2]. The adapted SANDE questionnaire is a visual analogue scale quantifying the severity and frequency of dry eye symptoms for each eye separately from 0 to 100 [3]. These were self-administered by the patient on a printed A4 sheet of paper.

ii. Visual acuity assessment

A standard vision chart mounted on the wall was used to assess high-contrast vision in logMAR units, calibrated at 3 m.

iii. Oculus Keratograph 5M, Part 1 (Optikgerlite GmBH, Wetzlar, Germany)

Number of total and partial blinks in 30 seconds, tear meniscus height measured using in-built calipers in the Oculus Keratograph 5M software (average of 3 measures), objective non-invasive tear break up time (average of 3 measures), subjective lipid layer pattern grading (1: Open meshwork; 2: Closed meshwork; 3: Wave pattern; 4: Amorphous pattern; 5: Colour fringe pattern) [4], and automated bulbar conjunctiva and limbal redness grading (0: none to 4: severe in 0.1 increments) were assessed using this instrument.

iv. Laser Doppler flowmetry

This is an instrument developed by the Aston Institute of Photonics Technology (Aston University, Birmingham, United Kingdom) which uses a small probe lightly touching the skin to analyse blood flow in small vessels [5, 6]. This was adapted and mounted on the slit lamp to assess the blood flow of the external cutaneous skin of the central lower eyelid of each side.

v. Slit lamp biomicroscopy

The number of blocked or plugged Meibomian gland orifices were assessed under white light of the slit lamp biomicroscope (CSO SL9900 Digital LED Slit Lamp, Worcestershire, United Kingdom). Subjective grading of telangiectasia in the lower lid

margin (from 0 signifying no telangiectasia, to 3 representing telangiectasia crossing orifices across ≥ 50% of the lid margin) was also conducted [7]. The number of eyelashes with any presence of Demodex mite around the base of the lashes were assessed by random selection of two lashes from each eyelid and using a lateral traction method as described previously [8]. Diagnostic Meibomian gland expression was conducted using a Meibomian Gland Evaluator (TearScience, North Carolina, United States) across five glands temporally, five glands centrally and five glands nasally. The expressibility and meibum quality were graded with the Pflugfelder scale (0: all 5 glands expressing; 1: 3 to 4 glands; 2: 1 to 2 glands; 3: no glands expressing) and Bron scale (0: clear fluid; 1: cloudy fluid; 2: cloudy particulate fluid; 3: inspissated), respectively [9], and averaged across the 15 glands assessed.

vi. Oculus Keratograph 5M, Part 2

This instrument was also used for imaging sodium fluorescein and Lissamine green staining, graded using the Oxford scale (from 0, absent to 5, severe) for the overall cornea and bulbar conjunctiva [10]. For each participant, a fluorescein sodium impregnated dry strip (Bio Fluoro, Bio-Tech Vision Care, Gujarat India) was moistened with a drop of saline solution (sodium chloride 0.9%), and the excess fluid shaken off, before applying on the lower temporal palpebral conjunctiva after slightly everting the lower eyelid. The same was done with a lissamine green impregnated dry strip (Contacare Ophthalmics and Diagnostics, Gujarat, India), but with the excess fluid allowed to fully coat the whole strip, before a large droplet was instilled in the same manner. Imaging was conducted following this using the in-built blue light and yellow filter, and then the white light for lissamine green staining. Following upper and lower lid eversion, lid wiper epitheliopathy was also assessed by grading the width (0: < 25 %; 1: 25 to 50 %; 2: 50 to 75 %; 3: > 75 %) and length (0: < 2 mm; 1: 2 to 4 mm; 2: 5 to 9 mm; 3: > 10 mm) of the staining [11], and an average of the width and length grading taken as the lid wiper epitheliopathy grading for each of the upper and lower eyelid. Meibography of the upper and lower lids were performed and graded using the meiboscale for the percentage area of gland loss or atrophy compared to the whole tarsal area (0: around 0%; 1: ≤ 25 %; 2: 26 to 50 %; 3: 51 to 75 %; 4: > 75 %) [12].

vii. In-vivo corneal confocal microscopy

The Heidelberg Retinal Tomograph III with Rostock Corneal Module (Heidelberg Engineering GmbH, Heidelberg, Germany) was used to image the sub-basal nerve plexus of the central cornea and inferior whorl. One drop of anaesthetic eyedrop (proxymethacaine hydrochloride 0.5% minims, Bausch & Lomb, Surrey, United Kingdom) used routinely in clinical practice was administered in both eyes for comfort and to minimise blinking for image capturing. Analyses of the total corneal nerve length (in mm/mm^2) using a semi-automated method in NeuronJ, a plugin of Image J (National Institutes of Health, Maryland, United States of America), and density of immune cells [$cells/mm^2$ of putative epithelial T cells (small, immune cells with no visible dendrites) and of dendritic cells (larger cells with dendritiform shape and apparent dendrites)] [13] using the Cell Counter plugin within ImageJ, from the central corneal (4 images randomly selected) were conducted. Total inferior whorl nerve length was also measured using NeuronJ from 1 image of the inferior whorl, an anatomical landmark where the sub-basal corneal nerves traverse towards 1 to 2 mm inferonasal to the central cornea.

Statistical Analysis

Two-factor repeated measures analysis of variance (ANOVA) was used for statistical analysis to assess changes over time across the multiple visits between each treatment group. One-way repeated measures ANOVA was used to assess changes over time for overall measures including OSDI scores, DEQ-5 scores, visual acuity, and blink rate. The Greenhouse-Geisser correction was applied to the one-way and two-factor repeated measures ANOVA results to adjust for the lack of sphericity. Paired t-test for normally distributed data or Wilcoxon signed-rank test for non-normally distributed data was used to assess differences between the two treatment modalities within the same visit.

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