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Intense Pulsed Light for the Treatment of Dry Eye Owing to Meibomian Gland Dysfunction

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Author Comments:	Dear JoVE Editorial team, as previously indicated by Nandita Singh, we are going to submit the invited manuscript titled "Intense Regulated Pulsed Light for the Treatment of Dry Eye Owing to Meibomian Gland Dysfunction" The article deals with a recently developed and commercialized non-surgical non-laser procedure for the treatment of one of the most common ocular surface discomfort syndrome nowaday. Thank you in advance for your cooperation. Sincerely your, Giuseppe Giannaccare
Additional Information:	
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Bologna,30th December 2017

To the kind attention of
Editor-in-Chief of
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Dear Editor,

as Corresponding Author I enclose herewith the invited manuscript titled “Intense Regulated Pulsed Light for the Treatment of Dry Eye Owing to Meibomian Gland Dysfunction”. The article deals with a recently developed and commercialized non-surgical non-laser procedure for the treatment of one of the most common ocular surface discomfort syndrome nowaday.

Our study is a prospective interventional clinical study conducted at Carones Ophthalmological Center, Milan, Italy. Methods of the procedure are herein deeply described for ophthalmologists who would like to approach to this novel tecnhique in order to extend their currently available options for the management of their patientis affected by dry eye owing to meibomian gland dysfunction.

Thank you in advance for your cooperation,

sincerely your,

Giuseppe Giannaccare, MD, PhD
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TITLE:

Intense Pulsed Light for the Treatment of Dry Eye Owing to Meibomian Gland Dysfunction

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KEYWORDS:

Dry eye, meibomian gland dysfunction, intense pulsed light, noninvasive break-up time, lipid layer thickness, noncontact meibography, tear osmolarity, ocular surface disease index

SHORT ABSTRACT:

Dry eye disease is an increasingly common condition, which strongly impair patients' life quality. Recently, a new device employing intense pulsed light, specifically designed for the periocular area, has been shown to improve tear film stability and ocular discomfort symptoms in dry eye disease owing to meibomian gland dysfunction.

LONG ABSTRACT:

Dry eye disease (DED) is an increasingly common condition and one of the most common complaints of patients. The vast majority of DED is caused by the so-called "evaporative" subtype, that is mainly caused by meibomian gland dysfunction (MGD). Intense pulsed light (IPL) devices employ high intensity pulses of polychromatic lights with a broad range of wavelength (515-1200 nm). IPL treatment has been utilized for years in the field of dermatology, and then its use was applied to ophthalmology for the treatment of MGD. Recently, a new device employing IPL was specifically designed for the periocular application. This procedure determines the thermal selective coagulation and ablation of superficial blood vessels and telangiectasias of the eyelids skin, reducing the release of inflammatory mediators and tear cytokines levels, and improving meibomian glands outflow. IPL treatment is noninvasive and easy to perform, lasts for only a few minutes and can be conducted in an office setting. In the present study, 19 patients underwent 3 sessions of IPL treatment. After treatment, both mean noninvasive break-up time and lipid layer thickness grade significantly

increased, as a result of an improvement of tear film stability and quality, respectively. Conversely, no statistically significant changes were found for meibomian gland loss and tear osmolarity. Furthermore, the vast majority of the treated patients (17/19; 89.5% of the total) perceived an improvement of their ocular discomfort symptoms after IPL treatment. Although IPL treatment provides an improvement of both ocular surface parameters and ocular discomfort symptoms after one cycle of three sessions, regular repeated treatments are usually required to maintain the persistence over the time of its beneficial effects.

INTRODUCTION:

Dry eye disease (DED) is an increasingly common condition and one of the most common reasons for clinical visits to an eye doctor[1]. The main symptom complained by patients affected by DED differs from various grades of redness and ocular discomfort to a chronic foreign body sensation, stinging, burning, itching, excessive tearing, pain, recurrent infections and transient visual disturbances[2]. The impact of DED on patients' quality of life has been compared to that caused by moderate to severe angina or dialysis treatment[3], and is associated with a restriction in daily activities and a loss of work productivity[4]. The recent definition of dry eye disease formulated by the TFOS DEWS II highlights its multifactorial nature, since different complex and heterogeneous alterations play a significant role in the onset and the maintenance of the disease[2].

The vast majority of dry eye is caused by the so-called "evaporative" subtype that is mainly caused by meibomian gland dysfunction (MGD), a condition that affects up to 70% of the population in particular regions of the world[5]. Meibomian gland dysfunction is caused by a chronic alteration of the meibomian glands that are located inside the upper and lower eyelids. The disease is characterized by hyperkeratinization of the external duct of the glands and the obstruction of the orifices with insufficient, not functional, production of the external lipid layer of the tear film, resulting in tear instability[6-8].

Currently, several different therapeutic strategies are available aiming at interrupting the vicious spiral of dry eye, and consist mainly of antibiotics, anti-inflammatory drugs, eyelid hygiene, warm compresses and tear substitutes[9]. However, these therapies are chronic and provide often only partial or short-term relief of symptoms, with subsequent compliance issues. Therefore, novel treatments with high efficacy and tolerability are desirable. In recent years, intense pulsed light (IPL) therapy has been widely used in dermatology for the treatment of different skin diseases such as acne, rosacea, telangiectasias and vascular and pigmented lesion (*e.g.*, hemangiomas, venous malformations, port-wine stains)[10]. When the light is applied to the skin, it is absorbed by pigmented structures, such as blood cells and teleangectasias, with subsequent heat production that coagulates and destroys the abnormal blood vessels[11]. Recently, this technique was applied to the ophthalmic field for the treatment of DED owing to MGD. Several mechanisms have been postulated to explain the therapeutic effect of the procedure. Firstly, IPL treatment acts inducing thermal coagulation and selective ablation of superficial blood vessels and telangiectasias of the eyelids skin. Furthermore, the procedure reduces the release of inflammatory mediators and the levels of tear cytokines, which may promote the keratinization of mebomian glands terminal duct[12,13]. Secondly, the light energy

transformed into heat causes the warming and liquefying of meibomian glands secretions, with subsequent melting and improved outflow[11,14]. More recently, other mechanisms such as the enhancement in collagen synthesis and connective tissue remodeling, the reduction in skin epithelial cell turnover, and the modulation of cellular inflammatory markers have also been hypothesized[15].

In this study, we describe the use and the therapeutic effects of a recently commercialized IPL device, developed specifically for the treatment of MGD, in which IPL emission has been "regulated" in a multiple polychromatic train of calibrated and homogenously sequenced pulses. Therefore, the aim of this study is to describe the treatment procedure in detail, in order to spread it to the scientific medical community, giving the opportunity to ophthalmologists to expand their current armamentarium for the treatment of MGD.

PROTOCOL:

All participants were provided both verbal and written informed consent before any study procedure. The protocol of the study was carried out in accordance with the Declaration of Helsinki and was approved by the local Institutional Review Board.

1. Automated Ocular Surface Workup

1.1. Noninvasive break-up time (BUT) and lipid layer thickness (LLT) evaluation

1.1.1. Download the "I.C.P. by SBM Sistemi" application on a tablet computer (download link: https://itunes.apple.com/us/app/i-c-p-by-sbm-sistemi/id1002330054?mt=8).

1.1.2. Apply the device to the tablet.

1.1.3. Open the application and insert the patient's data.

1.1.4. Select the exam to be performed in the menu: select **NIBUT** to measure the noninvasive BUT or **Interferometry** to evaluate the lipid layer type and thickness grade.

1.1.5. Place the camera lens in front of the patient's eye at a distance of 1-2 cm.

1.1.6. Ask the patient to blink twice, and then stare without blinking for as long as possible.

1.1.7. Keep the instrument still and click the proper button to acquire the video. The system will automatically analyze and provide the value of noninvasive BUT (time in s) or LLT (grade from 1 to 7).

Note: These exams are completely noninvasive, and it is not necessary to use any topical anesthetic or vital staining for the acquisitions and measurements.

1.1.8. Repeat the same procedure for the other eye, if required.

1.2. **Meibomian gland loss (MGL) assessment**

1.2.1. Download the "I.C.P. by SBM Sistemi" application on a tablet computer (download link: https://itunes.apple.com/us/app/i-c-p-by-sbm-sistemi/id1002330054?mt=8)

1.2.2. Open the application and insert patient's data.

1.2.3. Select **Meibomian gland** in the menu of the exams.

1.2.4. Connect the device to the tablet by cable or Wifi connection.

1.2.5. Gently evert the lower eyelid using a cotton swab.

1.2.6. Acquire an infrared image of the inner part of the lower eyelid in order to obtain a good quality image of the meibomian glands, which appear whitish.

1.2.7. Draw the margins of the eyelid area on the touchscreen and click to fill the area.

1.2.8. Draw the margins of the meibomian glands on the touchscreen and click to fill the area.

Note: The system automatically calculates the percentage of MGL area in the total area of the eyelid. In addition, it is possible to use ImageJ software (National Institute of Health; http://imagej.nih.gov/ij) to analyze the images, and measure the MGL value as the percentage of gland loss in relation to the total tarsal area of the eyelid, as previously described[16].

1.2.9. Repeat the same procedure for the other eye, if required.

1.3. **Tear osmolarity measurement**

1.3.1. Clip the single use test card with a microchip into the top of the pen. The pen confirms when the test card is correctly attached.

1.3.2. Gently move down the lateral lower eyelid using a cotton swab to create a little space between the eyeball and the eyelid.

1.3.3. Place the tip of the test card in this space to collect a sample of tear fluid (50 nL) from the inferior lateral meniscus of the tear film. The pen confirms when the tear fluid sample has been properly collected.

Note: It is recommended to collect tear fluid at the outermost area of the eyelid to minimize the risk of corneal injuries.

1.3.4. Dock the pen in the reader unit. The system reader measures and displays on the screen

the osmolarity of the tear in mΩ/L.

1.3.5. Repeat the same procedure for the other eye, if required.

Note: Separate test cards are needed for each eye.

1.4. **Subjective symptoms assessment**

1.4.1. Administer the Ocular Surface Disease Index (OSDI) questionnaire.

1.4.2. Ask the patient the 12 questions of the OSDI questionnaire, and circle the number in the box that best represents each answer.

Note: The patient needs to answer 12 questions and give a score from 0 to 4 to each answer.

1.4.3. Calculate the sum of the scores of each question, and the total number of questions answered.

1.4.4. Assess the severity of patient's ocular discomfort symptoms using the specific chart.

1.5. **Fitzpatrick skin phototype assessment**

1.5.1. Determine the patient's Fitzpatrick skin phototype score basing on the color of the patient's skin and its reaction to sun exposure, as previously described by Fitzpatrick[17].

Note: Patient's Fitzpatrick skin phototype score is necessary to determine the proper treatment parameters for each patient.

2. Intense Pulsed Light (IPL) treatment

2.1. Seat the patient comfortably in a treatment chair or lying down on a couch in office.

2.2. Press the start button on the LCD touchscreen of the control unit to activate the device.

2.3. Select the treatment for MGD by pressing the proper button on the LCD touchscreen.

2.4. Select the desired treatment energy level using the up and down arrows on the LCD touchscreen and then press on the **Tick** button to confirm the choice. Six different energy levels can be chosen, ranging from 9 J/cm^2 to 13 J/cm^2.

Note: Treatment energy level is determined basing on the Fitzpatrick skin phototype grading scale from I to V (grade VI is not suitable for IPL treatment). Darker skins need lower energy levels.

2.5. Validate each of the six safety features by pressing the six icon-buttons one by one and then press the **Tick** button to confirm that the procedure is performed in total safety.

Note: A summary with all the selections made will be displayed on the LCD touchscreen.

2.6. Press the **Tick** button to start the device and wait a few seconds until the device is ready for the treatment.

2.7. Put the eyewear or the eye mask on the patient's eyes in order to protect eyes from the emitted therapeutic lights.

2.8. Wear safety glasses to protect the eyes from the emitted light (200-1400 nm).

2.9. Apply a thick layer of optical gel over the skin under the lower eyelid, from the edge of the nose up to the temple, in order to conduct the light and help to spread the energy homogeneously.

Note: The gel layer should be at least 1 cm thick.

2.10. Take the device handpiece from the central unit and gently push its head in direct contact with the skin of the area to be treated.

2.11. Press the **Start** button over the handpiece to emit a single treating IPL pulse.

2.12. Apply 5 single IPL pulses distributed along the area, starting from the inner canthus and progressing to the temporal area, trying to be as close as possible to the lower eyelid margin in order to obtain the greatest effect. **(Figure 1)**

Note: The upper eyelids are not treated directly because of the risk of light penetration through the eyelid with possible light absorption and subsequent structural damage and inflammation of the intraocular pigmented structures (*i.e.*, iris tissue, ciliary body, uveal tissues).

2.13. Remove gently the optical gel from the treated skin area.

2.14. Place a warm compress over the eyelids of both eyes for 2-3 min.

2.15. Repeat the same procedure for the other eye, if required.

Note: The total time session lasts only few minutes (about 10 min). Based on the physician's choice, the meibomian gland of the lower eyelid of both eyes could be manually expressed after IPL treatment using expressor forceps or two cotton swabs.

3. Post-treatment Therapy

3.1. Instill antibiotic plus steroid eye drops immediately after the procedure.

3.2. Prescribe topical steroid eye drops 2 times per day for the following 10 days after the first session of IPL.

Note: Patients are encouraged to continue warm compresses and topical lubricants use at least twice daily during the treatment period.

3.3. Repeat IPL treatment after 15 and 45 days to complete the starting treatment protocol.

Note: The standard protocol consists of 3 sessions at day 0, day 15 and day 45. Another additional optional session could be performed at day 75, based on the physician and the patient's preferences. Repeated treatments could be necessary at regular follow-up to maintain the efficacy after the 3 initial sessions (loading-phase), depending on the single clinical case.

4. Post-treatment Assessment

4.1. Examine carefully the patient' eyes and eyelids at the slit lamp.

4.2. Repeat noninvasive BUT, LLT, meibography and tear osmolarity to evaluate the treatment efficacy.

4.3. Administer the OSDI questionnaire to the patient to evaluate the patient's symptoms.

4.4. Ask the patient whether he perceived improvements from his baseline ocular discomfort symptoms according to a 5-grade scale: none = 0, trace = 1, mild = 2, moderate = 3, high = 4.

Note: It is recommended to wait at least 15 days after the third session before evaluating the clinical results obtained with the procedure.

REPRESENTATIVE RESULTS:

Nineteen patients (7 males and 12 females, mean age 39.3 ± 7.0 years) (mean ± standard deviation) underwent IPL treatment between September 2016 and June 2017. Fifteen days after the third IPL treatment session, noninvasive BUT significantly increased from 7.6 ± 0.6 s to 9.8 ± 0.7 s (mean ± standard error of the mean) (p = 0.017), and LLT grade significantly improved from 2.3 ± 0.1 to 3.4 ± 0.3 (p = 0.003). No statistically significant changes were found for MGL and tear osmolarity (23.9 ± 3.6% vs 25.4 ± 2.6% and 304.5 ± 2.4 mΩ/L vs 300.6 ± 2.4 mΩ/L, respectively; always p>0.05) **(Figure 2)**. In addition, mean OSDI score did not differ significantly before and after the last treatment session (p>0.05).

Seventeen patients (89.5% of the total) showed an improvement of ocular discomfort symptoms after the treatment (mean grade 2.0 ± 1.2 out of 4). **Figure 3** shows the distribution of patients' perceived improvement in symptoms according to the 5-grade scale. The perceived

improvement in symptoms was significantly correlated with the improvement of LLT after the treatment (r = 0.476, p = 0.039).

FIGURE LEGENDS:

Figure 1. Treatment area. The treatment area includes the lower eyelid, from the inner to the lateral canthus, the cheekbone and the temporal zone. Each red rectangle schematically represents the site of a single IPL pulse application. The numbers indicate the sequence of IPL pulse applications.

Figure 2. Automated ocular surface workup before and after the treatment. Noninvasive BUT, lipid layer thickness (LLT), meibomian gland loss (MGL) and tear osmolarity before and 15 days after the third intense pulsed light treatment session. Error bars represent the standard error of the mean.

Figure 3. Patients' perceived improvement in dry eye symptoms after the treatment. The distribution of the patients according to the 5-grade scale about their perceived improvement in symptoms assessed 15 days after the third intense pulsed light treatment session.

DISCUSSION:

Intense pulsed light devices employ xenon gas-filled flash lamps to produce non-laser high intensity pulses of polychromatic non-coherent lights in a broad wavelength spectrum, from visible (515 nm) to infrared (1200 nm)[18]. The light energy pulse is released by the head of the handpiece by means of a sapphire or quartz block, and so directly applied onto the skin surface. The mechanism of action of IPL systems is based on the principle of selective thermolisis, according to which certain targets, called chromophores, are capable of absorbing and then convert the light into heat energy[12].

The particular characteristics of IPL devices make them capable of the simultaneous emission of different wavelengths lights (green, yellow, red and infrared), which allow to target at the same time the two main chromophores present in human skin, namely hemoglobin and melanin[14,18]. Intense pulsed light treatment has been utilized for years in the field of dermatology, and recently, its use was translated to ophthalmology for the treatment of MGD, as a result of the improvement of dry eye symptoms in patients who underwent IPL treatment for facial rosacea[19].

We describe the use and the therapeutic effects of a recently commercialized IPL device that was developed specifically for the treatment of MGD. In this device, IPL emission have been "regulated" in a multiple polychromatic train of calibrated and homogenously sequenced pulses.

To date, previous studies reported some improvements in terms of lid margin features (*e.g.*, lid thickening and vascularity, telangiectasias, the number of plugged glands) and the secretion quality and expressibility of the meibomian glands after IPL treatment[20-24]. However, these measures are subjective, and prone to observer bias due to a low degree of standardization.

Conversely, in order to overcome these drawbacks and improve the objectivity of the data, we used an automated quantitative analysis of ocular surface to evaluate the dry eye disease course after IPL treatment [25,26].

In our patients, noninvasive BUT significantly increased after IPL treatment, as previously reported by other research groups[11,20-23,27], as a result of an improvement of tear film stability and a reduction in the tear evaporation rate. In addition, we found a significant increase of the tear film characteristics and the quality measured by LLT, in agreement with Craig, *et al*.[27].

No significant changes were found for the tear osmolarity after IPL treatment, in agreement with other studies[21,23,27]. Although the recent TFOS DEWS II identified evaporation-induced tear hyperosmolarity as the core mechanism of dry eye disease[8], MGD alone, without other ocular surface abnormalities, perhaps may not be sufficient to alter this parameter, particularly in milder or early stages of the disease. In fact, it should be highlighted that in our study, as well as in other MGD populations, tear osmolarity values were within the normal range[21,26,28,29]. Furthermore, the area of MGL did not change after IPL treatment in our patients. Conversely, the only previous study, which evaluated this parameter by noncontact infrared meibography, reported a 5% decrease of MGL after IPL treatment, suggesting a possible effect of this therapy upon meibomian gland parenchima[30].

We investigated the patients' subjective ocular discomfort by administering the OSDI questionnaire and a five-grade scale specifically focused on the patients' perceived improvement in symptoms after the treatment. Despite the lack of a significant decrease of OSDI score after IPL, the vast majority of patients reported an overall improvement of ocular discomfort symptoms, and in about one third of the patients, this improvement was classified as moderate or high. These findings are in agreement with previous studies, which employed both validated questionnaires[21-24,27,31] and specific scales of satisfaction[11,20].

A careful patient selection is extremely important to obtain the best results from IPL. In fact, the treatment selectively acts on meibomian glands, whose dysfunction is the cause of evaporative DED, the most common subtype of dry eye. However, since DED is a multifactorial condition, the therapeutic approach should be dynamic, aiming at treating the dominating mechanism during the natural course of the disease. Therefore, IPL could be taken into consideration also in mixed forms of dry eye but in combination with other therapeutic options available.

IPL treatment provides an improvement of ocular surface parameters and discomfort symptoms. Regular repeated treatments may be required after the 3 starting sessions to maintain the beneficial effects of the procedure over the time. However, there is still no consensus about the time interval among treatments, which conversely should be customized according to the patient's satisfaction and changes of objective parameters.

The major limitation of the present study is represented by the lack of a control group. Additionally, the relatively small size of population might hamper the detection of further significance in case of small differences among parameters.

In conclusion, IPL for the treatment of patients with dry eye owing to meibomian gland dysfunction improved noninvasive break-up time and lipid layer thickness, as well as subjective symptoms.

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DISCLOSURES:
The authors have nothing to disclose.

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Figure 1

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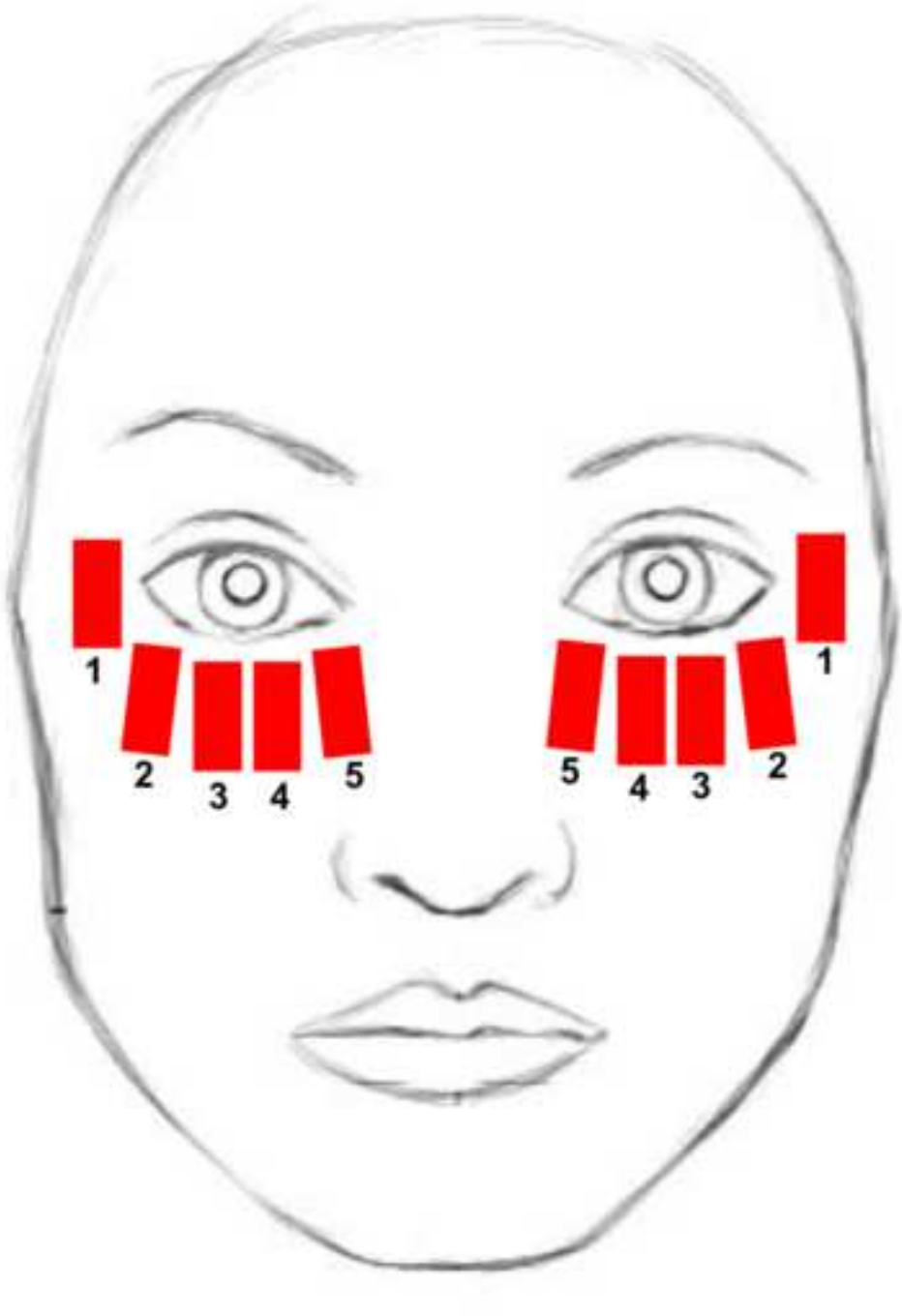

Figure 2

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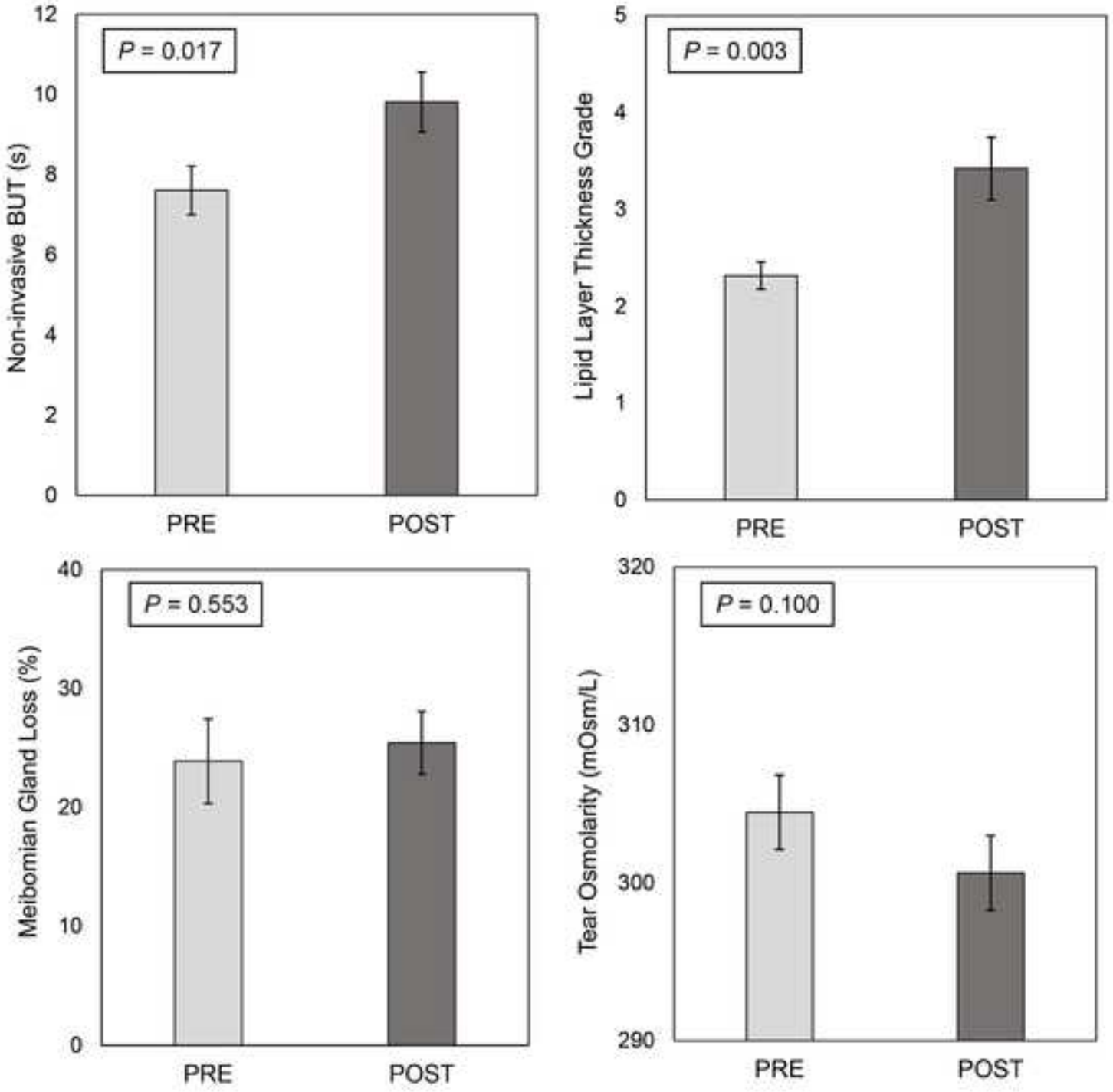

Figure 3

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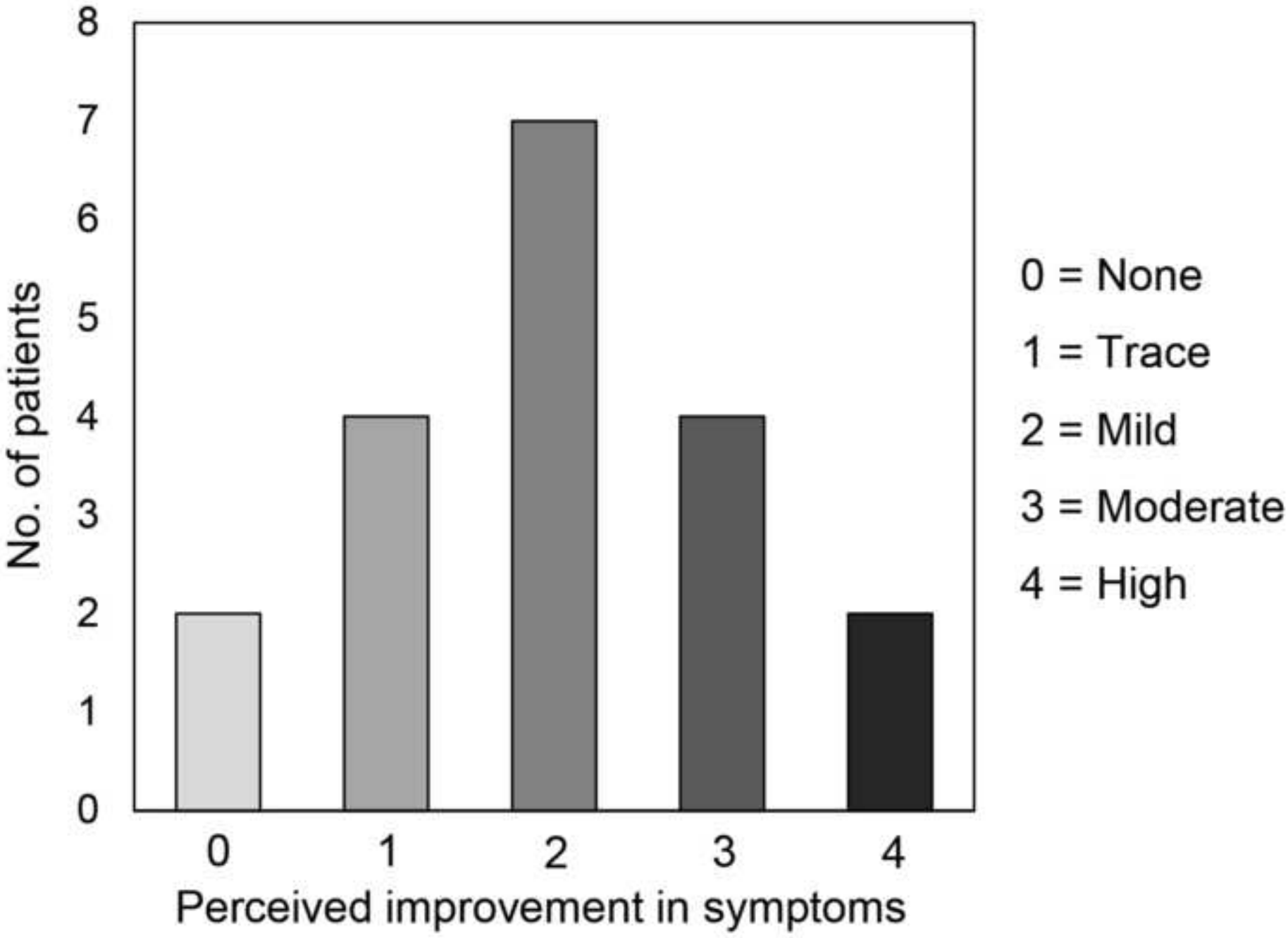

Materials Table

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Name of Material/ Equipment	Company	Catalog Number
I.C.P. Tearscope	SBM Sistemi, Turin, Italy	1340864/R
I.C.P. MGD	SBM Sistemi, Turin, Italy	15006
TearLab Osmolarity System	TearLab Corporation, San Diego, CA, US	83861QW
E>Eye	E-Swin, Paris, France	
BM 900 Slit Lamp Biomioscropy	Haag-Streit, Koeniz, Switzerland	BM 900
Tobradex eye drops	Alcon Inc., Fort Worth, TX, USA	S01CA01

Comments/Description

Device for noninvasvive break-up time and lipid layer thickness evaluation
Device performing infrared meibomography and meibomian gland loss evaluation

Device for the measurement of tear osmolarity
Intense pulsed regulated light treatment device

Slit Lamp Biomiscroscopy
Eye drops instilled immediately after the procedure in office

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Title of Article: INTENSE REGULATED PULSED LIGHT FOR THE TREATMENT OF DRY EYE OWING TO MEIBOMIAN GLAND DYSFUNCTION

Author(s): LUCA VIGO, GIUSEPPE GIANNACCARE, STEFANO SEBASTIANI, MARCO PELLEGRINI, FRANCESCO CARONES

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REBUTTAL COMMENTS to manuscript 57811_R0 "Intense Pulsed Light for the Treatment of Dry Eye Owing to Meibomian Gland Dysfunction"

We appreciate the opportunity to revise our work for consideration for publication on Journal of Visualized Experiments journal, and we thank the Editor and the Reviewers for their helpful comments. We agree with the points raised and carefully addressed them as follows:

Author's responses are written in red.

Editorial comments #1:
The manuscript has been modified and the updated manuscript, 57811_R0.docx, is attached and located in your Editorial Manager account. Please use the updated version to make your revisions.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
A native English speaker revised and proofread the manuscript, as suggested.

2. For in-text referencing, please put the reference number before a comma or a period.
We modified and corrected all in-text references according to your indication.

3. Please revise the text in Protocol to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).
We reviewed the Protocol section and eliminated the use of personal pronouns.

Editorial comments #2:
The manuscript has been modified and the updated manuscript, 57811_R0.docx, is attached and located in your Editorial Manager account. Please use the updated version to make your revisions.

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Yes, done.

2. Please ensure that the references appear as the following:
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For more than 6 authors, list only the first author then et al.
Yes, ok. We corrected the references list according to your indication.

3. Please define all abbreviations before use.
Yes, done.

4. Please revise the table of materials to include all essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file.
Yes, we revised the table of materials, including all essential supplies used in the protocol section.

5. Please use standard SI unit symbols and prefixes such as µL, mL, L, g, m, etc., and h, min, s for time units.
Yes, done.

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Yes, we do not use any figure from previous publications.

7. Figure 2: Please define the error bar.
Yes, ok. We defined the error bar in the legend of the figure 2.

8. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.
Yes, done.

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Yes, ok. We deleted all references to commercial materials and products from the manuscript.

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Yes, ok. We used a more generic language in manuscript trying to avoid the use of commercial names. We only leave the name of the application downloadable on the App Store.

11. Step 1.1.5: Please specify how far is the lens from patients' eyes. How long should the patients keep their eyes open?
Thank you for your indications that allow us to better describe this point of the protocol section. We added the following sentence in the protocol section (items 1.1.5 and 1.1.6): "at about 1-2 cm of distance." (line 134) and "1.1.6) Ask the patient to blink twice and then stare without blinking for as long as possible." (line 136).

12. 1.2.4: How to evert the lower eyelid? By hand?
We used a cotton swab to evert the lower eyelid. We added the following sentence to the item 1.2.4 of the protocol section: "using a cotton swab." (line 158).

13. 1.3.2 How to move down the eyelid?
Also in this case we used a cotton swab to gently move down the lower eyelid. We added the following sentence in the protocol section: "using a cotton swab" (line 182).

14. 1.4, 1.5: This step cannot be filmed. Please do not highlight.
Yes, ok.

Reviewers' comments:

Reviewer #1:
This reviewer has several questions and comments related to the article, entitled "Intense regulated pulsed light for the treatment of dry eye owing to meibomian gland dysfunction."
*In the Introduction, line 74, the phrase "Tear Film Ocular Surface" should be change to "TFOS."
Yes, we agree and we corrected the sentence according to your indication.

*In the Introduction, line 80, the authors state "MGD is caused by a chronic alteration and inflammation of the lipid sebaceous glands." The authors should replace "lipid sebaceous" with "meibomian." All sebaceous gland release lipid, and the authors need to distinguish between the non-meibomian sebaceous glands and the meibomian glands (MGs), all of which are in the lid. Also, a global consensus is that obstructive MGD is due to hyperkeratinization of the MG external duct and a reduced quality of meibum (Knop E et al, Invest Ophthalmol Vis Sci 2011;52:1938-1978; Bron et al, Ocul Surf 2017;15:438-510).
We agree that “meibomian” is a more appropriate term to describe the particular glands of the lid involved in this disease. We corrected the sentence and replaced the term as suggested.

*The authors should rephrase line 80. There is no peer reviewed evidence of any inflammation within the meibomian glands in obstructive MGD (e.g. Knop E et al, Invest Ophthalmol Vis Sci 2011;52:1938-1978; Bron et al, Ocul Surf 2017;15:438-510). If present, inflammation occurs in the periglandular conjunctiva (i.e. posterior blepharitis).
We agree that inflammation in MGD is mainly located in the periglandular conjunctiva. We modified the sentence in question according to your corrections as follows: “Meibomian gland dysfunction is caused by a chronic alteration of the meibomian glands that are located inside the upper and lower eyelids. The disease is characterized by hyperkeratinization of the external duct of the glands and obstruction of the orifices with insufficient, not functional, production of the external lipid layer of the tear film, resulting in tear instability.” (line 77-81). We also added the two important references that you suggested.

*In items 1.3.6 and 2.15 the task should read "Repeat the same procedure in the other eye."
Thank you for your correction. We corrected the points 1.3.5 and 2.15 according to your suggestions. In addition, we also added the sentences “Repeat the same procedure to the other eye, if required.” as point 1.1.8 and 1.2.8, to indicate that all the examinations should be performed for both eyes, if required.

*If the authors are concerned about safety of the IRPL treatment (see 4.1), why do they wait until 15 days after therapy to check? Why not immediately after the procedure?
Safety of the procedure is not a major concern since it is well tolerated and no adverse events were reported in any of the previous studies. We modified the item 4.1 as follows: “4.1) Examine carefully patients’ eye and eyelid at the slit lamp at any follow-up visit.” We think it is not necessary to examine the patient immediately after the procedure.

*Are the values in Figures 2 and 3 mean ± SE or SD?

In Figure 2 the values are now expressed as mean ± standard error of the mean (we modified the error bars to standard error of the mean as suggested by Reviewer #2).
Figure 3 shows the number of patients who refer each one of the 5 grade of the subjective improvement scale. We described the mean value (± standard deviation) of subjective improvement in the text (lines 328-329): ”Seventeen patients (89.5% of the total) showed an improvement of symptoms after treatment (mean grade 2.0 ± 1.2 out of 4) (mean ± standard deviation)”.

*In the Discussion, lines 376-379, the authors speculate that tear osmolarity may not be altered in MGD. In contrast, the recent TFOS DEWS II Pathophysiology Subcommittee concluded that the core mechanism of dry eye disease is evaporation-induced tear hyperosmolarity, which is the hallmark of the disease (Bron et al, Ocul Surf 2017;15:438-510). Further, obstructive MGD is the major cause of dry eye disease (Knop E et al, Invest Ophthalmol Vis Sci 2011;52:1938-1978; Bron et al, Ocul Surf 2017;15:438-510), and osmolarity measurements are recommended for the evaluation of dry eye disease in patients with MGD (Wolffsohn et al, Ocul Surf 2017;15:539-574). In effect, it would appear that IRPL does not alter tear osmolarity (i.e. instead of tear osmolarity not reflecting MGD). Would the authors balance their speculation?

Yes, we agree that the core mechanism of dry eye disease is evaporation-induced tear hyperosmolarity, as stated in the TFOS DEWS II (Bron et al, Ocul Surf 2017;15:438-510), and we modified the discussion section accordingly: “Although the recent TFOS DEWS II identified evaporation-induced tear hyperosmolarity as the core mechanism of dry eye disease, probably MGD alone, without other ocular surface abnormalities, may not be sufficient to alter this parameter, particularly in milder or early stages of the disease.” (lines 389-392).
However, not all patients with evaporative dry eye have pathological values of tear osmolarity (particularly those affecred by mild dry eye), as occurred in our series.
As reported by Sullivan et al. (Sullivan et al, Invest Ophthalmol Vis Sci 2010;51:6125-30), mild/moderate dry eyes have average tear osmolarity values of approximately 315 ± 10 mOsm/L. Tear osmolarity values are even slightly lower in our treated patients (before: 304.4±2.3 mOsm/L; after 300.6±2.3 mOsm/L). Currently, 308 mOsm/L is proposed as a sensitive threshold for discriminating between normal eyes and those presenting with early stages of DED (Bron et al, Ocul Surf 2017;15:438-510). According to this threshold, almost the totality of our patients has tear osmolarity values within normal limits both before and after the procedure. It is conceivable that patients with more severe MGD compared to those enrolled in our study may present higher (pathological) tear osmolarity values. However, it is also true that the procedure seems to not alter tear osmolarity, as yourself stated.

*In the Discussion, line 396, the authors might consider noting that the reduced tear cytokine levels might lessen the impact of inflammatory mediators, that may be promoting keratinization of the MG terminal duct.
We agree that the reduction of tear film cytokine levels might reduce the keratinization of the mebiomian gland terminal ducts promoted by the inflammatory mediators. We modified the sentence as follows: “Furthermore, the procedure reduces the release of inflammatory mediators and the levels of tear cytokines, which may promote the keratinization of mebomian glands terminal duct.” We also moved the sentence in the introduction section as suggested by Reviewer #2 (lines 97-98)

*In the Discussion, lines 404-405, the authors suggest that IPL stimulates the parasympathetic nerve pathway to alleviate the obstruction in MGD. Would they provide a reference to support this statement?
Yes, we agree that to date there is no scientific evidence supporting this theory that conversely was formulated by the manufacturers. As consequence, we deleted the sentence in question from the discussion section.

*Throughout this article there are numerous instances of awkward phraseology, misspelled words, and incorrect use of words. It would be very helpful if the authors revised this manuscript with the assistance of an English editor.
We agree that some typos and grammatical errors were present in the text. A native English speaker reviewed the manuscript making corrections along the entire manuscript.

*Would the authors report the source of funding for the research reported in this article?
We did not receive any founding for this research project.

Reviewer #2:
Manuscript Summary:
The manuscript is generally well written, although would benefit from some copyediting to improve the grammatical structure of some sentences. With the interest in IPL use for MGD, this paper will be a valuable addition to the literature.

Major Concerns:
No major concerns.

Minor Concerns:
Introduction: Copyeditting required in general, but more specifically the link between inflammation and MGD (touched on line 80) and the proposed mechanism of action of IPL (line 92) could be expanded, as this justifies the method over other treatments. Perhaps some of the discussion regarding mechanism could be moved from discussion (from lines 392) to intro?
Yes, we agree that the proposed mechanism of IPL action in the introduction section could be expanded. As proposed, we moved the paragraph in question from the discussion section (from line 392) to the introduction section (lines 93-103).

While I realise this may be due to claims made by this specific IPL manufacturer (wanting to differentiate their device and their 'sculpted' wavelengths), the usage of IRPL is uncommon, especially when compared to 'IPL'. As all IPL light systems are regulated to some degree and this is really just unnecessary marketing jargon. I would suggest replacing all usages of IRPL to IPL to improve consistency, familiarity and searchability. The detail regarding this in the conclusion could also be removed, as the citation provided does not compare the efficacy of different wavelengths of light.
Yes, we agree with your considerations. As suggested we replaced “IRPL” with “IPL” along the entire manuscript.

Methods: Well written, I only have minor notes:

"Download the "I.C.P." application in your tablet". may not be specific enough, as when I tried searching for this it found a group of musicians. Is there any way to link directly to the download link?
Thank you for your question. We agree that could be difficult to find the "I.C.P. by SBM Sistemi" application, that is available on the App Store and is compatible with Ipad. The app is free and the direct link for downloading it is the following: https://itunes.apple.com/us/app/i-c-p-by-sbm-sistemi/id1002330054?mt=8.
We added the complete name of the application and the direct download link in the protocol section: item 1.1.1 (lines 124-125) and item 1.2.1 (lines 149-150).

Line 127: remove 'perfectly', as impossible when handheld.
Ok, done.

Line 266: Q-tips is a brand name which is not internationally available (or familiar). Suggest cotton swab as replacement.
Thank you for your correction. We agree that "cotton swab" is more appropriate and we modified it along the entire manuscript, as suggested.

Line 270: May need to expand why antibiotic cover is required. Is this doxy/azith PO or gtts (may require justification/citations). Is there literature supporting the use of AB and steroid post-IPL?
There are no guidelines regarding post-IPL therapy. Therefore, as explained in the text we instilled one drop of steroid-antibiotic combination eye drops (Tobradex, Alcon Inc., TX, USA) only immediately after the procedure; then we prescribed one drop of hydrocortisone eye drops (Cortivis, Medivis, Italy) twice daily for the following 10 days.

Conclusions:
This section could benefit from some restructuring and perhaps consolidation. For example: The paragraph beginning line 407 seems highly relevant for a methods paper, yet is below the theoretically mechanism of action section (starting line 392), for example.
Yes, we agree that some part of the discussion section could be restructured and consolidated. We made some corrections to improve this issue. We moved lines 392-405 from the discussion section to the introduction section (lines 93-103), as you suggested before. In addition, we modified the lines 407-411 as following: "A careful patient selection is extremely important to obtain the best results from IPL. In fact, the treatment selectively acts on meibomian glands, whose dysfunction is the cause of evaporative DED, the most common subtype of dry eye.
However, since DED is a multifactorial condition, the therapeutic approach should be dynamic, aiming at treating the dominating mechanism during the natural course of the disease. Therefore, IPL could be taken in consideration also in mixed forms, in combination with the other therapeutic options available." (lines 406-412).

Line 358: Consider removing, as no evidence this is true (and others get similar results with different IPL systems). Does this claim only come from the manufacturer?
We agree that other IPL devices achieved similar results in the previous published data. Therefore, we removed the sentence (lines 358-360) as suggested.

Line 403: Citation required (again, not just manufacturers claim).

To date there is no scientific evidence supporting this theory that conversely was supported by unpublished data from the manufacturers. As consequence, we deleted the sentence in question from the discussion section.

Figures: What are the error bars? For the purposes of a methods paper, I would suggest that group comparisons are more relevant so the error bars should reflect standard error of the mean (or even better, 95% confidence intervals of the mean).
We agree that standard error of the mean is more appropriate to depict the error bars in Figure 2. We modified the errors from "standard deviation" to "standard error of the mean" both in Figure 2 and in the text (lines 323-326), as you suggested. We changed accordingly Figure 2 legend: "Error bars represent the standard error of the mean." (lines 344-345).

Reviewer #3:
Manuscript Summary:
See below

Major Concerns:
There are no details about the Methods used. Methods should be described clearly and in detail.
Please see the Protocol section where we described in detail the preoperative examination performed and the treatment procedure.

The Results section is incomplete.
We improved the result section by including also not statistically significant results, please see lines 324-327.

Also, there are results presented in the Figure Legends.
We agree and we modified figure legends accordingly. In particular we modified Figure 2 legend to: "**Figure 2. Automated ocular surface workup before and after treatment.** Noninvasive BUT, lipid layer thickness (LLT), meibomian gland loss (MGL) and tear osmolarity before and 15 days after the last intense pulsed light treatment session. Error bars represent the standard error of the mean." (lines 342-345).

The Discussion section is long and wordy and filled with speculation unsupported with references.
As suggested, we shortened the discussion section and removed the speculations not supported by published data: "…ranging from 580 nm to 1200 nm wavelengths spectrum, controlled by a proprietary algorithm, which was named Intense Regulated Pulsed Light (IRPL). This technical improvement permits to best apply the treatment to the periocular area, minimizing the risks and maximizing the therapeutic effects." (lines 357-360); "Furthermore, the newly developed IPL settings and parameters employed by the device used in the present study, seems to be also able to stimulate the parasympathetic nerve pathway, resulting in the unblocking of meibomian glands ducts and in the improving of lipid secretion quality." (lines 402-405).

Minor Concerns:
Suggestions
Ln35 delete "to be able"
Yes, we agree and we did it.

Ln36 delete "sessions of"
Yes, we agree and we did it.

Ln40 delete "actually"
Yes, we agree and we did it.

Ln41 place a period after "complaints." And delete "at Ophthalmic practice"
Yes, done.

Ln47 place a period after "application." And delete "and commercialized"
Yes, we agree and we did it.

Ln52 sentence would read better as follows "IPL treatment is noninvasive, lasting only a few minutes, can be conducted in an office setting and is easy to perform. "
Yes, we agree and we modified the sentence as you suggested.

Ln56 suggest "improvement in tear film stability and quality respectively."
Yes, we agree with your suggestion and we modified the sentence accordingly.

Ln57 unsure what "area" means?
Meibomian gland loss area indicates the percentage of MGL in relation to the total area of the eyelid. Please see lines 167-173.

Ln59 delete "of" and substitute "in"
Yes, done.

Ln60 how was "rapid" measured?
We deleted “rapid” from the sentence.

Ln62 how was the "beneficial effects" measured?
We measured the effects of the treatment both objectively by analyzing ocular surface parameters (namely, noninvasive break-up time, lipid layer thickness, meibomian glan loss and tear osmolarity) and subjective symptoms scale, as described in detail in the protocol section.

Ln67 should this be "clinic" or another descriptor e.g. eye doctor?
Yes, we modified to eye “doctor”.

Ln68 suggest "symptom complaint in patients affected by DED differs from"
Yes, we agree and we modified the sentence as you suggested.

Ln70 add a "," after "tearing" and delete "and up to severe"
Yes, done.

Ln85 delete "in" and substitute "of"
Yes, we did it.

Ln87 delete "needing" substitute "need"
Yes, we did it.

Ln89 delete "the last" and substitute "recent"
Yes, we did it.

Ln90 add "such" after "diseases"
Yes, done.

Ln91 add "such" after "lesions"
Yes, done.

Ln92 add "it" after "skin," and add "such" after "structure,"
Yes, we made the changes suggested.

Protocol:
Ln105 add "were" after "participants"
Yes, we did it.

Ln111 what is I.C.P.
I.C.P. is the name of the application developed to control the device and acquire the images for noninvasive BUT, lipid layer thickness and meibomian gland loss analysis. It is downloadable from the App Store directly on the Ipad.

This whole section needs a detailed narrative explanation of the Methods.
The entire protocol section is a detailed description of methods applied for both examining patients (before and after the procedure) and performing the procedure itself.

Ln304 Why are the results only presented as "representative results"
We followed the Authors Instructions detailed by JoVE manuscript template (downloadable from JoVE site). In the template the result section is already titled as “REPRESENTATIVE RESULTS” and we did not modify the titles of the section as indicated by JoVE journal.

Ln305 What "study period" must be defined?
We added the study period in the result section: “between September 2016 and June 2017” (line 321).

Ln210 What symptoms, please define in detail?
As explained in the introduction, the main symptom complained by patients affected by DED differs from various grades of redness and ocular surface discomfort to a chronic foreign body sensation, stinging, burning, itching, excessive tearing, pain, recurrent infections and visual disturbances. However, we did not investigate one symptom in particular but we asked to the patients an overall judgment of the changes of their ocular discomfort symptoms, after the procedure. We added “ocular discomfort” (lines 328) to better characterize the features of the symptoms.

Ln312 Which 5 grade scale?
Please see item 4.4: “4.4) Ask the patient whether he perceived improvements from his baseline ocular discomfort symptoms according to a 5-grade scale: none = 0, trace = 1, mild = 2, moderate =

3, high = 4.”

Figure Legends
Ln319 delete "interests" and substitute "includes"
Yes, ok.

Ln322 delete "s" from "pulses"
Yes, ok.

The explanations in the figure captions should be worked into the Results section.
Yes, we modified the figure captions according to your indications.

Discussion
Note that this section is far to long for the minimal amount of data presented in this paper. The discussion needs to be focused and condensed to be suitable for publication.
Ln336 delete "the"
Yes, ok.

Ln337 delete "the" after "to"
Yes, ok.

Ln341 delete "to" and enter "of"
Yes, ok.

Ln341 change "absorb" to "absorbing" and delete "to" after "then"
Yes, done.

Ln344 enter "it" after "allow"
Yes, ok.

Ln359 delete "to best" and expand "apply" to "application of"
Yes, done.

Ln363 add "lid" before "thickening"
Yes, ok.

Ln370 reword to read "findings (list of reference numbers) and indicating an improvement in tear film…"
The author should carefully describe what each of these authors did. I do not believe the "authors" confirmed these findings in each of these papers.
We reviewed all the references cited in the text that reported an improvement of break-up time after the procedure. We confirmed that they are correct.

Ln371 change to "reduction in its evaporation rate."
Yes, done.

Ln374 delete "On the other hand" and begin sentence with "No"
Yes, done.

Ln376 delete "probably" and enter "perhaps"
Yes, ok.

Ln379 change to "normal"
Yes, done.

Ln381 describe how this decrease was measured.
Yin et al. (Curr Eye Res, 2017) examined meibomian glands using a noncontact infrared meibography system (Keratograph, OCULUS, German) and measured meibomian gland loss area using ImageJ software. We added in the text “by noncontact infrared meibography system” (line 395).

Ln382 delete "these" and substitute "this"
Yes, ok.

Ln383 delete "also upon" and change to "on"
Yes, done.

Ln385 which questionnaire?
We used two questionnaires: 1) OSDI validated questionnaire; 2) five-grade scale questionnaire focused on patients’ perceived improvement in symptoms after treatment.

Ln388 delete "the"
Yes, ok.

Ln392 delete "its" and enter "are promising" after the word "results" and enter the word "of" after "action" and delete "through which" and "acts"
Yes, we did the suggested changes.

Ln402 which settings and parameters?
Ln403 who said? What proof?
We deleted the entire sentence in question.

Ln407 this paragraph appears to be speculation and should be described that way. Moreover, this is not part of the study and if it is should be put in the Methods section.
We removed it.

Ln412 this should be described and entered into the Methods section.
Please see 1.5 (lines 215-220).

Ln417 it is not clear how this relates to the present study.
We removed it.

Ln419 a reference is needed here.
We removed it.

Ln422 who measured the improvement? Also a reference is needed.

The improvement of ocular surface parameters, namely noninvasive break-up time and lipid layer thickness, represents the results of the present work.

Ln429 enter "this patient" before "population"
Yes, ok.

Ln431 use abbreviation "IRPL"
Yes, done.

Ln433 consider deleting the last sentence.
Yes, done.

The Discussion needs a major rewrite before it is suitable for publication.
We modified the discussion as yourself suggested above.