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Intense Pulsed Light Therapy for Patients with Meibomian Gland Dysfunction and Ocular *Demodex* Infestation*

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Summary: To observe the clinical changes of meibomian gland dysfunctipn (MGD) and ocular *Demodex* infestation after intense pulsed light (IPL) treatment to further examine the mechanism of IPL treating patients with MGD and ocular *Demodex* infestation. The medical records of 25 patients (49 eyes) with MGD treated with IPL, were retrospectively examined to determine outcomes. Associated ocular-surface parameters (ocular surface disease index, OSDI; lipid layer thickness, LLT; noninvasive first breakup time, NIF-BUT; noninvasive average breakup time, NIAvg-BUT; tear film breakup area, TBUA; Schirmer I Test, SIT; corneal fluorescein staining, CFS), eyelid margin abnormalities, meibum quality and expressibility, MG morphological parameters (macrostructure and microstructure), and the number of *Demodex* infestation were examined before and after treatment. The MG microstructure and the *Demodex* infestation were examined via *in vivo* confocal microscopy (IVCM). The results showed that there were statistically significant differences in associated ocular-surface parameters (all $P<0.05$) before and after IPL treatment, except SIT ($P=0.065$). Eyelid margin abnormalities, meibum quality and expressibility obviously improved in upper and lower eyelid after IPL treatment (all $P<0.0001$). MG macrostructure (MG dropouts) decreased in upper ($P=0.002$) and lower eyelid ($P=0.001$) after IPL treatment. The nine parameters of MG microstructure in upper and lower eyelid all distinctly improved after IPL treatment (all $P<0.0001$). The mean number of *Demodex* mites on the upper lid margin (6.59±7.16 to 3.12±3.81/9 eyelashes) and lower lid margin (2.55±2.11 to 1.29±1.53/9 eyelashes) significantly reduced after IPL treatment (all $P<0.0001$). The *Demodex* eradication rate was 20% (8/40) in upper lid margin and 34.15% (14/41) in lower lid margin. These findings indicate that IPL shows great therapeutic potential for patients of MGD and ocular *Demodex* infestation.
Key words: intense pulsed light; meibomian gland dysfunction; *Demodex*

Meibomian gland dysfunction (MGD) is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion[1]. MGD may be symptomatic in its own right or give rise to symptoms through its contributions to ocular surface damage or to dry eye, such as ocular surface burning and stinging sensation, photophobia, redness, pain, visual blurring, and so on. The clinical features of MGD include orifice plugging, duct obstruction and dilatation, gland atrophy and dropout, qualitative and quantitative changes in secretions, lid margin hyperemia and telangiectasia. MGD patients suffer from abnormalities of function and morphology of Meibomian gland (MG). The superficial location of the MG in the tarsal plates permits it to be quantified via noncontact infrared meibography and *in vivo* confocal microscopy (IVCM). MG dropout refers to the loss of acinar tissue detected by meibography, and several investigators have elucidated that MG dropout is a useful index of obstructive MGD[2–4]. From a microscopic point of view, IVCM has been reported to be useful in describing the morphologic alterations of MG in MGD, such as the decrease of MG acinar density, the enlargement of acinar diameter, and the diminution of MG orifices[5, 6].

The ocular *Demodex* infestation can cause blepharitis and ocular surface disease[7], leading to eye discomfort, so it has attracted more and more attention of ophthalmologists. In recent years, many investigators have reported that *Demodex* infestation was often

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associated with MGD[8–12]. Ocular *Demodex* mite can give rise to inflammation of anterior lid margin, which can lead to properties alteration or discharge disorder of the MG secretion when the inflammation develops continuously until involving posterior lid margin, especially the region of the MG orifice. Thus MGD occurs, or the degree of MGD is aggravated.

The exact cause of MGD is unknown, so it does not have an exact treatment. Commonly applied current therapies include artificial tears, warm compresses, manual gland expression, lid hygiene, omega-3 fatty acid supplementation[13], topical cyclosporine[14], serum tears, topical azithromycin[15], oral doxycycline[15], moisture chambers, and others. Therapies above not only provide transient relief, but also the effect that is not obvious, greatly reducing the quality of life of patients, which is considered afflictive by clinicians and suffering patients.

It was found that in patients receiving intense pulsed light (IPL) for treatment of rosacea, their ocular surface condition was improved concurrently, and then IPL therapy begins to be considered as a potential treatment for MGD. It has been demonstrated that IPL is a safe and effective approach for MGD management[16–20]. Nevertheless, the mechanism of IPL in the treatment of MGD remains unclear. Several conjectures, such as photothermal effect, closing abnormal vasculature, relief of inflammatory and neurogenic pain and reducing bacteria loads, remain under discussion[17, 21]. Despite many reports outlining efficacy of IPL treatment in MGD, research quantifying the alterations of MG morphology before and after IPL treatment is still sparse, and the difference of ocular *Demodex* infestation under IVCM between pretherapy and post-treatment of IPL is not reported yet.

In this study, we evaluated the alteration of MG function and morphology in MGD patients after receiving IPL therapy. At the same time, we used IVCM to observe ocular *Demodex* infestation and took count of them before and after IPL therapy. The results reported in this study will contribute to further exploring the potential mechanisms of IPL treating MGD and discerning whether IPL treatment can serve part functions on eradication of *Demodex* mite.

1 MATERIALS AND METHODS

1.1 Subjects

This study was approved by the Ethics Committee of Union Hospital affiliated to Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, and was conducted in a strict accordance with the Helsinki Declaration. All participants provided written informed consent for IPL treatment.

The medical records of 25 MGD patients (49 eyes) treated with IPL between July 2018 and November 2018 were retrospectively examined to determine outcomes. Demographics, ocular and systemic histories were reviewed. In our study, patients who were diagnosed with MGD (>stage 1)[1] and failed in or refused to (because of side effects, cost, or other reasons) conventional treatments for at least 3 months were treated with IPL. Patients were excluded if one of the following conditions was recorded in the medical records: (1) eye or body suffering from acute inflammation, (2) being on local (eg. eye drops or eye ointment) or systemic medication, (3) having ocular trauma, ocular deformity scar, exophthalmos, eyelid insufficiency, (4) having undergone eye surgery within 3 months, (5) having diabetes mellitus, rheumatism, immune diseases and other serious systemic diseases, (6) eyelid and periorbital dermatosis within 1 month, (7) excessive sun exposure in 1 month, (8) skin Fitzpatrick scale V/VI[22], (9) pregnant or lactation patients. In additing, outcomes of OSDI scores, slit-lamp examinations and IVCM of included patients at baseline and 1 week after the last IPL treatment were also reviewed.

1.2 IPL Treatment

The IPL Machine (Icon Aesthetic System, USA) was used for all patients. This IPL device emitted energy in a band from a base of the visible (500 nm) to near infrared spectrum (1200 nm), and the energy range of the treatment was 30–34 J/cm^2. Potential IPL candidates underwent Fitzpatrick skin typing to classify their skin response to ultraviolet exposure by the degree of burning and tanning[22]. Fitzpatrick skin types Ⅰ, Ⅱ, Ⅲ, and Ⅳ were included as recommended by the manufacturer, and Ⅴ and Ⅵ were excluded, for darker skins cannot tolerate IPL and ware relatively prone to side effects such as depigmentation[17]. Before treatment, the patients received topical ophthalmic oxybuprocaine hydrochloride eye drops (Santen Pharmaceutical Co., Ltd, Japan) into the conjunctival sac three times. Then protective metal shields were placed over the cornea and sclera of each therapeutic eye. After generous application of ultrasonic gel to the periocular skin of upper and lower eyelids, IPL treatment was administered in 6 treatment areas from the nasal to the temporal side on each eyelid, for a total of 8–12 overlapping IPL pulses approximately each eye, with each pulse action area was 10 mm×15 mm or 4 mm×4 mm (When the upper eyelid skin was bumpy or the upper eyelid edge was very close to the eyebrows). Each course of IPL treatment was conducted within 42 days, with four separate treatment sessions on days 1, 7, 21, and 42 according to the manufacturer recommendations (the specific interval date was adjusted slightly by natural scabbing). During the treatment period, all patients used sodium hyaluronate eye drops (Santen Pharmaceutical Co., Ltd, Japan) four times a day.

1.3 Evaluation Parameters

Before and after the last IPL treatment, each patient had a full ocular evaluation, with both upper eyelid and lower eyelid included.

1.3.1 Associated Ocular-surface Parameters (1) Ocular surface disease index (OSDI): the OSDI was a valid and reliable scale for assessing the severity of dry eye disease, with 3 subscales (12-item) of vision-related function, ocular symptoms, and environmental triggers. According to the severity of dry eye symptoms, the OSDI score ranges from 0 to 100, with asymptomatic patients scoring 0 and the most symptomatic patients scoring 100[23]. (2) Tear film lipid layer thickness (LLT): LLT was detected by LipiView® Ocular Surface Interferometer (TearScience, Inc., USA). An interferometric color unit (ICU) (1 ICU=1 nm) represented the lipid distribution, and the ICU value 1 means the tear film LLT being 1 nm[24]. Because the exact value of LLT cannot be measured precisely or shown by this instrument if the LLT value exceeds 100 nm, we artificially set the LLT to 100 nm in the cases where LLT was ≥100 nm. (3) Noninvasive first breakup time (NIF-BUT), noninvasive average breakup time (NIAvg-BUT) and tear film breakup area (TBUA): we used a noninvasive method via Sirius (Sirius system CSO, Italian) to measure these three indicators to assess the stability of the tear film. The total duration of shooting was 17 s. (4) Schirmer I Test (SIT): SIT was used to evaluate the tear amount of each eye. the process lasted for 5 min without providing topical anesthesia. (5) Corneal fluorescein staining (CFS): the cornea was divided into 4 quadrants. Each quadrant was graded on a scale of 0 to 3. Total CFS of 4 quadrants ranged from 0 to 12.

1.3.2 Eyelid Margin Abnormalities[25] (1) Vascularity of lid margins was graded by a 4-point scale: 0=No or slight redness in lid margin conjunctiva and no telangiectasia crossing meibomian gland orifices; 1=Redness in lid margin conjunctiva and no telangiectasia crossing meibomian gland orifices; 2=Redness in lid margin conjunctiva and telangiectasia crossing meibomian gland orifices with a distribution of less than half of the full length of the lid; 3=Redness in lid margin conjunctiva and telangiectasia crossing meibomian gland orifices with a distribution of half or more of the full length of the lid. (2) Plugging of meibomian gland orifices was graded by a 4-point scale: 0=No plugging of gland orifices; 1=plugging of fewer than 3 gland orifices; 2=plugging of 3 or more gland orifices with a distribution of less than half of the full length of the lid; 3=pluggings of 3 or more gland orifices with a distribution of half or more of the full length of the lid. (3) Lid margin irregularity was assessed by a 3-point scale: 0=No lid margin irregularity; 1=Fewer than 3 lid margin irregularities with shallow notching; 2=Three or more lid margin irregularities or deep notching. (4) Lid margin thickening: 0=No lid margin thickening; 1=Lid margin thickening with or without localized rounding; 2=Lid margin thickening with diffuse rounding.

1.3.3 Meibum Quality and Expressibility[26] (1) Meibum quality: MG glands in the central parts of the eyelid were assessed using a scale of 0–3 : 0, clear fluid; 1, cloudy fluid; 2, cloudy particulate fluid; and 3, inspissated, like toothpaste. (2) Expressibility: Five MG glands in the central part were evaluated on a scale of 0–3: 0, all glands expressible; 1, 3–4 glands expressible; 2, 1–2 glands expressible; and 3, no glands expressible.

1.3.4 MG Morphological Parameters (1) MG macrostructure parameters: the MG dropouts were observed via a noncontact infrared meibography system (Sirius system, CSO, Italian), with the ratio automatically calculated via its own system. (2) MG microstructure parameters: *in vivo* confocal microscopy (HRTIII Corneal Rostock Module, Heidelberg Engineering GmbH, Germany) was performed on all subjects. The MG acinus and MG orifice were examined, and the corresponding images were collected and stored. Three non-overlapping, high-quality IVCM images of MG were randomly selected from the nasal, central and temporal sides of each eyelid (a total of 9 images per eyelid). The following variables were quantitatively determined: (1) MG acinar density (MAD) (The acinus manually marked inside each 400 μm×400 μm frame and the density was automatically calculated using HRT3 cell counting system), (2) MG acinar longest diameter (MALD), (3) MG acinar shortest diameter (MASD), (4) MG orifice area (MOA) (the area was calculated automatically by ImageJ software, developed by Wayne Rasband, National Institutes of Health, Bethesda, USA), (5) severity of MG fibrosis (MF), (6) MG acinar irregularity (MAI), (7) meibum secretion reflectivity (MSR), (8) inhomogeneous appearance of walls (AWI), and (9) periglandular interstices of acinar units (API), (10) the number of *Demodex* mite. The MF was scored on a 3-point scale, with no fibrosis listed as 0, fibrosis in less than half of the lower eyelid as 1 and fibrosis in more than half of the lower eyelid as 2[27]. The MSR was evaluated on a 4-point scale, as reported in a previous study from Villani *et al*[28], with black color of secretion listed as 0, dark gray color as 1, light gray color as 2 and white color as 3. The inhomogeneity of interstices or walls of acinar units was rated on a 4-point scale, with absence of punctate reflecting elements listed as 0, slight presence of punctuate reflecting elements as 1, moderate presence as 2, higher presence as 3. The MAI was assessed on a 4-point scale, with virtually round or elliptical shape as 0, minimal presence of lobulated shaped acinar units as 1, moderate presence as 2 and heavy presence as 3[28, 29].

1.3.5 The Number of *Demodex* Mite in the Eyelash

The instrument and method used for observing the *Demodex* mite in the eyelash follicles were similar to those used for detection of MG microstructure parameters. The total number of *Demodex* mite at the root of 9 eyelashes per eyelid was calculated. Greater than or equal to 1 was defined as *Demodex*-positive and a reduction in the count to 0 after treatment as "eradication"[30].

1.4 Statistical Analysis

Data were analyzed using statistical software GraphPad Prism 7.0 (GraphPad Software, Inc, CA). Descriptive statistics for all patient data were obtained. Data were expressed as mean±standard deviation (SD). Continuous variables were tested for normality with the Shapiro-Wilk test. Analysis between 2 different time points (pre- and post-treatment) for single variable data was performed using a paired-samples test. Normally distributed continuous data were analyzed with the paired samples *t*-test and nonparametric data with the Wilcoxon signed rank test. Results were considered statistically significant for $P<0.05$.

2 RESULTS

2.1 Population Demographics

As shown in table 1, the mean patient age was 56.80±15.95 (median, 56; range 24–86) years. As expected, the majority (68%) of the patients were women. The median duration of dry eye disease was 3 years (range, 1–30). More than half (52%) of the patients had undergone previous ocular disease and/or ocular surgery. A small number (24%) of the patients had other eye conditions, such as eyeliner tattooing, high myopia.

2.2 Associated Ocular-surface Parameters

As shown in table 2, OSDI score, LLT, NIF-BUT, NIAvg-BUT, TBUA and CFS improved significantly after treatment (all $P<0.05$). Although SIT improved after treatment, there was no statistically difference ($P=0.065$).

2.3 Eyelid Margin Abnormalities

Vascularity of lid margins, plugging of meibomian gland orifices, lid margin irregularity and lid margin thickening improved in both upper eyelid and lower eyelid after treatment, and there were significantly statistical significances (all $P<0.0001$, table 3).

2.4 Meibum Quality and Expressibility

Meibum quality and MG expressibility improved both in upper eyelid and lower eyelid with statistical significance after treatment (all $P<0.0001$, table 3).

2.5 MG Morphological Parameters

As shown in table 3, there was mild improvement in MG dropouts in both the upper eyelid (△MG dropouts =–5.70±11.76, $P=0.002$) and the lower eyelid (△MG dropouts= –6.30±15.46, $P=0.001$). However, the MG microstructure (MOA, MAD, MALD, MASD, MAI, MSR, AWI, API, MF) in both the upper eyelid and the lower eyelid significantly improved after treatment (all $P<0.0001$).

2.6 The Number of *Demodex* Mite

The mean number of *Demodex* mite on the upper lid margin (6.59±7.16 to 3.12±3.81/9 eyelashes) and

Table 1 Population demographics of MGD patients

Demographic factors	Patients (n=25)	Percentage of patients (%)
Age, mean±SD (range), years	56.80±15.95 (24–86)	
Gender		
Female	17	68
Male	8	32
Duration, median (range), years	3 (1–30)	
Previous ocular disease* (%)	13	52
Previous ocular surgery† (%)	13	52
Other conditions‡ (%)	6	24

*: cataract, glaucoma, blepharospasm, conjunctivochalasis; †: cataract extraction, intraocular lens placement, blepharoplasty, laser *in situ* keratomileusis, lacrimal duct plug implantation, YAG laser iris circumcision; ‡: eyeliner tattooing, high myopia

Table 2 Comparison of associated ocular-surface parameters before and after IPL treatment (mean±SD)

Parameters	Baseline	After treatment	*P* value	Difference△
OSDI score	32.57±15.80	18.72±12.62	0.0003	–13.85±15.40
LLT (nm)	61.31±19.03	69.96±22.75	0.003	8.65±17.26
NIF-BUT (s)	6.06±3.78	8.51±4.44	0.005	2.46±5.90
NIAvg-BUT (s)	8.18±3.53	9.98±3.69	0.017	1.80±5.09
TBUA (%)	8.21±9.18	3.09±5.48	<0.0001	–5.12±10.64
CFS	2.92±2.31	0.65±1.07	<0.0001	–2.27±2.51
SIT (mm)	5.47±3.29	8.00±6.66	0.065	2.53±7.08

OSDI=Ocular surface disease index; LLT=Tear film lipid layer thickness; NIF-BUT=Noninvasive first breakup time; NIAvg-BUT=Noninvasive average breakup time; TBUA=Tear film breakup area; SIT=Schirmer I Test; CFS=Corneal fluorescein staining; △The difference between post-treatment and pre-treatment

Table 3 Comparison of meibomian gland before and after IPL treatment (mean±SD)

Parameters	Eyelid	Baseline	After treatment	*P* value	Difference△
Eyelid margin abnormalities					
Vascularity	Upper eyelid	2.73±0.53	1.49±0.68	<0.0001	–1.24±0.75
	Lower eyelid	2.24±2.90	0.71±0.71	<0.0001	–1.53±0.84
Plugging	Upper eyelid	2.94±0.24	1.08±0.61	<0.0001	–1.86±0.61
	Lower eyelid	2.90±0.31	0.65±0.63	<0.0001	–2.24±0.72
Irregularity	Upper eyelid	1.37±0.67	0.82±0.67	<0.0001	–0.55±0.54
	Lower eyelid	1.18±0.67	0.39±0.49	<0.0001	–0.80±0.74
Thickening	Upper eyelid	1.71±0.46	1.35±0.56	<0.0001	–0.37±0.49
	Lower eyelid	1.51±0.51	0.88±0.63	<0.0001	–0.63±0.67
Meibum quality	Upper eyelid	1.76±0.52	0.80±0.41	<0.0001	–0.96±0.45
	Lower eyelid	1.71±0.46	0.86±0.35	<0.0001	–0.86±0.46
Expressibility	Upper eyelid	2.35±0.75	0.43±0.50	<0.0001	–1.92±0.70
	Lower eyelid	2.43±0.65	0.63±0.57	<0.0001	–1.80±0.71
MG macrostructure					
MG dropouts (%)	Upper eyelid	35.56±15.08	29.86±12.42	0.002	–5.70±11.76
	Lower eyelid	46.77±19.99	40.47±16.64	0.001	–6.30±15.46
MG microstructure					
MOA (μm^2)	Upper eyelid	1901.63±1052.33	4027.84±1638.59	<0.0001	2126.20±1744.00
	Lower eyelid	2084.67±1487.17	3887.82±1724.60	<0.0001	1803.14±2203.27
MAD (/mm^2)	Upper eyelid	85.57±42.39	120.10±37.59	<0.0001	34.53±49.47
	Lower eyelid	87.45±30.79	145.08±46.85	<0.0001	57.63±46.27
MALD (mean±SD,μm)	Upper eyelid	126.76±37.49	79.97±18.18	<0.0001	–46.79±44.76
	Lower eyelid	115.45±29.06	75.88±15.19	<0.0001	–39.57±31.88
MASD (μm)	Upper eyelid	48.90±21.71	31.63±10.33	<0.0001	–17.27±22.13
	Lower eyelid	41.12±16.05	25.97±9.93	<0.0001	–15.15±17.72
MAI	Upper eyelid	1.92±0.86	1.00±0.74	<0.0001	–0.92±0.98
	Lower eyelid	2.29±0.79	1.33±0.97	<0.0001	–0.96±1.12
MSR	Upper eyelid	2.12±0.53	1.31±0.58	<0.0001	–0.82±0.70
	Lower eyelid	1.94±0.56	1.14±0.58	<0.0001	–0.80±0.84
AWI	Upper eyelid	2.00±0.68	0.96±0.41	<0.0001	–1.04±0.79
	Lower eyelid	1.96±0.71	1.02±0.48	<0.0001	–0.94±0.90
API	Upper eyelid	1.39±0.70	0.53±0.58	<0.0001	–0.86±0.89
	Lower eyelid	1.37±0.73	0.41±0.57	<0.0001	–0.96±0.87
MF	Upper eyelid	1.04±0.50	0.49±0.51	<0.0001	–0.55±0.58
	Lower eyelid	1.33±0.55	0.71±0.61	<0.0001	–0.61±0.67

MOA=MG orifice area; MAD=MG acinar density; MALD=MG acinar longest diameter; MASD=MG acinar shortest diameter; MAI=MG acinar irregularity; MSR=Meibum secretion reflectivity; AWI=Inhomogeneous appearance of walls of acinar units; API=Inhomogeneous appearance of periglandular interstices of acinar units. MF=Severity of MG fibrosis. △The difference between post-treatment and pre-treatment

lower lid margin (2.55±2.11 to 1.29±1.53/9 eyelashes) significantly reduced after IPL treatment (P<0.0001, fig. 1). The *Demodex* eradication rate was 20% (8/40) in upper lid margin and 34.15% (14/41) in lower lid margin (table 4).

3 DISSCUSSION

In this retrospective study, our main finding was the number of *Demodex* mite detected and counted via IVCM significantly reduced after IPL. At the same time, MG microstructure obviously improved. We evaluated the effect of IPL on eyes with MGD from symptoms, ocular surface, MG function, MG structure and *Demodex* infestation, to clarify the specific mechanisms by which IPL treats MGD. In our study, all other evaluation parameters except SIT responded positively to the treatment. And the IPL can be applied

Table 4 Comparison of *Demodex* infestation before and after treatment

Eyelid	Baseline (*n*)		After treatment (*n*)		Eradication rate (%)
	Demodex negative	*Demodex* positive	*Demodex* negative	*Demodex* positive	
Upper eyelid	9	40	17	32	20.00
Lower eyelid	8	41	22	27	34.15
Total	17	81	39	59	27.16

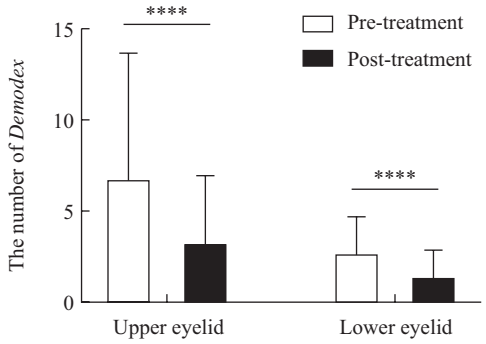

Fig. 1 Comparison of the mean number of *Demodex* mite/9 eyelashes before and after treatment (****P<0.0001)

to not only the lower eyelid but also the upper eyelid, and this was a great progress when compared with previous studies.

It was widely accepted that the hyposecretion of meibomian lipids results in a thinning of the lipid layer and an instability of the tear film, thus leading to the evaporation of the aqueous component[31]. However, LLT measurement was significantly affected by many factors, including MGD type[32]. So in order to reduce the influence of this factor, all patients included in our study were obstructive MGD. In our study, the significantly increased LLT following treatment suggested that outflow of meibum from the glands had been facilitated by the IPL treatment, which is accordant with other study[20], although it used the lipid layer grade through tear film interferometry (Tearscope Plus; Keeler, UK) to evaluate the change of tear film. Accordingly, NIF-BUT, NIAvg-BUT and TBUA, which reflect the stability of tear film, all significantly increased in the treated eyes. We used a new index, TBUA, to evaluate the changes of tear film, and an decrease in mean TBUA in the treated eye from (8.21±9.18)% to (3.09±5.48)% represented a meaningful clinical improvement, and the improvements showed a high degree of conformity with changes of NIF-BUT and NIAvg-BUT. In addition, the current study confirmed improvements in ocular surface injury (CFS) and the symptom score of patients (OSDI), which suggested at least some of those improvements seen in LLT, BUT and CFS translated into improvements of subjective symptoms. These results agree with those found by several other researchers[18, 20, 33, 34].

In our study, we used a new grading scale for MGD, which was described by Arita *et al*[25]. Four types of lid margin abnormality, including vascularity of the lid margin, plugging of meibomian gland orifices, lid margin irregularity and lid margin thickening, were evaluated and were found to be significantly improved after IPL treatment. The IPL device emitted energy that was preferentially absorbed by chromophores in hemoglobin, resulting in coagulation of blood in abnormal vessels of telangiectasia in the eyelid margin and adjacent conjunctiva and eventually in closure of the vessels and reduced vascularization[35, 36]. When the telangiectasia reduced in the eyelid margin, local release of inflammatory factors reduced, and the irregularity and thickening of the lid margin may also improve. IPL produced heat that was transferred to the thin periocular skin of the eyelid, which allowed the softening of meibum, and melted pathologically dysfunctional secretions. Plugging of meibomian gland orifices would therefore be ameliorated[17].

Our results were in agreement with several trials, which had demonstrated the efficacy of IPL for the improvement of meibum quality and expressibility[17–19, 37–39]. IPL therapy was believed to be effective for MGD in part through its heating of the eyelid, just like eyelid warming at home, which however found to be transiently effective for treatment of MGD[14]. Eyelid temperature influences not only the secretion but also the delivery of meibum to the ocular surface[40]. Arita *et al*[41] found that the temperature of the eyelid skin was found to increase to 34°C during the application of a warming device but then to decrease rapidly over 10 min after device removal, whereas IPL had been found to increase the temperature of small vessels in the targeted skin area to between 45°C and 70°C[42], which was likely sufficient to increase the temperature of eyelid skin and the tarsal conjunctiva adjacent to the meibomian glands and thereby to melt

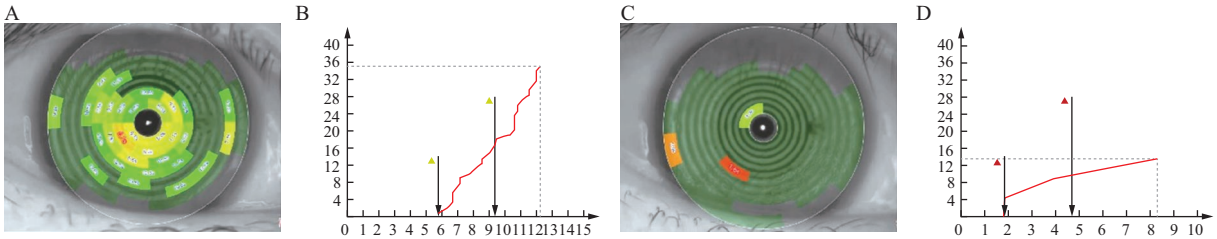

Fig. 2 Noninvasive tear film breakup images observed by Sirius from a 45-year-old female meibomian gland dysfunction (MGD) patient before and after three simple intense pulsed light (IPL) treatments
Noninvasive fist breakup time (NIF-BUT), noninvasive average breakup time (NIAvg-BUT) decreased, but tear film breakup area (TBUA) distinctly decreased after treatment. A, B: Before treatment, NIF-BUT was 5.7 s, NIAvg-BUT was 9.4 s, TBUA was 35%. C, D: After treatment, NIF-BUT was 1.8 s, NIAvg-BUT was 4.6 s, TBUA was 3.5%.

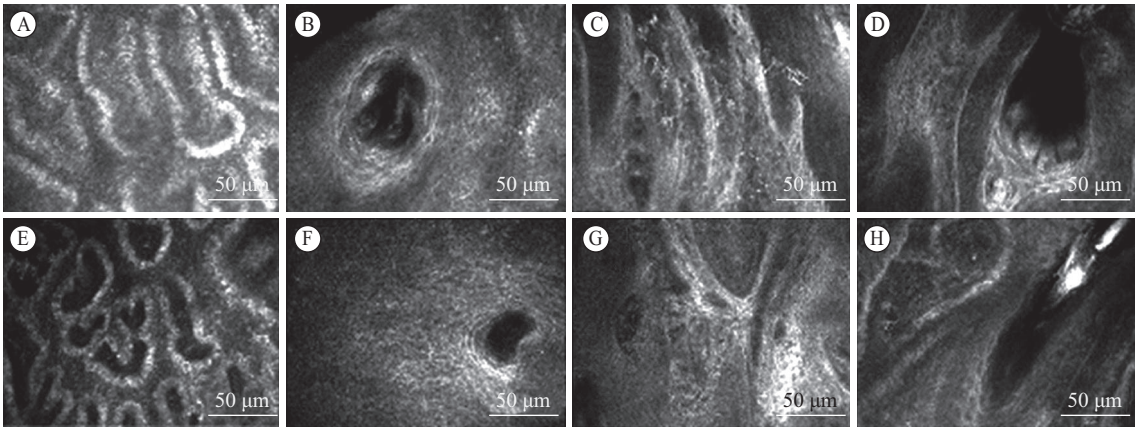

Fig. 3 Meibomian gland (MG) and lash follicle images of upper eyelid observed by *in vivo* confocal microscopy (IVCM) from a 70-year-old female meibomian gland dysfunction (MGD) patient before and after three simple intense pulsed light (IPL) treatments
A, E: MG acinus images before and after treatment, respectively. MG acinar density (MAD) increased from 61/mm^2 to 126/mm^2, MG acinar longest diameter (MALD) decreased from 192.77 μm to 113.72 μm, MG acinar shortest diameter (MASD) decreased from 34.93 μm to 18.89 μm, MG acinar irregularity (MAI) decreased from 3 to 1, meibum secretion reflectivity (MSR) decreased from 2 to 0, inhomogeneous appearance of walls (AWI) decreased from 2 to 1, periglandular interstices of acinar units (API) decreased from 1 to 0. B, F: MG orifice images before and after treatment, respectively. MG orifice area (MOA) decreased from 6596 μm^2 to 3287 μm^2. C, G: MG fibrosis images before and after treatment, respectively. Severity of MG fibrosis (MF) decreased from 2 to 1. D, H: lash follicle images before and after treatment, respectively. The number of *Demodex* of this lash follicle decreased from 4 to 0.

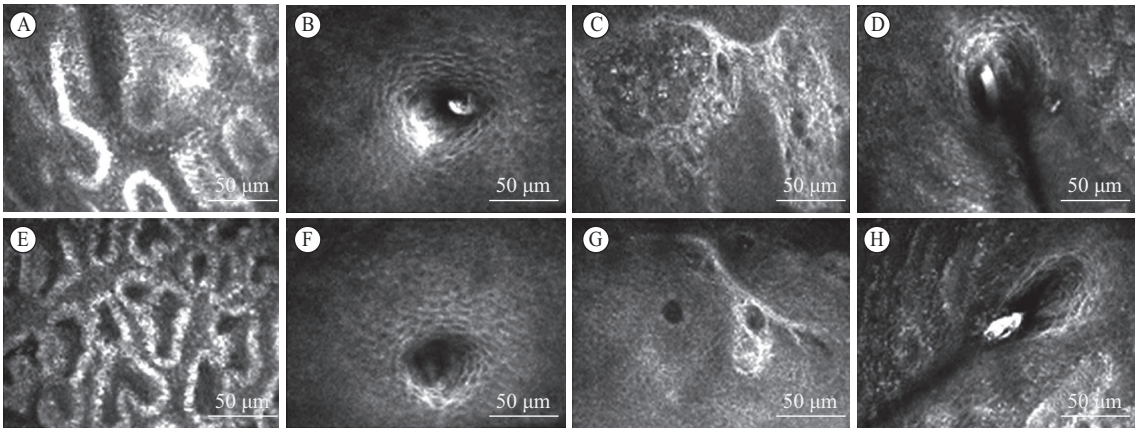

Fig. 4 Meibomian gland (MG) and lash follicle images of lower eyelid observed by *in vivo* confocal microscopy (IVCM) from a 56-year-old male meibomian gland dysfunction (MGD) patient before and after three simple intense pulsed light (IPL) treatments
A, E: MG acinus images before and after treatment, respectively. MG acinar density (MAD) increased from 41/mm^2 to 138/mm^2, MG acinar longest diameter (MALD) decreased from 142.29 μm to 85.59 μm, MG acinar shortest diameter (MASD) decreased from 37.64 μm to 22.80 μm, MG acinar irregularity (MAI) decreased from 3 to 1, meibum secretion reflectivity (MSR) decreased from 3 to 0, inhomogeneous appearance of walls (AWI) decreased from 3 to 1, periglandular interstices of acinar units (API) decreased from 3 to 1. B, F: MG orifice images before and after treatment, respectively. MG orifice area (MOA) decreased from 2622 μm^2 to 4085 μm^2. C, G: MG fibrosis images before and after treatment, respectively. Severity of MG fibrosis (MF) decreased from 2 to 1. D, H: lash follicle images before and after treatment, respectively. The number of *Demodex* of this lash follicle decreased from 2 to 0.

meibum[33]. Of course, it was conducive to the excretion of eyelid lipid and the improvement of the quality of eyelid lipid.

When it comes to the MG morphological change before and after treatment, differences were obvious not only in upper eyelid but also in lower eyelid, especially in microstructure. Although the research about the observation of MG microstructure was sparse, our study was highly consistent with the results of one previous study[39]. But the evaluation parameters of MG microstructure in that study were only MALD, MASD and MAD, and our study included not only 7 items about MG acinar unit but also 1 item about MG orifice and 1 item about MG fibrosis, which may

be more comprehensive and persuasive to describe changes in microstructure. These indicators had been used many times in other studies related to the microstructure of MGD[27–29, 39], and was worthy of evaluation of the therapeutic effect of IPL. Yin *et al*[39] speculated that the particular improvement in MG microstructure was induced by the photomodulation effect of IPL, which stimulates acinar cell activity, thus improving MG microstructure. Our study may confirm the above hypotheses.

Very few studies evaluated the effect of IPL by counting ocular *Demodex* mite via IVCM, which explains why no previous data can be found to compare with our results. For the high magnification of IVCM (800 times), as well as the curved eyelid margin, no special markers can be found to track the hair follicles and glands of the same parts. In order to reduce this part of the error, we divided the detection range into three areas, namely the nasal side, the central side and the temporal side, to observe three hair follicles in each side, with a total of nine hair follicles. In addition, in order to compare the changes of the number of *Demodex* mite before and after treatment more accurately, we analyzed separately the infestation of the upper lid margin and that of the lower lid margin. In this study, a small number of enrolled patients were tested with inconsistent negative and positive infestation of *Demodex*, which may be caused by the following reasons: firstly, although *Demodex* mite was an infectious surface parasite, it is not 100% infectious. Therefore, if the upper eyelid was infected with *Demodex*, the lower eyelid may not be infected. Secondly, it may be the limitation and error of the detection method. After all, currently IVCM cannot detect all hair follicles. Our study found that the mean number of *Demodex* mite/9 eyelashes distinctly decreased in upper lid margin (P<0.0001) and in lower lid margin (P<0.0001) after treatment, although the *Demodex* eradication rate was not so high (20% and 34%). Prieto *et al*[43] found that in sun-damaged patients treated with IPL, coagulated mites could be detected on the skin surface and lymphocyte infiltration in the epidermis was reduced. They considered that *Demodex* organisms contained chromophores that rendered them more sensitive to the energy delivered by IPL, thus inducing coagulation necrosis of *Demodex* organisms, but without damaging the surrounding hair follicles. Zhang *et al*[30] epilated the eyelashes to evaluate *Demodex* counts before and after treatment with IPL and tea tree oil, and found that IPL treatment showed therapeutic potential for ocular demodicosis. It was known to us that *Demodex* would not grow and develop well under 0°C or above 37°C, with 54°C being the lethal temperature and 58°C being the effective temperature required to eliminate *Demodex*[44]. They speculated that the heat generated by IPL, which can increase the temperature of small vessels in the targeted skin area to between 45°C and 70°C[42], reached the temperature required to eliminate mites effectively[30].

From our point of view, the primary mechanisms for the treatment effect of IPL for MGD are as follows: (1) IPL improves the functions of MG and ocular surface through heating, (2) IPL can directly repair the structure of MG to treat MGD, (3) IPL treated MGD via reducing the damage of *Demodex* to the MG structure.

There were several limitations of this study. Firstly, this study is retrospective and the sample size is small. These preliminary data allow us to plan for more rigorous prospective case-controlled studies with long-term follow-up. Secondly, the treatment in this study is only IPL, not combined with meibomian gland expression (MGX), for most patients previously receiving MGX counseling cannot tolerate the pain associated with MGX, and Craig *et al*[20] have shown that IPL treatment alone can also improve the symptoms and signs of patients with dry eyes. Thirdly, ocular *Demodex* includes two types: *Demodex* folliculorum living in the lash follicles and *Demodex* brevis living deep in the MGs and the sebaceous glands of the lash. IVCM cannot accurately distinguish those two types, so our study only quantify *Demodex* in the lash follicles. Fourthly, this study only verified the possible mechanisms of IPL treatment at the clinical observation stage, further cytological and molecular studies are required to comprehensively and precisely elucidate the mechanisms involved in IPL treating MGD.

In conclusion, our results suggest that IPL treatment improves symptoms, ocular surface condition, eyelid margin abnormality, MG function, MG macrostructure as well as MG microstructure. Especially, IPL treatment can significantly reduce the infestation of ocular *Demodex*. IPL thus has the potential to help many patients with MGD and is a promising treatment modality for MGD with ocular *Demodex* infestation in particular.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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