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Case Reports

Real-Time Video Microscopy of *In Vitro* Demodex Death by Intense Pulsed Light

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Abstract

Objective: To directly observe the *in vitro* real-time effects of intense pulsed light (IPL) on a Demodex mite extracted from an eyelash of a patient with ocular rosacea.
Background: Demodex is a risk factor in the pathogenesis of oculofacial rosacea, meibomian gland dysfunction (MGD), and dry eye disease (DED). Recent studies suggested IPL to control or eradicate Demodex organisms in the periocular area. Despite encouraging reports, the direct effect of IPL on Demodex is not well understood.
Methods: An eyelash infested with Demodex was epilated from a 62-year-old female patient with oculofacial rosacea. Following isolation and adherence of a mite onto a microscope slide, real-time video microscopy was used to capture live images of the organism before, during, and after administration of IPL pulses. IPL pulses were delivered with the M22 IPL (Lumenis), with IPL settings used for treatment of DED due to MGD (the ''Toyos protocol''). A noncontact digital laser infrared thermometer was used to measure the temperature of the slide.
Results: Before the IPL pulses, legs of the Demodex mite spontaneously moved in a repetitive and semicircular motion. During administration of IPL, spontaneous movements of the legs continued. Immediately after administration of five IPL pulses, the temperature of the slide increased from room temperature to 49°C. Immediately afterward, the Demodex mite became completely immobilized. The legs appeared retracted, smoother, less corrugated, bulkier, and less well-defined. Movement of the Demodex mite was not observed at the hourly inspections for 5 h and after 24 h following the application of IPL pulses.
Conclusions: Our video directly demonstrates the effect of IPL on a live Demodex mite extracted from a freshly epilated eyelash. The results suggest that IPL application with settings identical to those used for treatment of DED due to MGD causes a complete destruction of the organism.

Keywords: dry eye, intense pulsed light, demodex, ocular rosacea, meibomian gland disease, blepharitis

Introduction

Demodex folliculorum and *Demodex brevis*, collectively known as Demodex, are a normal part of the ocular and facial microbiome.[1–3] An increase in Demodex mite colonization is a strong risk factor in the pathogenesis of oculofacial rosacea, meibomian gland dysfunction (MGD), and dry eye disease (DED).[4,5] Treatment of DED using intense pulsed light (IPL) has been extremely successful in MGD patients,[6–9] but the mechanisms of action are still not well understood. One of the potential mechanisms is the control or elimination of demodicosis.[9–11]

Prieto et al. took 2-mm punch biopsies from the facial skin of subjects before and after IPL treatment and showed histologic evidence of coagulative death of Demodex organisms.[10] More recently, complete eradication of Demodex mites within eyelashes of MGD patients was observed after treatment with IPL.[11] Another study found that the density of Demodex organisms significantly decreased in treated rosacea patients with pulsed dye laser, another light-based approach.[12] While these studies collectively support the hypothesis that IPL is beneficial for MGD patients by reducing the density of Demodex mites, the immediate and real-time response of these organisms to IPL has not been demonstrated before. In this case study, we present video microscopy of a Demodex organism exposed to a series of IPL pulses, showing real-time evidence of Demodex kill. The IPL settings used in this case study are identical to those developed by the group of Toyos, which was recently reported as effective for treatment of DED due to MGD.[13–16]

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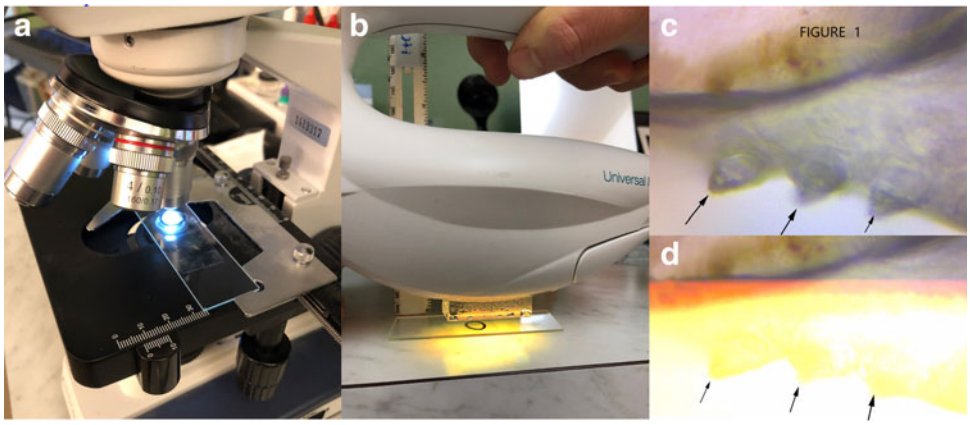

FIG. 1. Experimental setup showing experimental protocol. **(a)** Upright video microscopy showing extracted eyelash mounted on a slide and positioned on microscope stage. **(b)** The rectangular M22 light guide is shown positioned ~5 mm above the eyelash mounted on a glass slide. The slide was briefly removed from the microscope stage to be treated with the IPL light guide. **(c)** A still image from real-time video of Demodex immediately before administration of an IPL pulse using the Toyos settings (Fluence: 11 J/cm^2). **(d)** Same as **c**, during the IPL pulse. The legs of the Demodex mite are indicated with arrows. IPL, intense pulse light.

Case Report

A 62-year-old female with a history of oculofacial rosacea, hordeola, and DED presented to the clinic. An upright light microscope (Fig. 1a) (AmScope 40X-2500X LED Biological Binocular Compound Microscope) was used to confirm the presence of ocular demodicosis at the base of an eyelash epilated from the upper eyelid of the patient. The epilated lash was adhered to the adhesive surface of clear tape and then mounted directly onto a borosilicate glass microscope slide. Video microscopy with a USB Digital Camera Imager attached to the eyepiece of the microscope was then used to image the live Demodex organism.

IPL exposure of the Demodex mite was implemented with the IPL module of an M22 device (Lumenis Ltd., Yokneam, Israel) using treatment parameters shown in Table 1. Just before IPL application, the microscope slide onto which the Demodex mite was mounted was briefly removed from the microscope platform, and the IPL light guide was positioned ~4–5 mm parallel to the surface of the slide (Fig. 1b). Then, five IPL pulses were fired at intervals of 1–2 sec, each pulse with settings identical to those developed by the Toyos' group (Wavelengths: 590 nm to 1200 nm, Pulse structure: triplet of subpulses; Duration per subpulse: 6 msec; fluence per pulse: 12 J/cm^2). The microscope slide was returned to the microscope platform within 25 sec, and video microscopy was resumed. Figure 1c is a snapshot captured just before application of the IPL pulses. The snapshot zooms in on three legs of the Demodex mite protruding from its body (bottom third of the panel). Figure 1d shows a similar snapshot captured during application of an IPL pulse. Due to the strong intensity of the IPL signal, this panel is saturated with yellow light, and details on the Demodex body are lost.

A noncontact digital laser infrared thermometer temperature gun (Nubee NUB8380) was used to measure the temperature of the slide. A temperature of 49°C was measured immediately after the five IPL pulses.

Figure 2 shows individual frames from real-time video of Demodex treated by the IPL pulses. To illustrate the video

Table 1. IPL Treatment Parameters for Demodex Mite

Manufacturer	*Lumenis*
Model identifier	M22 with IPL handpiece
Year produced	2018
Number and type of emitters (laser or LED)	Xenon lamp
Wavelength and bandwidth (nm)	590–1200
Pulse mode (CW or Hz, duty cycle)	Triplet pulse
Beam spot size at target (cm^2)	5.25
Irradiance at target (mW/cm^2)	N/A
If pulsed peak irradiance (mW/cm^2)	N/A
Exposure duration (sec)	N/A
Radiant exposure (J/cm^2 per pulse)	12
Radiant energy (J per pulse)	63
Number of points irradiated	1
Area irradiated (cm^2)	N/A
Application technique	Application of IPL light guide 5 mm perpendicular to a microscopic slide (on which eyelash with specimen was mounted)
Number and frequency of treatment sessions	1
Total radiant energy over entire treatment course (J)	315 (5 pulses × 63 J/pulse)

IPL, intense pulse light; N/A, not available.

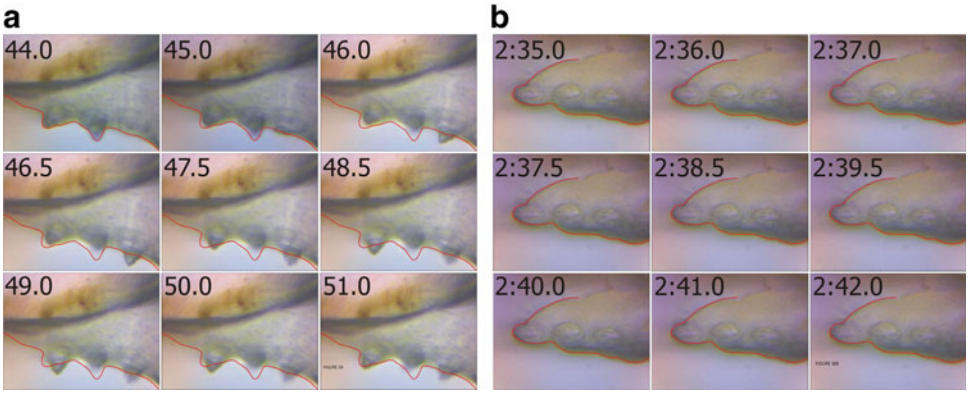

FIG. 2. (a) Individual frames from the movie are sequentially presented (0.5 sec apart) from real-time video of Demodex. In the first frame of the sequence, the Demodex legs are outlined with a red border in the first panel, and this red line was duplicated, unchanged, on all subsequent frames to illustrate the relative movement of the legs in subsequent frames. Images captured before IPL pulses showing the robust activity of the Demodex. **(b)** Individual frames from the movie are sequentially presented (0.5 sec apart) from real-time video of Demodex. In the first frame of the sequence, the Demodex legs are outlined with a red border in the first panel and this red line was duplicated, unchanged, on all subsequent frames to illustrate the relative movement of the legs in subsequent frames. Images captured after five IPL pulses, showing complete and absolute cessation of any movement of the legs of the Demodex. No leg movement was seen at hourly microscopic observation intervals for 5 h and then at 24 h.

movie in a static format, individual frames from the movie are sequentially presented in Fig. 2a (before an IPL pulse) and b (after an IPL pulse). Both figures should be read from left to right within each row and progressing from top to bottom between the rows; adjacent frames are shown in time steps of 1.0 sec, as indicated in the time stamps at the top left corner of each frame. The top left frame shows three legs of the Demodex mite at the beginning of each time sequence. To emphasize the motion of these legs in subsequent frames, a red line was superimposed on the contour of the three legs in the top left frame. This contour line was duplicated, unchanged, on all subsequent frames.

Figure 2a shows a static representation of the video movie captured several seconds before the IPL application. In this sequence of frames, the three legs of the Demodex mite spontaneously move in a repetitive and semicircular motion, with an average irregular rate of about 5 μm/sec. The three legs are not phase locked and appear to move independently from each other.

Figure 2b shows a sequence of frames captured $\sim$25 sec after application of five IPL pulses. The figure shows a complete and absolute cessation of any movement of the legs of the Demodex mite.

Figure 3a and b show a digital magnification of the Demodex mite before and after the IPL application, respectively. Comparison between the two panels shows definite structural changes of the Demodex exoskeleton, following IPL application: the legs appear smoother, less corrugated, and receded. The eyelash (insets) appears to remain intact, although some shrinkage may be evident. No pedal movement was observed hourly for 5 h and after 24 h following the application of IPL pulses (not shown here).

Discussion

IPL is a technique well known for treating facial rosacea and has recently become a recognized nonpharmacologic alternative for ocular rosacea and DED.[6,9,17] Numerous publications have shown the ability of IPL to treat the clinical signs of inflammation associated with DED, and the speculated mechanism includes photocoagulation of abnormal telangiectatic vessels, photobiomodulation of mitochondrial metabolism, and photoimmunomodulatory effects on IL-4, IL-6, IL-10, IL-17A, and TNF-α.[9,14]

However, it is intriguing to consider whether the improvement in the signs and symptoms of DED after IPL treatment could result, in part, from the elimination of Demodex. Indeed, pharmacological eradication of Demodex in patients with ocular rosacea, including tea tree oil, oral Ivermectin, and hypochlorous acid sprays, has been shown to improve symptoms of DED and ocular surface discomfort.[18–20] While Demodex in low numbers is considered part of the normal ocular microbiome, uncontrolled proliferation of Demodex, as occurring in facial rosacea, may represent a dysbiosis in the parasitic infestation, eventually leading to eyelid inflammation and blepharoconjunctivitis.[21,22] Since IPL is effective against demodicosis, as the current study suggests,

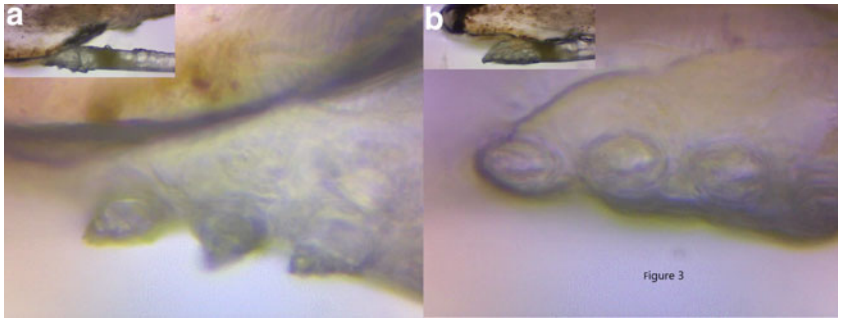

FIG. 3. Digitally magnified images before **(a)** and after **(b)** five IPL pulses with the Toyos settings. The inset shows that a larger perspective of the organism adhered to the eyelash.

at least part of the mechanism by which IPL treatment benefits MGD patients could be attributed to its coagulative effects on Demodex.

Thus far, research studies showing the effect of IPL treatments on Demodex have been limited by indirect evidence using either direct microscopic observation of a few random epilated lashes or skin punch biopsies with histologic analysis. To our knowledge, real-time evidence that IPL is directly microbiocidal has not been shown before. This case report shows real-time video microscopic evidence that IPL pulses (with the same settings as the Toyos protocol, which is used for treatment of DED due to MGD) kill Demodex organism in an *in vitro* environment. While the biochemical mechanism of demodex death and histological confirmation of cellular apoptosis and necrosis remain to be determined, we use the same video microscopic analysis that was established by Tseng and coworkers to support Demodex death or at the very least inactivation.[18]

Several lines of evidence indicate that the death of Demodex induced in our case study is caused by coagulative necrosis. Absorption of IPL energy by chromophores intrinsic to Demodex and the closed cylindrical shape of the Demodex may cause the rapid accumulation of thermal energy and surrounding heating without the possibility of rapid dissipation of heat through its exoskeleton. Our video microscopic observation showing ''smoothed and retracted'' feet (Fig. 3b) is consistent with coagulative necrosis following IPL indicating that the accumulated thermal energy was high enough to be lethal. Demodex thrives between optimal growth temperatures of 16–20°C, but temperatures above 54°C are damaging to Demodex, and temperatures above 58°C are considered lethal.[23] Using the digital laser infrared thermometer, we found that the temperature of the slide after the IPL application was 49°C. While this measurement is a few degrees below the lethal threshold, the temperature of the glass slide during and immediately after the IPL application was probably higher, since there were a few seconds delay between the end of the IPL pulse sequence and the temperature measurement.

Conclusions

In summary, this work shows that standard Toyos dry-eye IPL settings are sufficient to kill the Demodex mite on an epilated lash. Our sequential video images showing complete inactivation are strong evidence that IPL directly and rapidly kills Demodex, presumably by coagulative necrosis, although additional histologic analysis is needed to confirm this mechanism. Because definitive evidence that IPL kills Demodex is still scarce, this case report is relevant for advancing our understanding of the possible role of IPL in eliminating Demodex in rosacea and MGD patients. Further, it brings us closer to understanding the interplay between IPL, Demodex, and the improvement of symptoms in DED.

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Permissions

The patient in this case study has signed a permission allowing the eyelash to be used in this article.

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