Case Reports

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

Harvey A. Fishman, MD, PhD,1 Laura M. Periman, MD,2 and Ami A. Shah, MD3

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.8,9,11

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

1FishmanVision, Palo Alto, California.
2Oracle Eye Institute, Seattle, Washington.
3Mobile Eyes, Newark, California.
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/cm²). 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 administration of an IPL pulse using the Toyos settings (Fluence: 11 J/cm²). (d) Same as c, during the IPL pulse. The legs of the Demodex mite are indicated with arrows. IPL, intense pulse light.

Table 1. IPL Treatment Parameters for Demodex Mite

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Lumenis</td>
</tr>
<tr>
<td>Model identifier</td>
<td>M22 with IPL handpiece</td>
</tr>
<tr>
<td>Year produced</td>
<td>2018</td>
</tr>
<tr>
<td>Number and type of emitters</td>
<td>Xenon lamp</td>
</tr>
<tr>
<td>(laser or LED)</td>
<td></td>
</tr>
<tr>
<td>Wavelength and bandwidth (nm)</td>
<td>590–1200</td>
</tr>
<tr>
<td>Pulse mode</td>
<td>Triplet pulse</td>
</tr>
<tr>
<td>(CW or Hz, duty cycle)</td>
<td></td>
</tr>
<tr>
<td>Beam spot size at target (cm²)</td>
<td>5.25</td>
</tr>
<tr>
<td>Irradiance at target (mW/cm²)</td>
<td>N/A</td>
</tr>
<tr>
<td>If pulsed peak irradiance (mW/cm²)</td>
<td>N/A</td>
</tr>
<tr>
<td>Exposure duration (sec)</td>
<td>N/A</td>
</tr>
<tr>
<td>Radiant exposure (J/cm² per pulse)</td>
<td>12</td>
</tr>
<tr>
<td>Radiant energy (J per pulse)</td>
<td>63</td>
</tr>
<tr>
<td>Number of points irradiated</td>
<td>1</td>
</tr>
<tr>
<td>Area irradiated (cm²)</td>
<td>N/A</td>
</tr>
<tr>
<td>Application technique</td>
<td>Application of IPL light guide 5 mm perpendicular to a microscopic slide (on which eyelash with specimen was mounted)</td>
</tr>
<tr>
<td>Number and frequency of treatment sessions</td>
<td>1</td>
</tr>
<tr>
<td>Total radiant energy over entire treatment course (J)</td>
<td>315 (5 pulses × 63 J/pulse)</td>
</tr>
</tbody>
</table>

IPL, intense pulse light; N/A, not available.
movie in a static format, 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.

**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 photoimunomodulatory effects on IL-4, IL-6, IL-10, IL-17A, and TNF-\(\alpha\).\(^9,14\)

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,
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 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 heat 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.

Acknowledgments

The authors thank Dr. Yair Manor for his editorial expertise in preparing this article.

Author Disclosure Statement

H.A.F. has received past research support and lecture honoraria from Lumenis, but the clinical patient data and work in this article are a financially independent project performed at FishmanVision. H.A.F. has received research support from Eyedetec and is a nonpaid medical consultant for MiboMedical. H.A.F. is cofounder of TearBio, a dry eye genetics start-up. Other consulting arrangements that are not related to this work include 23&me, Google verify.

L.M.P. has received past research support and lecture honoraria from Lumenis. L.M.P. is an advisor for Eyedetec. There are no other relevant financial disclosures.

Permissions

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

Funding Information

There was no funding provided for this article.

References


Address correspondence to:
Harvey A. Fishman, MD, PhD
FishmanVision
706 Webster Street
Palo Alto, CA 94301

E-mail: drfishman@fishmanvision.com

Received: August 8, 2019.
Accepted after revision: September 23, 2019.
Published online: January 28, 2020.