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In Vitro and In Vivo Visualization of Perfluorohexyloctane, an Eye Drop for Dry Eye Disease, Using Infrared Emissivity

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Purpose: To visualize the behavior of perfluorohexyloctane (PFHO), an eye drop to treat dry eye disease (DED), on the surface of saline in vitro and on the human ocular surface using infrared emissivity.

Methods: Emissivity videos were used to measure the spreading and disappearance rates of PFHO on saline (with and without mucin for spreading rate) and layered over a 125 nm film of meibum on the surface of saline using a TearView camera. Ocular surface emissivity was videoed in a volunteer without DED before and after instillation of 1 drop of PFHO. Videos were exported and converted to still photographs, and grayscale levels measured.

Results: PFHO formed a layer over saline that spread at a mean (SD) rate of 0.89 (0.5) cm^2/s and disappeared at 0.0760 (0.0055) μL/min, consistent with reported rates of evaporation for PFHO. Mucin in the subphase did not alter spreading rate ($P > 0.2$). In vitro, a single drop of PFHO spread over the top of a 125-nm thick film of meibum remaining for 3.3 hours. In the volunteer, an increase in emissivity was detected on the ocular surface for ≥5 hours.

Conclusions: PFHO quickly spread to form a layer over the surface of saline or meibum in vitro and was detected on the ocular surface in vivo for ≥5 hours after topical administration. This supports findings that PFHO forms a long-lasting barrier to evaporation at the air–liquid interface of the tear film and thus reduces signs and symptoms of DED.

Received for publication May 15, 2024; revision received August 11, 2024; accepted August 21, 2024.

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Funding was provided by Bausch & Lomb.

The authors have indicated that they have no conflicts of interest regarding the content of this article. The study was funded by Bausch & Lomb.

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Key Words: dry eye, emissivity, cornea

(*Cornea* 2025;44:350–359)

Perfluorohexyloctane (PFHO; trade name MIEBO; Fig. 1A) is Food and Drug Administration approved for the treatment of the signs and symptoms of dry eye disease (DED). DED is defined as "a multifactorial disease of the ocular surface, characterized by loss of homeostasis of the tear film and accompanied by ocular symptoms, in which an etiological role is played by instability and hyperosmolarity of the tear film, inflammation and damage to the ocular surface, and neurosensory abnormalities".[1] Meibomian gland dysfunction is commonly associated with compositional changes[2] and reduced quality of the tear film lipid layer (TFLL),[3,4] which are characteristics of evaporative DED. One phase 2 and two phase 3 studies demonstrate that PFHO[5–7] provides relief of dry eye signs and symptoms, and the results support its mechanism of action as being a substitute/supplement for the TFLL in patients with evaporative DED.[5–12]

Structurally, PFHO is a chain of 6 fluorinated carbons connected to a chain of 8 protonated carbons (Fig. 1A). The fluorinated chain is both lipophobic/aerophilic and hydrophobic and is more hydrophobic compared with the lipophilic protonated chain.[13] As an eye drop, PFHO has a long residence time (up to 6 hours) in rabbit tears and meibomian glands (through at least 8 hours)[14]; is biologically, thermally, and chemically stabile[15,16]; has an optical absorbance less than emulsion eye drops[17]; and is unlikely to be a barrier to oxygen reaching the cornea when applied to the eye because of its oxygen carrying capacity.[18] An 11-μL drop of PFHO applied to the surface of saline (~1.8 cm^2) inhibited the evaporation rate 4 times more than human meibum from a healthy volunteer.[19]

The objective of this study was to gain more insight into the mechanism of action of PFHO, by investigating its distribution and residence time on the surface of saline or meibum in vitro and comparing these results with its distribution and residence time when applied to the human ocular surface. High-resolution infrared detection technology, TearView, was used because it allows dynamic visualization of the spread of PFHO on the surface of saline and ocular surface. This technology detects very long wavelength infrared that is naturally emitted (infrared emissivity). The emissivity depends on composition, structure, temperature,

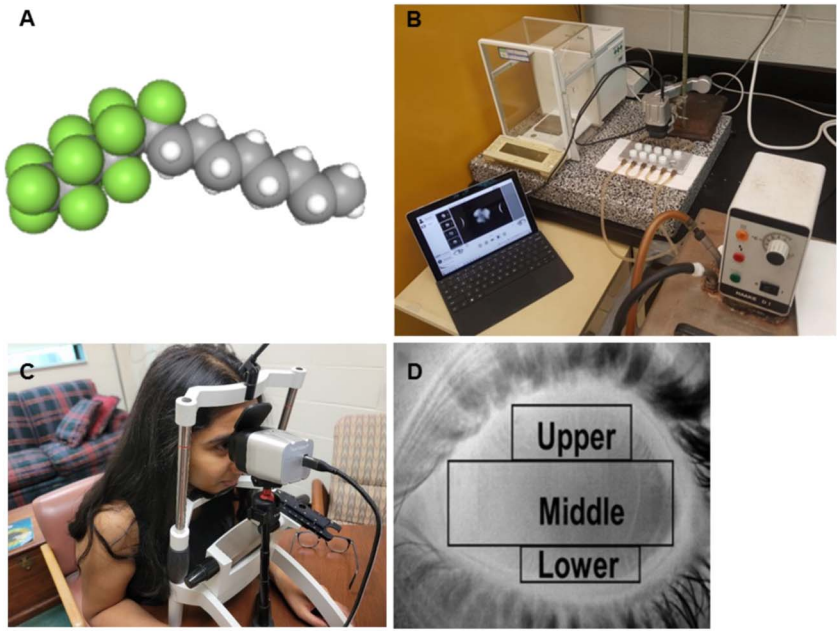

FIGURE 1. A, Space-filling model of PFHO (white spheres, protons; gray spheres, carbon atoms; green spheres, fluorine atoms). B, i) Bench set up for collecting in vitro infrared emissivity images using a TearView camera. ii) Computer for collecting images and controlling the TearView emissivity camera. iii) TearView emissivity camera. iv) Aluminum sample block with temperature-controlled water circulating through it and plastic sample containers within the block. v) Temperature control bath that provides temperature-controlled water to the sample block. C, Chin rest for the measurement of ocular surface emissivity using a TearView camera seen on the tripod. D, Regions of the eye that were cropped and used for quantification of emissivity. PFHO, perfluorohexyloctane.

and thickness of materials, and so is different for different materials at the same temperature.[20] The sensitivity of the TearView camera means that PFHO can be distinguished from the saline surface and from features of the ocular surface.[21] When observing the eye, the sensitivity of TearView means that the ocular surface and dynamic spread of the tear film over the whole ocular surface can be seen in real time repeatedly without interference. Unlike other methods typically used for tear film evaluation, it requires no light to be shone into the eye (bright light causes tearing); it is not restricted to observation of a small part of the ocular surface such as the cornea; it does not require a specific blinking regime (natural blinking is observed); no dye is required to be added to the tear film; and no sampling of the tear film is needed. However, this technique is limited in that the position of the cornea is not seen, and corneal staining is not observed.

MATERIALS AND METHODS

Materials

Phosphate-buffered saline and mucin from bovine submaxillary glands, Type 1S, were obtained from Sigma Chemical Company (St Louis, MO). The TearView camera was obtained from Beyond 700, Castle Hill, New South Wales, Australia. Meibum was collected by medically qualified personnel as described below. The protocols for collection were in accordance with the tenets of the Declaration of Helsinki and approved by the University of Louisville Institutional Review Board (#11.0319, October 2021).

Meibum Collection

Written consent was obtained from a 68-year-old White man volunteer without DED. Briefly, meibum was expressed from all 4 eyelids of the volunteer using an ILUX instrument (Alcon, Fort Worth, TX) according to the manufacturer's instructions, except that the lids were not anesthetized beforehand.[22] A total of approximately 0.5 mg of meibum was expressed from all 4 lids and collected with a platinum spatula then immediately dissolved into 0.5 mL of $CDCl_3$ in a 9-mm glass microvial with a Teflon cap (Microliter Analytical Supplies Ind., Suwanee, GA) and stored at −70° C until use. The cholesterol ester to wax ester molar ratio and amounts of esters of the expressed meibum were evaluated using ^{1}H-nuclear magnetic resonance analysis as described previously.[2,23]

In Vitro PFHO Emissivity Studies

In all experiments, the ambient temperature was 21°C and relative humidity was 31% to 36%. The grayscale infrared emissivity intensity (Ie; 0, completely black; 255, completely white) was determined for various materials (Table 1) placed on an aluminum block using a TearView camera and compared with published emissivity values.[24,25] To emulate the ocular surface temperature during the experiments, the aluminum block was heated by water circulating through it so that the saline in the plastic containers was 35°C. The temperature of the circulating water was controlled with a Haake-B water bath (Haake Manufacturing, DeSoto, MO; Fig. 1B). Samples were applied to the surface of the saline at 35 °C.

To evaluate the behavior of PFHO in vitro, 1.5-mL samples of saline were placed into open plastic containers 0.8-cm deep with an inside diameter of 1.5 cm, giving a surface area equivalent to that of the exposed surface of a human eye.[26,27] This system was used previously for evaporation measurement studies.[28–30]

TABLE 1. Grayscale Ie for Various Materials Using a TearView Camera Compared With Ie Reported in the Literature

	Grayscale Ie, (255 White, 0 Black)	Emissivity from literature[24,25] (1 White, 0 Black)
Cholesteryl behenate	222	
Water	210	0.95
Perfluorohexyloctane (35°C)	210.7 ± 4.3 (3)	
Human meibum (35°C)	205.0 ± 4.8 (7)	
Saline (35°C)	201.5 ± 1.5 (5)	
Perfluorohexyloctane	199	
Stearyl palmitate	198	
Paper	194	0.68
Mucin	148	
Styrofoam	79	0.61
Aluminum	40	0.05

25°C unless indicated. One sample measured unless indicated by the number of samples in parentheses. Ie, emissivity intensity.

The expansion or spreading rate of 2-μL aliquots of PFHO added to saline or saline containing 1, 10, 25, or 50 mg/mL of mucin (35°C) was measured. Larger volumes of PFHO spread too quickly to be measured. The spreading rate was calculated by measuring the diameter of the PFHO emissivity image 6 to 8 times between 0 and 40 seconds after adding PFHO.

To determine if PFHO interacts with human meibum, 35 μL of human meibum (20.2 μg) in $CDCl_3$ was applied to the center of a saline sample at 35°C. This amount of meibum was estimated to produce a 125-nm thick layer of meibum once spread over the surface of the saline sample. After approximately 10 minutes, the $CDCl_3$ had evaporated, as evident from the Ie video, and then 1- or 2-μL aliquots of PFHO were applied to the center of the meibum-on-saline sample. The Ie was recorded for 3 minutes. The experiment was repeated 3 times.

For the above experiments, Ie was video recorded at indicated times with a TearView camera. Videos were exported to an avi format using the software included with the TearView camera, and individual images from the videos were converted to a still photograph in tif format using QuickTime 7.7.5 software (Roland Corporation, Nakagawa, Hamamatsu, Japan). The tif images were subsequently exported to Corel Photo-Paint 12 (Corel Corporation, Ottawa, Canada) and cropped to encompass an area of 1.06 cm by 1.06 cm (or 1.125 cm^2) within the walls of the container. The Ie within cropped images were then determined using the histogram algorithm from Corel Photo-Paint 12.

To determine the rate of disappearance of PFHO from the surface of saline, aliquots (5, 10, 15, 30, 50, and 100 μL) of PFHO were applied to saline samples (equilibrated to 35°C) and 2-second Ie videos were recorded at about 15 minutes intervals in the early part of evaluation and more extended time intervals later. This was to ensure adequate coverage over the whole time interval. For accuracy, the actual times of the recordings were 13, 31, 45, 61, 77, 90, 104, 123, 144, 170, 201, 248, 287, and 321 minutes. The rate of disappearance of PFHO was estimated from the difference in the time where PFHO was observed to be completely absent in the emissivity images.

In Vivo PFHO Emissivity Study

To measure PFHO in vivo on the ocular surface, the same TearView emissivity camera from the in vitro studies was used. The volunteer's head was held still by placing their chin on a chin rest (Fig. 1A), and the TearView camera, mounted on a tripod, was moved to be level with and focused on the center of the right eye (Fig. 1A). As a preliminary study, a 2-minute infrared emissivity video was taken before and every hour for 1 to 6 hours after 1 11-μL eye drop of PFHO was administered. As with in vitro experiments, the infrared emissivity videos were exported to an avi format and converted to still photographs. Only tif images at 1, 2, 3, 4, and 5 seconds after blinking were exported to Corel Photo-Paint 12 for quantitation. Ie was quantified for the upper, middle, and lower ocular surface regions (Fig. 1D). The distribution of the grayscale pixels for the same regions were also quantified as a measure of pixel heterogeneity (H). Images for the middle region of the ocular surface were cropped to the top and bottom of the medial canthus extending to but not including the temporal lid margin. The upper and lower ocular surface regions were cropped such that the lid margins were not included. Ocular surface region images were cropped to the identical dimensions each time to ensure the accuracy of Ie and H quantitation.

RESULTS

The emissivity of various materials including PFHO was assessed with the TearView camera for validation. The Ie measured by the grayscale value followed the order: water = paper > styrofoam > aluminum, which was consistent with the order of published emissivity values (Table 1). The Ie for PFHO and human meibum were similar ($P = 0.49$) on the surface of saline at 35°C, suggesting that it could be difficult for the camera to differentiate between PFHO and meibum (Table 1).

In Vitro PFHO Emissivity Study

Emissivity images showed PFHO (2 μL) quickly spread over saline at a mean (SD) rate of 0.89 (5) cm^2/s at 35°C

TABLE 2. Spreading (Expansion) Rate of PFHO With and Without Mucin

Mucin Concentration (mg/mL)	Average Spreading Rate ± Standard Error of the Mean (cm^2/s)	Average Correlation Coefficient (r)	Number of Trials/ Data Points	X Intercept (cm^2)	Spreading Rate *P* versus 0 mg/mL Mucin Samples
0	0.89 ± 0.5	0.905	4/57	6.98 ± 5.40	—
1	0.96 ± 0.11	0.916	3/17	4.79 ± 5.48	0.6
10	1.33 ± 0.14	0.911	3/21	1.52 ± 7.98	0.0005
25	1.31 ± 0.13	0.900	4/26	3.08 ± 8.19	0.0005
50	1.42 ± 0.19	0.862	3/20	1.42 ± 4.01	0.033

P value determined using the Student *t* test. PFHO, perfluorohexyloctane.

(Table 2 and Fig. 2). The spreading rates of PFHO were linear between 0 and 50 seconds for all experimental samples, with a correlation coefficient (r) of 0.0905 when 4 trials were combined. There were no significant differences between the spreading rate of PFHO on saline containing 0 to 50 mg/mL of mucin: $P < 0.05$ (Table 2 and Fig. 2) below 20 seconds. The spreading rate of PFHO on saline increased in the presence of 10–50 mg/mL of mucin: $P \leq 0.033$ above 20 seconds (Table 2 and Fig. 2).

Emissivity images of a 35-μL solution of meibum in $CDCl_3$ showed an initial darker region because of the evaporation of $CDCl_3$ (Fig. 3 and see Video 1) leaving behind a light, more diffuse film of meibum. The Ie at the center of meibum island (Fig. 3) was significantly lighter ($P = 0.0006$) with a mean (SD) grayscale value of 205.0 (12.8; 7 samples), compared with the surrounding saline 177.3 (6.7; 6 samples).

When PFHO (2 μL) was applied to a meibum film on saline (Fig. 4 and see Video 2), it was easily distinguishable despite having a similar Ie. It spread over the top of the meibum film. Importantly, PFHO did not mix with meibum under these static conditions as indicated by a distinct boundary of the spreading PFHO, and the meibum film was not displaced by the spreading PFHO. Instead, PFHO spread on top of the meibum. Light manual swirling of the dish also did not cause the meibum and PFHO to mix.

The persistence of PFHO on saline at 35°C was investigated. Disappearance time of PFHO versus volume of PFHO applied to the surface of saline was linear ($P < 0.05$), with correlation coefficient (r) of 0.975 and slope of 0.0760 ± 0.0055 μL/min (Fig. 5, inset). Doubling the volume of PFHO spread on the surface from 15 μL to 30 μL (~2 drops) doubled the time before it disappeared from the surface from 3.3 hours to 6.6 hours. Temporal variation of mean surface emissivity was due to convection currents (Fig. 5, labeled "0-μL PFHO").

In Vivo PFHO Emissivity Study

Ocular Surface Emissivity of a 27-Year-Old Asian Woman Before Adding PFHO

There was no reporting or evidence of dry eye or ocular surface disease in this subject, and they were not using ocular medications. A dark horizontal line (arrow) of meibum is seen moving up to the upper eyelid nearly as quickly as the eyelid moves up, with new tear film forming behind it (see Video 3). The mean grayscale Ie versus time

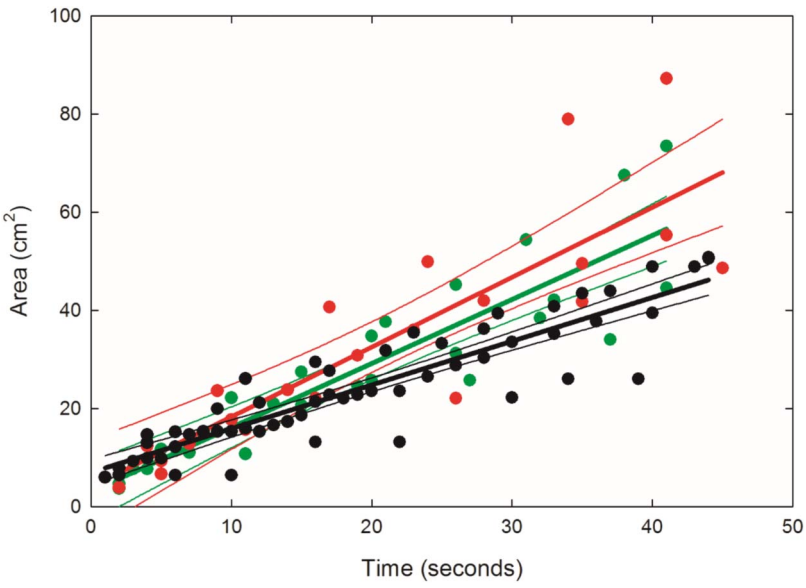

FIGURE 2. Area of a 2-μL sample of PFHO on saline alone (black) or containing 25-mg/mL mucin (green) or 50-mg/mL mucin (red). Three to 4 trials were combined, and linear regression data are provided in Table 2. Bold lines are the best fit of the linear regression lines. Thin lines are the 95% confidence limits. Note that there were no differences between samples with and without mucin below 20 seconds ($P > 0.05$). PFHO, perfluorohexyloctane.

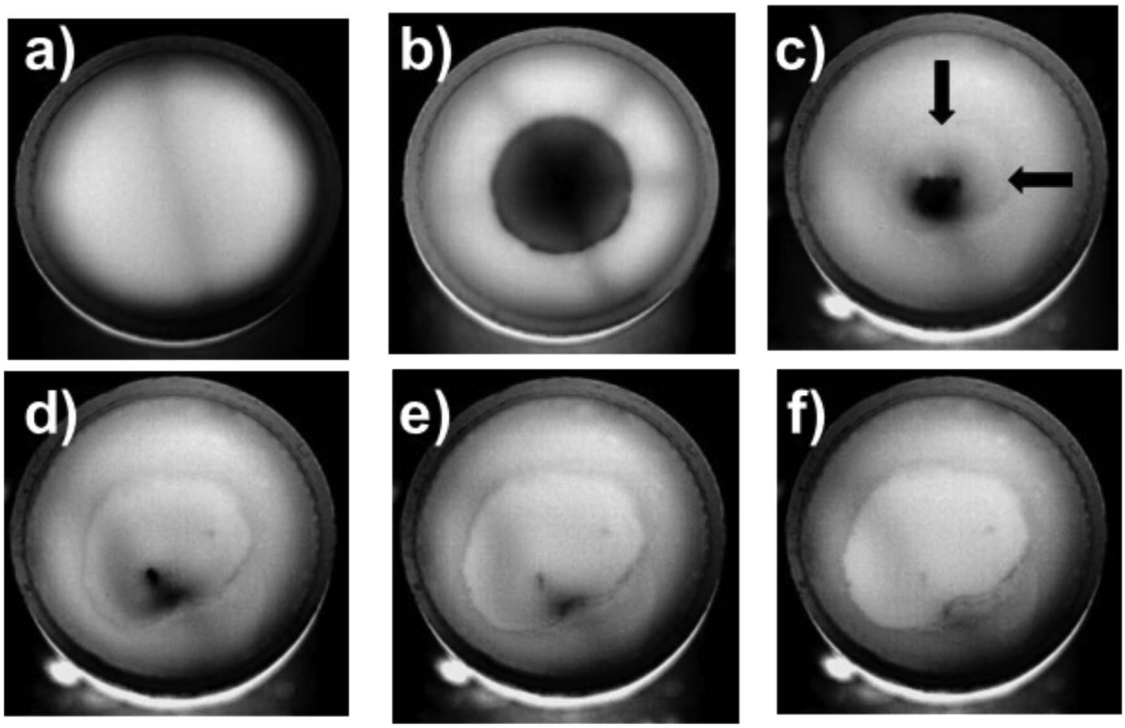

FIGURE 3. TearView emissivity images of (A) 1.5-cm diameter sample container with saline; (B) appearance of a 35-μL drop of $CDCl_3$ containing human meibum (dark spot) immediately after application to the saline; (C) meibum seen spreading out (arrows) on the surface of saline as the $CDCl_3$ evaporates; and (D) 1; (E) 2; and (F) 3 seconds thereafter.

after blink before adding PFHO was 16% higher ($P < 0.0001$) in the upper region and 5% lower ($P = 0.0043$) in the lower region compared with the middle region (Fig. 6A). Ie decreased significantly ($P < 0.01$) after blinking for all 3 eye regions. The H versus time after blink was about 69% to 85% ($P = 0.0005$) higher for the middle region compared with the lower and upper regions (Fig. 7A). One can observe that Ie was highest in the medial canthus regions (Fig. 8A).

H was highest in the middle region compared with the upper and lower regions, which were similar (Fig. 7A).

Ocular Surface Emissivity of a 27-Year-Old Asian Woman After Adding PFHO

Increased Ie was noted through 5 hours after instillation of a single drop of PFHO (see Video 4 and Video 5). Ie increased over the 1- to 6-hour time interval after administration of PFHO by 30% to 39%, 17%, and 18% to 25% for the lower, middle, and upper regions, respectively ($P < 0.0001$; Figs. 6B–D, 8A–C). For the middle region, the Ie peaked 5 hours after PFHO administration, and after 6 hours, it returned to a value similar to that before PFHO administration (Figs. 6C, 8).

In the upper region, H increased 1 to 6 hours after administration of PFHO by 10% to 13% ($P = 0.0004$; Fig. 7B). In the middle region, H decreased slightly by −8.6% ($P = 0.0004$) 1 to 6 hours after administration of PFHO (Fig. 7C). H was elevated in all regions 6 hours after administration of PFHO compared with the average 1 to 6 hours after administration ($P < 0.013$; Fig. 7B–D).

DISCUSSION

Insight into the clinical mechanism of action of an eye drop, PFHO, recently approved by the Food and Drug Administration, was gleaned by dynamically recording at high resolution natural infrared being emitted from the ocular surface and comparing these data with controlled in vitro experiments. Importantly, pilot data from a single volunteer indicate that PFHO does not spill out of the eye and seems to spread over the tear film, forming a protective barrier that lasts for more than 5 hours. The in vitro experiments showed that it was able to spread over both a meibum film with no mixing and an aqueous (saline) subphase. Its eventual removal was due to evaporation of the PFHO. Clinically, this supports that PFHO has a novel action compared with other eye drops in that it acts as an antievaporative film to improve tear stability. As demonstrated in the clinical development studies, this results in healing of the ocular surface and a reduction in dry eye symptoms caused by deficiencies in the lipid and/or aqueous layer of the tear film. A limitation of this pilot experiment for testing the feasibility of the TearView camera to assess the presence of PFHO on the ocular surface was that the subject had a normal tear film, and so the effects on subjects with defective tear films still need to be investigated with this technology.

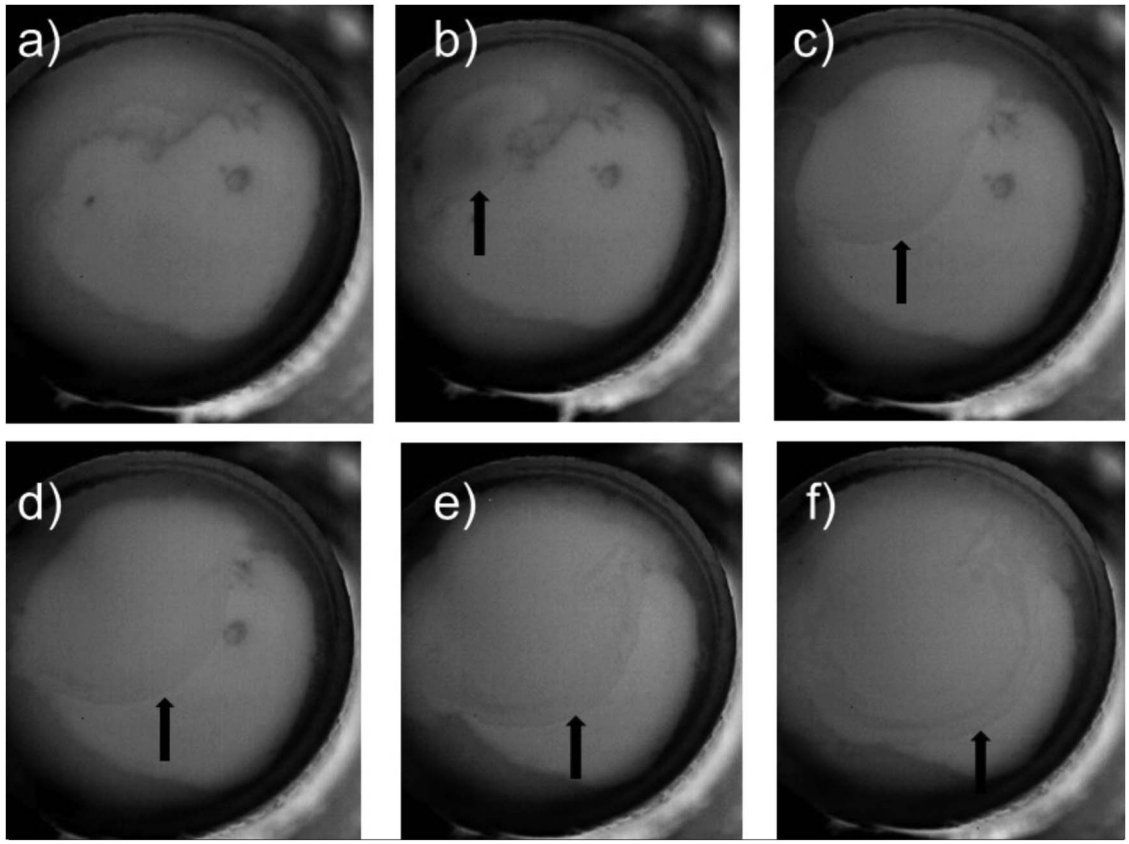

FIGURE 4. TearView emissivity images at 35˚C. A, 125-nm thick layer of human meibum layered on saline. B–F, 7 to 73 seconds after adding 2-μL PFHO to A. Arrows show edge of PFHO layer spreading over time on top of the meibum layer, and it never fully covers the meibum film, but it does cover the surface of the hole in the meibum film (D and E). PFHO, perfluorohexyloctane.

A useful advantage of this technology is that semi-quantitative measurements of emissivity can be made, enabling measurement of dwell time and distribution of PFHO. These emissivity measurements were validated by comparing the TearView grayscale pixel values of various objects with literature values for emissivity.

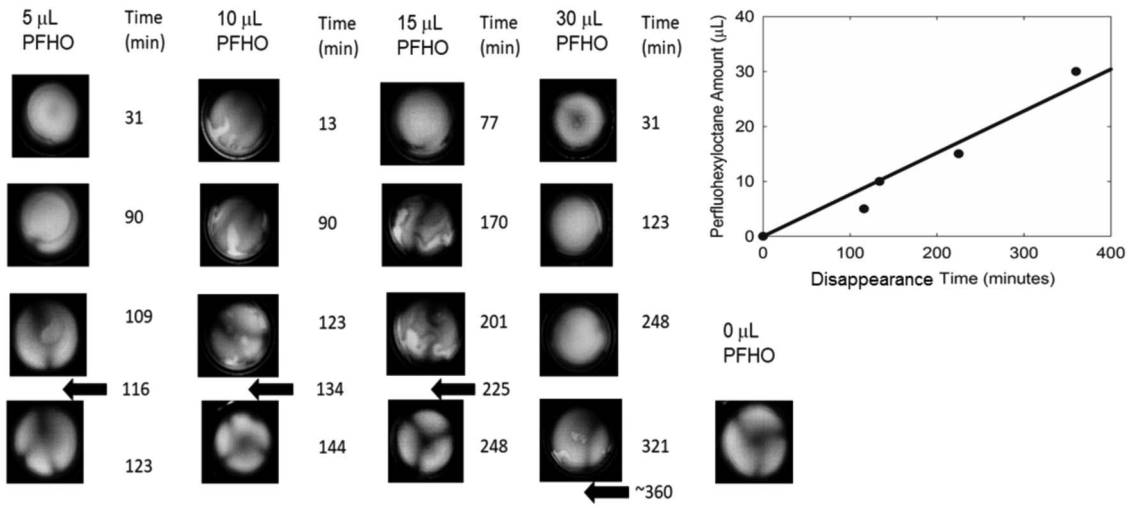

FIGURE 5. TearView emissivity images of PFHO layered over saline at 35˚C to determine the disappearance time of PFHO. Images were selected from a total of 13 images for each PFHO concentration taken between 31 and 321 minutes. The large arrows show the time that PFHO was no longer detectable (inset upper right). PFHO on saline evaporated at a rate of 0.0760 ± 0.0055 μL/min, r = 0.975. PFHO, perfluorohexyloctane.

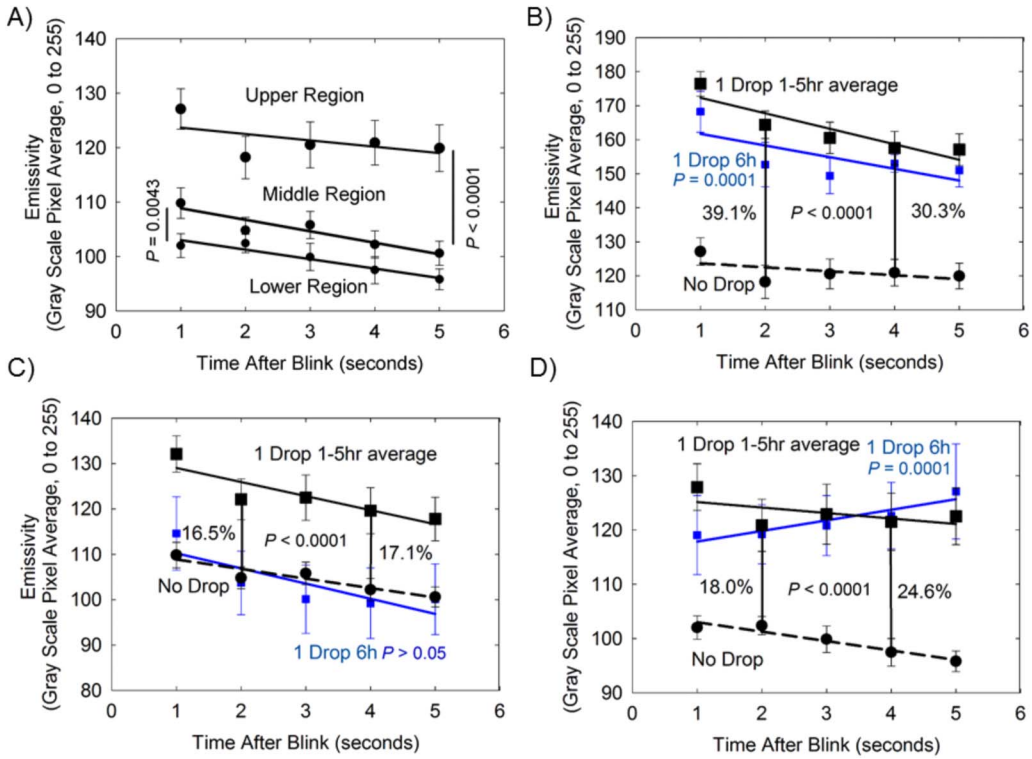

FIGURE 6. Emissivity intensity (Ie) from the videos of an eye from a 27-year-old female Asian without dry eye. A, Regional differences before adding PFHO. B–D, Comparison of Ie before (dashed line) and after a single instillation of PFHO (solid black line, 1- to 5-hour average; blue line at 6-hour postinstillation in B, lower region; C, middle region; and D, upper region. *P* values were obtained from the paired Student *t* test. *P* values for the 6-hour plot were obtained by comparing the 6-hour data with the 1- to 5-hour data. For (A) and the "no drop" plots in (B–D), data from 9 blinks were averaged. For the "1- to 5-hour average" plots in (B–D), data from 44 blinks were averaged. For the "6-hour" plots in (B–D), data from 12 blinks were averaged. PFHO, perfluorohexyloctane.

PFHO 15 μL, 0.0205 g, on saline, in vitro, at 35°C disappeared after 3.3 hours, slightly longer than the 2.3 hours predicted from the evaporation rate of PFHO alone, 0.82 μm/min[19] In comparison, the Ie measured for PFHO on the ocular surface was elevated for at least 5 hours, close to the timeframe that PFHO has been shown to be present in a rabbit pharmacokinetic study.[14] The residency time of PFHO on the ocular surface is critical to its antievaporative[19] and ameliorative effects on dry eye symptoms.[5–11] The longer residency time of PFHO in vivo versus in vitro is not unreasonable in that the turnover of the TFLL is 10 times slower (0.93%/min) compared with the aqueous portion of tears (10.3%/min).[31] Much like the TFLL, PFHO floating on the aqueous surface would not be expected to enter the tear duct far below. It is interesting that in vivo, after administering PFHO, Ie was found to be highest in the medial canthus regions. It is likely that the nasal flow of the tear aqueous below the more stationary overlaying TFLL/PFHO layer migrates to the canthus region. The canthus and lid margin regions, which also have a higher Ie, may serve as reservoirs for PFHO and tear lipids,[24] increasing their longevity on the ocular surface. PFHO has a residency time of at least 8 hours in rabbit meibomian glands.[19] Although levels of PFHO are lower in the meibomian glands as compared with the tears, the meibomian glands could also serve as a reservoir of PFHO. In humans, if PFHO does enter the meibomian glands, it must diffuse into the glands against the outward flow of meibum with every blink. Further studies on humans are needed to test this hypothesis.

Effect of Mucin and Meibum on PFHO Spreading

As the tear film evaporates, the possibility that PFHO will encounter soluble and membrane-bound mucin on the surface of the cornea becomes greater. If PFHO interacts with membrane mucin as it does with a high concentration of soluble mucin used in the current study, it is unlikely that the spreading rate of PFHO on the ocular surface will be affected significantly by mucin, as in the current study, mucin did not affect the spreading rate below 20 seconds after adding PFHO, and tears are replenished after every blink, which occurs every 13 seconds for adults without dry eye.[32] It should be noted that above 20 seconds, which might not be relevant physiologically, large concentrations of mucin increased the spreading of PFHO. Under static conditions in vitro, with PFHO in contact with mucin for up to 2 hours, the antievaporative effect of PFHO was reduced, albeit minimally, and PFHO is expected to inhibit the excessive

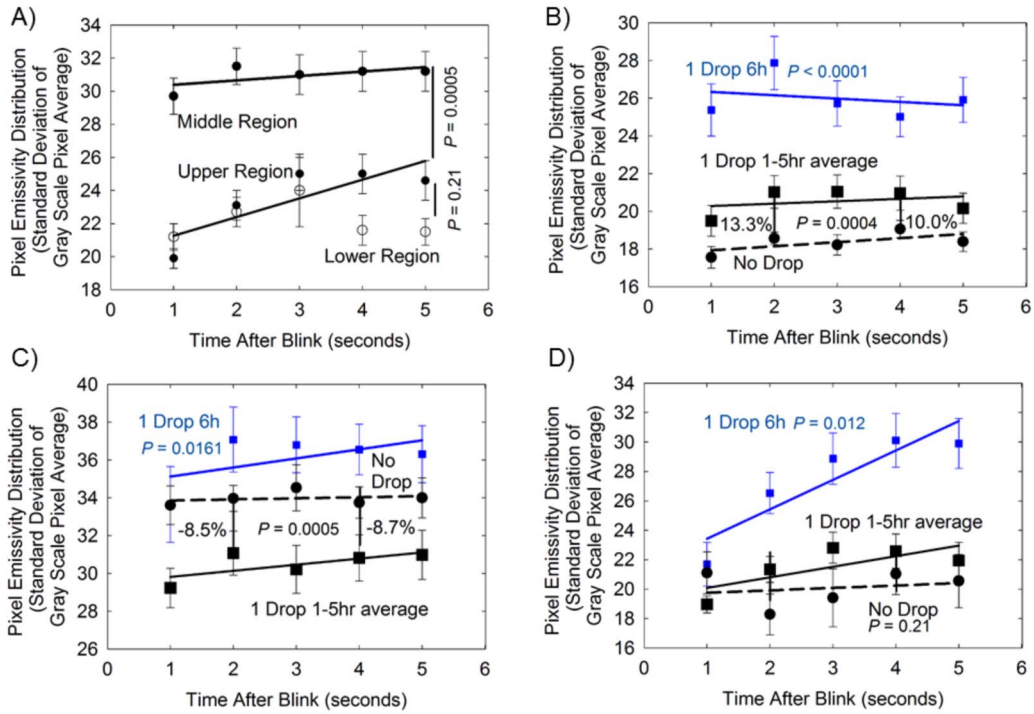

FIGURE 7. Pixel emissivity distribution, a measure of heterogeneity, from the videos of an eye from a 27-year-old female Asian without dry eye. A, Regional differences before adding PFHO. B–D, Comparison of H before (dashed line) and after a single instillation of PFHO (solid black line, 1- to 5-hour average; blue line at 6-hour postinstillation in B) lower region, (C) middle region, and (D) upper region. *P* values were obtained from the paired Student *t* test. *P* values for the 6-hour plot were obtained by comparing the 6-hour data with the 1- to 5-hour data. For (A) and the "no drop" plots in (B–D), data from 9 blinks were averaged. For the "1- to 5-hour average" plots in (B–D), data from 44 blinks were averaged. For the "6-hour" plots in (B–D), data from 12 blinks were averaged. PFHO, perfluorohexyloctane.

evaporation of the tear film layer significantly even in the presence of tear mucins.[19]

In vitro, PFHO spread over the top of a layer of meibum and no mixing of meibum and PFHO was observed. One would expect that, initially upon administration, PFHO would settle on the surface of the TFLL before mixing because of blinking. Our recent unpublished rheological and physical studies[17,24] indicate that PFHO does mix and is miscible with disordered esters, and

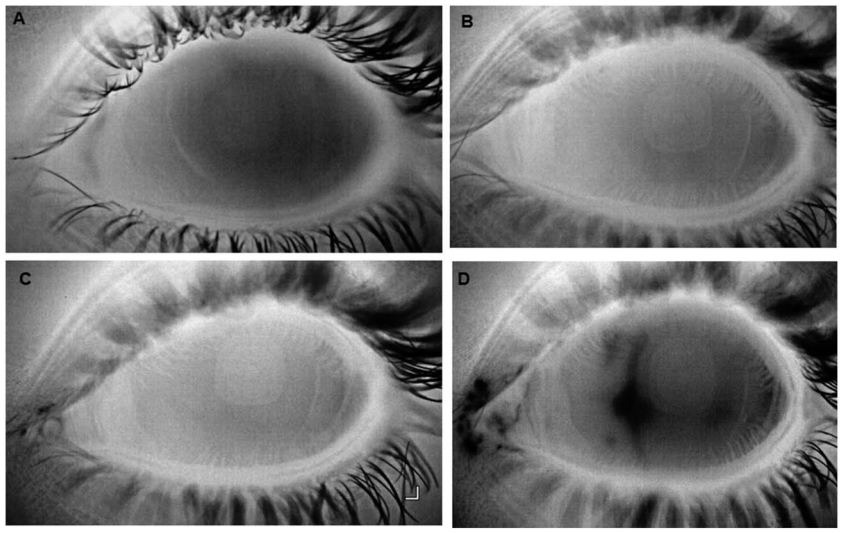

FIGURE 8. Normal eye from a 27-year-old (A) before and (B) 3 hours, (C) 5 hours, and (D) 6 hours after administration of 1 drop of PFHO. Images were taken 5 seconds after blinking. Note how much brighter (more PFHO/lipid) (B, C) is compared with (A, D). PFHO, perfluorohexyloctane.

some PFHO is seen on the surface of the film using Brewster angle spectroscopy, even after PFHO and meibum are mixed.

Ocular Surface Emissivity In Vivo

The major findings of the in vivo study are that changes in Ie and H related to blink time can be quantitated and comparisons tested statistically. In vivo emissivity data was included in the current study to evaluate the potential of measuring tear-lipid/PFHO interactions on the ocular surface. The major finding in a single healthy volunteer was that Ie of the TFLL followed the regional order upper > middle > lower region. H was higher in the middle region compared with the upper and lower regions. Ie decreased linearly in all regions 1 to 5 seconds after blinking, whereas H remained constant. The decrease in Ie after blinking may be related to tear breakup time and possibly due to the thinning of the lipid layer.

After administration of PFHO, Ie increased significantly, and the increase was sustained for at least 5 hours after administration. For the 1 individual studied, the increase in Ie after administration of PFHO is likely due to an increase in the emissivity because of the added PFHO on the surface, the thickness of the tear lipid layer,[4,33] and perhaps an increase in temperature. It is interesting that at less than 15 minutes after the administration of PFHO, the ocular surface temperature decreased slightly by 0.15 °C.[34] However, there was no difference between the ocular surface measured 60 minutes after administration of saline or PFHO. One might expect from these results that because of cooling, Ie would be lower 5 to 60 minutes and would rise 1 to 5 hours after administration of PFHO. The current study suggests that the TearView camera could be a valuable tool for measuring TFLL and PFHO dynamics on the ocular surface. However, no conclusions can be made regarding the location and longevity of PFHO on the ocular surface from this current data in only 1 participant. Studies to evaluate tear-lipid/PFHO relationships using the TearView camera are underway in a larger number of human participants with and without dry eye.

The current study demonstrates that PFHO forms a long-lasting layer over saline and meibum in vitro, consistent with its antievaporative mechanism of action. Visualization of the dynamics of the TFLL and prolonged detection of PFHO after a single instillation in a human volunteer using the TearView camera suggest that this instrument is a valuable tool for measuring TFLL and PFHO dynamics on the ocular surface.

REFERENCES

1. Craig JP, Nichols KK, Akpek EK, et al. TFOS DEWS II definition and classification report. *Ocul Surf.* 2017;15:276–283.
2. Nguyen A, Naidoo KK, Ajouz L, et al. Changes in human meibum lipid composition related to the presence and severity of meibomian gland dysfunction. *J Ocul Pharmacol Ther.* 2024;40:562–570.
3. Georgiev GA, Eftimov P, Yokoi N. Structure-function relationship of tear film lipid layer: a contemporary perspective. *Exp Eye Res.* 2017;163: 17–28.
4. Borchman D. Lipid conformational order and the etiology of cataract and dry eye. *J Lipid Res.* 2021;62:100039.
5. Tauber J, Wirta DL, Sall K, et al. A randomized clinical study (SEECASE) to assess efficacy; safety; and tolerability of NOV03 for treatment of dry eye disease. *Cornea*. 2021;40:1132–1140.
6. Tauber J, Berdy GJ, Wirta DL, et al. NOV03 for dry eye disease associated with meibomian gland dysfunction: results of the randomized Phase 3 GOBI study. *Ophthalmology*. 2023;130:516–524.
7. Sheppard JD, Kurata F, Epitropoulos AT, et al, MOJAVE Study Group. NOV03 for signs and symptoms of dry eye disease associated with meibomian gland dysfunction: the randomized Phase 3 MOJAVE Study. *Am J Ophthalmol.* 2023;252:265–274.
8. Steven P, Scherer D, Krösser S, et al. Semifluorinated alkane eye drops for treatment of dry eye disease--A Prospective; multicenter noninterventional study. *J Ocul Pharmacol Ther.* 2015;31:498–503.
9. Steven P, Augustin AJ, Geerling G, et al. Semifluorinated alkane eye drops for treatment of dry eye disease due to meibomian gland disease. *J Ocul Pharmacol Ther.* 2017;33:678–685.
10. Schmidl D, Bata AM, Szegedi S, et al. Influence of perfluorohexyloctane eye drops on tear film thickness in patients with mild to moderate dry eye disease: a randomized controlled clinical trial. *J Ocul Pharmacol Ther.* 2020;36:154–161.
11. Eberwein P, Krösser S, Steven P. Semifluorinated alkane eye drops in chronic ocular graft-versus-host disease: a prospective; multicenter; noninterventional study. *Ophthalmic Res.* 2020;63:50–58.
12. Agarwal P, Khun D, Kroesser S, et al. Evaluating the lubricating effect of semifluorinated alkanes on the ocular surface. *Invest Ophthalmol Vis Sci.* 2018;59:3282.
13. Mukerjee P. Fluorocarbon—hydrocarbon interactions in micelles and other lipid assemblies, at interfaces, and in solutions. *Colloids Surf A: Physicochem Eng Aspects*. 1994;84:1–10.
14. Kroesser S, Spencer E, Grillenberger R, et al. Ocular and systemic distribution of ^{14}C-perfluorohexyloctane following topical ocular administration to rabbits. *Invest Ophthalmol Vis Sci.* 2018;59:2656.
15. Postel M, Riess JG, Weers JG. Fluorocarbon emulsions—the stability issue. *Artif Cell Blood Substit Immobil Biotechnol*. 1994;22:991–1005.
16. Riess JG, Krafft MP. Fluorinated materials for in vivo oxygen transport (blood substitutes); diagnosis and drug delivery. *Biomaterials*. 1998;19: 1529–1539.
17. Borchman D, Vittitow J, Ewurum A, et al. Spectroscopic study of perfluorohexyloctane human meibum interactions. *Invest Ophthalmol Vis Sci.* 2022;63:1525.
18. Stolowich N, Vittitow J, Kissling R, et al. Oxygen carrying capacity of perfluorohexyloctane, a novel eye drop for dry eye disease. *Curr Ther Res Clin Exp.* 2023;98:100705.
19. Vittitow J, Kissling R, DeCory H, et al. In vitro inhibition of evaporation with perfluorohexyloctane, an eye drop for dry eye disease. *Curr Ther Res Clin Exp.* 2023;98:100704.
20. Trefil JS. *The Nature of Science: An A-Z Guide to the Laws and Principles Governing Our Universe*. Boston: Houghton Mifflin Harcourt; 2003.
21. Kokkinakis J, Schuett BS, Millar TJ. Effects on the human tear film of applying skin lipids to the ocular surface. *Cornea*. 2023;42:1562–1571.
22. Tauber J, Owen J, Bloomenstein M, et al. Comparison of the ILUX and the LipiFlow for the treatment of meibomian gland dysfunction and symptoms: a randomized clinical trial. *Clin Ophthalmol.* 2020;14:405–418.
23. Ewurum A, Ankem A, Georgiev G, et al. A spectroscopic study of the composition and conformation of cholesteryl and wax esters purified from meibum. *Chem Phys Lipids*. 2021;238:105088.
24. Borchman D, Vittitow J, Kissling R, et al. Spectroscopic characterization of perfluorohexyloctane, an eye drop for dry eye disease. *Invest Ophthalmol Vis Sci*. 2023;64:3975. Presented at the ARVO Conference, May 2023, New Orleans, LA.
25. American Society of Heating, Refrigerating, and Air-Conditioning Engineers. *2009 ASHRAE Handbook: Fundamentals: I-P Edition*. Atlanta: American Society of Heating, Refrigerating, and Air-Conditioning Engineers, 2009.
26. Crahay FX, Debellemanière G, Tobalem S, et al. Quantitative interocular comparison of total corneal surface area and corneal diameter in patients with highly asymmetric keratoconus. *Sci Rep.* 2022;12:4276.
27. Millar TA. Surface area of the exposed eye. *Invest Ophthalmol Vis Sci.* 2021;62:18.
28. Sledge SM, Khimji H, Borchman D, et al. Evaporation and hydrocarbon chain conformation of surface lipid films. *Ocul Surf.* 2016;14:447–459.

29. Borchman D, Foulks GN, Yappert MC, et al. Factors affecting evaporation rates of tear film components measured in vitro. *Eye Contact Lens*. 2009;35:32–37.
30. Borchman D, Yappert MC, Milliner SE, et al. ^{13}C and ^{1}H NMR ester region resonance assignments and the composition of human infant and child meibum. *Exp Eye Res.* 2013;112:151–159.
31. Mochizuki H, Yamada M, Hatou S, et al. Turnover rate of tear-film lipid layer determined by fluorophotometry. *Br J Ophthalmol.* 2009;93:1535–1538.
32. Nosch DS, Pult H, Albon J, et al. Relationship between corneal sensation, blinking, and tear film quality. *Optom Vis Sci.* 2016;93:471–481.
33. Habbe KJ, Frings A, Saad A, et al. The influence of a mineral oil cationic nanoemulsion or perfluorohexyloctane on the tear film lipid layer and higher order aberrations. *PLoS One*. 2023;18:e0279977.
34. Delicado-Miralles M, Velasco E, Díaz-Tahoces A, et al. Deciphering the action of perfluorohexyloctane eye drops to reduce ocular discomfort and pain. *Front Med (Lausanne)*. 2021;8:709712.