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In Vitro Inhibition of Evaporation with Perfluorohexyloctane, an Eye Drop for Dry Eye Disease ☆

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ABSTRACT

Objective: Perfluorohexyloctane (PFHO) MIEBO™, formerly (NOV03) is a single component, water-free eye drop approved by the Food and Drug Administration in the United States for the treatment of dry eye disease. We evaluated the in vitro inhibitory effect of PFHO on the evaporation rate (R_{evap}) of saline.
Methods: Evaporation rates were measured gravimetrically at 25°C or 35°C. The evaporation rate (R_{evap}) of phosphate-buffered saline (PBS) was measured following the application of 11-200 µL PFHO or 100 µL artificial tears (Soothe XP [Bausch + Lomb, Bridgewater, New Jersey], Systane Balance [Alcon, Fort Worth, Texas], and Systane Ultra [Alcon]). The effect of PFHO on the R_{evap} of PBS was further evaluated following the addition of 50 mg/mL mucin to PBS and compared with that of meibum lipid collected from a 68 year-old White volunteer.
Results: At 25°C the mean (SEM) R_{evap} of PBS alone or PFHO alone was 4.06 (0.06) and 0.137 (0.004) µm/min, respectively. Layering 100 µL PFHO over PBS inhibited the R_{evap} of PBS by 81% ($P < 0.0001$), whereas artificial tears had no effect. The presence of mucin attenuated the inhibition of the R_{evap} of PBS by PFHO by 17% ($P < 0.0001$). At 35°C, the R_{evap} of PBS was inhibited by 88% when layering 100 µL PFHO over PBS and 28% when applying a single 11 µL drop of PFHO (P value < 0.0001 for both). Meibum lipid inhibited the R_{evap} of PBS by 8% at this temperature, whereas the combination of a drop of PFHO plus meibum inhibited the R_{evap} of PBS by 34%.
Conclusions: PFHO significantly inhibited the R_{evap} of saline in this in vitro model. The data support the idea that PHFO may form an antievaporative layer on the tear film surface and may be a functional substitute for the native tear-film lipid layer in patients with dry eye disease.

Introduction

Dry eye disease (DED) is a common presentation in eye care and can be attributed to reduced tear production, increased tear evaporation, or a combination thereof.[1] The majority of DED is evaporative in nature, and the primary cause of evaporative DED is Meibomian gland dysfunction (MGD).[2,3] In MGD, qualitative changes and/or reductions in meibum secretion are believed to lead to excessive tear evaporation, which, if uncontrolled, leads to a loss of tear film homeostasis and tear film instability.[2,4,5]

Perfluorohexyloctane (MIEBO™, formerly NOVO3, henceforth abbreviated PFHO), a semifluorinated alkane (Figure 1) is approved by the Food and Drug Administration in the United States as a prescription treatment for DED.[6–8] It has also been used as a component of artificial blood,[9–15] in drug delivery,[16–22] as an endotamponade,[23,24] and is registered as a medical device outside the United States.[25–30] PFHO is an amphiphilic molecule, consisting of 6 perfluorinated and 8 hydrogenated carbon atoms. The hydrogenated carbon chain of PFHO has a much smaller cross-sectional area, 18.5 Å, than the perfluorinated carbon chain, at 28 Å

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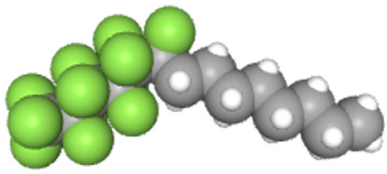

Figure 1. Space filling model of perfluorohexyloctane. White balls are hydrogen atoms, gray balls are carbon atoms and the green balls are fluorine atoms.

(Figure 1),[31] which gives PFHO its unique physical properties compared with nonfluorinated or completely fluorinated hydrocarbons. Semifluorinated alkanes are immiscible with hydrocarbons or water and, unlike hydrocarbons, do not form ordered liquid crystalline phases when the number of perfluorocarbons in the semifluorinated alkane is <8.[32] The immiscibility may arise because perfluorinated hydrocarbons are much more hydrophobic compared with alkanes[33] and because fluorine is less polarizable than hydrogen. Computational studies indicate that "relatively weak intermolecular forces in perfluoroalkanes compared to alkanes are their ground-state geometries, which are increasingly unsuitable for intermolecular interactions as the carbon chain length increases, and their rigidity, which makes deformation from the ground-state geometries unfavorable."[34] Thus, the physical properties of, and cohesive energy between perfluorinated carbon chains is much less compared with protonated carbon chains resulting in a lower surface tension.[35]

PFHO does not scatter or absorb visible light, transmitting >99% of visible light, but absorbs harmful higher energy ultraviolet light.[36] Infrared spectroscopy,[36] differential scanning calorimetry,[37] and molecular volume measurements[38] indicate that unlike most semifluorinated alkanes, PFHO is a liquid above 0°C, and no conformational or structural phase transitions were observed. As a consequence of it being a liquid at physiological temperatures with a positive spreading coefficient,[24] and surface rheology,[39] PFHO would be expected to readily spread across the ocular surface on instillation. One would expect that PFHO layered on top of the tear film would prevent evaporation of the aqueous layer underneath.[25] However, until now, there were no published articles quantifying the antievaporative properties of PFHO.

The aim of the current study was to explore the antievaporative mode of action of PFHO further. An in vitro assay incorporating the approximate area of the ocular surface was used to measure the evaporation rate (R_{evap}) of phosphate-buffered saline (PBS) following the application of PFHO compared with common over-the-counter artificial tears. As well, the inhibition of evaporation of PBS with PFHO was evaluated in the presence of mucin added to PBS to simulate how the antievaporative properties of PFHO might be influenced when the tear film has evaporated down close to the mucin layer. Finally, the inhibitory effect of a single drop of PFHO on the evaporation of PBS was evaluated at a temperature close to that of the eyelid and compared with that of meibum from a healthy volunteer.

Methods

Materials

PFHO (NovaTears lot TA0310A) was obtained from Novaliq, Heidelberg Germany. The following artificial tears were obtained from Walmart, San Bruno, California: Soothe XP (Bausch and Lomb, Bridgewater, New Jersey), Systane Balance (Alcon, Fort Worth, Texas), and Systane Ultra (Alcon). PBS and mucin from bovine submaxillary glands, Type 1S were obtained from Sigma Chemical Company (St Louis, Missouri).

Meibum was collected by medically qualified personnel as described below. All protocols were in accordance with the tenets of the Declaration of Helsinki and approved by the University of Louisville Institutional Review Board (No. 11.0319; October 2021).

Meibum collection

Written consent was obtained from a 68-year-old male volunteer without DED. Briefly, meibum was expressed from all 4 eye lids using an ILUX instrument (Alcon) according to the manufacturer's instructions other than that the lids were not anesthetized beforehand.[40] Approximately 0.5 mg meibum was collected from all 4 lids with a platinum spatula and immediately dissolved into 0.5 mL chloroform-d in a 9-mm glass microvial with a polytetrafluoroethylene cap (Microliter Analytical Supplies Inc, Suwanee, Georgia) and stored in the freezer until use.

NMR spectroscopy

A 700 MHz ^{1}H-NMR was used to analyze the cholesterol ester (CE) and wax ester (WE) composition of collected meibum. Analyses were performed with 1024 scans, 45^0 pulse width, and a 1.000 s relaxation delay between 0 and 11 ppm. Chemical shifts were referenced to the chloroform-d resonance at 7.25-ppm resonance. GRAMS/386 software (Galactic Industries, Salem, New Hampshire) was used to analyze all spectra. The molar ratios of CE to WE, were calculated from Eqs. 1–3 below as described previously.[41]

$$\text{CE/WE molar ratio} = I_{4.6} \text{ X } 2/I_{4.1} \quad (1)$$

$$\text{CE/WE molar ratio} = (I_1 + I_{6.3})/I_{4.1}/3 \quad (2)$$

$$\text{Average of CE/WE molar ration from equation 1 and 2.} \quad (3)$$

Where *I* is the area of the resonance (ppm) that appears as a subscript.

Calculation of the amount of WE and CE collected.

The amount of WE and CE collected was determined similar to a previous study.[41] Standard solutions of cholesteryl stearate and stearyl stearate in chloroform-d were prepared at the following concentrations: 0, 0.01, 0.05, and 0.1 mg/mL. A ^{1}H-NMR spectrum was measured for each sample. A WE standard curve was made by plotting the ratio of the WE resonance intensity at 4.01 ppm/the chloroform-d resonance intensity at 4.25 ppm for the *y*-axis, vs the milligrams per milliliter standard on the *x*-axis. A CE standard curve was made by plotting the ratio of the CE resonances at 1 and 0.65 ppm / the chloroform-d resonance intensity at 4.25 ppm for the *y*-axis vs the milligrams per milliliter standard on the *y*-axis.

R_{evap} studies

R_{evap} was measured gravimetrically as described previously using a Mettler-Toledo AT261 analytical balance (Columbus, Ohio).[42–44] The balance was calibrated and certified by a Mettler technician. Unless stated otherwise, R_{evap} measurements were conducted at 25°C. To control the temperature of the experiments, samples were placed on an aluminum block, the temperature of which was controlled by water circulating through it from a Haake-B water bath (Haake Manufacturing, DeSoto, Missouri). To measure the R_{evap} of PBS, PFHO, and artificial tears alone, 1 mL samples were placed into a plastic container 0.8-cm deep with an inside diameter of 1.5 cm as reported elsewhere.[42–44] At these dimensions, the surface area of samples within the container, 1.77 cm^2, is similar to that of the human ocular surface, of 1.3 cm^2 to 2 cm^2.[45,46] Sample weight was recorded to 5 decimal places once every 10 minutes over a period of 100 minutes. PBS was used as

a surrogate for actual tears, given the R_{evap} of human tears is the same as that of PBS alone[43] and the length of time that would have been required to collect the volume of tears needed prohibited using human tears in the current study.

To evaluate the effect of PFHO and artificial tears on the R_{evap} of PBS, aliquots of PFHO (11–200 µL) or artificial tears (100 µL) were placed on the surface of 1 mL samples of PBS, and total sample weight was recorded to 5 decimal places every 10 minutes over 100 minutes. Similarly, to evaluate the effect of meibum lipids with and without PFHO compared with PFHO only on the R_{evap} of PBS, meibum lipids (34 µL containing 20.2 µg meibum esters in chloroform-d) was applied to the surface of the 1-mL samples of PBS at 35 °C to form a 125-nm thick layer of meibum lipids. After a 30-minute equilibration period, aliquots of PFHO (11 or 100 µL) were added to the appropriate meibum lipids/PBS samples or PBS-only samples and the total sample weight was recorded to 5 decimal places every 10 minutes over 100 minutes. To evaluate the effect of mucin, dissolved in PBS, on the inhibition of the R_{evap} of PBS by PFHO, mucin (50 mg/mL) dissolved in PBS was used as the subphase and aliquots of PFHO (100 µL) were added to the mucin/PBS samples or PBS-only samples. Again, the total sample weight was recorded to 5 decimal places every 10 minutes over 100 minutes. The R_{evap} for PBS alone was included in every experiment as a control.

In all experiments, the total R_{evap} for each sample was obtained from the slope of the best-fit line obtained by least squares linear regression analysis expressed in units of grams per minute. To convert R_{evap} in grams per minute to R_{evap} in micromoles per minute, the following equation was used:

$$R_{evap}(g/min)/density(g/mL) \times 1cm^3/mL/area(cm^2) \times 10^4 \mu m/cm$$

Where 'area' is the surface area of the samples within the container exposed to the air, or 1.766 cm^2. A density of 1.000 g/mL was used for PBS (actual density of water is 0.997 g/cm^3 at 25 °C) and artificial tears, and a density of 1.331 g/mL was used for PFHO. For experimental samples in which PFHO was added to PBS, or PBS and meibum, the R_{evap} of PBS was determined by subtracting the R_{evap} of pure PFHO at the relevant temperature from the total R_{evap} of the combined sample to generate an adjusted R_{evap}. This correction was not necessary when artificial tears were added as the difference between the R_{evap} of the artificial tears alone and PBS alone was not significantly different except for Systane Balance, where the difference was minimal (<10%). The percent inhibition of the R_{evap} of PBS, because of the added PFHO, was calculated by subtracting the product of the adjusted R_{evap} multiplied by 100 and divided by the R_{evap} of PBS alone from 100.

R_{evap} data are presented as mean (SEM). *P* values to test for significance were measured using a 2-tailed Student *t* test. A *P* value < 0.05 was considered statistically significant.

Results

^{1}H-NMR spectroscopy of human meibum lipids

The ^{1}H-NMR spectrum of meibum lipids collected from a 68-year-old White man was measured and was typical of human meibum spectra (Figure 2, Table 1). Standard curves were prepared as outlined in the Methods section and used to calculate the amount of meibum lipids collected. In 649 µL chloroform-d there was 1.7 mg meibum lipids per milliliter-pooled meibum. The slopes and correlation coefficients of the standard curves used to make the calculation were 0.688, $r = 0.9941$; and 0.458, $r = 0.9923$, for cholesteryl stearate and stearyl stearate, respectively.

Proton NMR analysis showed the CE/WE molar ratio of the meibum lipids sample used for the current study was 0.75, 0.68, 0.71, as calculated based on Eqs. 1–3 of the Methods, respectively, and was characteristic of previous meibum and meibum lipids samples collected from the same donor.

Table 1
Assignments for resonances for human meibum.

Resonance No.*	Chemical shift, ppm	Proton resonance assignment†
1	5.36	Cholesterol carbon #6
2	5.33	Hydrocarbon =CH- *cis*
3	5.125	Squalene
4	4.6	Cholesteryl ester carbon #3
5	4.29-4.10	Tri- and di-glycerides
6	3.9	Wax ester $-CH_2$-O-(C=O)-
7	1.55	$-CH_2$-
8	1.24	$CHCl_3$
9	0.998	Cholesterol carbon # 19
10	0.906	Cholesterol carbon #21
11	0.897	Cholesterol carbon #21
12	0.878	Straight-chain
13	0.868	Straight-chain
13	0858	Straight-chain
14	0.853	Iso-branched
14 right shoulder	0.850	Anteiso-branched
15	0.843	Iso-branched
15 right shoulder	0.839	Anteiso-branched
16	0.829	Anteiso-branched
17	0.821	Anteiso-branched
18	0.663	Cholesterol carbon #18

* Corresponds to resonance numbers in Figure 2.
† The carbon number refers to the protons on that carbon.

Table 2
Evaporation rates of samples alone.

Sample	Evaporation rate,* µm/min	Number of determinations
		25 °C†
Phosphate-buffered saline	4.06 (0.06)	37
Perfluorohexyloctane	0.137 (0.04)	12
Systane Ultra‡	3.77 (0.1)	6
Systane Balance‡	3.2 (0.02)	8
Soothe XP§	3.65 (0.07)	4
		35 °C
Perfluorohexyloctane	0.82 (0.12)	6
Phosphate buffered saline	10.47 (0.008)	16

* Values are presented as mean (SEM).
† Values are presented as n.
‡ Alcon, Fort Worth, Texas.
§ Bausch + Lomb, Bridgewater, New Jersey.

R_{evap}

In all experiments, the room temperature was 25 °C, and the relative humidity varied between 31% and 35%. R_{evap} was linear over the 100-minute testing period for all experimental samples based on correlation analysis of the best-fitted line of the plot of weight vs time ($r > 0.999$ for all).

The mean (SEM) (number of determinations) R_{evap} for PBS alone, PFHO alone, and for artificial tears alone are given in Table 2. The R_{evap} of PFHO alone was significantly different from that of PBS alone ($P < 0.0001$). Of the artificial tears, only Systane Balance alone demonstrated a slightly lower, significantly different R_{evap} vs PBS alone ($P < 0.0001$).

Layering PFHO (50, 100, 150, and 200 µL) over PBS inhibited the R_{evap} of PBS by 71%, 81%, 82%, and 87%, respectively (Figure 3 and Table 3). Regardless of the amount of PFHO added, the R_{evap} of PBS was significantly lower in the presence of PFHO compared with PBS alone ($P < 0.0001$ for all PFHO aliquots).

The effect of layering PFHO on the R_{evap} of PBS was compared with that of artificial tears. Results (Figure 4) showed that while the R_{evap} of PBS was significantly inhibited by the addition of 100

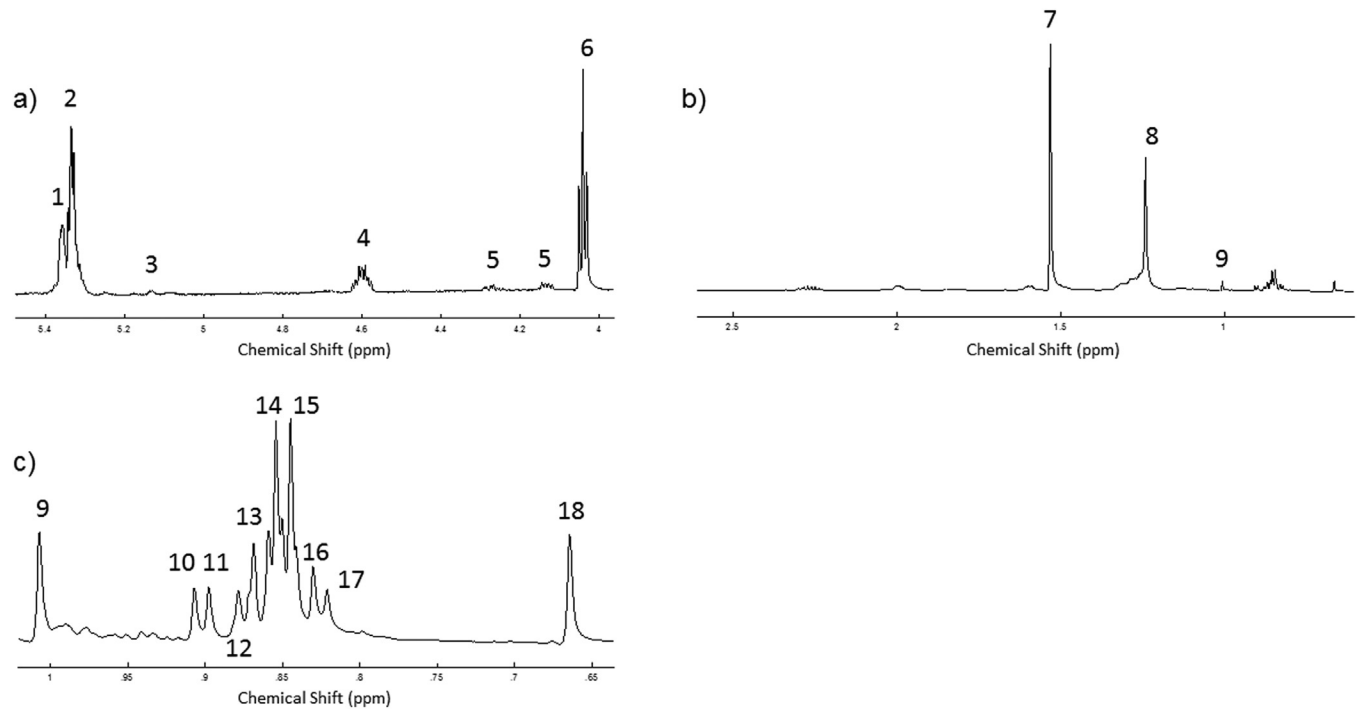

Figure 2. [1]H-NMR spectra (a, b, c) of human Meibum collected from a 68-year-old White man without dry eye disease. Numbers refer resonance assignments in Table 1.

Table 3
Evaporation rate for phosphate-buffered saline with increasing amounts of perfluorohexyloctane (PFHO) layered on top at 25 °C.*

Parameter	PFHO added, µL				
	0 (n = 37)	50 (n = 7)	100 (n = 12)	150 (n = 8)	200 (n = 4)
Evaporation rate, g/min‡	0.00072 (0.00001)	0.00024 (0.00002)	0.00017 (0.00002)	0.00016 (0.00004)	0.000129 (0.000003)
Adjusted evaporation rate (g/min)†	NA	0.00021 (0.00002)	0.00014 (0.00001)	0.00013 (0.00003)	0.000097 (0.000003)
% Inhibition		71	81	82	87
Evaporation rate, µm/min‡	4.06 (0.06)	1.4 (0.1)	0.78 (0.07)	0.9 (0.2)	0.73 (0.01)

NA = not available.
* Values are presented as mean (SEM).
† Minus evaporation due to PFHO at 0.00005 g/min.
‡ $P \leq 0.001$ for all data compared with 0 µL PFHO added.

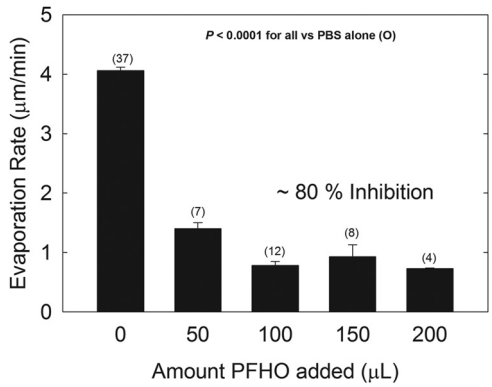

Figure 3. Mean (SEM) evaporation rates of phosphate-buffered saline alone and with increasing amounts of perfluorohexyloctane (PFHO) layered on top at 25 °C. Numbers in parentheses are the number of determinations. *P* values to test for significance were measured using the Student *t* test.

µL PFHO ($P < 0.0001$), the addition of 100 µL artificial tear eye drops had no influence on the R_{evap} of PBS ($P \geq 0.13$ vs PBS alone). Specifically, the mean (SEM) R_{evap} of PBS was 3.9 (0.2) µm/minute with Systane Ultra, 3.8 (0.1) µm/minute with Systane Balance, 3.77 (0.08) µm/minute with Soothe XP, and 0.78 (0.07) µm/minute with PFHO layered on top.

The effect of layering PFHO on the R_{evap} of PBS containing mucin is shown in Figure 5. The mean (SEM) R_{evap} for PBS alone and PBS containing mucin were 4.43 (0.07) and 4.34 (0.08) µm/minute, respectively, and were not statistically different. When 100 µL PFHO was layered on top of PBS alone or PBS containing added mucin, the mean (SEM) R_{evap} of PBS was decreased to 0.78 (0.07) and 1.40 (0.09) µm/minute ($P < 0.0001$), corresponding to a percent inhibition of the R_{evap} of PBS by PFHO of 82% and 68%, respectively. Thus, the inhibition of the R_{evap} of PBS by PFHO was decreased by 17% in the presence of mucin compared with PBS without mucin.

The effect of layering a single 11-µL drop or 100 µL PFHO on the R_{evap} of PBS at 35 °C in comparison to that of a 125 nm layer of meibum lipids or the combination of PFHO with a 125 nm layer of meibum lipids is shown in Figure 6. The mean (SEM, number of determinations) R_{evap} for PBS alone, PFHO alone, PBS with 100 µL PFHO layered on top, and PBS with 1 11-µL drop of PFHO (ie, the actual volume of a dispensed drop) layered on top were 10.47 (0.08, 16), 0.82 (0.12, 6), 1.3 (0.3, 8), and 7.6 (0.9, 6) µm/minute, respectively, corresponding to an inhibition of the R_{evap} of PBS by PFHO of 88% and 28% for the 100 µL aliquot and the single 11-µL drop, respectively, at this temperature ($P < 0.0001$ vs PBS alone).

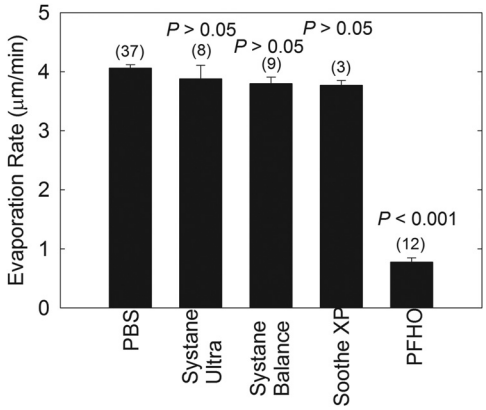

Figure 4. Mean (SEM) evaporation rates of phosphate-buffered saline alone (PBS) and with 100 µL various over-the-counter eye drop samples and perfluorohexyloctane (PFHO) placed on top at 25 °C. Numbers in parentheses are the number of determinations. *P* values to test for significance vs buffer alone were measured using the Student *t* test. Systane Ultra (Alcon, Fort Worth, Texas). Systane Balanced (Alcon, Fort Worth, Texas). Soothe XP (Bausch + Lomb, Bridgewater, New Jersey).

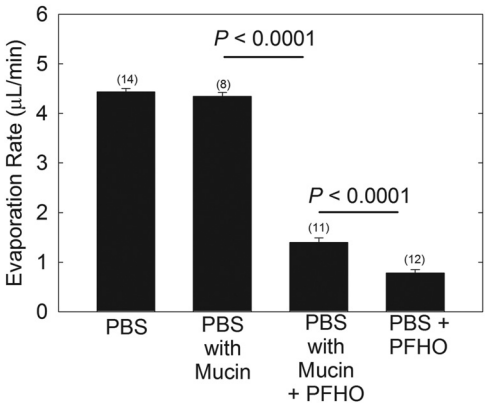

Figure 5. Mean (SEM) evaporation rates for phosphate-buffered saline (PBS) or PBS containing 50 mg/mL mucin (PBS with mucin) alone and each with 100 µL perfluorohexyloctane (PFHO) layered on top at 25 °C. Numbers in parentheses are the number of determinations. *P* values to test for significance were measured using the Student *t* test.

In contrast, the mean R_{evap} for PBS with a layer of meibum lipids was 9.66 (0.15, 6) µm/minute corresponding to an inhibition of the R_{evap} of PBS by meibum lipids of only 8%; whereas the mean R_{evap} for PBS with a layer of meibum in combination with a 11-µL drop or 100 µL PFHO layered on top were 6.9 (0.6, 8), and 1.82 (0.22, 8) µm/minute, respectively, corresponding to an inhibition of the R_{evap} of PBS of 34% and 83%, respectively ($P < 0.0001$ vs PBS alone). Thus, a single 11-µL drop of PFHO inhibited the R_{evap} of PBS nearly 4 times more than meibum lipids alone, whereas the addition of 100 µL PFHO inhibited the R_{evap} of PBS approximately 10 times more than meibum lipids alone. There was no difference between the R_{evap} of PBS with only PFHO layered on top compared with both meibum lipids and PFHO layered on top ($P > 0.5$).

Discussion

PFHO was recently approved by the Food and Drug Administration in the United States for the treatment of DED. PFHO is a preservative-free, water-free, single-component ocular drop that is believed to be a functional substitute for the lipid layer of the tear film, inhibiting tear evaporation.[25] We evaluated the in vitro inhibitory effect of PFHO on the evaporation rate of PBS in comparison to artificial tears and in the presence of mucin added to PBS.

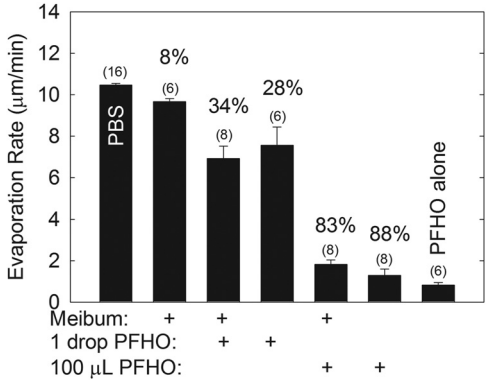

Figure 6. Mean (SEM) evaporation rate (R_{evap}) of phosphate-buffered saline (PBS) alone, PFHO alone, and PBS overlaid with human meibum with and without the addition of either an 11-µL drop of or 100 µL PFHO layered on top at 35 °C. Meibum was obtained from a 68-year-old White man. Percentage values are percent inhibition of the R_{evap} of PBS. Numbers in parentheses are the number of determinations. *P* values to test for significance were measured using the student's *t* test.

Our in vitro model mimicked the area of the surface of the eye. At 25 °C, a 100-µL sample of PFHO layered on top of PBS inhibited the evaporation rate of PBS by 82%, whereas artificial tears failed to inhibit the evaporation of PBS. PFHO had a similar effect on the rate of evaporation of PBS at 35°C, a temperature very close to that of the eyelid,[47] inhibiting the evaporation rate of PBS by 88% at this temperature. The inhibition of evaporation of PBS by PFHO was robust and only slightly attenuated when mucin was added to the PBS.

At 35 °C the in vitro R_{evap} of PFHO alone was 0.82 µm/minute, 13 times slower than that of PBS alone at that temperature. At this rate of evaporation, with no other attenuating factors, 1 drop (11 µL or 0.015 g) of PFHO is expected to be present on the eye inhibiting tear evaporation for 1.7 hours. However, PFHO has been shown to be present in tears for at least 6 hours following ocular application in rabbits,[48] indicating that, in vivo, PFHO likely interacts with tear moieties (ie, polar and nonpolar lipids), and is retained on the ocular surface much longer than under in vitro static conditions. An in vitro nuclear magnetic resonance spectroscopic study indicates that meibum is miscible in PFHO, and an in vivo emissivity study visualizing a drop of PFHO on the ocular surface of a human volunteer showed it to be present for the full 2-hour evaluation period. Notably, PFHO has been shown to be present in Meibomian glands of rabbits even longer,[48] suggesting that Meibomian glands or lipids on the eye lids[49] may function as reservoirs replenishing PFHO on the ocular surface as it evaporates. Studies evaluating the ocular residence time of PFHO in human volunteers and patients with DED are ongoing.

The average thickness of the tear film is 4.8 µm (0.9).[50] At 35 °C, PBS was found to evaporate at 10.5 µm/minute. As the in vitro R_{evap} of human tears containing native tear lipids/meibum has been shown to be the same as that of PBS alone,[43] it follows that, based on this rate, half the tear film is expected to evaporate in 14 seconds in vivo, consistent with the average tear film breakup time of a person without DED who is older than age 50 years,[51] and it would take approximately half a minute to completely evaporate a 5-µm thick tear film.

A recent literature review reported that, in vivo, the R_{evap} of tears increases with various forms of DED.[52] The absolute value of the R_{evap} for normal individuals varies over orders of magnitude.[52] Similarly, the average percent increase in the R_{evap} of tears in patients with DED varied widely among the 33 studies reported.[52] In the current study, 1 drop of PFHO inhibited R_{evap} of PBS by 28% at 35°C. The inhibition of R_{evap} of PBS was even greater, 88%, with 100 µL PFHO added. One would expect that the inhibition of R_{evap}

of tears by PFHO would ameliorate much of the excessive evaporation of tears observed in DED.[52]

Tear mucins serve as a barrier, preventing apical cell-surface adhesion, protecting against pathogen colonization, clearing molecules and microorganisms, and hydrating and lubricating the corneal surface.[53] Membrane-associated mucins have glycosylated portions that extend 200 to 500 nm above the ocular surface.[54] Because the glycosylated portion is hydrophilic, it is unlikely that the membrane-associated mucins interact with hydrophobic PFHO. Mucins 2 and 5AC are gel-forming soluble mucins found in tears.[54] It has been reported that the hydrophobic portion of soluble mucins do interact with hydrophobic waxes and meibum.[55,56] Thus, it is possible that the hydrophobic region of mucins may also interact with hydrophobic PFHO.

To provide insight into the antievaporative action of PFHO when the tear film evaporates down to the mucin layer, PFHO was added on top of a solution of PBS containing mucin 1S, as a representative soluble mucin, and the R_{evap} of PBS was measured. The addition of mucin did not affect the R_{evap} of PBS itself, in agreement with a previous study.[43] However, PFHO was found 17% less effective in inhibiting the R_{evap} of PBS in the presence of added mucin. It should be noted that the amount of soluble mucin such as MUC5AC ranges from undetectable to 0.2 mg/mL in human tears, 250 times less than the amount of soluble mucin used in the current study.[57] Thus, it is expected that, in vivo, the antievaporative effect of PFHO may be reduced, albeit minimally. PFHO is expected to inhibit the excessive evaporation of the tear film layer significantly even in the presence of tear mucins.

A major finding of the current study is that, in vitro, PFHO was found to inhibit the R_{evap} of PBS 4 (with 1 11-µL drop,) to 10 (with 100 µL) times more than human meibum. In contrast, in our study, a 125-nm thick film of meibum lipids only inhibited the R_{evap} of PBS by 8%. This is consistent with another study[58] and is in relative agreement with yet other studies that show that, in vitro, meibum lipid does not inhibit the R_{evap} of saline significantly.[42,43,58–60] The finding also brings into question the degree to which the native tear film lipid layer inhibits the R_{evap} of tears in vivo. In this regard, a review of the literature since 1980 shows that in vivo the mean (SD) R_{evap} of tears from 54 healthy volunteers without dry eye is 1.0 (0.7) µg/cm^2/s (see Supplemental Table 1 in the online version at doi:xxxxxxxx), similar to the R_{evap} of tears from 59 patients with dry eye, or 1.08 (0.68 µg/cm^2/second) (see Supplemental Table 2 in the online version at doi:xxxxxxxx). Along the same lines, we previously found the R_{evap} of human reflex tears to be 0.88 µg/cm^2/second (12.3 µm/minute),[43] whereas in the current study, the R_{evap} of PBS was 0.75 µg/cm^2/second (10.5 µm/minute) at 35 °C.

A limitation of the current study is that the influence of PFHO on R_{evap} was not measured in vivo, in an environment where blinking and tear film composition is more complex than the current in vitro study. Thus, an in vivo study of the R_{evap} of tears after instillation of a drop of PFHO is warranted. However, given the order of magnitude differences between the measurements of in vivo evaporation rates measured in different studies, a large number of measurements and patients will need to be studied for the results to be meaningful.

Tear break-up time is associated with the R_{evap} of tears, and tear break-up time is much shorter with dry eye. In addition, there is evidence that PFHO treatment increases the tear film thickness, which is related to R_{evap} and breakup time.[26,29] Exciting is the possibility that PFHO on the surface of the eye decreases the R_{evap} of tears. It follows that, PFHO likely ameliorates ocular surface inflammation resulting from desiccation stress and facilitates corneal healing. Thus, on the eye, in the presence of PFHO, tear turnover via blinking is expected to restore and spread the tear aqueous layer long before tear evaporation dries the ocular surface. A restored tear film, in turn, will attenuate the inflammatory response at the surface and permit healing of the ocular surface epithelium. Indeed, results of 3, large randomized controlled US registry studies evaluating PFHO in patients with DED associated with MGD have shown significant reductions of total corneal fluorescein staining at day 57, following treatment with PFHO administered 4 times a day compared with saline.[6,7,8] A significant decrease from baseline in total corneal fluorescein staining compared with saline was demonstrated by day 15 of PFHO treatment, the first on-treatment clinic visit, in all of these studies.[6–8] Furthermore, it has been suggested that the lubricating effect of PFHO on the ocular surface provides symptomatic relief to patients with dry eye,[62] as also attested to in clinical studies. Thus, treatment with PFHO not only led to a significant change from baseline in dryness score (based on the visual analog scale) compared with saline treatment both at day 15 and day 57, but PFHO also had substantial effects on the visual analog scale burning/stinging and other dry eye symptoms, including foreign body sensation, itching, and sensitivity to light and pain.

An unstable tear film lipid layer leads to a pathological cycle of evaporation, inflammation, and ocular surface damage.[2,3] PFHO may be considered a functional substitute for the native tear film lipid-layer in patients with DED associated with MGD based on its ability to provide relief of dry eye signs and symptoms,[6–8] its optical properties,[36] its ability to provide oxygen to the cornea,[61] and as shown in the current study its superior ability to inhibit evaporation as compared to meibum lipids and aqueous artificial tears. As a topical administered eye drop, PFHO likely reduces surface friction[62] and forms a long lasting antievaporative barrier on the ocular surface, facilitating surface healing.

Declaration of Competing Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.curtheres.2023.100704.

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