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Comparison of Collection Methods for the Measure of Human Meibum and Tear Film-Derived Lipids Using Mass Spectrometry

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Abstract

Purpose/aim: To assess the effectiveness of polytetrafluoroethylene (PTFE) tubes in the collection of human tears and meibum.

Materials and methods: This was a prospective study that enrolled 10 healthy human subjects. Both the tear film and meibum were sampled using PTFE tubes in the right eye of all subjects. In the left eyes, either 5-μL or 1-μL glass microcapillary tubes were used to collect tears, and 0.5-μL glass microcapillary tubes were used to collect meibum. The lipids from the samples were extracted and analyzed using mass spectrometry (SCIEX TripleTOF 5600, Framingham, MA, USA). The absolute peak intensities of the omega-acyl hydroxy fatty acids (OAHFA), cholesterol esters (CE), and wax esters (WE) obtained for both methods were summed and compared between collection methods.

Results: A total of 10 subjects completed the study (five female, mean age: 35.7 ± 7.9 years). Using the mass spectrometer output, the median (first quartile, third quartile) summed intensity units of OAHFA, CE, and WE collected associated with tears using PTFE were 516 (125, 1315), 7946 (2571, 19,915), and 38,892 (139,630, 174,082), all of which were significantly higher (all p 0.04) than those collected from glass microcapillaries (91 (41, 408), 2463 (1389, 6042), and 11,109 (7465, 37,371), respectively). The median summed intensity units of OAHFA, CE, and WE associated with meibum (1958 (1417, 3502), 11,726 (8434, 87,691), and 84,771 (52,657, 206,050), respectively) using PTFE were not significantly different (all p 0.13) than those associated with glass microcapillaries (1502 (699, 4407), 10,781 (3287, 38,205), and 77,381 (26,590, 178,213), respectively).

Conclusions: PTFE tubes, which are thought to be lipophilic, were associated with more measurable lipids from the tear film than glass microcapillaries. There was no difference between collection methods in lipid profiles when used with meibum.

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Declaration of interests

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Keywords

Tear film; lipids; mass spectrometry; meibomian gland; dry eye

Introduction

Dry eye disease (DED) is a highly prevalent (5–50%) condition affecting many countries around the world.[1] The symptoms of DED may present as ocular stinging, burning, or irritation, especially during extended hours of reading,[2] computer use,[3] or in desiccating environments.[4] The pathological changes and dysregulation of the lacrimal gland, meibomian glands, or the ocular surface and tear film homeostasis are thought to be the cause of DED.[5,6] The definition of DED was recently updated in 2017 by the Tear Film and Ocular Surface Society (TFOS) Dry Eye Workshop II (DEWS II) to be "… a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles".[7]

The tear film is a thin film (approx. 3–5 μm),[8,9] consisting of a superficial lipid layer,[10] and accompanied by an aqueous-mucin phase underneath.[11] The tear film is responsible for protecting the ocular surface[11] and providing a smooth refractive surface for the eye.[10] The lacrimal glands, meibomian glands, and the cells of the ocular surface all function in unity to maintain the healthy structure of the tear film.[12] Ocular surface disease may result when one or more components of this system are compromised. For example, individuals with DED have altered tear film composition compared to normal individuals,[13] which may arise as a result of improperly functioning lacrimal or meibomian glands.[14]

The meibum and lipids of the tear film have been studied extensively.[10,15,16] They function to form the superficial lipid layer, which serve as a barrier against evaporation, and to stabilize and reduce the surface tension of the tear film.[16,17] The lipid layer is predominantly composed of nonpolar lipids such as wax esters (WE), cholesteryl esters (CE), and diesters.[18–20] To form a stable interaction between these nonpolar lipids and the polar aqueous-mucin phase, the presence of polar lipids (e.g., phospholipids[21]) is believed to be essential. Recently, a new class of polar lipids, omega-acyl hydroxy fatty acids (OAHFA) was identified in relatively higher amounts in the tear film than phospholipids and may contribute a greater role in maintaining stability of the interface.[20,22,23] The relative amount of OAHFA has been reported to range from 2.9–3.5%[24–26] of total lipids in meibum, and 2.9%[26] to 4.4%[25] of the total lipids in tears.

To study the role of the various lipids associated with the meibum and tear film, it is necessary to reliably collect and quantify the various lipid classes from the tears and meibum. The different methods of lipid sampling from the tears and/or meibum include Schirmer's strips, glass microcapillary tubes, Dacron swabs, cytological microbrushes, and metallic spatulas.[25–27] The small volume of lipids collected and sample contamination are two issues that must be addressed with improved sampling techniques. Some of these methods may be capable of collecting more lipids than others, due to larger surface area or greater invasiveness, yet samples may be prone to contamination from surrounding tissues,

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debris, and cells.-[27] In contrast, other methods may be associated with lower levels of contamination, but a direct result of this may be lower collected volumes.[26] Of course, patient safety and comfort relative to invasiveness is also a key criteria relative to the selection of an optimal collection method.

The aim of this study was to develop an approach that improves sampling of meibum and tear film lipids, beyond that obtained in prior collection studies.[27] Thus, a sampling tube that was non-invasive, yet which was hydrophobic and also resistant to degradation from chloroform (for downstream lipid extraction) was desired.[28] Inspired by the application in mass spectrometry, polytetrafluoroethylene (PTFE) met these desired features. During preliminary in vitro experiments, PTFE tubes were found to be capable of drawing up oil (Figure 1A) to a greater degree than they did water (Figure 1B); furthermore, the tubes were also capable of separating oil from water (Figure 1C). Based on these preliminary findings, it is hypothesized that PTFE tubes will be associated with greater measurable lipid profiles than glass microcapillaries when used for collecting lipids from tears and meibum.

Materials and methods

This study was conducted in compliance with the principles of the Declaration of Helsinki, and approval was obtained from The University of Alabama at Birmingham Institutional Review Board. Informed consent was obtained from all study subjects prior to conducting procedures.

Tears and meibum were collected from both eyes of 10 healthy subjects. In the right eye, tears and meibum were collected using PTFE tubes (Component Supply, Sparta, TN, USA). The tubing had a nominal inner diameter of 0.56 mm and wall size of 0.30 ± 0.05 mm and length of 30 mm. In the left eye, depending on ease of collection either a 5-μL or 1-μL glass microcapillary tube (Drummond Scientific Company, Broomall, PA, USA) was used for collecting tears, and a 0.5-μL glass microcapillary tube (Drummond Scientific Company, Broomall, PA, USA) was used for collecting meibum. The specific procedures for collecting tears and meibum using PTFE and glass tubes are outlined below. After collection, each individual tube was placed into a glass amber vial and stored at −20°C. Storage at −20°C was believed to be appropriate since the samples were stored for a brief duration of time, and because the samples were held within small diameter tubes, the exposure to air and subsequent degradation was minimal.[20]

The personnel involved with sample collection have had extensive training and experience in collecting tear samples using a variety of techniques. To maintain consistency with collection technique, one clinician (JFZ) collected the majority of samples. The process of tear collection was very similar for the clinician (the clinician knows which tube they were holding) and indistinguishable for the subject.

Collecting tears with PTFE tubes

The end of a PTFE tube was gently applied to the temporal inferior tear meniscus of the right eye. Gentle lateral motions were used to facilitate entry of tear lipids into the tube. Subjects were asked to blink frequently to prevent desiccating the ocular surface and to

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prevent reflex tearing. This study aimed to collect a minimum length of 0.5 mm of tear lipids within the PTFE tube.

Collecting meibum with PTFE tubes

The inferior meibomian glands of the right eye were expressed by applying digital pressure along the inferior eyelid margin. The eyelid margin was pulled away slightly from the ocular surface to avoid mixing the meibum with tears. The end of the PTFE tube was placed over the pools of expressed meibum, or was used to gently scoop meibum into the tube. This study aimed to collect a minimum length of 1 mm within the tube.

Collecting tears with glass microcapillary tubes

A 5-μL glass microcapillary tube was gently applied to the temporal inferior tear meniscus of the left eye. Subjects were asked to blink frequently to prevent desiccating the ocular surface and inducing reflex tearing. Tears were drawn into the glass microcapillary tube by capillary action. This study aimed to collect a minimum length of 5 mm of tears within the glass microcapillary tube. For two subjects, 1-μL glass microcapillary tubes were used.

Collecting meibum with glass microcapillary tubes

The inferior meibomian glands of the left eye were expressed by applying digital pressure along the lower eyelid margin. The eyelid margin was pulled away slightly from the ocular surface to minimize mixing of the expressed meibum with tears. As with the PTFE tubes, the opening of the 0.5-μL glass microcapillary tube was placed over the pools of expressed meibum to collect or scoop meibum into the tube. This study aimed to collect a minimum length of 1 mm within the glass microcapillary tube.

Mass spectrometry analysis

Chemicals—Chloroform (HPLC grade, > 99.9%, with amylene as the stabilizer), methanol (LC-MS grade, > 99.9%), water (CHROMASOLV™ LC-MS grade) and ammonium hydroxide solution (25%, eluent additive for LC-MS, Fluka) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Extraction—The meibum collected in glass microcapillary tubes was transferred to sample vials directly with a stainless steel wire as described previously.[20] For tears collected in glass microcapillary tubes, the tears were first dispensed into the sample vial, followed by rinsing the capillary tubes with the 2:1 chloroform–methanol mixture. Meibum and tears collected in PTFE tubes were transferred to sample vials by expressing and rinsing the tube in a 2:1 chloroform–methanol mixture using a microcapillary pipette bulb assembly (Sigma-Aldrich, St. Louis, MO, USA).

Folch extraction[29] was used to remove non-lipid components from the meibum and tear samples. In short, the samples were mixed with chloroform, methanol, and water in a ratio of 8:4:3, followed by vortexing and standing. This yielded two phases; the lower phase (containing lipids), and the upper phase. The lower phase was removed and mixed with an equal volume of methanol and 1% of 2.5% ammonium hydroxide solution as the additive to facilitate mass spectrometry analysis.

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The mass spectrometry analysis was conducted in a similar manner as previously reported to minimize interference from carryover and impurities from the environment.[19,20] The working solution as described above was directly injected into a TripleTOF 5600 mass spectrometer (SCIEX, Framingham, MA, USA), in either positive or negative ion mode. The MS spectra were acquired for 3 minutes in each mode.

The MS spectra were processed with Peakview (SCIEX, Framingham, MA, USA). The signals were averaged and a list of the peaks were exported. The raw peak intensities corresponding to four known OAHFA-class molecules (*m/z* 729.677, *m/z* 755.693, *m/z* 757.709, *m/z* 785.740) were summed for each collection procedure. In addition, the raw peak intensities of WE and CE commonly found in the meibum and tear film were also summed for each collection procedure. As the intended outcome was absolute lipid quantities, intensities were not adjusted for volume. Each lipid species and its *m/z* are detailed in Table A.1. The assignment of these lipid peaks was based on *m/z* values previously reported by MS and MS/MS lipid peaks.[19,20]

Statistical analysis

Statistical analysis was conducted using GraphPad Prism version 7.03 for Windows (GraphPad Software, La Jolla, CA, USA). Data distributions were non-normal as tested using the Shapiro–Wilk normality test at a threshold of $\alpha = 0.05$, and therefore reported as medians (first quartile, third quartile). The Wilcoxon signed rank test was used to test the difference in distribution of OAHFA, CE, and WE quantities (summed intensity units) collected from glass microcapillary tubes versus PTFE tubes. The differences in distributions are presented as medians of differences. The threshold for statistical significance was set at p 0.05.

Results

Tear and meibum collection was completed successfully on the 10 healthy volunteers (mean age ± SD = 35.7 ± 7.9 years, 5F). Mean volumes of 0.19 ± 0.15 µL and 0.09 ± 0.07 µL were associated with PTFE collection for tears and meibum, respectively. Mean volumes of 3.00 ± 1.98 µL and 0.02 ± 0.01 µL were associated with glass microcapillary collection for tears and meibum, respectively. Table 1 summarizes the volumes of samples collected from each subject.

Overall, PTFE tubes were associated with higher amounts of lipids from tears than glass microcapillaries. However, for meibum, the difference in amount of lipids collected between PTFE tubes and glass microcapillaries was undetectable. Tables 2 and 3 summarize the summed intensity values for OAHFAs, CEs, and WEs from tears and meibum, respectively.

Quantities of omega-acyl hydroxy fatty acids

The median (first quartile, third quartile) summed intensity of OAHFA from tears was 91 (41, 408) intensity units for glass microcapillaries, and was 516 (125, 1315) intensity units for PTFE. The median of differences was 443 intensity units, and the two distributions were significantly different ($p = 0.04$). The median summed intensity of OAHFA in meibum was 1502 (699, 4407) intensity units for glass microcapillaries, and was 1958 (1417, 3502)

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intensity units for PTFE. The median of differences was 429 intensity units, and the two distributions were not significantly different ($p = 0.56$). This information is summarized in Figure 2.

The mass spectrum plots demonstrated clear and strong OAHFA signals from tears associated with PTFE. In contrast, the OAHFA signals from samples associated with glass microcapillaries had relatively lower signal, and were difficult to discriminate from commonly known impurities peaks. The difference in mass spectrum OAHFA intensity between PTFE and glass microcapillaries for a single subject is demonstrated in Figure 3. Note the difference in scale.

Quantities of cholesterol esters

The median (first quartile, third quartile) summed intensity of CE from tears was 2463 (1389, 6042) intensity units for glass microcapillaries, and was 7964 (2571, 19,115) intensity units for PTFE. The median of differences was 5484 intensity units, and the two distributions were significantly different ($p = 0.01$). For meibum, the median summed intensity of CE was 10,781 (3287, 38,205) intensity units for glass microcapillaries, and was 11,726 (8434, 87,691) intensity units for PTFE. The median of differences was 2977 intensity units, and the two distributions were not significantly different ($p = 0.13$). A summary is displayed in Figure 4.

Quantities of wax esters

The median (first quartile, third quartile) summed intensity of WE from tears was 11,109 (7465, 37,371) intensity units for glass microcapillaries, and was 38,892 (13,963, 174,082) intensity units for PTFE. The median of differences was 25,672, and the two distributions were significantly different ($p < 0.01$). In meibum, the median summed intensity associated with microcapillary collection was 77,381 (26,590, 178,213) intensity units and was 84,771 (52,657, 206,050) intensity units for PTFE. The median of differences was 12,876 intensity units, and the two distributions were not significantly different ($p = 0.23$). Figure 5 summarizes this information.

For tears, PTFE collection was associated with a greater measurable quantity of each lipid class for the majority of subjects (8/10 subjects for OAHFA, 8/10 subjects for CE, 9/10 subjects for WE). In meibum, PTFE tubes were superior than glass in 6/10 subjects for OAHFA, 8/10 subjects for CE, 6/10 subjects for WE. A series of histograms showing the summed peak intensities recorded for each subject is shown in Figure 6.

Discussion

This study confirmed the hypothesis that PTFE tubes were associated with more measurable tear film-derived lipids than glass microcapillaries. However, for meibum, there did not appear to be any significant advantages of PTFE tubes over glass microcapillaries. To the best of our knowledge, there have been few publications that have detailed a variety of sampling techniques for tear film and meibum,[26,27,30,31] but the technique of using PTFE tubes to collect lipids from tears and meibum, including OAHFA lipids, is entirely novel.

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The mechanism for drawing up tears within glass microcapillary tubes is thought to be due to capillary action, where the adhesive force between glass and water draws the tear fluid into the tube.[32] However, the precise mechanism for which lipids enter either glass or PTFE tubes is not under-stood. It was apparent from preliminary experiments (Figure 1A, 1B) that hydrophobic interactions facilitate capillary uptake of lipids to occur within PTFE. However, the reason for this interaction to exist within PTFE is not clear.The constituent of PTFE, a tetrafluoroethylene -[C_2F_4]- unit contains net zero dipole,[33] which make polar interactions between the PTFE and sample less likely. Additionally, the fluorine atoms are highly electronegative and resists polarization,[34] which minimizes the ability of PTFE to partake in Van der Waals interactions. These two physical properties make PTFE extremely inert and suggest that the uptake of lipids should theoretically not be possible. Yet, the preliminary experiments (Figure 1) showed evidence of capillary attraction associated uptake of lipids into PTFE, in addition to the findings of predominant lipid profiles shown when using them as a means of collection, particularly for tear film-derived lipids. A possible explanation for this is that long lipid chains facilitate Van der Waals interactions between the lipids and the PTFE surface, which may facilitate adhesion and capillary action of lipids in PTFE. In contrast, the hydrophobicity of PTFE characterized by high contact angles[35] and low propensity to form hydrogen bonds with water[36] prevents water from being taken up into the tube, more selectively allowing for lipid uptake.

While lipids were also associated with glass microcapillaries for the tear-derived lipids, the amount was generally unpredictable since a large portion of that volume consisted of water. For instance, it is possible to collect tears within the entire length of glass microcapillary tube (32 mm), but yet still obtain very weak lipid signals. It is hypothesized that the speed of capillary action for glass in the uptake of tear film aqueous might hinder its ability to attract lipid. Conversely, the hydrophobic nature of PTFE may allow it to selectively attract lipids while simultaneously repelling water, leaving a concentrate of lipids within the tube. As a result of this, an amount as small as 0.02 μL can be sufficient to detect the different classes of lipids associated with the tear film (Table 1, Figure 6).

Given that PTFE and microcapillary glass tubes have different physical properties, it was anticipated to see differences in lipid profiles associated with each in the collection of meibum lipids. However, there was no evidence in the results to support the notion that PTFE was any better than glass microcapillary in this aspect. While there was a fourfold volume difference in PTFE over glass microcapillary, the mean absolute difference is only 0.07 μL. Additionally, the data in Table 3 where the median amount of OAHFA, CE, and WE collected using PTFE for meibum lipids all fell within the first and third quartiles of the glass microcapillary data, representing at most a 30% difference between the lipid classes. In contrast, the median amount of OAHFA, CE, and WE of tear film lipids associated with PTFE have all exceeded the third quartile of that in glass microcapillary, representing a difference of more than 200% between the lipids classes.

The uptake of meibum lipids into glass microcapillary tubes may have been facilitated by water within meibum. Since meibum consists mainly of lipids (and less water), the difference in lipid quantities between PTFE and glass microcapillaries was smaller and may have been harder to detect. It was also possible that there were larger quantities of lipids

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collected with both PTFE and glass microcapillaries for meibum, but ion suppression during mass spectrometry[37] may have minimized the difference in intensities between the two. This effect of ion suppression may occur when analyte concentration exceeds the upper limit allowable for an electrospray event.[38] The analyte saturation causes the linear response to level off and return an intensity quantity that is lower than actual.[38] However, based on volume alone, there were more lipids detected by mass spectrometry from 0.19 μL of tears in PTFE than there were in 3.00 μL of tears from the glass microcapillaries (Table 1, Figure 6). This highlights the utility of PTFE for the study of lipids from the tear film using mass spectrometry.

There are potential limitations to both approaches when collecting samples from human subjects, but PTFE collected samples exhibited overall higher lipids than glass microcapillaries for tear film samples, regardless of volume collected, suggesting that PTFE is the preferred method for lipid analyses of tears.

There may be a concern that the gentle lateral motions of the PTFE and microcapillary tubes to facilitate tear collection may have caused entry of ocular surface cells into the tubes. To determine if this was the case, the MS plots were analyzed and found a negligible presence of phospholipids for PTFE, but a slightly higher amount of phospholipids in glass tubes (data not shown). This is not expected to impact the study results since the lipids of interest in this current study (OAHFA, CE, and WE) were all specifically meibum-derived.

In conclusion, these data showed that PTFE collection was associated with higher values of tear film-derived lipids than glass microcapillaries when using electrospray ionization MS. However, there were no significant differences in lipid values associated with PTFE and glass microcapillaries for meibum. Therefore, PTFE can be used to maximize collection of lipid from the tear film, whereas either PTFE or glass microcapillary tubes may be used for collecting lipids from meibum.

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Appendix

Appendix 1.

Reference *m/z* values for cholesterol esters (CE) and wax esters (WE)

Cholesterol ester	*m/z*	Wax ester	*m/z*
CE(16:1)	640.6027	WE(32:0)	498.5244
CE(17:0)	656.634	WE(32:1)	496.5088
CE(18:0)	670.6496	WE(32:2)	494.4931

Cholesterol ester	*m/z*	Wax ester	*m/z*
CE(18:1)	668.634	WE(34:0)	526.5557
CE(19:0)	684.6653	WE(34:1)	524.5401
CE(20:0)	698.6809	WE(34:2)	522.5244
CE(20:1)	696.6653	WE(34:3)	520.5088
CE(20:2)	694.6496	WE(35:1)	538.5557
CE(21:0)	712.6966	WE(36:0)	554.587
CE(21:1)	710.6809	WE(36:1)	552.5714
CE(21:2)	708.6653	WE(36:2)	550.5557
CE(22:0)	726.7122	WE(36:3)	548.5401
CE(22:1)	724.6966	WE(37:0)	568.6027
CE(22:2)	722.6809	WE(37:1)	566.587
CE(23:0)	740.7279	WE(37:2)	564.5714
CE(24:0)	754.7435	WE(38:0)	582.6183
CE(24:1)	752.7279	WE(38:1)	580.6027
CE(25:0)	768.7592	WE(38:2)	578.587
CE(25:1)	766.7435	WE(38:3)	576.5714
CE(26:0)	782.7748	WE(39:0)	596.634
CE(26:1)	780.7592	WE(39:1)	594.6183
CE(27:0)	796.7905	WE(39:2)	592.6027
CE(27:1)	794.7748	WE(39:3)	590.587
CE(28:0)	810.8061	WE(40:0)	610.6496
CE(28:1)	808.7905	WE(40:1)	608.634
CE(29:0)	824.8218	WE(40:2)	606.6183
CE(30:1)	836.8218	WE(40:3)	604.6027
CE(30:2)	834.8061	WE(41:0)	624.6653
CE(32:1)	864.8531	WE(41:1)	622.6496
		WE(41:2)	620.634
		WE(41:3)	618.6183
		WE(42:0)	638.6809
		WE(42:1)	636.6653
		WE(42:2)	634.6496
		WE(42:3)	632.634
		WE(42:4)	630.6183
		WE(43:0)	652.6966
		WE(43:1)	650.6809
		WE(43:2)	648.6653
		WE(43:3)	646.6496
		WE(43:4)	644.634
		WE(44:1)	664.6966
		WE(44:2)	662.6809
		WE(44:3)	660.6653
		WE(44:4)	658.6496

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Cholesterol ester	*m/z*	Wax ester	*m/z*
		WE(45:1)	678.7122
		WE(45:2)	676.6966
		WE(45:3)	674.6809
		WE(45:4)	672.6653
		WE(46:0)	694.7435
		WE(46:1)	692.7279
		WE(46:2)	690.7122
		WE(46:3)	688.6966
		WE(46:4)	686.6809
		WE(46:6)	682.6496
		WE(47:1)	706.7435
		WE(47:2)	704.7279
		WE(47:3)	702.7122
		WE(48:0)	722.7748
		WE(48:1)	720.7592
		WE(48:2)	718.7435
		WE(48:3)	716.7279
		WE(48:4)	714.7122
		WE(49:1)	734.7748
		WE(49:3)	730.7435
		WE(50:1)	748.7905
		WE(50:2)	746.7748
		WE(50:3)	744.7592
		WE(50:4)	742.7435
		WE(50:6)	738.7122
		WE(52:2)	774.8061
		WE(52:3)	772.7905

References

1. Stapleton F, Alves M, Bunya VY, Jalbert I, Lekhanont K, Malet F, Na KS, Schaumberg D, Uchino M, Vehof J, et al. TFOS DEWS II epidemiology report. Ocul Surf. 2017;15(3):334–65. [PubMed: 28736337]
2. Miljanovic B, Dana R, Sullivan DA, Schaumberg DA. Impact of dry eye syndrome on vision-related quality of life. Am J Ophthalmol. 2007;143(3):409–15. doi:10.1016/j.ajo.2006.11.060. [PubMed: 17317388]
3. Portello JK, Rosenfield M, Bababekova Y, Estrada JM, Leon A. Computer-related visual symptoms in office workers. Ophthalmic Physiol Opt. 2012;32(5):375–82. doi:10.1111/j.1475-1313.2012.00925. x. [PubMed: 22775070]
4. Wolkoff P Ocular discomfort by environmental and personal risk factors altering the precorneal tear film. Toxicol Lett. 2010;199 (3):203–12. doi:10.1016/j.toxlet.2010.09.001. [PubMed: 20833234]
5. Stern ME, Gao J, Siemasko KF, Beuerman RW, Pflugfelder SC. The role of the lacrimal functional unit in the pathophysiology of dry eye. Exp Eye Res. 2004;78:409–16. [PubMed: 15106920]

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6. Chhadva P, Goldhardt R, Galor A. Meibomian gland disease: the role of gland dysfunction in dry eye disease. Ophthalmology. 2017;124(11S):S20–S26. doi:10.1016/j.ophtha.2017.05.031. [PubMed: 29055358]
7. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK, Liu Z, Nelson JD, Nichols JJ, Tsubota K, et al. TFOS DEWS II definition and classification report. Ocul Surf. 2017;15(3):276–83. doi:10.1016/j.jtos.2017.05.008. [PubMed: 28736335]
8. Azartash K, Kwan J, Paugh JR, Nguyen AL, Jester JV, Gratton E. Pre-corneal tear film thickness in humans measured with a novel technique. Mol Vis. 2011;17:756–67. [PubMed: 21527997]
9. Werkmeister RM, Alex A, Kaya S, Unterhuber A, Hofer B, Riedl J, Bronhagl M, Vietauer M, Schmidl D, Schmoll T, et al. Measurement of tear film thickness using ultrahigh-resolution optical coherence tomography. Invest Ophthalmol Vis Sci. 2013;54 (8):5578–83. doi:10.1167/iovs.13-11920. [PubMed: 23847319]
10. Bron AJ, Tiffany JM, Gouveia SM, Yokoi N, Voon LW. Functional aspects of the tear film lipid layer. Exp Eye Res. 2004;78:347–60. [PubMed: 15106912]
11. Dartt DA, Willcox MD. Complexity of the tear film: importance in homeostasis and dysfunction during disease. Exp Eye Res. 2013;117:1–3. doi:10.1016/j.exer.2013.10.008. [PubMed: 24280033]
12. Milner MS, Beckman KA, Luchs JI, Allen QB, Awdeh RM, Berdahl J, Boland TS, Buznego C, Gira JP, Goldberg DF, et al. Dysfunctional tear syndrome: dry eye disease and associated tear film disorders - new strategies for diagnosis and treatment. Curr Opin Ophthalmol. 2017;27(Suppl 1):3–47. doi:10.1097/01.icu.0000512373.81749.b7. [PubMed: 28099212]
13. Lam H, Bleiden L, de Paiva CS, Farley W, Stern ME, Pflugfelder SC. Tear cytokine profiles in dysfunctional tear syndrome. Am J Ophthalmol. 2009;147(2):198–205 e191. doi:10.1016/j.ajo.2008.08.032. [PubMed: 18992869]
14. Borchman D, Foulks GN, Yappert MC, Milliner SE. Differences in human meibum lipid composition with meibomian gland dysfunction using NMR and principal component analysis. Invest Ophthalmol Vis Sci. 2012;53(1):337–47. doi:10.1167/iovs.11-8551. [PubMed: 22131391]
15. Cwiklik L Tear film lipid layer: A molecular level view. Biochim Biophys Acta. 2016;1858(10):2421–30. doi:10.1016/j.bbamem.2016.02.020. [PubMed: 26898663]
16. McCulley JP, Shine WE. Meibomian gland function and the tear lipid layer. Ocul Surf. 2003;1:97–106. [PubMed: 17075642]
17. McCulley JP, Shine WE. The lipid layer of tears: dependent on meibomian gland function. Exp Eye Res. 2004;78:361–65. [PubMed: 15106913]
18. Butovich IA. Meibomian glands, meibum, and meibogenesis. Exp Eye Res. 2017;163:2–16. doi:10.1016/j.exer.2017.06.020. [PubMed: 28669846]
19. Chen J, Green KB, Nichols KK. Quantitative profiling of major neutral lipid classes in human meibum by direct infusion electrospray ionization mass spectrometry. Invest Ophthalmol Vis Sci. 2013;54(8):5730–53. doi:10.1167/iovs.12-10317. [PubMed: 23847307]
20. Chen JZ, Green-Church KB, Nichols KK. Shotgun lipidomic analysis of human meibomian gland secretions with electrospray ionization tandem mass spectrometry. Invest Ophth Vis Sci. 2010;51(12):6220–31. doi:10.1167/iovs.10-5687.
21. Rantamaki AH, Holopainen JM. The effect of phospholipids on tear film lipid layer surface activity. Invest Ophthalmol Vis Sci. 2017;58(1):149–54. doi:10.1167/iovs.16-20468. [PubMed: 28114572]
22. Butovich IA, Wojtowicz JC, Molai M. Human tear film and meibum. Very long chain wax esters and (O-acyl)-omega-hydroxy fatty acids of meibum. J Lipid Res. 2009;50(12):2471–85. doi:10.1194/jlr.M900252-JLR200. [PubMed: 19535818]
23. Schuett BS, Millar TJ. An investigation of the likely role of (O-acyl) omega-hydroxy fatty acids in meibomian lipid films using (O-oleyl) omega-hydroxy palmitic acid as a model. Exp Eye Res. 2013;115:57–64. doi:10.1016/j.exer.2013.06.016. [PubMed: 23792170]
24. Lam SM, Tong L, Yong SS, Li B, Chaurasia SS, Shui G, Wenk MR. Meibum lipid composition in Asians with dry eye disease. PLoS One. 2011;6(10):e24339. doi:10.1371/journal.pone.0024339. [PubMed: 22043274]
25. Brown SH, Kunnen CM, Duchoslav E, Dolla NK, Kelso MJ, Papas EB, Lazon de la Jara P, Willcox MD, Blanksby SJ, Mitchell TW. A comparison of patient matched meibum and tear lipidomes.

Invest Ophthalmol Vis Sci. 2013;54(12):7417–24. doi:10.1167/iovs.13-12916. [PubMed: 24135754]

26. Lam SM, Tong L, Duan X, Petznick A, Wenk MR, Shui G. Extensive characterization of human tear fluid collected using different techniques unravels the presence of novel lipid amphiphiles. J Lipid Res. 2014;55(2):289–98. doi:10.1194/jlr.M044826. [PubMed: 24287120]
27. Haworth KM, Nichols JJ, Thangavelu M, Sinnott LT, Nichols KK. Examination of human meibum collection and extraction techniques. Optom Vis Sci. 2011;88(4):525–33. doi:10.1097/OPX.0b013e318214ac0f. [PubMed: 21378589]
28. Polyfluor Plastics bv. Chemical resistance PTFE. 2018 [accessed 2018 Feb 5]. http://www.polyfluor.nl/en/chemical-resistance/ptfe/.
29. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957;226:497–509. [PubMed: 13428781]
30. Posa A, Brauer L, Schicht M, Garreis F, Beileke S, Paulsen F. Schirmer strip vs. capillary tube method: non-invasive methods of obtaining proteins from tear fluid. Ann Anat. 2013;195(2):137–42. doi:10.1016/j.aanat.2012.10.001. [PubMed: 23357333]
31. Stuchell RN, Feldman JJ, Farris RL, Mandel ID. The effect of collection technique on tear composition. Invest Ophthalmol Vis Sci. 1984;25:374–77. [PubMed: 6698755]
32. U.S. Geological Survey. Capillary action. 2016 [accessed 2018 Jan 18]. https://water.usgs.gov/edu/capillaryaction.html.
33. Chemical Entities of Biological Interest. CHEBI:53251 - poly(tetrafluoroethylene). 2014 [accessed 2018 Jan 18]. http://www.ebi.ac.uk/chebi/searchId.do?chebiId=53251.
34. Ebnesajjad S. Fluoroplastics. Volume 1: non-melt processible fluoropolymers - the definitive user's guide and data book. Vol. 1, 2nd. Amsterdam (Netherlands): Elsevier; 2015.
35. Zhao L, Cheng J. Analyzing the molecular kinetics of water spreading on hydrophobic surfaces via molecular dynamics simulation. Sci Rep. 2017;7(1):10880. doi:10.1038/s41598-017-11350-6. [PubMed: 28883662]
36. Dunitz JD, Taylor R. Organic fluorine hardly ever accepts hydrogen bonds. Chemistry-A Eur J. 1997;3(1):89–98. doi:10.1002/(ISSN)1521-3765.
37. Annesley TM. Ion suppression in mass spectrometry. Clin Chem. 2003;49:1041–44. [PubMed: 12816898]
38. Tang K, Page JS, Smith RD. Charge competition and the linear dynamic range of detection in electrospray ionization mass spectrometry. J Am Soc Mass Spectrom. 2004;15(10):1416–23. doi:10.1016/j.jasms.2004.04.034. [PubMed: 15465354]

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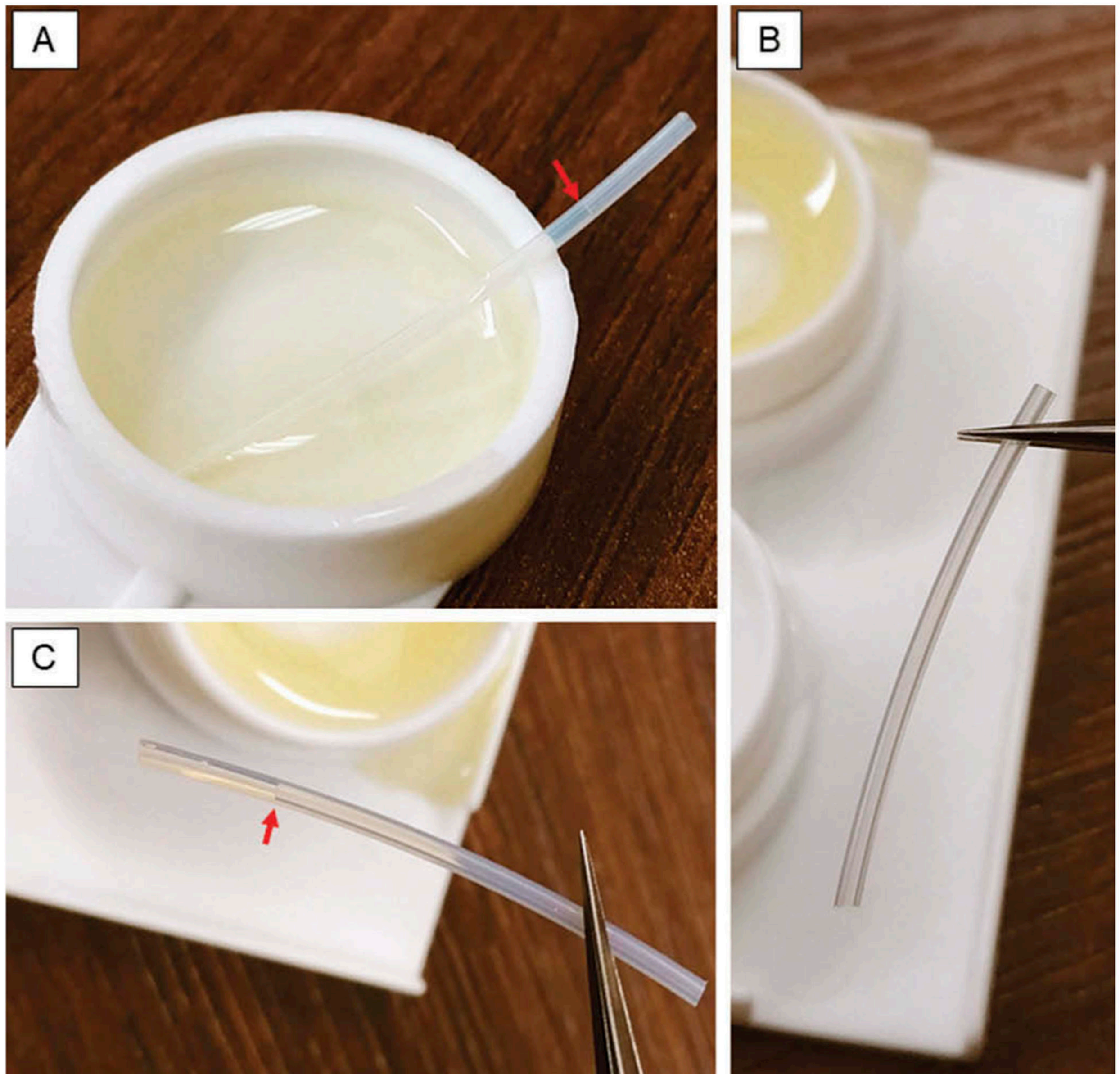

Figure 1.

Lipophilic activity of polytetrafluoroethylene (PTFE) tubes. (**A**) Lipids (canola oil) were drawn into the PTFE tube and the arrow indicates the oil level within the tube. (**B**) There was no entry of water into the PTFE tube. (**C**) Lipids from a mixture of canola oil and water were drawn into the PTFE tube (arrow).

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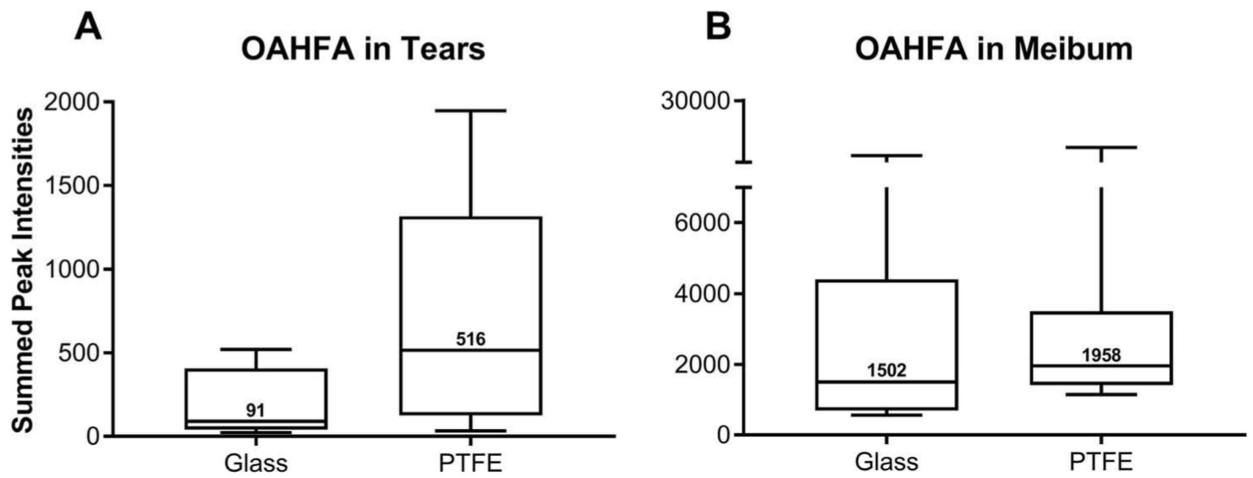

Figure 2.

A comparison of the amount of omega acyl hydroxyl fatty acids (OAHFA) in tears and meibum expressed in summed intensity units. (**A**) In tears, the summed intensity of OAHFA associated with polytetrafluoroethylene (PTFE) was significantly higher ($p = 0.04$) than glass microcapillaries. (**B**) In meibum, the summed intensity of OAHFA associated with PTFE was not significantly different ($p = 0.56$) than glass microcapillaries.

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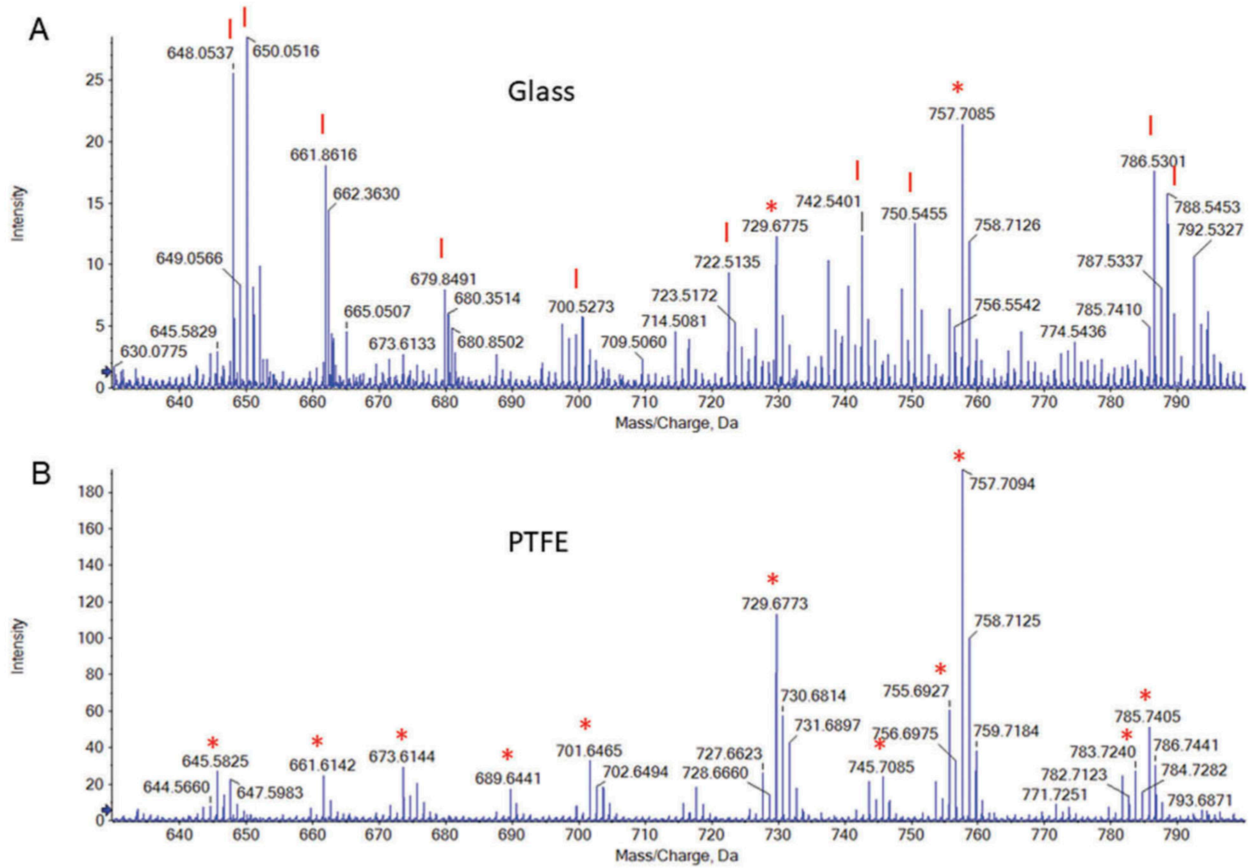

Figure 3.

Comparison of mass spectrum plots of omega acyl hydroxy fatty acids (OAHFA) peaks (*) detected in a subject for which tears were collected with a glass microcapillary and polytetrafluoroethylene (PTFE) tube. (**A**) Mass spectrum plot of OAHFA peaks associated with glass microcapillary. (**B**) Mass spectrum plot of OAHFA peaks associated with PTFE. The OAHFA peaks associated with PTFE are stronger than those associated with glass microcapillaries. Impurity peaks are labeled as I. Note the difference in intensity scale.

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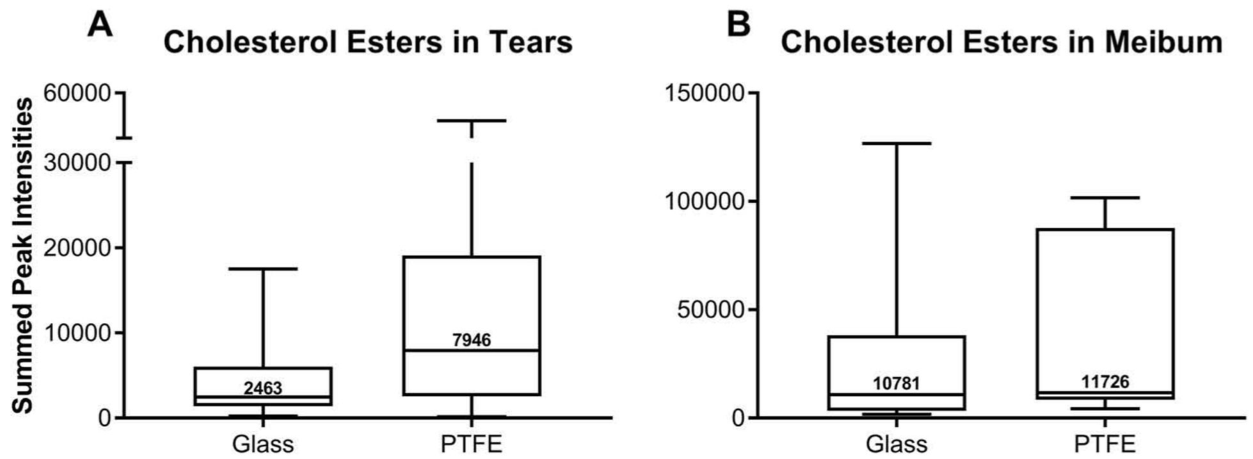

Figure 4.

A comparison of the amount of cholesterol esters (CE) in tears and meibum expressed in summed intensity units. (**A**) For tears, the summed intensity of CE associated with polytetrafluoroethylene (PTFE) was significantly higher ($p = 0.01$) than glass microcapillaries. (**B**) In meibum, the summed intensity of CE associated with PTFE was not significantly different ($p = 0.13$) than glass microcapillaries.

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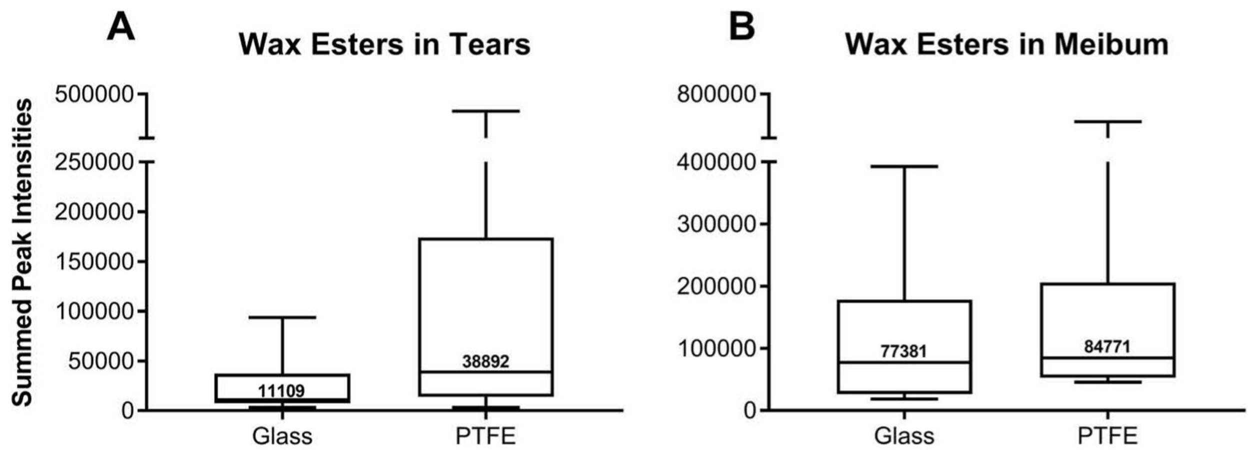

Figure 5.

A comparison of the amount of wax esters (WE) associated with tears and meibum expressed in summed intensity units. (**A**) For tears, the summed intensity of WE associated with polytetrafluoroethylene (PTFE) was significantly higher ($p < 0.01$) than glass microcapillaries. (**B**) In meibum, the summed intensity of WE associated with PTFE was not significantly different ($p = 0.23$) than glass microcapillaries.

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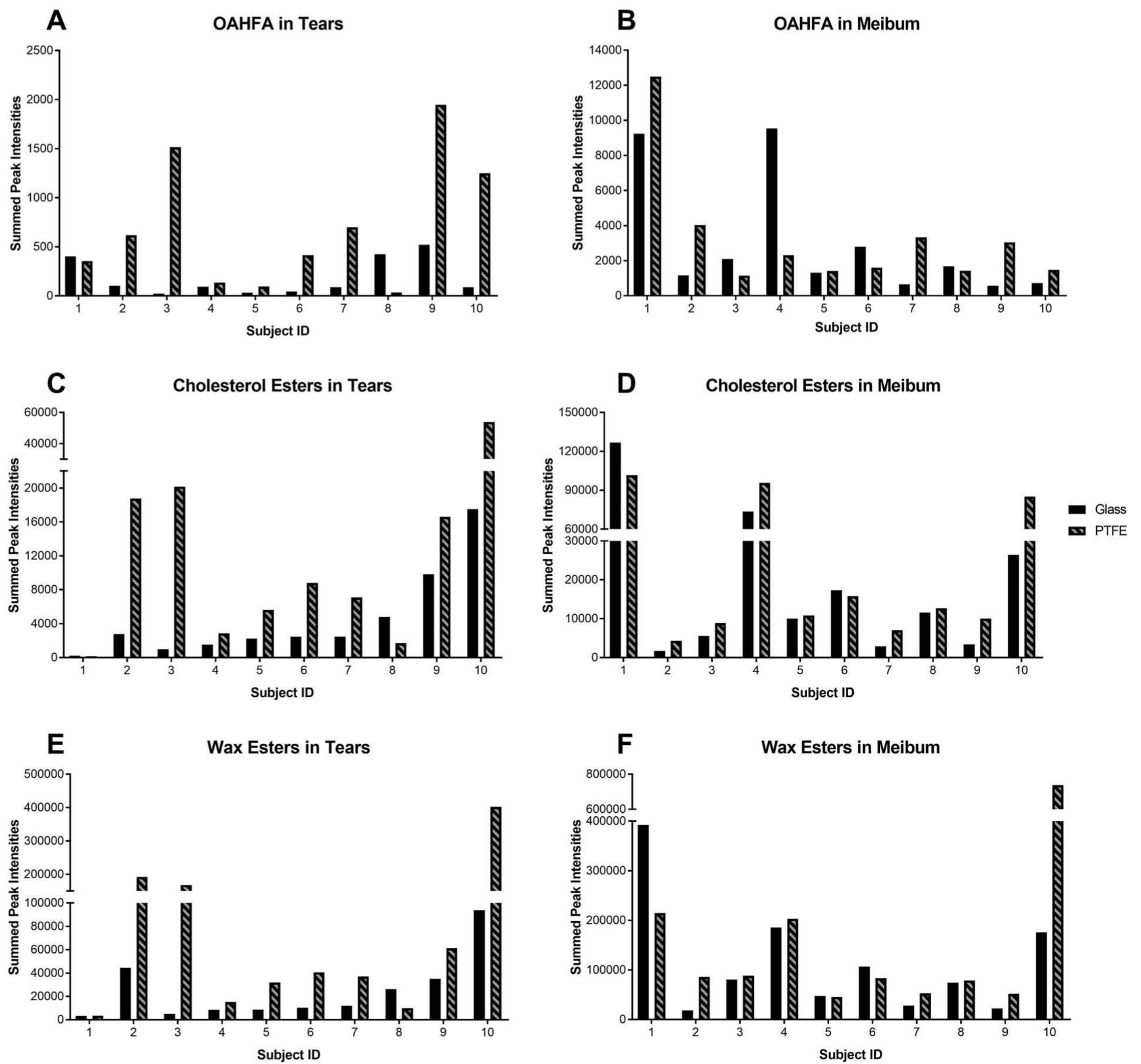

Figure 6.

A series of histograms of recorded summed lipid peak intensities by subjects. (**A, C, E**) For tears, in almost all subject cases, polytetrafluoroethylene (PTFE) was associated with a greater amount of omega acyl hydroxyl fatty acids (OAHFA), cholesterol esters (CE), and wax esters (WE) than glass microcapillaries. (**B, D, F**) However, with meibum, the PTFE and glass microcapillaries were equivocal for all lipid classes.

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Table 1.

Volume* of tears and meibum collected from each individual subject.

	Tears (µL)		Meibum (µL)	
Subject no.	**Glass microcapillary**	**Polytetrafluoroethylene**	**Glass microcapillary**	**Polytetrafluoroethylene**
1	5.0	0.25	0.03	0.12
2	1.0	0.12	0.02	0.05
3	5.0	0.25	0.01	0.02
4	0.5	0.02	0.03	0.12
5	2.5	0.25	0.02	0.05
6	5.0	0.25	0.03	0.12
7	2.5	0.49	0.02	0.25
8	5.0	0.25	0.01	0.02
9	3.4	0.02	0.03	0.12
10	0.1	0.02	0.01	0.02
Mean ± SD	3.00 ± 1.98	0.19 ± 0.15	0.02 ± 0.01	0.09 ± 0.07

*Volume of collected samples are calculated as follows:

1) $V_{microcap} = (L_{collected}/L_{tube}) \times V_{capacity}$

2) $V_{PTFE} = L_{collected} \times Pi \times r^2$

where $V_{microcap}$ and V_{PTFE} are volumes of samples collected in glass microcapillary and PTFE, respectively, $L_{collected}$ is the measured length of collected sample in the tube (mm), L_{tube} is the specified length of the microcapillary tube (32 mm), $V_{capacity}$ is the volumetric capacity of the microcapillary tube (1 µL or 5 µL), and r is the specified inner radius of the PTFE tube (0.28 mm).

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Table 2.

A comparison of summed intensity peaks (median, first quartile, third quartile) for omega acyl hydroxy fatty acids (OAHFA), cholesterol esters, and wax esters in the tear film, associated with the two different methods.

Tear film lipids	Glass microcapillary (n = 10)	Polytetrafluoroethylene (n = 10)	P-value
OAHFA	91 (41, 408)	516 (125, 1315)	0.04
Cholesterol esters	2463 (1389, 6042)	7946 (2571, 19,115)	0.01
Wax esters	11,109 (7465, 37,371)	38,892 (139,630, 174,082)	< 0.01

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Table 3.

A comparison of summed intensity peaks (median, first quartile, third quartile) for omega acyl hydroxy fatty acids (OAHFA), cholesterol esters, and wax esters in meibum, associated with the two different methods.

Meibum lipids	Glass microcapillary ($n = 10$)	Polytetrafluoroethylene ($n = 10$)	P-value
OAHFA	1502 (699, 4407)	1958 (1417, 3502)	0.56
Cholesterol esters	10,781 (3287,38,205)	11,726 (8434, 87,691)	0.13
Wax esters	77,381 (26,590, 178,213)	84,771 (52,657, 206,050)	0.23