RESEARCH ONLINE

University of Wollongong
Research Online

Faculty of Science - Papers (Archive)

Faculty of Science, Medicine and Health

2010

Detection and quantification of tear phospholipids and cholesterol in contact lens deposits: the effect of contact lens material and lens care solution

Jennifer Saville
University of Wollongong, jts02@uow.edu.au

Zhenjun Zhao
Eye Research Institute, Sydney, NSW Australia

Mark D.P. Willcox
Eye Research Institute, Sydney, NSW Australia

Stephen J. Blanksby
University of Wollongong, blanksby@uow.edu.au

Todd W. Mitchell
University of Wollongong, toddm@uow.edu.au

Publication Details

Saville, J., Zhao, Z., Willcox, M., Blanksby, S. J. & Mitchell, T. W. (2010). Detection and quantification of tear phospholipids and cholesterol in contact lens deposits: the effect of contact lens material and lens care solution. Investigative Ophthalmology and Visual Science, 51 (6), 2843-2851.

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

RESEARCH ONLINE

Detection and quantification of tear phospholipids and cholesterol in contact lens deposits: the effect of contact lens material and lens care solution

Abstract

PURPOSE. To examine the deposition of tear phospholipids and cholesterol onto worn contact lenses and the effect of lens material and lens care solution. METHODS. Lipids were extracted from tears and worn contact lenses using 2: 1 chloroform: methanol and the extract washed with aqueous ammonium acetate, before analysis by electrospray ionization tandem mass spectrometry (ESI-MS/MS). RESULTS. Twenty-three molecular lipids from the sphingomyelin (SM) and phosphatidylcholine (PC) classes were detected in tears, with total concentrations of each class determined to be 5 +/- 1 pmol/mu L (similar to 3.8 mu g/mL) and 6 +/- 1 pmol/mu L (similar to 4.6 mu g/mL),respectively. The profile of individual phospholipids in both of these classes was shown to be similar in contact lens deposits. Deposition of representative polar and nonpolar lipids were shown to be significantly higher on senofilcon A contact lenses, with similar to 59 ng/lens SM, 195 ng/lens PC, and 9.9 mu g/lens cholesterol detected, whereas balafilcon A lens extracts contained similar to 19 ng/lens SM, 19 ng/lens PC, and 3.9 mu g/lens cholesterol. Extracts from lenses disinfected and cleaned with two lens care solutions showed no significant differences in total PC and SM concentrations; however, a greater proportion of PC than SM was observed, compared with that in tears. CONCLUSIONS. Phospholipid deposits extracted from worn contact lenses show a molecular profile similar to that in tears. The concentration of representative polar and nonpolar lipids deposited onto contact lenses is significantly affected by lens composition. There is a differential efficacy in the removal of PC and SM with lens care solutions. (Invest Ophthalmol Vis Sci. 2010; 51: 2843-2851) DOI:10.1167/iovs.09-4609

Keywords

lens, deposits, effect, quantification, material, tear, care, phospholipids, detection, cholesterol, solution, contact

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

Saville, J., Zhao, Z., Willcox, M., Blanksby, S. J. & Mitchell, T. W. (2010). Detection and quantification of tear phospholipids and cholesterol in contact lens deposits: the effect of contact lens material and lens care solution. Investigative Ophthalmology and Visual Science, 51 (6), 2843-2851.

This journal article is available at Research Online: http://ro.uow.edu.au/scipapers/415

Detection and Quantification of Tear Phospholipids and Cholesterol in Contact Lens Deposits: The Effect of Contact Lens Material and Lens Care Solution

Jennifer T. Saville,[1] *Zhenjun Zhao,*[2,3] *Mark D. P. Willcox,*[2,3] *Stephen J. Blanksby,*[*,1] *and Todd W. Mitchell*[*,4]

Purpose. To examine the deposition of tear phospholipids and cholesterol onto worn contact lenses and the effect of lens material and lens care solution.

Methods. Lipids were extracted from tears and worn contact lenses using 2:1 chloroform:methanol and the extract washed with aqueous ammonium acetate, before analysis by electrospray ionization tandem mass spectrometry (ESI-MS/MS).

Results. Twenty-three molecular lipids from the sphingomyelin (SM) and phosphatidylcholine (PC) classes were detected in tears, with total concentrations of each class determined to be 5 ± 1 pmol/μL (~3.8 μg/mL) and 6 ± 1 pmol/μL (~ 4.6 μg/mL), respectively. The profile of individual phospholipids in both of these classes was shown to be similar in contact lens deposits. Deposition of representative polar and nonpolar lipids were shown to be significantly higher on senofilcon A contact lenses, with ~59 ng/lens SM, 195 ng/lens PC, and 9.9 μg/lens cholesterol detected, whereas balafilcon A lens extracts contained ~19 ng/lens SM, 19 ng/lens PC, and 3.9 μg/lens cholesterol. Extracts from lenses disinfected and cleaned with two lens care solutions showed no significant differences in total PC and SM concentrations; however, a greater proportion of PC than SM was observed, compared with that in tears.

Conclusions. Phospholipid deposits extracted from worn contact lenses show a molecular profile similar to that in tears. The concentration of representative polar and nonpolar lipids deposited onto contact lenses is significantly affected by lens composition. There is a differential efficacy in the removal of PC and SM with lens care solutions. (*Invest Ophthalmol Vis Sci.* 2010;51:2843–2851) DOI:10.1167/iovs.09-4609

The tear film covers the outer mucosal epithelial layers of the eye, cornea, and conjunctiva. Tears provide these layers with a hydrophilic covering, protect them from insult by microbes or other substances and providing a smooth surface for good visual acuity. The tear film is thought to be composed of three layers, a lower mucin layer closest to the eye, an aqueous layer (containing salts, proteins, and other components), and an external lipid layer.[1] It is believed that the outer lipid layer (farthest from the eye) reduces evaporation of tear film and reduces the ability of the more polar lipids in the sebum to interact with the mucosal surfaces of the eye.[2]

Compositional analyses of tear film lipids are limited and controversial. Lipids are thought to be secreted primarily from the meibomian gland, and the profile of meibomian gland secretions has been examined (Butovich IA, et al. *IOVS* 2009; 50:ARVO E-Abstract 2545)[3]; however, there is still some debate as to whether the meibomian profile is representative of the lipids present in tears themselves. In 1990, Wollensak et al.[4] determined the human tear lipid composition to consist primarily of wax and cholesterol esters, with phospholipids, free cholesterol, and other lipids also detected. In 1995, Greiner et al.[5] reported the quantification of 17 phospholipids in meibomian gland secretions from rabbits, with phosphatidylcholine (PC), sphingomyelin (SM), and phosphatidylethanolamine (PE) making up almost 80% of the total phospholipid content.[5] Shine and McCulley[6] later identified these same phospholipid classes in human meibomian gland secretions. These results, however, have recently been challenged by Butovich et al. (*IOVS* 2009;50:ARVO E-Abstract 2545)[7,8] who reported an upper limit of 0.01% phospholipid in meibomian samples. Furthermore, recent studies have shown that the profile of nonpolar lipids in meibomian gland secretions differs from the profile in tears,[9–12] suggesting that the meibomian gland is not the sole provider of lipids to the tear film. Given the suggested importance of phospholipids in acting as a surfactant to spread hydrophobic lipids across the aqueous phase and prevent excess evaporation,[13] these inconsistencies highlight the need for a more detailed analysis into the lipid profile of tears, with a particular focus on the polar lipid classes.

Tear film components deposit rapidly onto the surface of a contact lens after insertion.[14] These deposits can lead to spoliation of the contact lens, a problem that may decrease lens performance and result in discomfort and infection.[15,16] Research into the deposition of proteins onto contact lenses has been extensive.[17–20] Green-Church and Nichols[19] analyzed the deposition of proteins onto contact lenses and found a total concentration of 7.32 to 9.76 μg/lens; however, the concentration and type of protein deposited varied with the material composing the contact lens. Although the profile and quantification of proteins has been well documented, there is only limited information on the deposition of lipids.

Electrostatic binding of lipids to contact lenses may be a contributing factor in the thinning of lipid layers in the tear film and the increased evaporation of the aqueous phase.[21] In vitro studies have shown that hydrogel lenses constructed from

From the [1]School of Chemistry and [4]School of Health Sciences, University of Wollongong, Wollongong, New South Wales, Australia; the [2]Institute for Eye Research, Sydney, New South Wales, Australia; and the [3]School of Optometry and Vision Science, University of New South Wales, Sydney, New South Wales, Australia.

Supported by the Australian Research Council (Linkage Project Grant LP0989883), Institute for Eye Research. JTS is the recipient of a postgraduate scholarship from the Institute for Eye Research.

Submitted for publication September 7, 2009; revised December 3, 2009; accepted January 1, 2010.

Disclosure: **J.T. Saville** , None; **Z. Zhao**, None; **M.D.P. Willcox**, None; **S.J. Blanksby**, None; **T.W. Mitchell**, None

*Each of the following is a corresponding author: Todd Mitchell, School of Health Sciences, University of Wollongong, NSW 2522, Australia; toddm@uow.edu.au.
Stephen Blanksby, School of Chemistry, University of Wollongong, NSW 2522, Australia, blanksby@uow.edu.au.

Copyright © Association for Research in Vision and Ophthalmology

poly(2-hydroxyethyl methacrylate), poly(methy1 methacrylate)-poly(vinyl alcohol), or poly(2-hydroxyethyl methacrylate)-poly(viny1 pyrrolidone)-poly(methacrylic acid) can all adsorb lipids from solution and that lenses made from poly(methy1 methacrylate)-poly(vinyl alcohol) tend to adsorb slightly more lipids.[22] Lord et al.[23] demonstrated that adsorption of cholesterol to poly(HEMA) lenses may collapse the hydrogel and induce the expelling of water, whereas binding to PMMA lenses is simply an adsorption process. In vitro experiments by Carney et al.[24] involved soaking several types of contact lenses in solutions of fluorescently tagged PE, or cholesterol, to quantify the lenses' adsorption over time. They determined that the concentration of lipid adsorbed was affected by the contact lens material and that cholesterol adsorption was significantly higher than PE in all lens types (cholesterol, 3–24.1 μg/lens; PE, 0.4–5.1 μg/lens). Iwata et al.[25] confirmed the ability of the silicone hydrogels to bind cholesterol in relatively high levels and also demonstrated that these lens types could bind squalene, cholesterol esters, and wax esters. Overall, the lipids bind to the silicone hydrogel at levels between 0.4 and 7.6 μg/lens. Initial in vivo studies demonstrated that lipid is present in contact lens deposits, with the principal lipid type being cholesterol esters.[26] White spots, a form of deposit found on nonregularly replaced hydrogel lenses, have been shown to be predominantly lipids.[27,28] These deposits have a distinct structural stratification, with a lipid layer providing the interface between the contact lens surface and the deposit superstructure. This initial interfacial layer is made from cholesterol, cholesterol esters, and unsaturated lipids.[27,28] Jelly bump deposits, another form of deposit on lenses, are composed of long and intermediate sized cholesterol esters, triglycerides, and waxy esters.[26]

The influence of contact lens material on total lipid deposition has been shown in vivo in surface fluorescence analyses, with vifilcon A contact lenses shown to deposit a higher abundance of lipids than do etafilcon A and netrafilcon A contact lenses.[29] Zhao et al.[30] found that total protein and cholesterol concentrations were affected by the lens polymer and lens care solution used. Although these studies have given some insight into the deposition of total lipids, little is known about the profile of the lipids or their comparison to the tear film lipid profile.

In this study, we enlisted sensitive and selective mass spectrometric methods to examine the deposition of tear film phospholipids and cholesterol onto worn contact lenses and the affect of lens material and lens care solution. The phospholipid profile obtained from contact lenses was compared to that of typical basal tears.

Materials and Methods

All phospholipid standards were synthesized by Avanti Polar Lipids (Alabaster, AL) and purchased from Auspep (Parkville, VIC, Australia); HPLC-grade chloroform and methanol and analytical grade ammonium acetate from Crown Scientific (Moorebank, NSW, Australia); analytical grade butylated hydroxytoluene (BHT) and D_6-cholesterol from Sigma-Aldrich (Castle Hill, NSW, Australia); total recovery vials with polytetrafluoroethylene (PTFE)/silicone septa from Waters (Rydalmere, NSW, Australia); and borosilicate capillary tubing from SDR Clinical Tech (Middle Cove, NSW, Australia).

Contact Lens Samples

All patients signed an informed consent form before enrollment in the study, which was conducted in compliance with the tenets of the Declaration of Helsinki and was approved by the Human Ethics Review Panel of the Institute for Eye Research. Patients (10 men and 13 women; mean age, 36.5 years) wore either balafilcon A or senofilcon A contact lenses daily for a 30-day period, removing them each night and soaking them in commercially available cleaning and disinfection solutions (Table 1). At the time of collection, all contact lenses were removed with sterile gloves, rinsed with a commercial saline solution, and stored at −80°C.

Tear Samples

Tears were collected from non–contact-lens–wearing patients by the method described in Sack et al.[31] Briefly, basal tears were collected with a glass capillary at the lower lid margin without stimulating reflex tears. The samples were placed in glass vials and stored at −80°C.

Lipid Extraction from Contact Lenses

Contact lenses were placed in small glass beakers, and a methanol internal standard solution (50 μL), containing PC (19:0/19:0), 0.8 μM; SM (d18:0/12:0), 0.8 μM; PS (17:0/17:0), 0.5 μM; PE (17:0/17:0), 0.5 μM; PG (17:0/17:0), 1 μM; and PA (17:0/17:0) 0.5 μM, was added to the concave surface and then dried under nitrogen. Chloroform:methanol (3 mL; 2:1 vol/vol) containing 0.01% BHT was added to cover the contact lens, and the samples were mixed on an orbital shaker at low speed for 15 minutes. The solution containing the desorbed lipids was removed and placed in a glass test tube, with care taken not to break the contact lens. A biphasic lipid extraction was performed on the solution according to the method described by Folch et al.,[32] with the exception that aqueous ammonium acetate (0.15 M) was substituted for sodium chloride as described previously.[33] In brief, 500 μL of 0.15 M ammonium acetate was added to the chloroform:methanol solution of desorbed lipids and centrifuged. The organic phase was removed, dried under nitrogen, and reconstituted in 500 μL chloroform:methanol (1:2 vol/vol; 0.01% BHT). All samples were stored at −80°C until analysis.

Lipid Extraction from Tears

Tear samples from six individuals (~5 μL each) were spiked with 2 μL of phospholipid standard mixture (as described earlier). Lipids were extracted as described, before being reconstituted in chloroform:methanol (1:2 vol/vol; 0.01% BHT) and dried under nitrogen to a final volume of 10 μL.

Mass Spectrometry of Polar Lipids

All samples were analyzed by mass spectrometry (QuattroMicro; Waters, Manchester, UK, with Micromass MassLynx ver. 4.0 software; Matrix Science, Boston, MA). The contact lens extracts were analyzed by electrospray ionization (ESI), and the tear extracts were analyzed by nanospray ESI (nanoESI). Aqueous ammonium acetate (1 M; 25 μL per 500 μL of sample) was added to samples before analysis, to enhance the formation of $[M+H]^+$ ions. Nitrogen was used as the drying gas at a flow rate of 320 L/h. For ESI, samples were analyzed at a flow rate of 10 μL/min. The capillary voltage was set to 3 kV and desolvation temperature to 120°C. For nanoESI, capillary voltage was set to 1.5 kV. Capillaries for nanoESI were prepared from borosilicate tubing with a micropipette puller (Flaming/Brown; Sutter Instrument Co., Novato, CA) and gold coated with a sputter coater (K500X; Emitech, East Sussex, UK). Approximately 3 μL of sample was loaded into the capillary for analysis. For all acquisitions (ESI and nanoESI) source

Table 1. Ingredients of the Lens Care Solutions

Solution	Surfactant	Preservative
A	Tetronic 1304*	Polyquad 0.001%, Aldox 0.0005%
B	Poloxamer 407, poloxamine 1107	Alexidine 0.00045%
C	Pluronic 17R4†	Hydrogen peroxide 3%

* Tetronic 1304; Alcon, Ltd., Fort Worth, TX.
† BASF Corp., Mount Olive, NJ.

temperature was set to 80°C and cone voltage to 35 V. Argon was used as the collision gas for all precursor ion scans at a pressure of 3 mTorr and a collision energy offset of 35 eV.

Spectra were obtained over a range of *m/z* 640 to 860. Precursor ion scans for the *m/z* 184 fragment were used to identify lipids with a phosphocholine head group.[34,35] For each spectrum, 300 acquisitions were combined, before background subtraction and smoothing with a Savitsky-Golay algorithm. The instrument's limit of detection (LOD) and limit of quantification (LOQ) were determined each day based on the statistical method described in Armbruster et al.[36] In brief, the total ion count for a region of noise was calculated for each spectrum, and the mean and standard deviation of the noise were used to calculate the LOD (mean $+3 \times$ SD) and LOQ ($10 \times$ SD). Ions presenting below these limits were excluded from the analysis. Quantification was achieved by comparison of peaks with head group–specific internal standards, after correction for isotope contribution.[33]

MS of Cholesterol

GC-MS (QP5050; Shimadzu, Kyoto, Japan) was used to quantify cholesterol in all samples by direct-insertion electron ionization mass spectrometry (DI/EI-MS) according to the method described by Deeley et al.[33] In brief, approximately 10 μL of sample was dried on the end of a sealed glass capillary tube, which was directly inserted into the source of the mass spectrometer. A temperature program was used to heat the tube from 40°C to 250°C at 80°C/min. Selected ion monitoring (cholesterol: *m/z* 386, 368, and 353; D_6-cholesterol: *m/z* 392, 374, and 359) was used (Fig. 1). The ion intensity ratios of the D_6-cholesterol and cholesterol in each sample were used in conjunction with a standard curve, to calculate the concentration of cholesterol in each sample. This method measures free cholesterol only, as cholesterol esters have a separate distinct elution profile under these conditions.

Statistical Analysis

Data were analyzed by a one-way ANOVA using contact lens material or lens care solution as a fixed factor, with a Student's *t*-test for comparison of means. All values are expressed as the mean $\pm$ SE, and $P < 0.05$ was considered statistically significant (JMP 5.1; SAS Institute, Cary, NC).

Results

Analysis of choline-containing phospholipids in human basal tears revealed several PC and SM molecules, including the elucidation of those differing by only one double bond in the fatty acid chains. Twenty-three molecular lipids were detected and identified in tears in SM and PC classes. The combination of low lipid concentrations and sample volumes did not allow for additional experiments to differentiate between SM and dihydrosphingomyelin (DHSM) or to determine individual fatty acid composition. The total concentration of SM in tears was calculated as 5 ± 1 pmol/μL, and the total concentration of PC was 6 ± 1 pmol/μL, with the two most abundant phospholipids observed in this analysis being SM (16:0) (*m/z* 703) and PC (34:2) (*m/z* 758). The degree of unsaturation of PC was greater than that of SM, which was dominated by SM (16:0). Comparison of phosphocholine lipid profiles showed that contact lens lipid extracts exhibited a profile similar to that in tear extracts (Figs. 2A, 2B). Comparison of individual molecules as a percentage of total SM or PC showed differences in the SM profile, with contact lens lipid extracts containing a significantly larger percentage of SM (16:0), but no significant differences in the PC profile (Figs. 2C, 2D).

Although the overall profile of both tear and contact lens lipid extracts was similar, there were differences observed in the phospholipids detected that were dependent on contact lens material and lens care solution. Table 2 lists the individual phospholipids that were observed in samples at ion counts above the calculated LOD. The greatest number of individual phospholipids were detected from senofilcon A lenses disinfected and cleaned with solution A, whereas the fewest number were detected in both tear extracts and balafilcon A lenses disinfected and cleaned with solution C.

Figure 3 shows the effect of contact lens material on the concentration of lipids. Senofilcon A contact lenses had a significantly greater concentration of total SM than did balafilcon A (78 ± 25 pmol/lens vs. 25 ± 3 pmol/lens; $P < 0.05$). Furthermore, several individual short-chain SM molecules were observed at significantly higher concentrations in senofilcon A

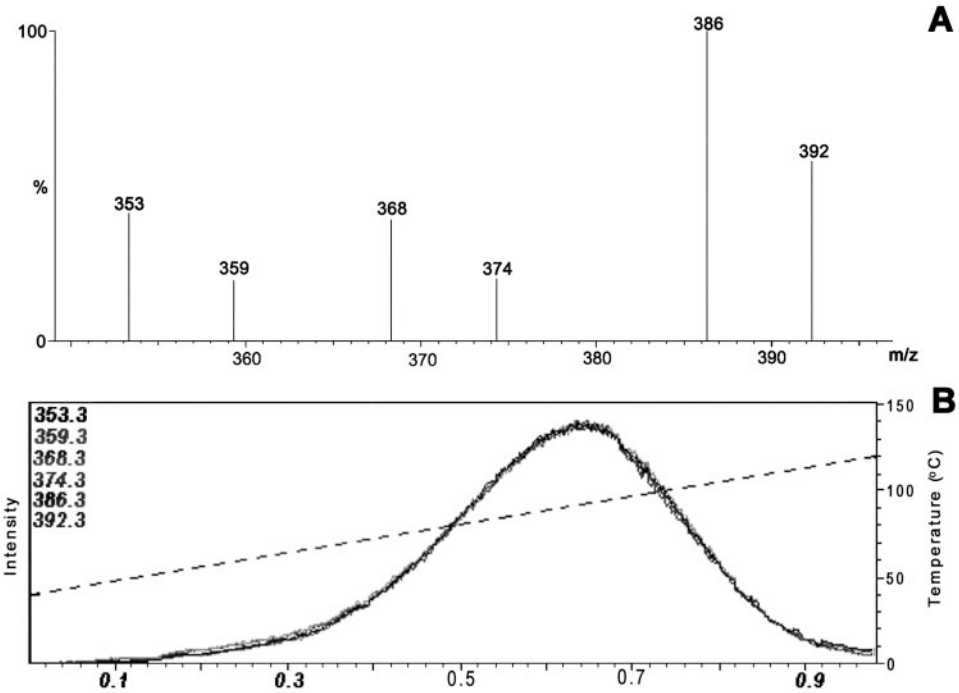
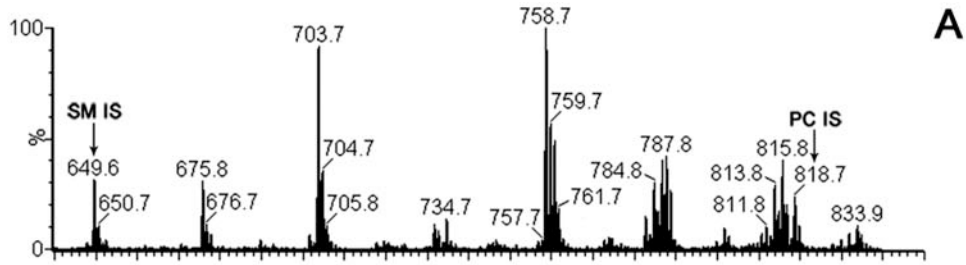
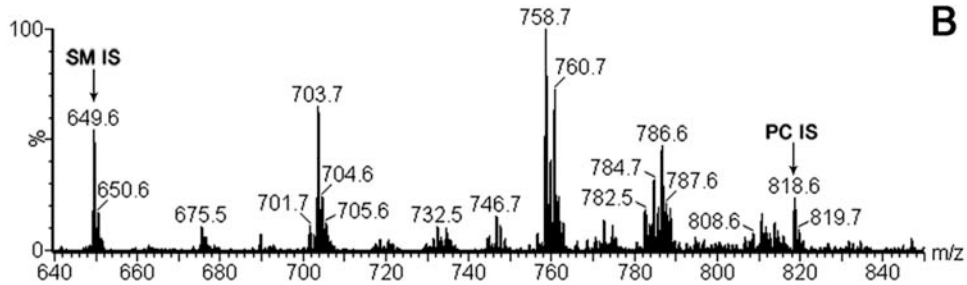
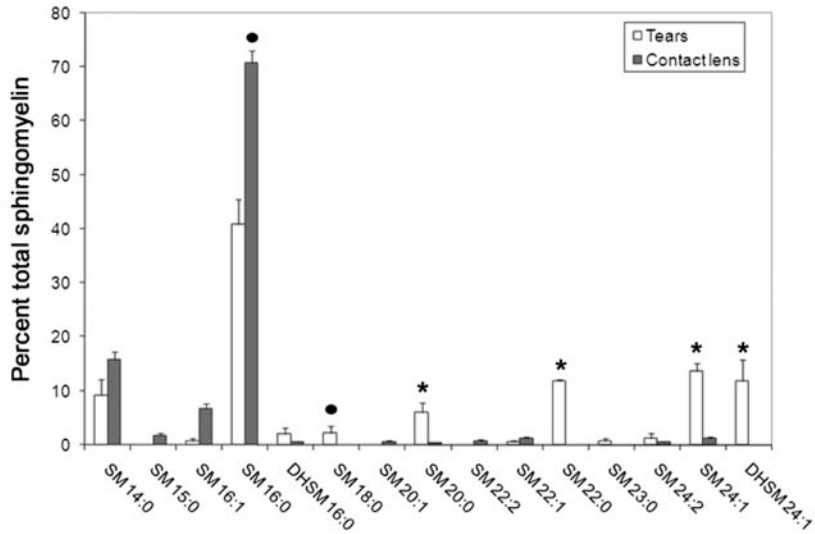


Figure 1. Typical data obtained from DI/EI-MS analysis of cholesterol from a contact lens extract. (**A**) Mass spectrum showing the characteristic ions for cholesterol (*m/z* 353, 368, and 386) and D_6-cholesterol (*m/z* 359 , 374, and 392). (**B**) Selected ion monitoring chromatogram showing the elution profile of cholesterol and D_6-cholesterol ions.







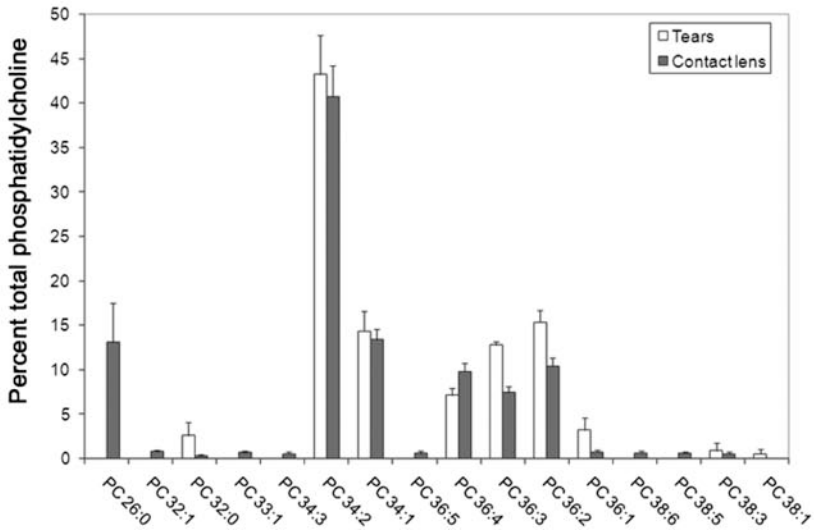


FIGURE 2. Comparison of tear and contact lens phosphocholine profiles. (**A**) A representative mass spectrum from a tear lipid extract. (**B**) A representative mass spectrum of an extract from worn senofilcon A contact lenses washed with solution A (see Table 1). Both spectra were obtained by performing a precursor ion scan for the *m/z* 184 ion using a triple-quadrupole mass spectrometer. (**C**) Concentration of individual SM and DHSM molecules in tear and senofilcon A contact lens extracts, shown as a percentage of total SM. (**D**) Concentration of individual PC molecules in tear and senofilcon A contact lens extracts, shown as a percentage of total PC. Data are presented as the mean ± SE: $^{\bullet}P < 0.01$; $^{*}P < 0.05$.

Table 2. Phospholipids Detected in Tear and Worn Contact Lens Lipid Extracts

Lipid*	*m/z* $[M+H]^+$	Tears	Senofilcon A Solution A	Balafilcon A Solution A	Balafilcon A Solution B	Balafilcon A Solution C
SM 14:0	675	√	√	√	√	√
SM 15:0	689	√	√			
SM 16:1	701		√		√	√
SM 16:0	703	√	√	√	√	√
DHSM 16:0	705	√	√	√	√	√
SM 18:1	729		√			
SM 18:0	731	√	√	√	√	√
SM 20:1	757		√		√	√
SM 20:0	759	√	√	√	√	√
SM 22:2	783		√	√	√	
SM 22:1	785	√	√	√	√	√
SM 22:0	787	√		√	√	√
SM 24:2	811	√	√	√	√	√
SM 24:1	813	√	√	√	√	√
*DHSM 24:1	815	√	√	√	√	√
PC 26:0	650		√	√		√
PC 30:1	704			√	√	√
PC 30:0	706	√	√		√	√
PC 32:1	732	√	√	√	√	√
PC 32:0	734	√	√	√		
PC 33:1	746		√			
PC 34:3	756		√			
PC 34:2	758	√	√	√	√	√
PC 34:1	760	√	√	√	√	√
PC 36:4	782	√	√	√	√	√
PC 36:3	784	√	√	√	√	
PC 36:2	786	√	√	√	√	√
PC 36:1	788	√	√	√	√	√
PC 38:6	806		√	√	√	
PC 38:5	808		√	√	√	
PC 38:4	810	√	√	√	√	√
PC 38:3	812	√	√	√	√	
PC 38:2	814	√		√	√	√

Only lipids with ion counts above the calculated LOD are included.

* SM and DHSM cannot be rigorously differentiated in this experiment because of the small samples. Assignment is based on natural occurrence. DHSM 24:1 is assigned based on its prevalence as an ocular lipid.[33]

extracts, including the most abundant SM (16:0) that alone constituted 44 ± 13 pmol/lens, whereas there was no significant difference in longer chain SM. A total PC concentration of 257 ± 77 pmol/lens was observed in senofilcon A contact lens extracts, which was significantly higher than that in balafilcon A contact lenses (25 ± 6 pmol/lens; $P < 0.01$). Five individual PC molecules were also observed at significantly higher concentrations in senofilcon A extracts, with the highest being PC (34:2), with a concentration of 92 ± 29 pmol/lens. Unlike SM, there did not appear to be any bias on chain length. Similar to the increased concentration of polar lipids extracted from senofilcon A lenses, the nonpolar lipid cholesterol was also found to be more abundant in the senofilcon A extracts. Figure 3C shows that 9.9 ± 2.2 μg of cholesterol was extracted from senofilcon A contact lenses compared with only 3.9 ± 0.9 μg from worn balafilcon A lenses ($P < 0.05$).

Lens care solution (Table 1) had no significant impact on the total SM, PC, or cholesterol extracted from the balafilcon A contact lenses (Fig. 4). Contact lenses soaked in solution C, however, showed significantly more SM (24:1) than did both solution A ($P < 0.05$) and solution B ($P < 0.01$), and significantly more DHSM (24:1) ($P < 0.01$) than did solution A, whereas lenses soaked in solution B showed significantly more SM (16:0) than did solution A ($P < 0.05$) and significantly more PC (34:2) than did solution C ($P < 0.01$).

Table 3 shows the abundance of total SM and PC molecules, normalized across the phosphocholine lipids. These data show that in tears, phosphocholine lipids were made up of approximately equal parts SM and PC molecules. This trend was also observed in extracts from balafilcon A contact lenses soaked in solution A. The concentration of PC was more than three times greater than SM in senofilcon A contact lens extracts, whereas extracts from balafilcon A soaked in solution C contained almost seven times the amount of SM than of PC.

Discussion

In this study we analyzed the profile of phospholipids in tears and deposited onto contact lenses and the effect of contact lens material and lens care solutions on the concentration of total and individual quantifiable phospholipids and cholesterol. Although several studies have established the profile of proteins in tears,[4,37,38] little work has been performed in the identification of phospholipids in tears, and the profile of nonpolar lipids is still controversial. Shotgun lipidomics, a technique that is now well established in the analysis of lipids in tissues,[33,39–42] identified 23 SM and PC molecules in tears. PC and SM have been reported in tears[12] and in their in vitro study, Peters and Millar[43] have suggested that PC increases the stability of the tear film by lowering surface tension. Other studies have associated the hydroxyl group present in SM with an increase in hydrogen bonding and therefore stable structure formation in the tear film.[44]

Results in work using thin layer chromatography (TLC) and high-pressure liquid chromatography with ultraviolet detection

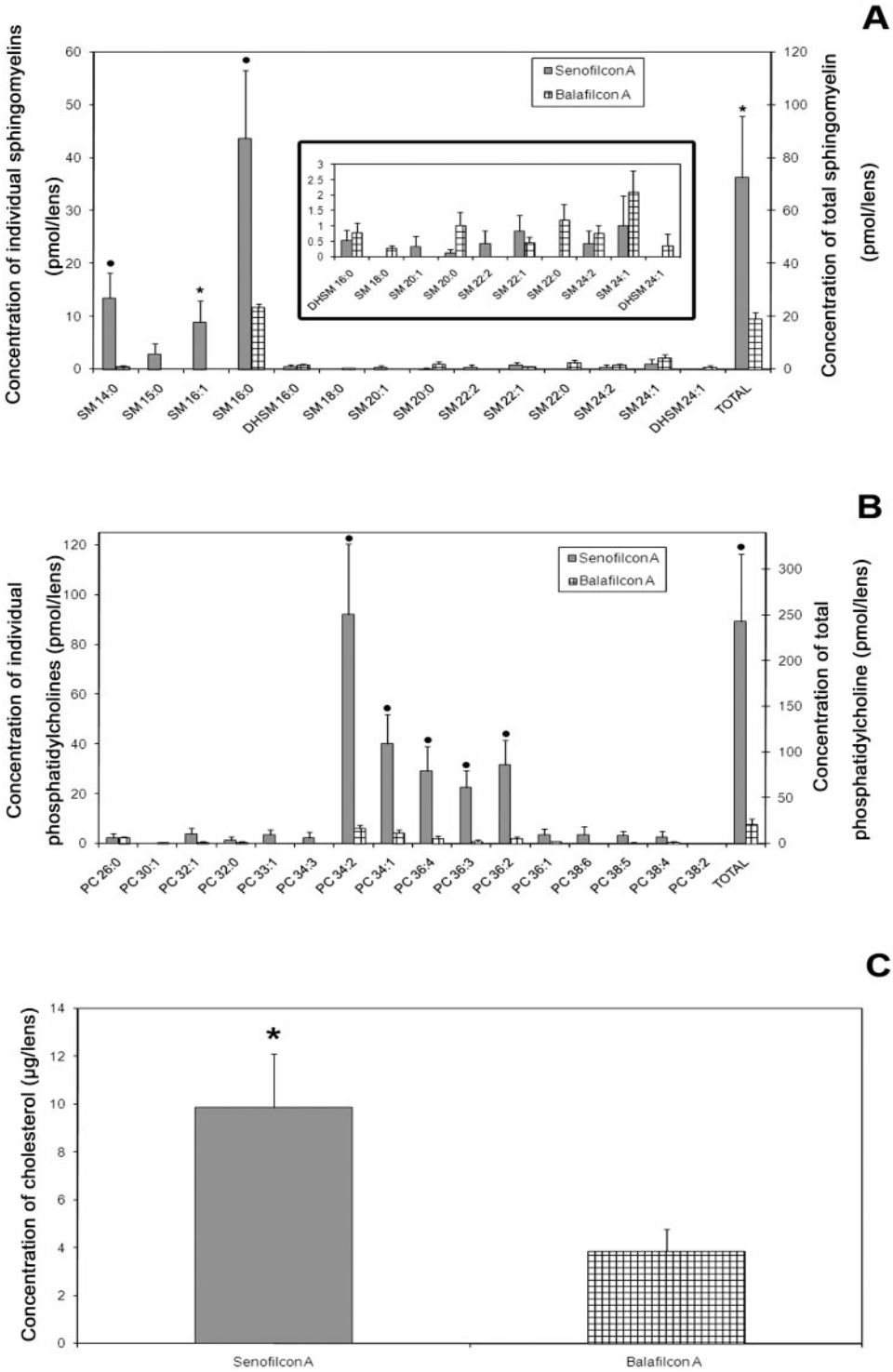


FIGURE 3. Concentration of (**A**) SM and DHSM, (**B**) PC, and (**C**) cholesterol extracted from senofilcon A and balafilcon A contact lenses washed with the commercial solutions in Table 1. Data are presented as the mean ± SE: $^{\bullet}P < 0.01$; $^{*}P < 0.05$. Senofilcon A, $n = 8$; balafilcon A, $n = 12$.

have suggested that the concentration of PC in meibomian gland secretions is approximately five times greater than SM[6]; however, the results shown herein suggest that the concentration of PC and SM in tears is approximately equal. This discrepancy may be further evidence of the differences between meibomian gland and tear film lipids, as has been suggested.[9–12] Other classes of phospholipids (i.e., PE, PS) have been reported in meibomian gland secretions[5,6,45] but were below the LOD in the current analysis of tears.

The results of this study suggest that the phosphocholine profile from contact lens lipid extracts is similar to that of tears, with the differences in SM attributable to contact lens material specificity. Several phospholipids were detected in contact lens extracts, but not in tear extracts. This diversity could arise from accumulation on the contact lens during wear, allowing the concentration to reach levels above the LOD. Contact lens material was an important factor in lipid deposition, with both polar (phospholipid) and nonpolar (cholesterol) lipids depositing in larger concentrations on senofilcon A contact lenses than on balafilcon A lenses. Furthermore, several individual phospholipids were detected only in senofilcon A lipid extracts. Senofilcon A is a U.S. Food and Drug Administration

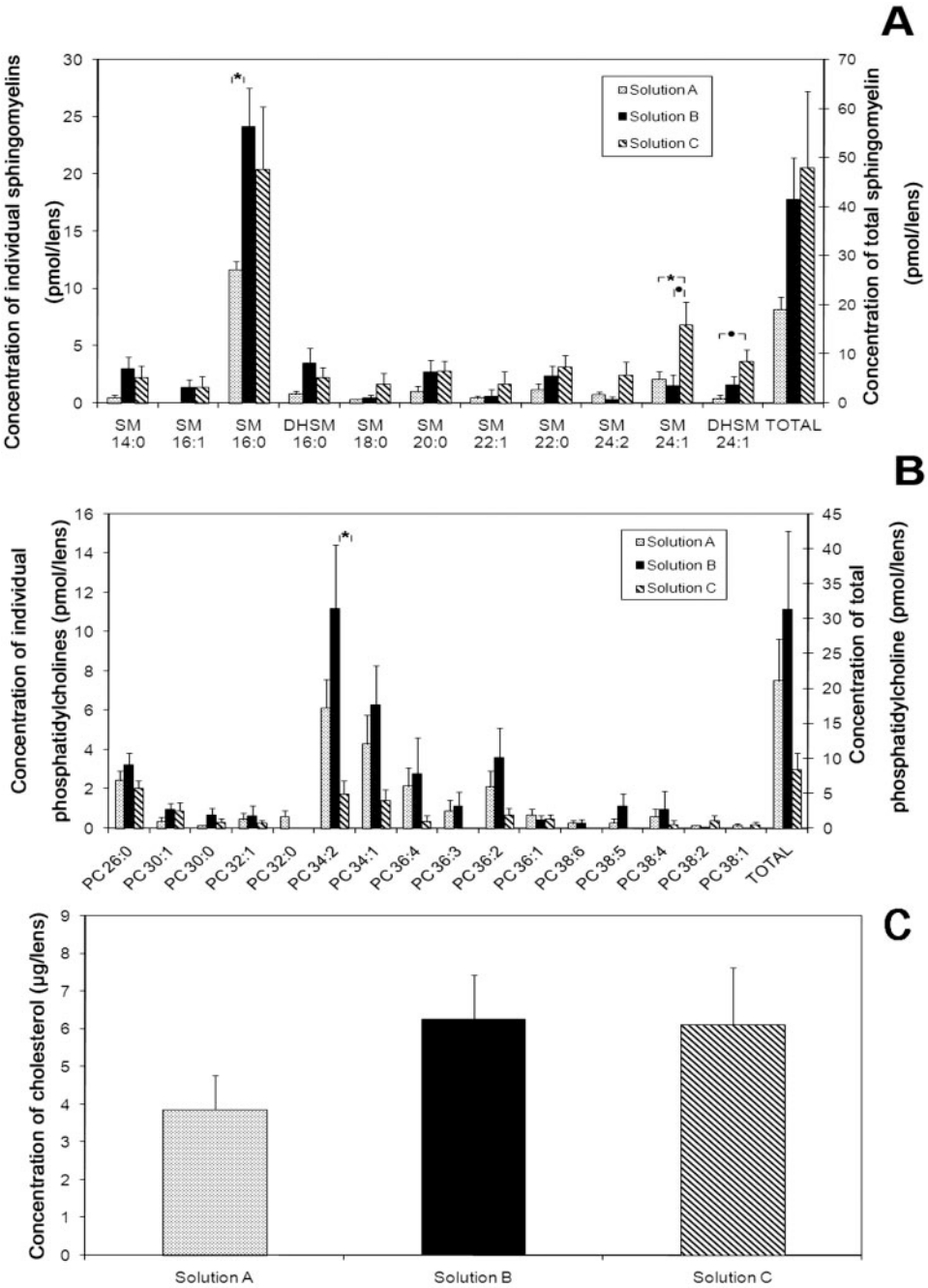


FIGURE 4. Concentration of (**A**) SM and DHSM, (**B**) PC, and (**C**) cholesterol extracted from worn balafilcon A contact lenses having been washed with the commercial solutions in Table 1. Data are presented as the mean ± SE: $^{\bullet}P < 0.01$; $^{*}P < 0.05$. Solution A, $n = 12$; solution B, $n = 11$, solution C, $n = 10$.

(FDA) group I material, meaning it has low water content and a nonionic polymer, whereas balafilcon A contact lenses are an FDA group III contact lens, with low water content and an ionic polymer. Studies have demonstrated that contact lenses containing a nonionic polymer show an increase in the deposition of lipids, whereas those containing an ionic polymer show a larger concentration of protein deposition but a smaller concentration of lipid deposits.[23,43,44] Lipids are soluble in the pyrrolidone derivatives that are found in both contact lenses, with senofilcon A containing polyvinyl pyrrolidone (PVP) and balafilcon A containing *N*-vinyl pyrrolidone (NVP).[46] The deposition of tear film components onto contact lenses has been linked to a large number of contact lens–related problems such as discomfort[15] and infection.[16] A sound knowledge of the individual molecules deposited may enable the manufacture of more suitable contact lenses or lens care solutions that specifically target the removal of these molecules.

Lens care solutions are an important part of contact lens health and many incorporate an antimicrobial agent, surfactant, and buffer system into a multipurpose solution. The efficacy of lipid removal by three commercially available lens care solutions was tested in this study, and the results indicated no significant differences in the total concentration of either polar or nonpolar lipid deposits on balafilcon A lenses using any of the three washes. Although contact lens material–lens care solution combinations investigated in this study showed no significant difference, some combinations analyzed by Zhao et al.[30] showed significantly higher deposition of proteins and cholesterol. The variation in cholesterol concentration in this study may be due to the differences in the method of analysis.

Table 3. Abundance of Targeted Lipid Classes in Tears and on Contact Lenses

Sample	$\frac{\Sigma SM_i}{\Sigma(SM_i + PC_i)}$	$\frac{\Sigma PC_i}{\Sigma(SM_i + PC_i)}$	Cholesterol (μg/lens)
Tears (n = 6)	0.49 ± 0.02	0.51 ± 0.02	Not measured
Senofilcon A Solution A (n = 8)	0.23 ± 0.09	0.77 ± 0.30	10 ± 2
Balafilcon A			
Solution A (n = 10)	0.50 ± 0.12	0.50 ± 0.18	3.9 ± 0.9
Solution B (n = 11)	0.63 ± 0.15	0.37 ± 0.16	6.3 ± 1.2
Solution C (n = 11)	0.87 ± 0.39	0.13 ± 0.05	6.1 ± 1.5

SM and PC abundances normalized across phosphocholine lipids.

Zhao et al. used thin-layer chromatography (TLC) with quantification by densitometry, as opposed to the quantification by mass spectrometry with internal standards described in this study. Perhaps co-eluting materials from either the wash or contact lenses gives rise to uncertainty in the densitometer reading in this case. Ensuring the compatibility of lens material and lens care solution in the removal of proteins and both polar and nonpolar lipids could be an advantage for eye care professionals in reducing contact lens–related problems.

The normalized amount of PC and SM showed that there were equal proportions of both classes of lipids in tears, and this finding was also reflected in the extracts from balafilcon A contact lenses soaked in solution A. Balafilcon A lenses soaked in solution B showed an increase in the proportion of SM relative to PC in extracts, with this bias further increased in extracts from balafilcon A lenses soaked in solution C. This may relate to the efficacy of the surfactant in removing PC over SM and may be amplified in solution C by the effect of hydrogen peroxide on the significantly more unsaturated PC molecules. Senofilcon A contact lenses soaked in solution A showed a greater proportion of PC than SM; however, it is unclear whether this finding represents an affinity between the material and the PC class or the more unsaturated fatty acid chains.

Acknowledgments

The authors thank John Korth for technical support.

References

1. Ohashi Y, Dogru M, Tsubota K. Laboratory findings in tear fluid analysis. *Clin Chim Acta.* 2006;369:17–28.
2. McDonald JE. Surface phenomena of the tear film. *Am J Ophthalmol.* 1969;67:56–64.
3. Mathers WD, Lane JA. Meibomian gland lipids, evaporation, and tear film stability. In: Sullivan DA, Dartt DA, Meneray MA, eds. *Lacrimal Gland, Tear Film, and Dry Eye Syndromes.* New York: Springer; 1998;2.
4. Wollensak G, Mur E, Mayr A, Baier G, Gottinger W, Stoffler G. Effective methods for the investigation of human tear film proteins and lipids. *Graefes Arch Clin Exp Ophthalmol.* 1990;228:78–82.
5. Greiner JV, Glonek T, Korb DR, Leahy CD. Meibomian gland phospholipids. *Curr Eye Res.* 1995;15:371–375.
6. Shine WE, McCulley JP. Polar lipids in human meibomian gland secretions. *Curr Eye Res.* 2003;26:89–94.
7. Butovich IA. The meibomian puzzle: combining pieces together. *Prog Retin Eye Res.* 2009;28:483–498.
8. Butovich IA, Uchiyama E, McCulley JP. Lipids of the meibomum: mass-spectrometric analysis and structural elucidation. *J Lipid Res.* 2007;48:2220–2235.
9. Butovich IA. On the lipid composition of human meibum and tears: comparative analysis of nonpolar lipids. *Invest Ophthalmol Vis Sci.* 2008;49:3779–3789.
10. Nagyova B, Tiffany JM. Components responsible for the surface tension of human tears. *Curr Eye Res.* 1999;19:4–11.
11. Borchman D, Foulks GN, Yappert MC, Ho DV. Temperature-induced conformational changes in human tear lipids hydrocarbon chains. *Biopolymers.* 2007;87:124–133.
12. Borchman D, Foulks GN, Yappert MC, Tang D, Ho DV. Spectroscopic evaluation of human tear lipids. *Chem Phys Lipids.* 2007;147:87–102.
13. Korb DR, Greiner JV, Glonek T. Tear film lipid layer formation: implications for contact lens wear. *Optom Vis Sci.* 1996;73:189–192.
14. Jones L, Mann A, Evans K, Franklin V, Tighe B. An in vivo comparison of the kinetics of protein and lipid deposition on group II and group IV frequent-replacement contact lenses. *Optom Vis Sci.* 2000;77:503–510.
15 Fonn D. Targeting contact lens induced dryness and discomfort: what properties will make lenses more comfortable. *Optom Vis Sci.* 2007;84:279–285.
16. Willcox MDP, Holden BA. Contact lens related corneal infections. *Biosci Rep.* 2001;21:445–461.
17. Garrett Q, Garrett RW, Milthorpe BK. Lysozyme sorption in hydrogel contact lenses. *Invest Ophthalmol Vis Sci.* 1999;40:897–903.
18. Garrett Q, Laycock B, Garrett RW. Hydrogel lens monomer constituents modulate protein sorption. *Invest Ophthalmol Vis Sci.* 2000;41:1687–1695.
19. Green-Church KB, Nichols JJ. Mass spectrometry-based proteomic analyses of contact lens deposition. *Mol Vis.* 2008;14:291–297.
20. Sack RA, Jones B, Antignani A, Libow R, Harvey H. Specificity and biological activity of the protein deposited on the hydrogel surface. relationship of polymer structure to biofilm formation. *Invest Ophthalmol Vis Sci.* 1987;28:842–849.
21. Nichols JJ, Sinnott LT. Tear film, contact lens, and patient-related factors associated with contact-related dry eye. *Invest Ophthalmol Vis Sci.* 2006;47:1319–1328.
22. Ho C-H, Hlady V. Fluorescence assay for measuring lipid deposits on contact lens surfaces. *Biomaterials.* 1995;16:479–482.
23. Lord MS, Stenzel MH, Simmons A, Milthorpe BK. The effect of charged groups on protein interactions with poly(HEMA) hydrogels. *Biomaterials.* 2006;27:567–575.
24. Carney FP, Nash WL, Sentell KB. The adsorption of major tear film lipids in vitro to various silicone hydrogels over time. *Invest Ophthalmol Vis Sci.* 2008;49:120–124.
25. Iwata M, Ohno S, Kawai T, Ichijima H, Cavanagh H. In vitro evaluation of lipids adsorbed on silicone hydrogel contact lenses using new gas chromatography/mass spectrometry analytical method. *Eye Contact Lens.* 2008;34:272–280.
26. Hart D. Lipid deposits which form on extended wear contact lenses. *Int Contact Lens Clin.* 1984;11:348–360.
27. Bowers R, Tighe B. Studies on the biocompatability of hydrogels: white spot deposits—incidence of occurrence, location and gross morphology. *Biomaterials.* 1987;8:89–93.
28. Bowers R, Tighe B. Studies of the ocular compatibility of hydrogels: white spot deposits-chemical composition and geological arrangement of components. *Biomaterials.* 1987;8:172–177.
29. Maissa C, Franklin V, Guillon M, Tighe B. Influence of contact lens material surface characteristics and replacement frequency on protein and lipid deposition. *Optom Vis Sci.* 1998;75:697–705.

30. Zhao Z, Carnt NA, Aliwarga Y, et al. Care regimen and lens material influence on silicone hydrogel contact lens deposition. *Optom Vis Sci.* 2009;86:251–259.
31. Sack RA, Tan KO, Tan A. Diurnal tear cycle: evidence for a nocturnal inflammatory constitutive tear fluid. *Invest Ophthalmol Vis Sci.* 1992;33:626–640.
32. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem.* 1957;226:497–509.
33. Deeley JM, Mitchell TW, Wei X, et al. Human lens lipids differ markedly from those of commonly used experimental animals. *Biochim Biophys Acta.* 2008;1781:288–298.
34. Brugger B, Erben G, Sandhoff R, Wieland FT, Lehmann WD. Quantitative analysis of biological membrane lipids at the low picomole level by nano-electrospray ionization tandem mass spectrometry. *Proc Natl Acad Sci USA.* 1997;94:2339–2344.
35. Cole MJ, Enke CG. Fast atom bombardment tandem mass spectrometry employing ion-molecule reactions for the differentiation of phospholipid classes. *J Am Soc Mass Spectrom.* 1991;2:470–475.
36. Armbruster DA, Tillman MD, Hubbs LM. Limit of detection (LOD)/limit of quantitation (LOQ): comparison of the empirical and the statistical methods exemplified with GC-MS assay of abused drugs. *Clin Chem.* 1994;40:1233–1238.
37. Fullard RJ, Snyder C. Protein levels in nonstimulated and stimulated tears of normal human subjects. *Invest Ophthalmol Vis Sci.* 1990;31:1119–1126.
38. McGill JI, Liakos GM, Goulding N, Seal DV. Normal tear protein profiles and age-related changes. *Br J Ophthalmol.* 1984;68:316–320.
39. Buyukpamukcu E, Hau J, Fay L-B, Dionisi F. Analysis of phospholipids using electrospray ionisation mass spectrometry. *Lipid Technol.* 2007;19:136–138.
40. Han X, Gross RW. Structural determination of picomole amounts of phospholipids via electrospray ionization tandem mass spectrometry. *J Am Soc Mass Spectrom.* 1995;6:1202–1210.
41. Mitchell TW, Ekroos K, Blanksby SJ, Hulbert AJ, Else PL. Differences in membrane acyl phospholipid composition between an endothermic mammal and an ectothermic reptile are not limited to any phospholipid class. *J Exp Biol.* 2007;210:3440–3450.
42. Nealon JR, Blanksby SJ, Abbott SK, Hulbert AJ, Mitchell TW, Truscott RJW. Phospholipid composition of the rat lens is independent of diet. *Exp Eye Res.* 2008;87:502–514.
43. Peters K, Millar TJ. The role of different phospholipids on tear break-up time using a model eye. *Curr Eye Res.* 2002;25:55–60.
44. Shine WE, McCulley JP. Keratoconjunctivitis sicca associated with meibomian secretion polar lipid abnormality. *Arch Ophthalmol.* 1998;116:849–852.
45. Greiner JV, Glonek T, Korb DR, Booth R, Leahy CD. Phospholipids in meibomian gland secretions. *Ophthalmic Res.* 1996;28:44–49.
46. Jones L, Evans K, Sariri R, Franklin V, Tighe B. Lipid and protein deposition of N-vinyl pyrrolidone-containing group II and group IV frequent replacement contact lenses. *CLAO J.* 1997;23:122–126.