Contact lens physical properties and lipid deposition in a novel characterized artificial tear solution

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Purpose: To characterize various properties of a physiologically-relevant artificial tear solution (ATS) containing a range of tear film components within a complex salt solution, and to measure contact lens parameters and lipid deposition of a variety of contact lens materials after incubation in this ATS.

Methods: A complex ATS was developed that contains a range of salts, proteins, lipids, mucin, and other tear film constituents in tear-film relevant concentrations. This ATS was tested to confirm that its pH, osmolality, surface tension, and homogeneity are similar to human tears and remain so throughout the material incubation process, for up to 4 weeks. To confirm that silicone hydrogel and conventional hydrogel contact lens materials do not alter in physical characteristics beyond what is allowed by the International Organization for Standardization (ISO) 18369–2. The diameter, center thickness, and calculated base curve were measured for five different lens materials directly out of the blister pack, after a rinse in saline and then following a two week incubation in the modified ATS. To test the ATS and the effect of its composition on lipid deposition, two lens materials were incubated in the ATS and a modified version for several time points. Both ATS solutions contained trace amounts of carbon-14 cholesterol and phosphatidylcholine, such that deposition of these specific lipids could be quantified using standard methods.

Results: This ATS is a complex mixture that remains stable at physiologically relevant pH (7.3–7.6), osmolality (304–306 mmol/kg), surface tension (40–46 dynes/cm) and homogeneity over an incubation period of three weeks or more. The physical parameters of the lenses tested showed no changes beyond that allowed by the ISO guidelines. Incubations with the ATS found that balafilcon A lenses deposit significantly more cholesterol and phosphatidylcholine than omafilcon A lenses ($p < 0.05$) and that removing lactoferrin and immunoglobulin G from the ATS can significantly decrease the mass of lipid deposited.

Conclusions: This paper describes a novel complex artificial tear solution specially designed for in-vial incubation of contact lens materials. This solution was stable and did not adversely affect the physical parameters of the soft contact lenses incubated within it and showed that lipid deposition was responsive to changes in ATS composition.

In vitro biomaterial models have been used extensively to analyze surface interactions that occur with an implanted medical device and their surroundings [1-5]. Contact lenses are similar to an implant in that they are a biomaterial that is exposed to a very complex biologic environment, in some cases more complex than permanently implanted biomaterials, such as a hip or knee replacement. Unlike these biomaterials, contact lenses are exposed to a continuously changing tear film composition and structure, induced by continuous blinking and drying of the lens surface, changes in environmental surroundings, systemic diseases, medications, alcohol consumption and diet [6-9].

The composition of the human tear film is complex and contains several layers, including a glycocalyx-like mucin layer covering the corneal epithelium, an aqueous layer rich in proteins, salts and electrolytes, and a lipid layer divided into both a polar and non-polar lipid component [10-12]. Although this layered tear film model is still favored, it is now believed that this structure is not as compartmentalized as previously thought and that the components from each layer can be found throughout the entire tear film [13-17]. Soft contact lens materials, once inserted into the eye, lie in the middle of this tear film structure and are known to readily adsorb many different tear film components, including lipids, proteins, and mucins [18-27].

Building an in vitro model to examine deposition of tear film components onto contact lens materials would allow for systematic and structured analysis of tear film interactions. These models could then be used to analyze various lens materials and their affinity for different tear film components, the conformation of proteins on contact lens materials, the exploration of tear film component interactions and competition, and the effectiveness of contact lens cleaning solutions to remove such deposits. These types of experiments would be difficult, if not impossible, to conduct in a controlled manner using in vivo or ex vivo studies. Therefore, in vitro models examining these interactions and processes can

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provide pertinent information to further our understanding of the ever growing field of contact lens material science.

In vitro models have many benefits over in-eye clinical studies. They allow for analysis of specific variables without the use of human or animal testing, the variables are easily and tightly controlled in laboratory settings, many different analysis techniques can be used that otherwise would not be available using in vivo or ex vivo based studies, allow for the examination of both simple and complex models, and lastly in vitro studies tend to require less financial support and time to conduct, since participant remuneration and ethics approval are not required.

Although in vitro models can never fully mimic the complex nature of human contact lens wear, they can be designed to be physiologically relevant and help understand the basic tear film interactions that occur. Many early in vitro contact lens deposition models involved incubating contact lens materials in a simple saline solution with one tear film component, such as a single protein or lipid [18,24,28-31]. This model is very simplistic and is not indicative of what is found in the human tear film. It is clear that there is a relative dearth of information on contact lens in vitro models, especially for lipid deposition.

More recently, researchers have started to increase the complexity of the artificial tear solutions used to mimic the tear film. Mirejovsky et al. [32] was the first to report on the use of a complex artificial tear film that contained a range of salts, proteins, and lipids. Artificial tear solutions used in in vitro studies must contain physiologically relevant components, maintain physiologically relevant solution properties and must not change the contact lens parameters during incubation, as alterations in these parameters can cause changes in the contact lens dimensions themselves. The contact lenses may swell/shrink, thicken/thin, or experience a change in their base curve if an inappropriate solution is used. These lens parameter changes could alter the deposition pattern and lens interactions with tear film components. If in vitro contact lens deposition models are to mimic human contact lens wear, then the artificial tear solutions used must be more complex than a single component system. Recent work from our laboratory has shown that an in vitro incubation solution consisting of a mixture of lipids, proteins, mucins and buffers is significantly different to that obtained in an in vitro model which uses single lipids alone [33]. In this study, we wanted to explore how sensitive the lipid deposition was to smaller changes in solution, such as adding or removing individual components.

Our laboratory has characterized a complex physiologically relevant artificial tear solution (ATS) designed for in vitro vial-enclosed incubation experiments. This solution has been tested to determine if the solution and contact lens parameters remain stable throughout contact lens incubation. Although this solution does not contain all of the individual human tear film components, it does contain a broad representation of the most abundant lipids, proteins, mucin, salts, and inorganics that are present.

METHODS

The ATS composition: ATS preparation required four main steps. These included preparation of the complex salt solution, lipid stock solution, adding lipids to the salt solution, and addition of the proteins and mucin to complete the solution.

The complex salt solution—The first step in making an ATS was the preparation of a complex salt solution (CSS). The composition of the CSS, which is used as the base of the ATS, is shown in Table 1. These specific salts and their relative concentrations are based on literature values [32, 34-36]. All CSS components were ACS grade and purchased from Sigma (Oakville, ON). The individual components were measured on an analytical balance and sequentially added to the desired volume of MilliQ water in the order that they are listed in table. Once all of the components had been added, ProClin 300 (Sigma, Oakville, ON), a preservative and antimicrobial agent, was added to the system. The use of ProClin 300 allows for incubation at 37 °C for prolonged periods of time with no fear of microbial contamination. After all the ingredients were added, the pH was approximately 7.15 and the osmolality was 305 mmol/kg. When the CSS was left at room temperature for three or more days it equilibrated naturally to the desired pH of 7.4, which is the typical pH of the human tear film [37]. However, if the solution was to be used immediately then purging with nitrogen gas equilibrated the solution to the desired pH much faster.

Concentrated lipid stock solution—The next step in the ATS preparation was to make a concentrated lipid stock. Here, a 2,000× concentrated lipid stock solution (LSS) was made to help facilitate dissolving the pure lipids into the CSS. Lipids, especially non-polar lipids, do not naturally dissolve into aqueous solutions, so dissolving them first into a solution of 1 hexane: 1 ether and then adding an aliquot of the hexane/ether LSS to the CSS helps facilitate the incorporation of lipids. To make a LSS, pure lipids were warmed up to room temperature and weighed out using an analytical balance (solid lipids) or pipetted using a positive displacement pipette (liquid lipids). The concentrated LSS was placed in an amber vial, sealed with Parafilm® (VWR, Mississauga, ON), wrapped in foil and stored at −20 °C until required. Table 2 shows the lipids used in the ATS, their characteristics, the lipid stock concentration and final ATS concentration used for each lipid. All pure lipids were purchased through Sigma (Oakville, ON). The lipids used in this ATS were chosen specifically so that a broad range of human tear film lipids were represented and their concentrations were chosen based on human tear film concentrations, artificial tear solution literature values, and lipid solubility in aqueous solutions [28,32,38-40].

Lipid artificial tear solution—The next step in making an ATS was to make the lipid artificial tear solution (LTS).

TABLE 1. ARTIFICIAL TEAR SOLUTION COMPLEX SALT SOLUTION COMPONENTS [35-38].

Salt component	Molecular formula	mM
Sodium chloride	NaCl	90.0
Potassium chloride	KCl	16.0
Sodium citrate	$Na_3C_6H_5O_7$	1.5
Glucose	$C_6H_{12}O_6$	0.2
Urea	$(NH_2)_2CO$	1.2
Calcium chloride	$CaCl_2$	0.5
Sodium carbonate	Na_2CO_3	12.0
Potassium hydrogen carbonate	$KHCO_3$	3.0
Sodium phosphate dibasic	Na_2HPO_4	24.0
Hydrochloric acid (10 molar)	HCl	26.0
ProClin 300 (Supelco 48912-U)		0.2 µl/ 1l
MilliQ Water		

TABLE 2. MOLECULAR AND EXPERIMENTAL DETAILS OF THE SPECIFIC LIPIDS USED FOR ALL LIPID INCUBATION SOLUTIONS [28,35,40-42].

Pqarameters	Triolein	Cholesterol	Oleic acid	Oleic acid methyl ester	Cholesteryl oleate	Phosphatidyl choline
Lipid type	Triglyceride	Sterol	Fatty acid	Fatty ester	Cholesteryl ester	Phospholipid
Formula	$C_{57}H_{104}O_6$	$C_{27}H_{46}O$	$C_{18}H_{34}O_2$	$C_{19}H_{36}O_2$	$C_{45}H_{78}O_2$	$C_{42}H_{82}NO_8P$
Molecular Weight (g/mol)	885.5	386.7	282.5	296.5	651	760.1
Lipid Stock Concentration (mg/ml)	32.0	3.6	3.6	24.0	48.0	1.0
Final ATS Concentration (mg/ml)	0.016	0.0018	0.0018	0.012	0.024	0.0005

TABLE 3. PROTEIN AND MUCIN CONCENTRATIONS AND DETAILS IN ATS [35,43-50].

Proteins	Molecular weight (kDa)	Concentration (mg/ml)	Sigma product number
Bovine albumin	66.4	0.20	A7888
Hen egg lysozyme	14.3	1.90	L6876
Bovine submaxillary mucin	3×10^5 to 4×10^7	0.15	M3895
Bovine colostrum lactoferrin	83.1	1.80	L4765
Bovine immunoglobulin G	161	0.02	I5506

This was accomplished by removing the LSS from the freezer and allowing it to warm up to room temperature in a dry dark place. The desired volume of room temperature CSS was placed into a glass septum jar and the required volume of LSS was added to the CSS. The cap was screwed onto the septum jar and the whole jar was placed into an ultra-sonic bath that was warmed to 37 °C. Two syringes were pierced through the septum, one large blunt syringe was placed into the solution and one smaller syringe was left sitting in the air space of the septum jar. The large syringe was connected to a nitrogen tank and the small syringe remained open to air to act as a vent. The LTS was sonicated at 90 W and purged with nitrogen gas at a pressure of 3 psi until the LSS was fully incorporated into the CSS and the odour of hexane:ether had dissipated. The LTS was now complete.

Incorporation of proteins and mucin to complete preparation of the ATS—The last step in preparing the ATS

was the addition of proteins and mucin. The specific proteins and mucin used and their concentrations in the final ATS are outlined in Table 3 and are based on literature values of the human tear film, literature ATS concentrations, and based on the cost of the component, as in the case of lactoferrin and IgG [32,41-48]. All proteins and mucin were purchased from Sigma. Bovine and hen-egg proteins were chosen for use in this ATS due to their cost and their similarities to human proteins in molecular weight, pI, amino acid chain length, and number of charged residues. The proteins and mucin were weighed out on an analytical balance and added to the LTS while stirring. When all components were incorporated fully, the complete ATS was sonicated at 37 °C for a maximum of 5 min, to prevent destruction of the proteins [49].

Solution properties:

pH and osmolality—To test the consistency of the ATS's pH and osmolality during in vitro incubations, a 28 day

TABLE 4. CONVENTIONAL HYDROGEL CONTACT LENS MATERIAL CHARACTERISTICS.

Material type	Conventional hydrogel	
USAN	Etafilcon A	Omafilcon A
Proprietary name	Acuvue®2	Proclear®
Manufacturer	Johnson & Johnson	CooperVision
Power (D)	−3.00	−3.00
Base Curve (mm)	8.7	8.6
Diameter (mm)	14.0	14.2
Monomers	HEMA, MA	HEMA, PhC
Surface Modification	None	None
Oxygen Transmissibility ($\times 10^{-9}$)	31.0	52.3
Water Content	58%	62%
FDA class	Group IV	Group II

USAN: United States adopted name; HEMA (poly-2-hydroxyethyl methacrylate); MA (methacrylic acid); PhC (phosphorylcholine).

study was performed. Clear borosilicate glass 6 mL vials were half filled with freshly made ATS with a starting pH of 7.35 and an osmolality of 305 mmol/kg. Vials were closed with PTFE-sealed screw caps, further sealed with Parafilm® and incubated at 37 °C for six different time points including: 1, 3, 7, 14, 21, and 28 days in triplicate. On the specific days, the vials were opened and the pH was measured using the SympHony SB20 pH meter (VWR, Mississauga, ON) and the osmolality was measured using the Wescor "Vapro" Vapor Pressure Osmometer 5520 (Discovery Diagnostics, Claremont, ON).

Surface tension and homogeneity of ATS—To test the surface tension and liposome homogeneity of the solution a 3.5 week study was conducted. Fresh ATS was made and tested for its surface tension and homogeneity and then the ATS was incubated for 3.5 weeks at 37 °C and tested again for the two parameters. Surface tension was measured using the Wilhelmy balance (CAHN Instruments, Madison, WI) using a platinum ring and the homogeneity of the solution was tested by staining liposomes in the ATS with Nile Red. To stain with Nile Red, the Nile Red was dissolved in acetone at 1 mg/ml, then 1 µl of the Nile Red solution was added to 100 µl of the test solution in a micro-centrifuge tube and shaken so the two components were well mixed [32]. Then 20 µl of the Nile Red test solution was then pipetted onto a slide (prewashed with methanol), and a coverslip was placed on top. The sample was then examined and photographed on the microscope at 10× and 40× magnifications using a green light filter. Samples of the complex salt solution and artificial tear solution were analyzed at several points in the preparation process and compared with the solution after 3.5 weeks of incubation. The distribution and diameter of the liposomes was analyzed for each sample.

Lens parameters: Five contact lens materials were tested in triplicate: Acuvue® 2 (etafilcon A; Vistakon, Jacksonville, FL), Proclear® (omafilcon A; CooperVision, Pleasanton, CA), Acuvue® OASYS™ (senofilcon A; Vistakon), Biofinity®

(comfilcon A; CooperVision), PureVision™ (balafilcon A; Bausch & Lomb, Rochester, NY). The material characteristics of all contact lens materials can be found in Table 4 and Table 5. All lens materials tested had a spherical power of −3.00 diopters (D) and had an approximate base curve of 8.6±0.2 mm. The individual lenses were measured on three separate occasions: out of the blister pack, after 40 h of soaking in CSS, and after a 2 weeks incubation at 37 °C in the ATS previously described. The center thickness was measured using a Rehder Development Co. E.T.-1 (Castro Valley, CA) and the diameter and sagittal height (Sag) of each lens was measured using the Optimec Soft Contact Lens Analyzer (Malvern, UK). The base curve was then calculated from the diameter and sagittal height. The data were analyzed using Statistica 9 using paired *t*-tests. The contact lens parameter measurements were taken so that comparisons could be made between the three parameters tested and was not meant to assess the contact lens parameter variability from their specified package dimensions.

Lipid deposition: As the last step of the ATS characterization process, the ATS was examined for its lipid deposition using a simple radioactive experiment previously developed by our laboratory. In this experiment, omafilcon A and balafilcon A lens materials were incubated in two different ATS solutions for three different time periods, as outlined in Figure 1. The first ATS solution composition was identical to the ATS described above (+LF/IgG) and the second ATS solution was a slightly simpler version with lactoferrin (LF) and immunoglobulin G (IgG) removed (- LF/IgG). To facilitate sensitive quantification of lipid deposition, both ATS solutions were prepared by adding a small aliquot of one of two radiolabelled lipids (Table 6); ^{14}C-cholesterol or ^{14}C-phosphatidylcholine. Lenses (n=3) were then incubated in each solution for 3, 7, and 20 days.

At the end of the incubation period, each lens was rinsed twice in saline and blotted on lens paper. The lenses were then placed in 20 ml glass scintillation vials with 2 ml of 2:1

TABLE 5. SILICONE HYDROGEL CONTACT LENS MATERIAL CHARACTERISTICS.

Material Type	Silicone Hydrogel		
USAN	Senofilcon A	Comfilcon A	Balafilcon A
Proprietary name	Acuvue® OASYS™	Biofinity®	PureVision™
Manufacturer	Johnson & Johnson	CooperVision	Bausch & Lomb
Power (D)	−3.00	−3.00	−3.00
Base curve (mm)	8.4	8.6	8.6
Diameter (mm)	14.0	14.0	14.0
Centre thickness (mm) −3.00D	0.07	0.08	0.09
Monomers	mPDMS, DMA, HEMA, siloxane macromer, EGDMA, PVP	M3U, FM0411M, HOB, IBM, NVP, TAIC, VMA	NVP, TPVC, NVA, PBVC
Surface modification	PVP as an internal wetting agent	None	Plasma oxidation
Oxygen transmissibility ($\times 10^{-9}$)	147	160	110
Modulus (MPa)	0.7	0.75	1.1
Water content	38%	48%	36%
FDA class	Group I	Group I	Group III

USAN: United States adopted name; DMA (N,N-dimethylacrylamide); EGDMA (ethyleneglycol dimethacrylate); FM0411M (2-ethyl [2-[(2-methylprop-2-enoyl)oxy]ethyl]carbamate); HEMA (poly-2-hydroxyethyl methacrylate); HOB ((2RS)-2-hydroxybutyl 2-methylprop-2-enoate); IBM (Isobornyl methacrylate); M3U (α-[[3-(2-[[2-(methacryloyloxy)ethyl] carbamoyloxy]ethoxy)propyl]dimethylsilyl]-ω-[3-(2-[[2-(methacryloyloxy)ethyl] carbamoyloxy]ethoxy)propyl]poly(([oxy[(methyl) [3-[ω-methylpoly(oxyethylene)oxy]propyl]silylene] /[oxy[(methyl)(3,3,3-trifluoropropyl)]silylene]/oxy (dimethylsilylene)])); mPDMS (monofunctional polydimethylsiloxane); NVA *N*-vinyl aminobutyric acid; NVP (N-vinyl pyrrolidone); PBVC (poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate]); PVP (poly(vinylpyrrolidone)); TAIC (1,3,5-triprop-2-enyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione); TPVC (tris-(trimethylsiloxysilyl) propylvinyl carbamate); VMA (N-Vinyl-N-methylacetamide).

chloroform:methanol extraction solution and were incubated for three hours each at 37 °C while shaking on an orbital shaker. Each lens was extracted in this way on two separate occasions and both extracts were pooled together in the same vial.

The extract vials were dried completely using nitrogen evaporation at 37 °C. All samples were re-suspended in 1 ml of chloroform, sonicated for one min, and 10 ml of Ultima Gold F scintillation cocktail (Perkin-Elmer, Woodbridge, ON) was added. The vials were submitted for liquid scintillation beta counting. Standard lipid samples were prepared and all data were analyzed and quantified using standard calibration curves.

RESULTS

pH and osmolality: When examining the stability of pH and osmolality of the ATS it was found that pH ranged from 7.35 to 7.49 and osmolality ranged from 305.0 to 303.7 mmol/kg, over the 28 days of incubation.

Surface tension and homogeneity of ATS: After the complex salt solution and ATS preparation was complete, several aliquots of each solution were stained with Nile Red examined microscopically at 200×-400× and photographed. Following a three week in-vial incubation, ATS aliquots were once again stained and photographed. Following staining with Nile Red, the CSS samples had no visible liposomes present in its solution, as expected. However, both ATS samples, freshly made and post incubation solutions, showed similar

distribution and sizes of liposomes stained by the Nile Red. The liposomes present in both ATS solutions ranged in size from 6 to 20 µm, with average sizes around 12 µm. Therefore, no discernible differences were found in fresh versus incubated ATS solutions in terms of its homogeneity.

The surface tension of the freshly prepared ATS was 51.5±0.38 dynes/cm and following the 25 days of incubation the surface tension fell to 45.05±1.25 dynes/cm. This is an average change of −6.46±1.30 dynes/cm.

Lens parameters: The center thickness of each lens material measured out of blister pack, following a saline soak, and after ATS incubation at 37 °C for two weeks can be graphically seen in Figure 2. One statistically significant difference was seen when analyzing the difference between the blister pack and post-incubation conditions. Omafilcon A lenses experienced a 1.0% average increase in center thickness following two week incubation in ATS. These changes in center thickness would not correlate to any significant clinically relevant changes in vivo.

The average contact lens diameter results measured out of blister pack, following a CSS soak, and following a two week incubation in ATS can been seen in Figure 3. Only etafilcon A had a statistically significant change in diameter following incubation in ATS, where the average diameter decreased by 0.81%. These changes in diameter are not considered to correlate to any clinically significant changes in vivo.
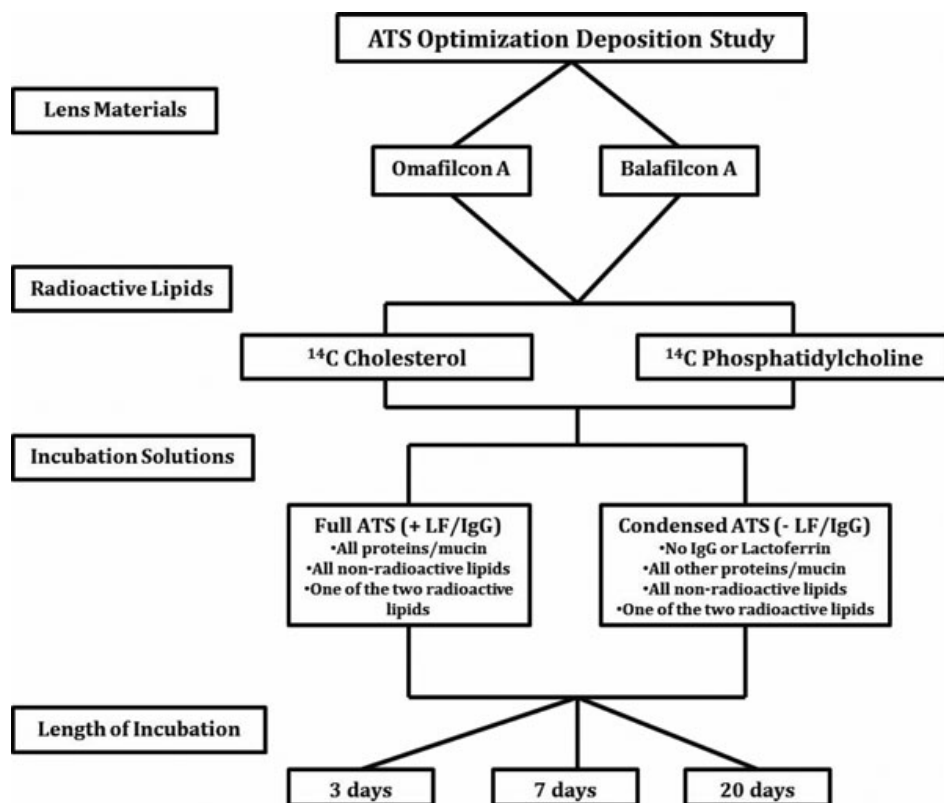
Figure 1. Lipid deposition study outline using the artificial tear solution (ATS) and two radiolabeled lipids.

	TABLE 6. RADIOACTIVE LIPID CHARACTERISTICS.		
Lipid	**Radiolabel**	**Molecular weight (g/mol)**	**Supplier**
Cholesterol [C]	4-^{14}C	386.6	Perkin-Elmer
L-α-DiPalmitoyl-Phosphatidylcholine [PC]	DiPalmitoyl-1-^{14}C	734.0	Perkin-Elmer

Average base curve results for each contact lens material after each lens treatment are displayed in Figure 4. No statistically significant differences were seen when comparing the blister pack measurements to the post-incubation in ATS measurements for any lens material.

Contact lens lipid deposition: The results of the radioactive cholesterol (C) and phosphatidylcholine (PC) kinetic uptake with and without the presence of lactoferrin and IgG can be seen in Figure 5 and Figure 6. As seen in the figures below, the silicone hydrogel lens material deposited more than the conventional hydrogel lens and that more cholesterol was deposited than phosphatidylcholine. The lipid uptake for all lens materials, especially the silicone hydrogels, was continuous throughout the 20 day period, with no plateau. The presence of lactoferrin and IgG in the ATS correlated with a statistically significant increase in cholesterol and PC deposition for balafilcon A at every time point (p≤0.001). Cholesterol deposition on omafilcon A in the presence of LF/IgG was greater than without, however the trend was not statistically significant for any time point (p>0.05). However,

PC deposition on omafilcon A did show statistically significant increases in the presence of LF/IgG for every time point (p≤0.008). Overall, there were statistically significant differences in the entire repeated measures ANOVA model, including all the variables and variable interactions for each lipid tested, as seen in Table 7 and Table 8.

DISCUSSION

In the creation of an in vitro model designed to analyze the dynamics of tear film interactions on a contact lens surface, the development of an appropriate artificial tear solution that is both physiologically relevant and stable is imperative. A handful of papers has been published using in vitro experimental models to examine contact lenses, their deposition and their tear film interactions. Many of these papers have used very simple in vitro solution models with single components for investigation, such as a single lipid or protein. These individual component model systems have been regularly used for the past 25 years and are continually being used. In the mid-1980s, Castillo et al. [50] used
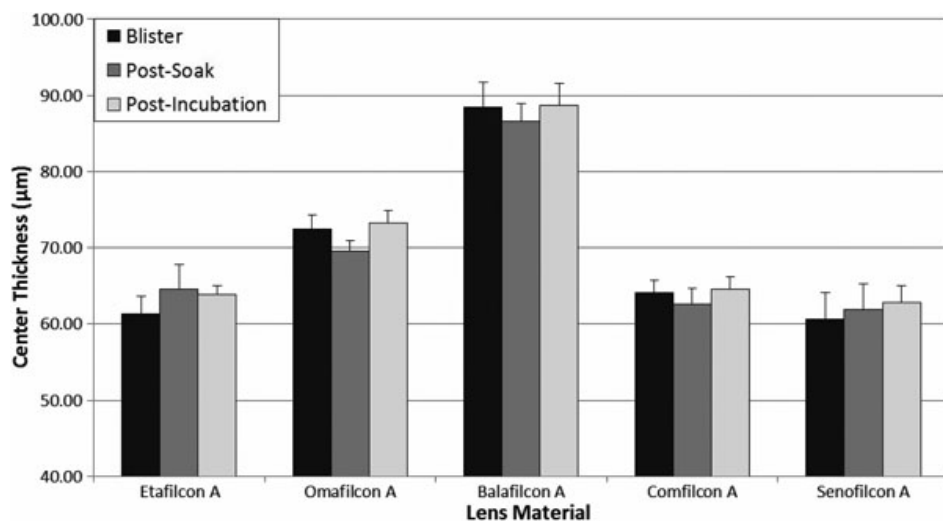
Figure 2. Average center thickness of all studied lens materials as measured directly from the blister pack, after a saline soak, and following 14 days incubation in ATS.
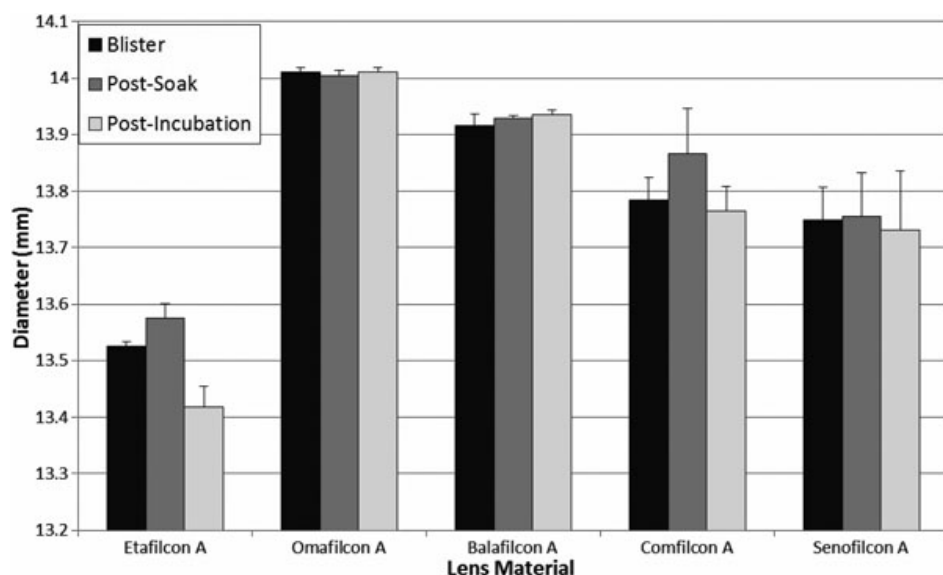

Figure 3. Average contact lens diameter of all studied materials as measured directly from the blister pack, after a saline soak, and following 14 days incubation in ATS.

lysozyme incubation solutions dissolved in a phosphate buffered saline (PBS) to examine conformational changes that occur on PHEMA materials fabricated using different methods via ATR-FTIR. Garrett et al. [24] and several studies from Jones et al. [18,29,30] used lysozyme or lactoferrin-only solutions in PBS for radiochemical studies examining lysozyme or lactoferrin adsorption and conformation onto various contact lens materials. Similar to proteins, there are several papers using single lipid in vitro systems, including Carney and colleagues work in 2008, where they examined kinetic uptake of lipid onto various contact lens materials using fluorescently labeled cholesterol and phosphatidylethanolamine solutions independently [28]. Most recently, Pucker et al. [31] published a similar paper examining the uptake of cholesterol oleate and phosphatidylcholine separately in an undisclosed buffer

solution. In most of these publications, a PBS solution with a single lipid or protein is used; however in many of the papers there is no information about the specific composition or concentrations of the PBS itself. Since there is no standardized composition of PBS, many of these papers are lacking important information regarding the ATS used.

There are several experimental papers where moderately complex in vitro artificial tear solutions were used. These solutions are mixtures of proteins or lipids dissolved into a saline base. Castillo et al. [51] and Bohnert et al. [52] both used an ATS which contained a mixture of several proteins dissolved into a saline solution to examine protein adsorption and conformation onto contact lens materials. Ho and Hlady examined lipid deposition using a mixture of several lipids dissolved into a more complex mixture of salts [53]. In each of these three examples, lipids and protein components were
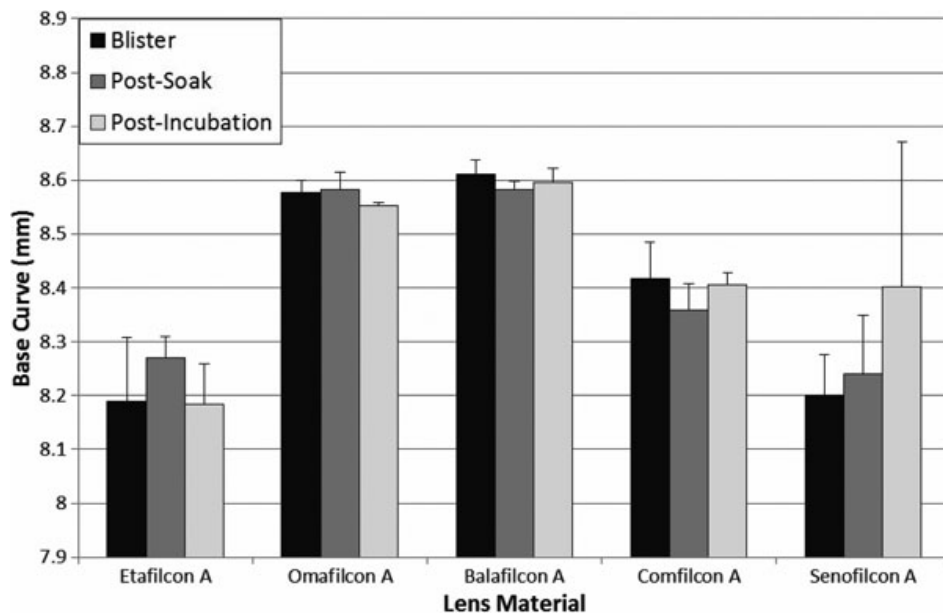
Figure 4. Average contact lens base curve of all studied materials as measured directly from the blister pack, after a saline soak, and following 14 days incubation in ATS.
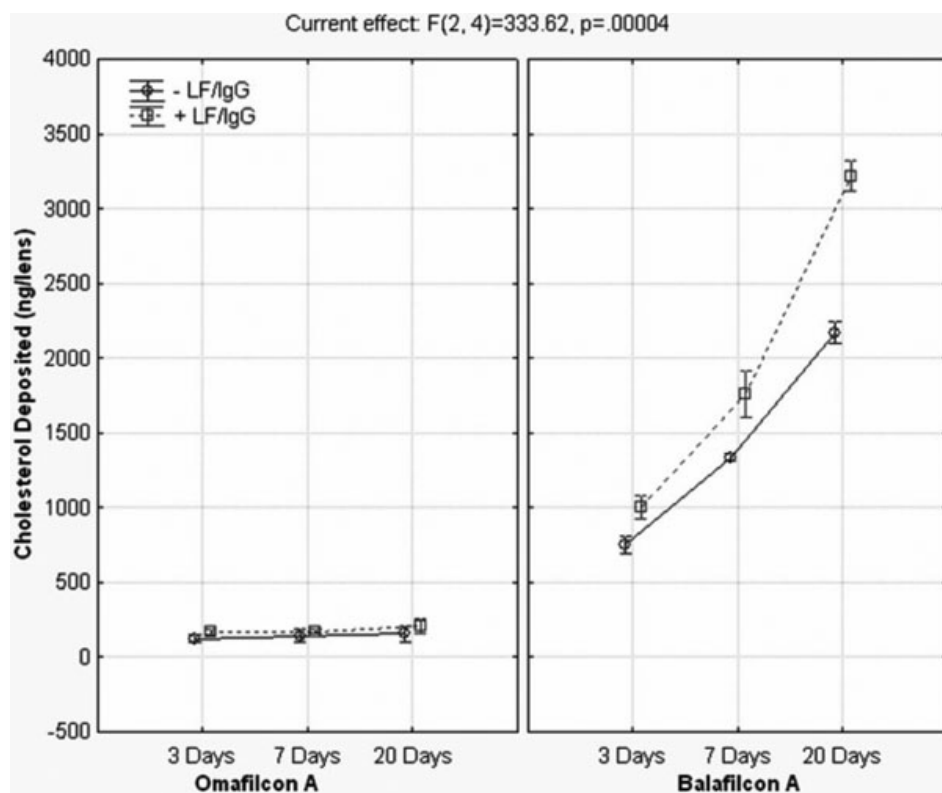

Figure 5. Cholesterol deposition with and without lactoferrin and immunoglobulin G for omafilcon A and balafilcon A. -LF/IgG=no lactoferrin and immunoglobulin G in the ATS. +LF/IgG=lactoferrin and immunoglobulin G were present in the ATS.

not mixed together within the ATS and there was no incorporation of mucin.

Recent work from our laboratory [33] and past work from Bontempo and Rapp [23,54] have found a dramatic difference in the amount of lipids and proteins deposited onto conventional and silicone hydrogel contact lens materials from an ATS of different complexities. Single component systems, moderately complex systems (no mixing of lipids and proteins together) and complex multiple lipid and protein systems have different deposition behaviors. Although simpler systems can be useful for particular experimental models, they are unsuitable to mimic human contact lens wear deposition and tear film interactions, due to their lack of complexity.
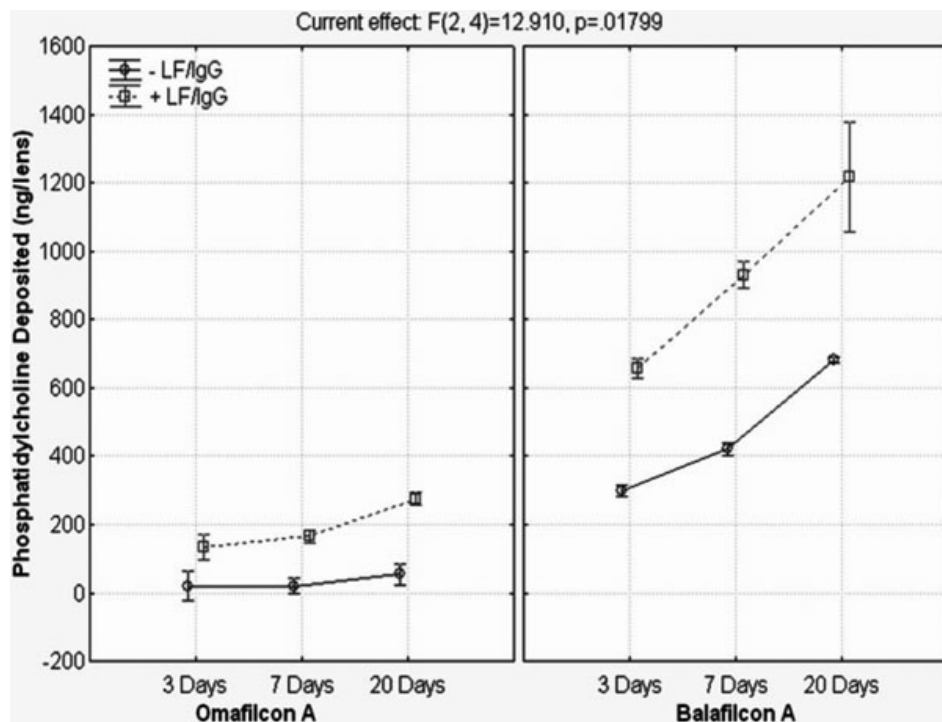
Figure 6. Phosphatidylcholine deposition with and without lactoferrin and immunoglobulin G for omafilcon A and balafilcon A. -LF/IgG=no lactoferrin and immunoglobulin G in the ATS. +LF/IgG=lactoferrin and immunoglobulin G were present in the ATS.

TABLE 7. CHOLESTEROL REPEATED MEASURES ANOVA RESULTS.

Variables	SS	DF	MSq	F	p
Time	5255742	2	2627871	2774	<0.0001
±LF/IgG	851579	1	851579	1739	0.0006
Lens	21480765	1	21480765	24367	<0.0001
Time * ±LF/IgG	266177	2	133089	196	0.0001
Time * Lens	4865540	2	2432770	1506	<0.0001
±LF/IgG * Lens	634230	1	634230	794	0.0013
Time * ±LF/IgG * Lens	254090	2	127045	334	<0.0001
Error	1523	4	381		

SS=sum of squares, DF=degrees of freedom, MSq=mean square, F=F statistic, p=probability.

Papers have been published introducing more complex in vitro artificial tear solutions. The first of these papers was by Mirejovsky et al. [32] in 1991, where lipids, proteins, mucin, and a variety of salts were all incorporated to form a complex tear solution. Mirejovsky's ATS contains a range of different proteins, lipids from different classification groups, and a non-physiologic biochemical buffer. It was more complex than many of the past solutions and the first to more accurately mimic human tear fluid with individualized concentrations for each component. Since the introduction of Mirejovsky's ATS, several other research groups have begun using a more complex ATS, including Prager and Quintana [25,44], Bontempo and Rapp [54,55], and Iwata et al. [56]. Prager and Quintana's [25,44] solution had the same protein portion as the Mirejovsky ATS and the lipid portion was similar, but instead of using a specialized blend of salts, Prager and Quintana used a Hank's Balanced Salt solution as their saline

base. The Bontempo and Rapp [54,55] ATS incorporated five tear film lipids, all incorporated in the same concentration, three tear film proteins, all incorporated in the same concentration, and a 0.9% saline base. The most recent solution of note is the one used by Iwata et al. [56] This solution used a mixture of four lipids, three proteins and a simplistic saline base [56].

It is common in in vitro ATS deposition models that the ATS is a homogenous composition with the proteins, lipids, and mucin mixed together throughout the solution. In other words, the solution is not in the layered biophysical structure as it is in the natural tear film. This is for several reasons; first, in-vial static aqueous incubations are not conducive to a lamellar structure, as the contact lens would not be exposed to all of the tear film components as they are in human contact lens wear. The blinking action, tear film mixing, tear film thinning and the eventual tear film breaking that occurs in

TABLE 8. PHOSPHATIDYLCHOLINE REPEATED MEASURES ANOVA RESULTS.

Variables	SS	DF	MSq	F	p
Time	476975	2	238488	737.92	<0.0001
±LF/IgG	889902	1	889902	1468.12	0.0007
Lens	3127668	1	3127668	2588.36	0.0004
Time * ±LF/IgG	30717	2	15358	21.80	0.0071
Time * Lens	221962	2	110981	424.64	<0.0001
±LF/IgG * Lens	212945	1	212945	440.59	0.0023
Time * ±LF/IgG * Lens	5516	2	2758	12.91	0.0180
Error	855	4	214		

SS=sum of squares, DF=degrees of freedom, MSq=mean square, F=F statistic, p=probability.

human contact lens wear exposes the lens to all layers and components of the tear film. The second reason for using a homogenous non-layered incubation solution is because this model is simpler to execute and has similar deposited masses of tear film components as ex vivo examined lenses [57,58]. Therefore, the biophysical arrangement of the ATS does not impact deposition to the same extent as the interactions that occur between the contact lens and tear film components. Therefore, even though the ATS structure is not necessarily identical to human tear film structure, it is still known to be a good model for deposition and tear film interaction research. Future models will incorporate a layered tear film analog and incorporate air exposure, mimicking the inter-blink period.

With the modified ATS solution introduced in this paper, we have tried to combine all of the necessary complexity by incorporating a variety of lipids, proteins, mucin, salts and also other prevalent tear film components such as physiologic buffers, glucose and urea, all within a stable system specially designed for in-vial incubations. All of these previously published variations on an ATS are indeed a great improvement over the more simplistic solutions based, primarily, on saline with a few added components. However, none of the papers described has shown the stability of the reported solutions, especially in terms of their pH and osmolality during the various contact lens incubations. Work in our laboratory during the development of this ATS clearly demonstrated the importance of reduced carbonates and increased phosphates in the complex saline solution, which was used as the base solution, to maintain pH and osmolality over time.

It is known that the pH and osmolality of a stable human tear film is 6.6–7.8 [37] and 305 mmol/kg [59], respectively, and that the surface tension of tears is 40–46 dynes/cm [60]. Therefore, we contend that the model ATS with the specific complex salt solution introduced in this paper is a suitable physical and chemical representation of the human tear film. The complex salt solution introduced in this paper was specially designed and extensively tested to confirm its stability. Many different combinations and concentrations of

salts and physiologic buffers were tested, however many of the test solutions did not remain stable in pH or osmolality over time. This was especially true for solutions with higher concentrations of carbonates, as carbonates tend to react with carbon dioxide in the air and therefore easily lead to a change in pH, especially if vials are not tightly sealed. This process was exacerbated when the ATS was incubated in plastic vials, instead of glass. All plastic vials tested, including low-density polyethylene, high-density polyethylene, super polyethylene, and Teflon-coated plastic vials all have intrinsic gas permeability and therefore the pH and osmolality of the ATS was constantly changing. Therefore, the final stable physiologically relevant complex salt solution modified by our laboratory contained only biologic buffers and a slightly reduced concentration of carbonates. This solution was specifically designed for closed in-vial incubations within borosilicate glass vials with screw caps with PTFE liners that are sealed with Parafilm®, so that ATS pH and osmolality remained stable throughout the incubation periods.

In all of these papers on in vitro model systems, only one of them has mentioned the lens parameter changes that occur upon incubation. Pucker et al. [31] admit that due to the incorporation of chloroform in their incubation solution, the lens materials do indeed swell. Most of the other systems do not have this chloroform addition and the extra solvents such as hexane that may be present from the use of a lipid stock are evaporated before lens incubation. None of the other papers has reported measuring the diameter, center thickness or base curve before incubation and following incubation in their ATS to know if the composition of the ATS is causing lens parameter changes beyond that which is considered allowable by the FDA. Contact lenses and their cleaning solutions are tightly regulated so that contact lens parameter changes do not occur. According to the ISO tolerance guidelines [61], contact lens materials are only allowed to change by ±0.20 mm in diameter and base curve, and by approximately ±18 µm in center thickness, depending on the specific lens material, during cleaning or contact lens wear. Swelling, stretching, shrinking and curvature changes could all induce power

changes, fitting changes, and comfort issues for the contact lens wearer. In an in vitro experiment, these changes can affect contact lens deposition and interactions with tear film components so that the contact lenses no longer react naturally to their surroundings.

In this experiment, the diameter, center thickness, and base curve of all contact lens materials were measured directly after removing them from the blister pack, following a soak in CSS, and after two weeks of incubation in the artificial tear solution described. The diameter, base curve and center thickness measurements all showed no clinically significant changes following incubation in the ATS and no parameter changes were found beyond what is allowed by 2006 ISO 18369–2 tolerance guidelines [61]. In a few instances, statistically significant changes in lens parameters were found between the blister pack measurements and following incubation in the ATS, however these changes were still well within ISO tolerances.

As the final step in the development of this ATS, the ATS was tested for its ability to deposit lipid onto both a conventional and silicone hydrogel contact lens material. Omafilcon A and balafilcon A lenses were chosen for the experiment, as previous research has shown that conventional hydrogels tend to deposit low amounts of lipid, whereas silicone hydrogel lenses, especially balafilcon A, are known to be more lipophilic and more likely to deposit lipid [28,56, 62]. Cholesterol and phosphatidylcholine were chosen for examination using a radiochemical experiment. Radiochemical experiments have been widely used in biomaterials research [63-68] including contact lens research, especially protein deposition research [18,24,25,29,30,69]. It has been shown to be a very sensitive, repeatable and reliable method of analysis and thus was chosen for this experiment. Cholesterol was selected as a representative non-polar lipid as it has been widely cited to be one of the most prevalent deposited lipids [57,62,70-72] and phosphatidylcholine was chosen as a polar lipid species, due to its presence in the tear film [11,73,74].

The results of the deposition experiment clearly showed that lipid deposition, especially on balafilcon A lenses, tend to continually deposit without a plateau effect throughout the 20 day incubation period, that the specific composition of the ATS will have a large impact on the deposition pattern for lipids, and that cholesterol tends to deposit more than phosphatidylcholine. Bontempo and Rapp [54] previously examined the impact that ATS composition has on lipid and protein deposition for conventional hydrogel lenses, but to date nothing has been published on silicone hydrogel lens materials.

This research supports the notion that the specific composition of an artificial tear solution will greatly impact the mass of tear film components that deposit. By simply removing two proteins from the ATS (lactoferrin and immunoglobulin G), lipid deposition significantly decreased. Data has established that the incubation volume (not shown) and lipid component concentrations [75] also affect the amount of lipid deposited. It is known that meibum, tear film, and deposited lipid concentrations and compositions can vary widely between individuals and that diet, medications, systematic diseases, and work environment can influence this deposition [6-9,76,77]. Therefore, it is very difficult to build an in vitro model to fully mimic all of the relationships and interactions that occur in human contact lens wear, so the first step is to begin unraveling the factors that may influence deposition.

When the deposited mass of lipids quantified in this experiment is compared with other in vitro and ex vivo data, it can be seen that differences do exist. In this experiment, after 7 and 20 days of incubation in the ATS solution (+ LF/ IgG), balafilcon A lenses deposited 1.80±0.06 and 3.22±0.04 µg of cholesterol and 0.93±0.02 and 1.22±0.07µg of phosphatidylcholine per lens, respectively. Omafilcon A lenses deposited 0.17±0.005 and 0.21±0.02 µg/lens of cholesterol after 7 and 20 days of incubation and similar masses of phosphatidylcholine at the same time points. Much of the other in vitro lipid work completed recently has quantified higher masses of cholesterol and phospholipids (either phosphatidylcholine or phosphatidylethanolamine) depositing on balafilcon A and on conventional hydrogel lens materials such as etafilcon A. In vitro work from Carney et al. [28], Iwata et al. [56], and Pucker et al. [31], all cited higher deposition values than the work presented here. However, these other in vitro studies had one or more of these main differences in their experimental design, which may account for increased deposition of lipids: the use of single lipid incubation solutions, higher concentrations of lipids in the ATS, altered incubation volumes, and replenishment of the ATS with fresh solution during incubation [28,31,56]. All of these factors may explain the higher deposition of cholesterol and phosphatidylcholine.

When the cholesterol deposition results found in this in vitro experiment are compared with recent ex vivo data it is found that results from the balafilcon A material are quite similar. Zhao et al. [57] quantified 4.1–8.2 µg/lens after 30 days of wear (depending on the cleaning solution used) and Saville et al. [78] found 3.9 µg/lens after 30 nights of wear. Saville [78] also examined phosphatidylcholine deposition and quantified 0.019 µg/lens following 30 nights of wear, which is lower than our quantified mass of 1.2 µg/lens on balafilcon A. Many of the recent in vitro and ex vivo studies were not completed with the same silicone hydrogel lens materials, did not include conventional hydrogel lens materials such as omafilcon A, and some of them examined different lipids than those quantified in this experiment.

It is clear that in vitro models do not always directly mimic what happens in vivo. Many times the masses

deposited are lower or higher than what is reported in human worn contact lenses. This may be due to the simplicity of the models being used, different ATS compositions and concentrations or an incomplete understanding of all of the interactions and influences that are present. The only way that in vitro models can be improved in their usefulness is to take a more in-depth look at the relationships that are occurring during human contact lens wear and then test and incorporate them into the in vitro models. It may transpire that the success of an in vitro model should not be measured according to the absolute mass deposited during human contact wear, as these values have large variations based on the populations tested, but should be examined to see if the hierarchy of deposition is consistent when comparing different lens materials and if the trends of wear are predictive of human wear. In the end, in vitro models must become more physiologically relevant so that their use can be validated and provide a basis for research and development of new and existing products.

As a first step in developing an in vitro model, the ATS developed in our laboratory has been shown to remain stable throughout incubation periods up to four weeks, the lens parameters show no significant changes following a two week incubation, and deposited lipids are in line with recent ex vivo data. The ATS solution introduced in this paper has the flexibility to be tailored to the individual needs of the specific in vitro experiment and can be used to mimic human worn lens interactions and depositions.

Conclusion—This paper has introduced a novel complex artificial tear solution specially designed for in-vial incubations. This solution maintains its own solution parameters and the parameters of the incubating contact lenses constant. This solution characterization is the first step in developing a new in vitro model for contact lens deposition and tear film interactions.

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