Modeling *In Vitro* Lipid Deposition on Silicone Hydrogel and Conventional Hydrogel Contact Lens Materials

by

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in fulfillment of the

thesis requirement for the degree of

Doctor of Philosophy

in

Vision Science

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

ABSTRACT

Purpose: To examine the variables that influence lipid deposition on conventional and silicone hydrogel contact lens materials and to build a physiologically relevant *in vitro* model of lipid deposition on contact lenses.

Methods: Lipid deposition on contact lens materials can lead to discomfort and vision difficulty for lens wearers. Using a variety of radiochemical experiments and two model lipids (cholesterol and phosphatidylcholine), a number of clinically significant parameters that may influence lipid deposition were examined.

- The optimization and characterization of a novel artificial tear solution (ATS) was examined (Chapter 3)
- Optimization of an extraction system to remove deposited cholesterol and phosphatidylcholine from various contact lens materials (Chapter 4)
- The influence of different tear film components on lipid deposition was researched (Chapter 5)
- The efficiency of hydrogen peroxide disinfecting solutions to remove deposited lipid from contact lenses was investigated (Chapter 6)
- The effect of intermittent air exposure on lipid deposition was examined through the use of a custom built "model blink cell" (Chapter 7)

Results: A novel complex ATS designed for in-vial incubations of contact lens materials was developed. This solution was stable and did not adversely affect the

physical parameters of the contact lenses incubated within it. An efficient extraction protocol for deposited cholesterol and phosphatidylcholine was optimized based on chloroform and methanol with the addition of water and acetic acid for phosphatidylcholine extraction. Overall, cholesterol and phosphatidylcholine deposition is cumulative over time and found to deposit in greater masses on silicone-containing hydrogels. Cholesterol and phosphatidylcholine deposition is influenced by the composition of the incubation medium and air exposure which occurs during the inter-blink period. Hydrogen peroxide disinfecting solutions were able to remove only marginal amounts of lipid from the contact lenses, with the surfactant containing solution removing more.

Conclusion: This thesis has provided hitherto unavailable information on the way in which lipid interacts with conventional and silicone hydrogel contact lens materials and the *in vitro* model built here can be utilized in various ways in the future to assess other aspects and variables of lipid and protein deposition on a variety of biomaterials.

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Thank you to the Centre for Contact Lens Research. You welcomed me with open arms into the CCLR as one of Lyndon's students. It is wonderful to be part of

such a tight knit family. The CCLR is a wonderful place to work and I hope to be part of it for many years to come.

DEDICATION

This thesis is dedicated to my incredible husband Nicholas and to my beautiful daughters Gabrielle and Natalie. You have never lost faith in me, especially during the difficult times, and you are always completely supportive of everything that I do or aspire to be. The end of this degree is the beginning of so much more in our life together and I am so glad to have all of you with me for the journey.

I would also like to dedicate this thesis to my parents. You have always believed that I have the capability to touch the stars and realize my dreams. That confidence and unending love has allowed me to strive for excellence in whatever I do.

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LIST OF SYMBOLS

θ	Angle
Å	Angstroms
cm	Centi-meters
Δ	Change
^{o}	Degrees
^{o}C	Degrees centigrade
D	Diopters
g	Grams
>	Greater than
hr(s)	Hour(s)
kDa	Kilo-daltons
kg	Kilo-grams
<	Less than
L	Liters
µg	Micro-grams
µL	Micro-liters
µm	Micro-meters
mg	Milli-grams
mL	Milli-liters
mm	Milli-meters
mmol	Milli-molar
min	Minutes

MPa	Megapascals
Ng	Nano-grams
nm	Nano-meters
Pa	Pascals
pI	Isoelectric point
%	Percent
±	Plus or minus
psi	Pounds per square inch
S	Seconds

LIST OF ABBREVIATIONS

2CM	(2:1) Chloroform: methanol
AA	Acuvue Advance (galyfilcon A)
AFM	Atomic Force Microscopy
ANOVA	Analysis of Variance
AO	AOSept
AOp	Air Optix Aqua (Lotrafilcon B)
APCI	Atmospheric Pressure Chemical Ionization
ARVO	Association of Research in Vision Science and Ophthalmology
ATR-FTIR	Attenuated Total Reflectance Fourier Transform Infrared Spectrometry
ATS	Artificial Tear Solution
AV2	Acuvue 2
AVA	Avaira (Enfilcon A)
Avg	Average
AvO	Acuvue Oasys (Senofilcon A)
AvT	Acuvue TruEye (Narafilcon A)
BAL	Balafilcon A (PureVision)
BIO	Biofinity (Comfilcon A)
C	Cholesterol

CC	ClearCare
CCLR	Centre for Contact Lens Research
CH	Conventional Hydrogels
CL	Contact Lens
Clr	Clariti (filcon II 3)
CM	(1:1) Chloroform: methanol
CMAW	(60:50:1:4) Chloroform: methanol: acetic acid: MilliQ water
CNRL	Control
CO	Cholesteryl Oleate
COM	Comfilcon A (Biofinity)
CPM	Counts per minute
CSS	Complex Salt Solution
CW	Continuous Wear
DF	Degrees of Freedom
DMA	N, N-dimethyl acrylamide
EGDMA	Ethyleneglycol dimethacrylate
ESI	Electrospray Ionization
ETA	Etafilcon A (Acuvue 2)
EW	Extended Wear
F	F statistic

FDA	Food and Drug Administration
FM0411M	2-ethyl [2-[(2-methylprop-2-enoyl)oxy]ethyl]carbamate
GC	Gas Chromatography
HEMA	2-hydroxyethyl methacrylate
HOB	(2RS)-2-hydroxybutyl 2-methylprop-2-enoate
HPLC	High-Performance Liquid Chromatography
IBM	Isobornyl methacrylate
IOVS	Investigative Ophthalmology and Visual Science
ISO	International Organization for Standardization
LASIK	Laser-Assisted In Situ Keratomileusis
LOB	Lotrafilcon B (Air Optix)
LSS	Lipid Stock Solution
LTS	Lipid Tear Solution
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)])
MA	Methacrylic acid
MALDI	Matrix Assisted Laser Desorption Ionization
MBC	Model Blink Cell

MGD	Meibomian Gland Dysfunction
MP	Mobile Phase
mPDMS	Monofunctional polydimethylsiloxane
MS	Mass Spectrometry
MSq	Mean Square
ND	Night & Day (Lotrafilcon A)
NMR	Nuclear Magnetic Resonance
NVA	*N*-vinyl aminobutyric acid
NVP	N-vinyl pyrrolidone
OA	Oleic Acid
OAME	Oleic Acid Methyl Ester
OMA	Omafilcon A (Proclear)
p	Probability
PBS	Phosphate buffered saline
PBVC	Poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate]
PC	Phosphatidylcholine
PhC	Phosphorylcholine
PHEMA	Poly hydroxyethyl methacrylate
PMMA	Polymethylmethacrylate
PO	Polymacon (Soflens 38)

PrC	Proclear (Omafilcon A)
PrO	PremiO (Asmofilcon A)
PrTS	Protein Tear Solution
PTFE	Polytetrafluoroethylene
PV	PureVision
PVA	Polyvinyl alcohol
PVP	Polyvinylpyrrolidone
RGP	Rigid Gas Permeable
SD	Standard Deviation
SEN	Senofilcon A (Acuvue OASYS)
Sag	Sagittal height
SEM	Scanning Electron Microscopy
SH	Silicone Hydrogel
SIMS	Secondary Ion Mass Spectrometry
SLTS	Single Lipid Tear Solution
SS	Sum of Squares
T	Triolein
TAIC	1,3,5-triprop-2-enyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione
TEM	Transmission Electron Microscopy
TI	(5:1) Toluene: isopropanol

TLC	Thin Layer Chromatography
TPVC	Tris-(trimethylsiloxysilyl) propylvinyl carbamate
TRIS	Trimethylsiloxy Siloxane
USAN	United States Adopted Names Council
UV	Ultraviolet
VMA	(N-Vinyl-N-methylacetamide)
XPS	X-ray Photoelectron Spectroscopy

This PhD thesis is an extension of my, Holly Lorentz's, MSc thesis. Therefore, this introduction and its contents is an updated and revised version of my MSc thesis introduction published as: Lorentz HI. Lipid deposition on hydrogel contact lenses. School of Optometry and Department of Chemistry, University of Waterloo. Master of Science Thesis, Waterloo, Ontario, 2006.

1 INTRODUCTION

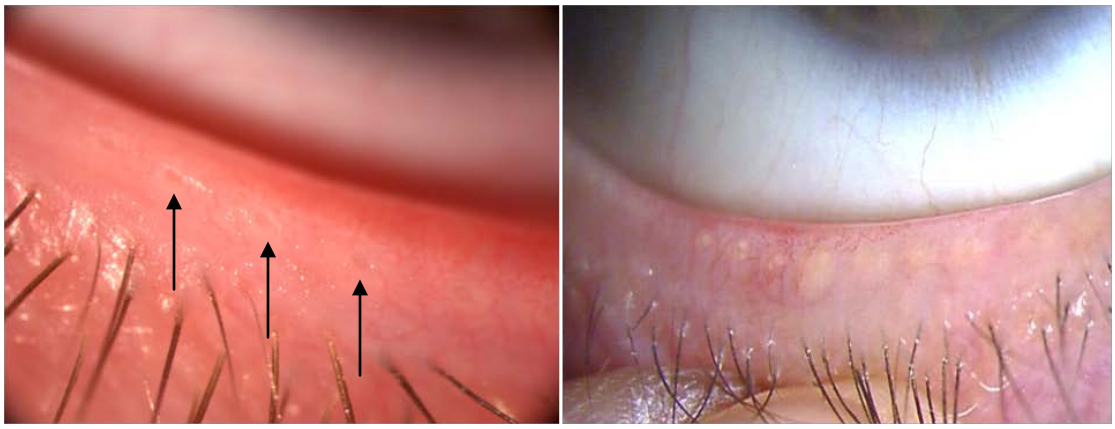
1.1 THE MEIBOMIAN GLANDS

1.1.1 STRUCTURE, FUNCTION AND PHYSIOLOGY

The meibomian glands, named after Heinrich Meibom who discovered them,[1] are sebaceous holocrine glands that produce and expel lipid through an orifice just anterior to the mucocutaneous junction. (Figure 1-1). The glands are found within the tarsal plate of the eyelid and are arranged in single parallel rows, with 20–30 individual glands in the lower lid and 25-40 glands in the upper lid (Figure 1-2).[2-9] The glands themselves range in size from 2-5.5mm in length, depending which lid they reside in, with the upper lid allowing for longer glands.[8] Individual glands are composed of 10-15 many small round acini, which are approximately 150-200 μm in diameter, that are attached to one central duct, which runs the entire length of the gland (Figure 1-3).[5, 7, 10] Each individual grape-like acinus produces both polar, non-polar and amphiphilic lipids.[2-5] It is the individual secretory cells called meibocytes that reside within the central part of the acini that produce the lipid components and then release them into the central duct.[10, 11] This process is called

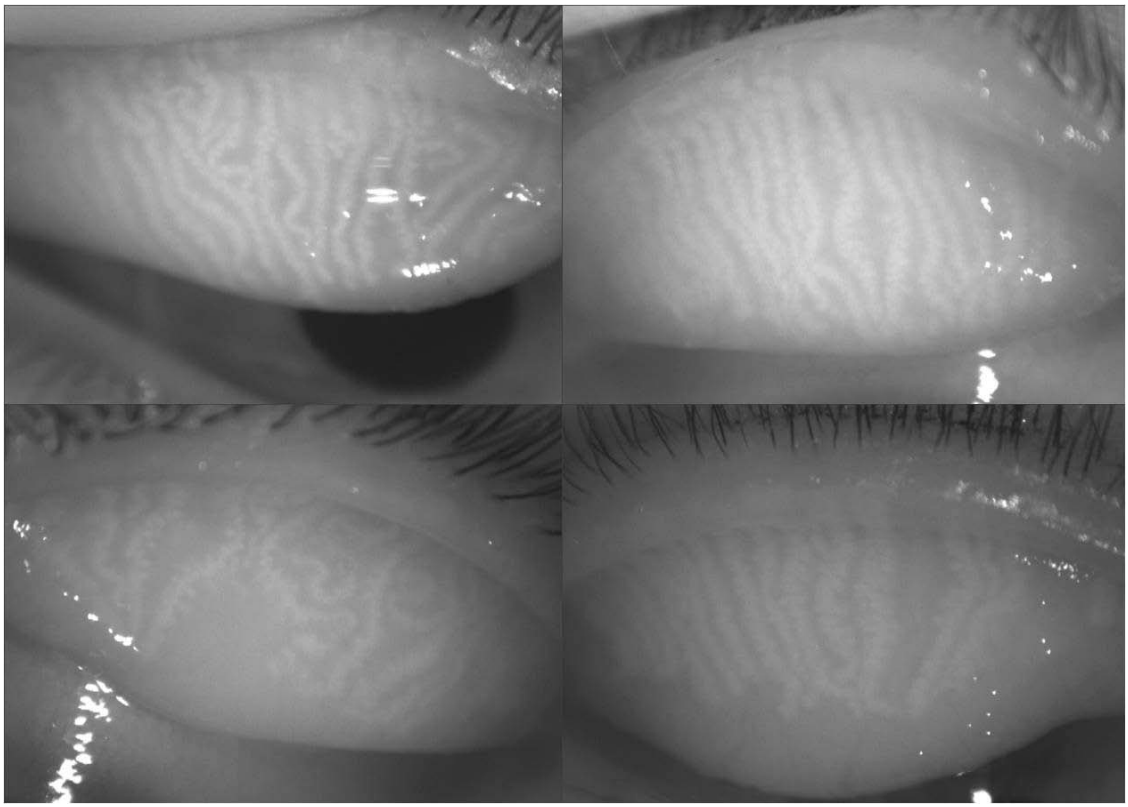
acinar cell degeneration.[5] The lipids secreted give protection by providing a hydrophobic barrier to reduce the chance of tear overflow onto the lid margin. The lipids also function to form a seal while the eye is closed during sleep and to reduce evaporation while the eye is open.[11] The lipids function as a lubricant while the eye blinks, and may provide a protective layer against bacterial infection.[5, 11]

FIGURE 1-1: TYPICAL APPEARANCE OF HEALTHY MEIBOMIAN GLAND ORIFICES WITHIN THE LOWER-LID.



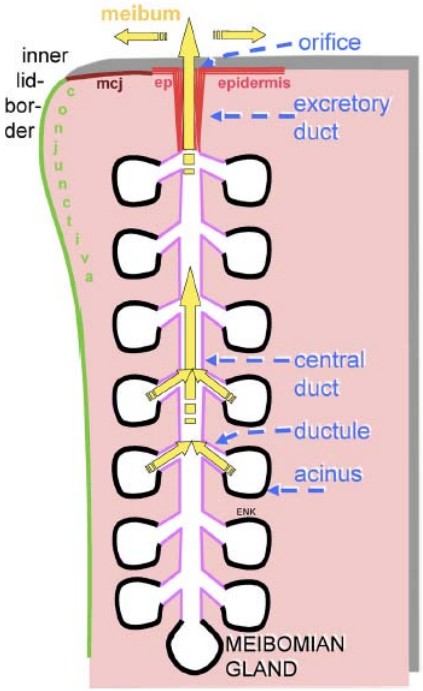
Thank you to Sruthi Srinivasan for the use of these pictures

FIGURE 1-2: REPRESENTATIVE IMAGES SHOWING MEIBOMIAN GLAND TORTUOSITY (TOP LEFT), PARTIAL AND TORTUOUS GLANDS (BOTTOM LEFT), HEALTHY GLANDS (TOP RIGHT), GLAND DROP OUT (BOTTOM RIGHT)



Thank you to Sruthi Srinivasan for the use of these pictures

FIGURE 1-3: STRUCTURE OF THE MEIBOMIAN GLAND



ep = epidermis, mcj= mucocutaneous junction

With kind permission from Springer Science+Business Media: Knop N, Knop E. [Meibomian glands. Part I: anatomy, embryology and histology of the Meibomian glands] Meibom-Drusen. Teil I: Anatomie, Embryologie und Histologie der Meibom-Drusen Ophthalmologe. 2009 Oct; 106(10):872-83, Figure 5.

Our current understandings of the mechanisms that regulate meibomian gland secretion are not well characterized. There have been several theories developed and tested over the past many years. Currently, it is thought that hormonal control through progesterone, androgens, and estrogen influences the acinar cells and thus meibomian gland secretion.[10, 12-17] Specifically, work from Sullivan *et al*. has revealed that androgens may stimulate meibomian gland secretion and influence the expressed lipid composition.[13, 18-23] In addition to this,

the thick innervations of the surrounding meibomian gland tissue and the presence of neurotransmitters indicate neuronal control of the meibomian glands.[10, 24-28]

By whatever means the glands are actually controlled and activated, the process to expel the lipid from the gland orifice remains the same. Two delivery methods exist, which are termed "active" and "passive". The active method occurs when the lipid is forced out of the meibomian gland orifice during a blink by compression of the tarsal plate.[29] The passive method is simply the continuous production of lipid which moves the lipid along the ducts and out the orifice. [10, 30] This build-up raises the intraductal pressure and causes the meibomian oil to exit the gland.[5, 10, 30-33] This accumulation is even greater during sleep when the eyelids are closed and there is a lack of blinking. This in turn, results in decreased secretion overnight and an accumulation of lipid in the duct until the eyes are opened and lipid can flow again, initially at an increased rate.[32, 33] The proposed normal rate of excretion is 6.7µl/hr per gland, or approximately 333µl/hr per eye.[5, 11] When the lid opens and closes, the meibomian oil moves along the entire ocular surface in a wave pattern.[5, 11]

1.1.2 THE MEIBUM COMPOSITION

Over the past 100 years, much work has been completed trying to categorize and quantify the various lipid families found within meibum.[11, 29, 34-54] Many different families of lipids have been identified in meibum, however absolute amounts or even ranges of each lipid family's presence in meibum are difficult to

ascertain due to variations in methodology, contamination, and inconsistencies in the literature. Despite this, many groups of lipids are consistently found in meibum including: free fatty acids, cholesterol and other sterols, sterol and cholesteryl esters, wax and wax esters, triacylglycerols, diacylglycerols, polar lipids and long chain alpha omegas.[11, 29, 34-54]

The cholesteryl esters and wax esters are thought to represent the bulk of lipid from meibomian gland secretions (≤60%) and are among the most non-polar lipids characterized.[55] The types of wax esters that have been identified in meibum range in their chain length, saturation, branching, fatty acid or alcohol base, and their isomeric forms.[36, 38-40, 52] The types of cholesteryl esters characterized in meibum have also been found to vary greatly and be of longer chain length than the wax esters.[35, 52]

Recently, there has been much discussion over the presence or absence of phospholipids in meibum.[5, 34, 38, 39, 49, 56-63] Phospholipids are surfactant amphiphilic molecules thought to be part of the polar lipid layer of the tear film, that function as a barrier between the surrounding layers.[55] It is now thought that phospholipids, if they are indeed present in meibum, are at a much lower concentration[38, 39, 56] and that previous reports of higher quantities of phospholipids may be due to the specific sampling, processing, contamination, instrumentation and analysis of those samples.[34, 39, 55, 64-67]

1.2 THE TEAR FILM

1.2.1 THE STRUCTURE OF THE TEAR FILM

The tear film is a complex multi-layered film that covers the anterior surface of the conjunctiva and cornea. It is thought to provide several unique roles and therefore its composition needs to be tightly regulated. The tear film is broadly described as having five main functions: it allows for a smooth optical surface by alleviating any small imperfections in the corneal epithelium; it protects the cornea from debris and foreign materials by forcing them away from the central cornea upon blinking; it provides oxygen and nutrition to the underlying corneal epithelium; it keeps the bulbar and palpebral conjunctiva moist and lubricated; and finally, the tear film contains various antibacterial and immunological agents to protect against ocular infection.[68]

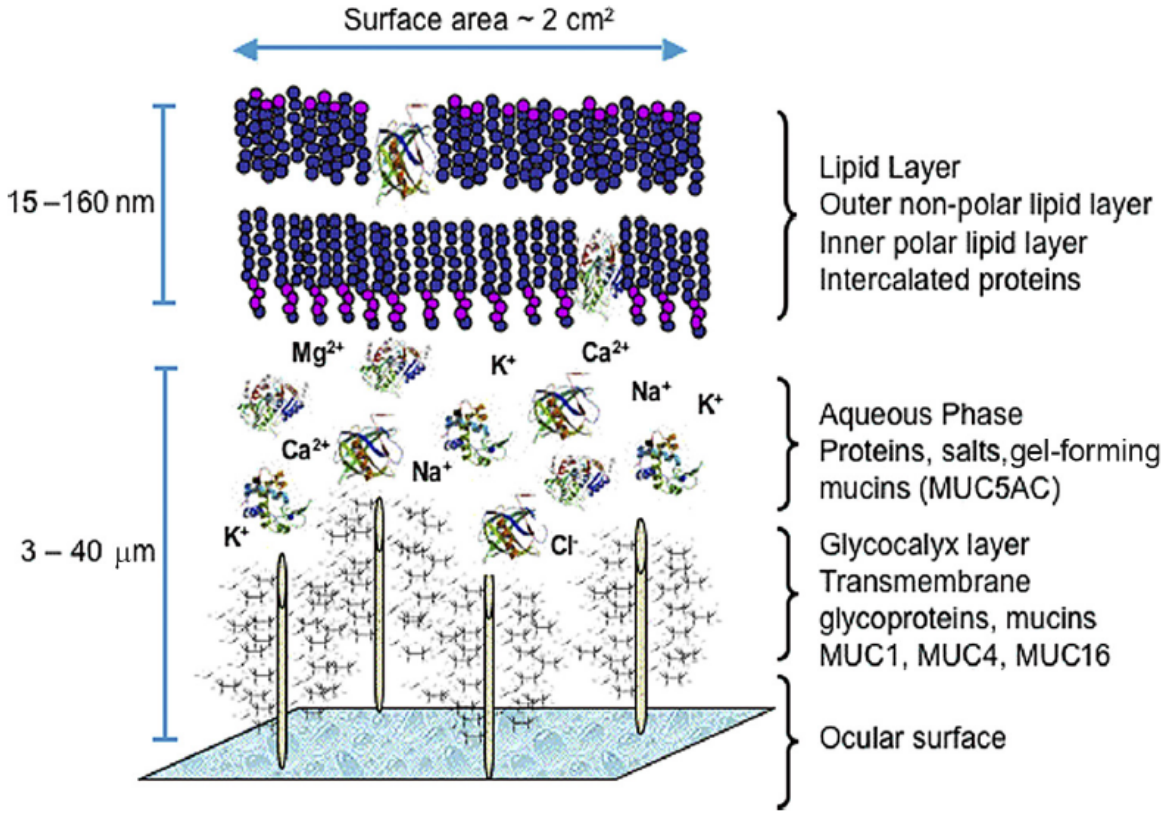
Previously, the tear film was believed to be a relatively simply structured film, consisting of 3-layers: an anterior lipid layer, intermediate aqueous layer and a mucin layer that covers the corneal epithelium.[9, 69, 70] Most of the original studies proposed that the mucin layer was the smallest layer, only representing 0.5% (0.02-0.05 μm) of the tear film, the middle aqueous layer represented the bulk of the tear film, possibly up to 98% (7 μm), and that the outermost lipid layer was 1-1.5% (0.1 μm) of the entire tear film thickness.[69-71] This simplistic tri-laminar model has been modified over the years to include a biphasic lipid layer and a more intricate "gel-like" mucin layer. In 1988, Tiffany suggested an update to the tear film model which incorporated these "layers". His model comprised of a non-polar

lipid layer at the air interface, polar lipid layer, absorbed mucoid, an aqueous layer rich in salts and proteins, and a mucin 'glycocalyx' layer covering of the corneal epithelial surface.[72-74]

Since 1988, the arrangement of the tear film has undergone some revision,[55, 67] and much research has been completed analyzing the thickness of the tear film and its layers,[75-81] the dynamics and organization of the layers,[82, 83] as well as the specific components of each layer.[84-88] The most current model of the tear film is described to include an outermost non-polar lipid layer, an inner polar lipid layer that contains intercalated proteins, an aqueous phase containing various proteins and gel-forming mucins, and finally a glycocalyx layer bordering on the corneal epithelium (Figure 1-4).[55, 67] Current research on the tear film approximates its thickness to be 3 μm, with decreasing thicknesses present in those individuals with dry eye.[75-81]

Just as the tear film as a whole has physiological and structural functions, each of the three broad layers of the tear film (lipid, aqueous and mucin layers), as described above, have their own unique and critical functions as well (Table 1-2).

FIGURE 1-4: UPDATED DIAGRAM OF THE TEAR FILM MODEL, AS PROPOSED BY THE MEIBOMIAN GLAND WORKSHOP[55]



Reprinted with permission from: Green-Church KB, Butovich I, Willcox M, Borchman D, Paulsen F, Barabino S, *et al*. The international workshop on meibomian gland dysfunction: report of the subcommittee on tear film lipids and lipid-protein interactions in health and disease. Invest Ophthalmol Vis Sci. 2011 Mar; 52(4):1979-93. © Association for Research in Vision and Ophthalmology 2011.

TABLE 1-1: TEAR FILM LAYERS AND FUNCTIONS

Layer	Origin	Components	Function of the Layer
Lipid Layer	Meibomian glands	Sterols, fatty acids, glycerides, esters, polar lipids	1. To prevent evaporation and to provide a barrier.[89] 2. To provide a smooth optical surface for the refraction light.[62, 90] 3. To act as a lubricant to aid the eyelid movement.[62] 4. To form a barrier against tear film contamination.[3] 5. To provide a surfactant layer between the non-polar lipid layer and the aqueous layer.[91] 6. To prevent tear overflow.[62]
Aqueous Layer	Lacrimal Glands	Proteins, lactoferrin, salts, glucose, urea, water	1. To create a favorable environment for the corneal epithelial cells, carry oxygen and nutrients to and from the cornea, and allow cell movement over the ocular surface.[92] 2. To wash away toxic substances and debris during blinking.[93] 3. To aid in antimicrobial activity through the tear film proteins (lipocalin, lactoferrin, lysozyme and IgA)[92-94] 4. Growth factors present in this tear film phase play a significant role in corneal physiology.[93]
Mucin Layer	Conjunctival goblet cells, Glands of Moll and Krasse	Glycoprotein	1. To act as a pathogen barrier using the ocular surface glycocalyx.[95] 2. Mucin is a lubricant, which allows the eyelid and conjunctiva to move smoothly over each another during blinking and ocular movements.[96] 3. Mucus threads protect the conjunctiva and cornea from injury by coating foreign bodies with a slippery mucus.[95] 4. Mucus aids in glycocalyx formation and wetting the ocular surface.[97] 5. Mucus helps overcome the hydrophobicity of the corneal surface.[96]

1.2.2 FORMATION OF THE LIPID LAYER OF THE TEAR FILM

Meibomian gland secretions have unique characteristics and properties (Table 1-3) that make it suitable to fulfill its functions as the lipid layer of the tear film, as previously described. For instance, the meibomian gland lipids have a low melting point, which allows for smooth delivery of the lipids through the ducts and orifices to reach the tear film.[5, 34, 56] Moreover, the integrity of the tear film, and therefore the overall function of the tear film, is dependent on the specific composition of lipids released from the meibomian glands.

TABLE 1-2: PHYSICAL PROPERTIES OF MEIBUM

Property	**Measurement**
Transition temperature (from a solid →liquid)	28-32^{o}C[56]
Viscosity	9.7-19.5 Pa·S[98]
Refractive Index	1.46-1.53 per the visible spectrum[99]
Volume of lipids at lid margin	300 μg[100]
Volume of lipids in tear film	9 μg[100]

The role that the meibomian gland lipids play in the tear film is complicated and not entirely understood. Lipids are found in several locations in the tear film, including the base of the tear film adjacent to the outermost corneal epithelium.[63] The corneal epithelium has microvilli protruding outwards and a unique covering drapes over the microvilli.[63] This covering is made up of a polar glycocalyx and

carbohydrates that separates the epithelium and the aqueous layer and anchors the aqueous-mucin layer.[73]

When lipid is excreted from the meibomian glands, in the form of meibum, it mixes with the tears found on the ocular surface. Once the lipid is incorporated within the tears, the composition of lipid is thought to change, by undergoing reactions with the other components of the tear film and the environment, thus creating new lipid types and/or concentrations. Comparative analysis between lipid profiles in tears and meibum have shown that the lipid composition in tears is more diverse and richer in lower molecular weight lipids and more polar phosphate containing esters than meibum.[34, 38, 39, 41, 56, 60, 66, 101] This increase in lower molecular weight lipids, such as wax esters, results in high chain order and therefore a higher phase transition temperature.[34, 41, 56, 91] All of these factors suggest that the lipoidal components in meibum and tears are indeed different.[55, 102] Despite these specific differences, the predominant lipid types found in the tear film are the same as meibum. These groups include: cholesteryl esters, wax esters, triglycerides, free fatty acids, monoacylglycerols, diacylglycerols, fatty sterols, and fatty alcohols.[49-51, 103-107] It has been estimated there are thousands of specific lipids which are contained in the tear film and meibum.[3, 108]

On the outermost surface of the tear film there is a lipid layer which contains two different lipid phases. The interior layer is believed to be a polar-surfactant phase and the outermost phase is a non-polar phase.[42, 109] Each phase of the lipid

layer of the tear film has unique characteristics and provides differing, yet crucial, functions.[63]

The polar lipid layer is thought to stabilize the non-polar layer by acting as an intermediate medium between the non-polar hydrophobic layer and the aqueous layer.[55] Due to the amphiphilic structure of the polar lipids, it is proposed that the molecules in this phase may orient themselves perpendicular to the tear film so that their hydrophobic tail is immersed in the nonpolar layer and their hydrophilic head is in contact with the aqueous layer.[55] It is thought that this layer may only be 7-20 molecules thick and be the smaller of the two lipid phases, with it only taking up 5-15% of the lipid phase.[35, 61, 67]

There are also a number of different types of polar lipids thought to be contained in this phase, including phospholipids, ceramides, sphingomyelin plus other polar lipids.[41, 42, 45, 73, 101, 110] Recently it has been found that (O-acyl)-ω-hydroxy fatty acids, a type of long chain amphiphilic lipid, has also been found to exist in meibum and reside in the polar lipid layer.[40]

The non-polar phase is thought to be the thicker lipid phase and contain primarily non-polar lipids, including free cholesterol, wax esters, cholesterol esters, triglycerides, and hydrocarbons.[73] It is thought that this layer helps to retard water evaporation.[42]

In addition to the lipids present in the outermost layers of the tear film, it is probable that surfactant proteins are a prominent presence within the lipid layers.[55,

[67] These proteins, mainly lysozyme, lipocalin, lactoferrin and mucin have been shown in *in vitro* experiments to associate with lipids in artificial films and therefore could readily integrate themselves in with the lipid layers of the tear film.[84, 86, 88, 91, 94, 111-113] This has brought about the updated structure of the tear film shown in Figure 1-4.

1.2.3 STABILITY OF THE TEAR FILM

For the purposes of this thesis the stability of the tear film and its component layers is defined as: the ability for the tear film to be resistant to change and to continue to perform its functions despite influences on its composition or structure.[114]

Research on the stability of the tear film in "healthy" individuals has been consistent throughout the years, showing that the tear film itself is considerably stable between blinks, even in the presence of particles and bubbles.[70, 115] When there is stability of the tear film between blinks, this is confirmation that the lipid layers are functioning to their full potential, with compression of the layer during blinking. During the down stage of a blink, if stability exists in the lipid layer, then the lipid layer will fold and the lipid will experience little mixing between layers.[116] The stability of the entire tear film is tied directly to the composition, integrity and surface tension of the lipids, proteins intercalated in the tear film lipid layer, mucin layers and the surface tension at the air interface.[84, 86, 88, 91, 117] Recent research has

also linked tear film instability to increased ocular surface temperature[118] and diurnal variations[119] in tear film quality. Furthermore, age, diet, medications, work atmosphere and the presence of contact lenses are just a few of the factors that can also alter the final composition of the tear film and possibly tear film stability.[68, 120-127]

In the past ten years, many studies have been completed to directly link the lipid layer of the tear film to the evaporation rate and tear film stability. Specifically, it is known that when the lipid layer is not structurally sound, thinned or absent there is a dramatic increase in evaporation and the film itself becomes unstable.[89, 128-130]

It has been shown that an unstable tear film can damage the ocular surface and cause symptoms of ocular discomfort or dry eye. The two main causes of instability of the tear film are a decrease in both the quantity and quality of tears. The causes of dry eye are directly linked to the types of dry eye: aqueous tear deficiency and evaporative dry eye. [131] Tear deficient dry eye occurs when the lacrimal gland does not function to produce an adequate tear flow or volume.[131] In evaporative dry eye, the lacrimal gland functions normally, but the tears are evaporating quickly from the ocular surface, which can be caused by numerous specific ocular disorders.[131-133] These disorders include meibomian gland dysfunction, lid/globe apposition, ocular surface disorders, blink disorders, lid aperture disorders, blepharitis, and tear film disorders.[131]

1.3 MEIBOMIAN GLAND DYSFUNCTION

Recently, a group of experts convened to classify and analyze our current knowledge of meibomian gland dysfunction (MGD) and all facets of the condition. All of the subcommittee reports are now available online through the journal: Investigative Ophthalmology and Visual Science (IOVS). The formal definition of MGD, as defined by the MGD workshop in IOVS is as follows:

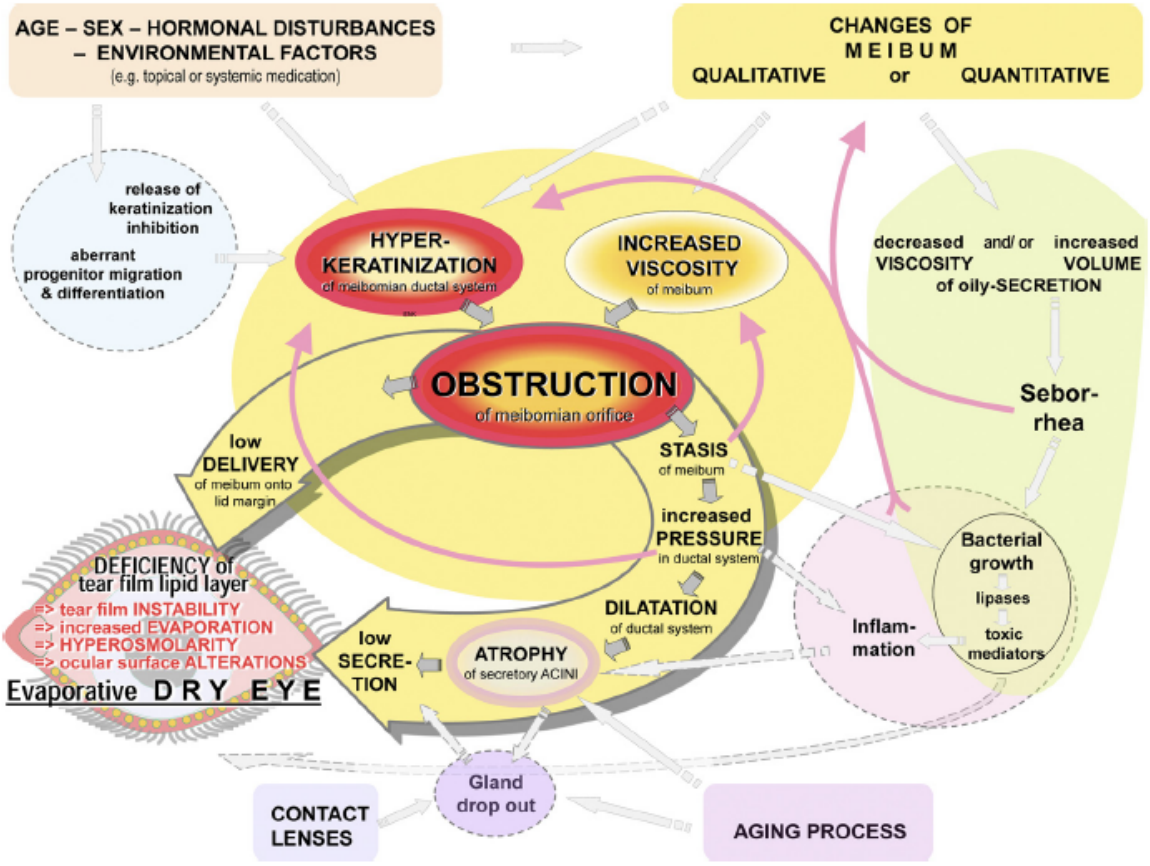
"Meibomian gland dysfunction (MGD) is a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. It may result in alteration of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease."[14, 134]

This definition attempts to convey the complexity of this condition, the possible physical changes to the glands, the resulting symptoms and ultimate outcomes that may occur. It is important to realize that this condition is multi-factorial and that MGD can manifest itself in many different ways. It has been found that MGD can be broken down into two broad categories: high delivery and low delivery of meibum.[14, 134] These two categories are further broken several more times to reflect possible changes to the glandular structure and the primary and secondary causes for each type of MGD.[14, 134]

Low delivery obstructive MGD, has been linked to many different triggers including: contact lens wear, gland drop out, hormonal disturbances, changes in meibum quantity and quality, just to name a few.[14, 134] A photograph of meibomian

gland dropout can be seen in Figure 1-2 in the bottom right corner. The pathophysiology of obstructive MGD can be found in Figure 1-5. This diagram demonstrates how changes in various life elements (i.e. age, hormones, medications) can induce a chain reaction, resulting in meibum changes, morphological gland changes, tear film instability and even evaporative dry eye.

FIGURE 1-5: OBSTRUCTIVE MEIBOMIAN GLAND DYSFUNCTION



With kind permission from Springer Science+Business Media: Knop E, Knop N. [Meibomian glands : part IV. Functional interactions in the pathogenesis of meibomian gland dysfunction (MGD)]. Meibom-Drusen : Teil IV: Funktionelle Interaktionen in der Pathogenese der Dysfunktion (MGD).Ophthalmologe. 2009 Nov;106(11):980-7. Figure 2.

1.4 LIPID AND CONTACT LENSES

The following section was published as follows:

1.4.1 TEAR FILM STABILITY AND CONTACT LENS WEAR

Contact lens wear causes changes in the structure of the tear film, particularly within the lipid layer.[68] Contact lenses lie within the aqueous layer of the tear film and therefore create a much thinner aqueous layer for the anterior lipid layer to cover. The presence of a contact lens also eliminates the smooth ocular surface over which the eyelid moves during a blink and also acts as a physical obstacle to destabilize the tear film. It is therefore much more difficult to reconstruct the tear film over this interface.[135] Due to these factors, there is only a thin lipid layer on the outer surface of a soft hydrogel lens and no lipid layer covering a rigid lens. [136] In order for a contact lens to remain "totally" biocompatible while being worn, the lens must form an overlying tear film that is structured similarly to that seen with no lens in place, which remains the ultimate goal in contact lens material research.

With little or no lipid layer present, the tear film easily becomes destabilized[136, 137] and the lipids come into direct contact with the lens material. While their interaction with rigid lenses may interfere with surface wetting, a

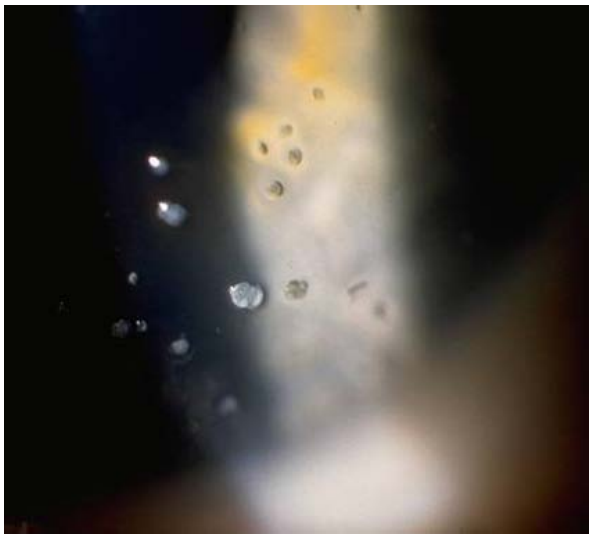
further problem exists with hydrogel lenses in that the materials are essentially semi-permeable membranes, which have an ability to both adsorb and absorb lipids, resulting in varying degrees of lipid deposition. While data on protein deposition on contact lens materials and its subsequent impact has been widely published,[138-146] there is a relative dearth of information on the interaction of lipid with contact lenses. The deposition of lipid is primarily driven by the hydrophobic lipids adhering to hydrophobic sites on the lens surface and the specific chemistry of the underlying lens material.

The following section was published as follows:

1.4.2 LIPID DEPOSITION ON CONVENTIONAL HYDROGEL CONTACT LENS MATERIALS

Some of the earliest observations of the interaction of lipid with hydrogel contact lenses was that of Hart and co-workers,[123, 147, 148] who examined lenses from both daily and overnight wearers, prior to the introduction of frequent replacement lenses. In one study,[147] Hart reported that 15% of hydrophilic extended wear contact lens wearers needed to replace their lenses due to obvious deposition, with the rate of deposition ranging from a few weeks to a few months and was highly subject-dependent. The deposition pattern commonly seen was a central deposition of "oily bumps", which Hart termed "jelly-bumps", "mulberry spots" or "lens calculi", as shown in Figure 1-6.[147] Hart demonstrated by various forms of microscopy and histochemical staining analysis that lipid was present in all deposits and was the prime component, with the principal lipid type being cholesteryl esters.[147] Scanning and scanning transmission electron microscopy found small amounts of calcium within the deposits, at much lower levels than the lipid. This was an important finding, as calcium was previously considered to be a major component of these nodular deposits,[149-155] which often are white in appearance. Hart also found that lipid deposits formed in an *in vitro* model were morphologically and histochemically similar to those formed *in vivo*.

FIGURE 1-6: LENS CALCULI (JELLY-BUMP) DEPOSITED UPON A HIGH WATER CONTENT SOFT CONTACT LENS AFTER 18 MONTHS OF WEAR.

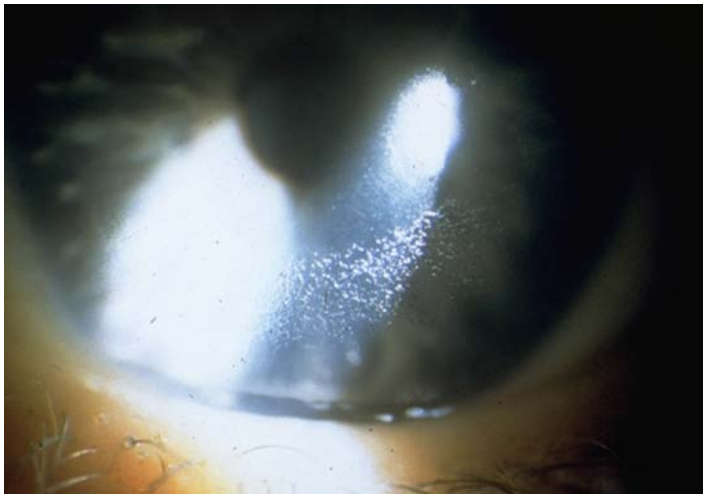


Reproduced with permission from: Lorentz H, Jones L. Lipid deposition on hydrogel contact lenses: how history can help us today. Optom Vis Sci 2007;84:286-95. ©The American Academy of Optometry 2007.

In a later study, [148] Hart determined that the jelly bump deposits had a fairly consistent composition of long and intermediate sized cholesteryl esters, triglycerides, and waxy esters. This composition is similar to the composition of lipids found in meibomian gland secretions. It was also found that individuals with higher deposition rates may have a lipid-rich tear film and a decreased tear flow, potentially resulting in "greasy" deposits on the front surface of their lenses (Figure 1-7). These lipids are insoluble in aqueous mediums and therefore showed some resistance to cleaning products.[148] Hart also examined lifestyle choices and their effect on lipid deposition of contact lenses.[123] Individuals who consumed larger amounts of alcohol, protein and fat exhibited increased lipid deposition on their

lenses. Patients with diabetes who were medicated with diuretics, anticholinergic or sympathomimetic drugs were found to have lower potassium levels in the tear film and this correlated with increased lipid deposition.[123] This was one of the first times that attention was drawn to the marked intersubject variability in lipid deposition patterns. Hart proposed that the reason such nodular deposits occurred were due to localised spots of drying, resulting in hydrophobic areas that attracted lipids, which then soaked into the lens material.[147] This area then acted as a larger non-wetting area, which acted as a nidus for more lipid deposition. This continuous cycle of dewetting and lipid deposition resulted in a lipid-based nodule forming.

FIGURE 1-7: HEAVY LIPID FILM DEPOSITED ON A LOW WATER CONTENT SOFT CONTACT LENS IN A PATIENT WITH MEIBOMIAN GLAND DYSFUNCTION.



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The work by Hart and colleagues in the US was closely mirrored by that of Bowers and Tighe in the UK. They focused on analyzing the gross morphology, chemical composition, and arrangement of "white spot deposits" that form on different contact lens materials.[156, 157] In their first experiment, they examined the occurrence, location, and gross morphology of elevated white spot deposition which formed on contact lenses taken from a controlled contact lens trial and randomly from a clinical setting.[157] Deposits were analyzed using several microscopy techniques including phase contrast, light, dark-field, and scanning electron microscopy (SEM). Additionally, stereo-microscopy was used to examine deposit occurrence. It was determined that there are three interactive sub-layers to the morphology of an elevated white spot deposit and that differences in lens material and wearing protocol do not affect this morphology. In contrast, the rate of deposition was markedly influenced by the lens materials and patient variability.[157] In their second experiment, Bowers and Tighe continued their previous white spot deposit analysis by examining their chemical composition and geological arrangement.[156] The deposits were found to have a well-formed tri-layer structure of lipid, where the primary or basal layer was composed of unsaturated lipids, while the secondary and tertiary layers were predominantly cholesterol and their esters.[156] Other tear components, like proteins, were present in the deposits, but were not found to play a role in the morphology of the deposits. The wearing schedule, lens material chemistry and individual differences in tear film structure did not influence the composition or location of these deposits.[156] They concluded from these results that it was the primary layer of unsaturated lipids that altered the

biological surface of hydrogel lens materials and thus cause decreased biocompatibility with the surrounding ocular environment.[156]

Throughout the 1990's, Franklin, Tighe, and colleagues set out to further their exploration into lipid deposition on contact lenses. They published a series of papers examining the influence that calcium, lens materials, and surfactant cleaners has on lipid deposition.[158-161] In their first paper, Bowers, Franklin and Tighe examined the formation of white surface films and the importance of the role of calcium.[161] Various contact lenses were collected, from a controlled clinical study and other clinical settings. During the controlled clinical study, the care solutions used were modified to increase the calcium concentration in the lens material in order to see the influence calcium has on deposit formation. They determined that these white surface films were morphologically different from elevated white spots, as these films have a heterogeneous structure where the lipid components are easily separated from the calcium portion. The lipid components were mainly cholesterol and cholesterol esters. The lens materials that were subjected to artificially raised calcium levels did not exhibit increased formation of elevated white spots. These results suggest that calcium may only have a secondary role in stabilizing lipids that have already been immobilized.[161] Franklin and Tighe next examined lipid and protein deposition on human worn lenses after 1 week and studied the effect of surfactant cleaning on these deposits. [160] Lipid and protein deposition was assessed using fluorescence spectroscopy at their respective wavelengths of emission and optimal excitation. This technique revealed that lipid deposition was largely

influenced by an individual's life-style, tear film composition and surrounding environment, whereas protein deposition was driven by the composition, charge and water content of the contact lens material. Individual tear film chemistry also influenced the effectiveness of surfactant cleaners on lipid deposition, making them only moderately helpful, especially within the first week of lens wear.[160] Other studies have indicated that some surfactant cleaners are more efficient at removing lipid and protein deposits than others, and that these cleaners are important in reducing reactive lipids that can accumulate further along in the deposition process.[159] Franklin and colleagues also examined the deposition of lipids onto a contact lens surface and the subsequent penetration into the lens matrix.[159] This experiment demonstrated that there is a dramatic range of lipid types that deposit on lenses, from polar to non-polar species and that this deposition is highly patient dependent. Surfactant cleaners are relatively helpful in minimizing lipid deposition and autooxidation of the lipids, but this is only temporary, as the lipid layer of the tear film is being constantly replenished.[159] One further study around this time period by Tighe and his group examined the different types of cleaners available on the market and their efficiency at removing *in vitro* doped lipid from the surface of a contact lens.[158] Soft contact lens surfactant cleaning solutions were compared with traditional chlorine-based and peroxide-based disinfectant systems. Surfactant cleaning solutions were found to vary widely in their ability to remove lipid from lens surfaces and disinfectant systems were found to remove virtually no lipid.[158]

In the early 1990's, Mirejovsky reported on an *in vitro* artificial tear solution that contained proteins, mucin and lipids.[162] This was a significant advance over previous doping solutions, which were almost exclusively based on proteins dissolved in buffer. The updated artificial tear solution better represented the range of components found within the tear film and therefore improved the usefulness of *in vitro* doping studies. Her work looked at both *in vitro* doped lenses and also investigated the ability of two histochemical stains (Nile Red and Oil-Red-O) to stain lipids. Mirejovsky showed that the Nile Red stain was far superior at detecting lipids and that the *in vitro* model solution produced a lipid deposition pattern that was similar to that obtained from human worn lenses. She also demonstrated that lipids could deposit onto hydrogel lenses either in isolation or bound to tear film proteins.[162]

Some of the most widely cited data on the interaction of lipids with hydrogel lenses was that undertaken during the early to mid 1990's by Rapp and colleagues, who completed a series of experiments examining lipid deposits on a wide variety of contact lens types.[144, 146, 163-165] In Rapp's first experiment, patient worn soft contact lenses were examined for lipid deposition and analyzed for various lipid types using thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) and gas liquid chromatography (GLC). Rapp showed that wax esters, fatty sterols, fatty alcohols, free fatty acids, and diglycerides were all detectable on hydrophilic lenses, whereas cholesterol, cholesteryl esters and triglycerides were not detectable. He concluded that the more polar lipids will deposit preferentially on hydrophilic

lenses when compared to non-polar lipids and that not all available lipids present in the tear film appear to deposit on hydrogel lenses.[165] Subsequent studies revealed that all lipid types interact with contact lens materials, but that the interaction is driven by both the lipid type and the chemical composition of the lens material.[144, 146, 163, 164] Rapp's work with Bontempo [144, 146, 163] was crucial in indicating that, within conventional hydrogel materials, FDA group II lenses deposit the most lipid, and FDA group III deposit the least. They also reported that non-ionic materials deposited more lipid than ionic materials, and that high water lenses deposited more lipid than low water materials.[163] This data led to the development of the "pull/push" theory of lipid deposition, in which the "pull" represents the polymer lens material adhering the lipid and the "push" represents the water in the lens material driving the lipid into the matrix.[163, 166] Further research has been undertaken to find the differences between monomeric compositions within the same FDA group,[167] which show that FDA classification alone is insufficient to accurately describe the pattern of lipid deposition that can occur.

Rigid gas permeable (RGP) lenses were also examined by Rapp,[163] and this work indicated that these materials generally deposit more lipid than many soft lens materials, probably due to the hydrophobicity of the lens. RGP lenses contain low amounts of water, and therefore the high lipid adherence is tied to the individual characteristics of the polymer. For instance, silicone-based RGP lenses deposit more lipid than fluorine-containing RGP lenses because the silicone addition increases the hydrophobicity of the lens, but the fluorine addition decreases the hydrophobicity

and thus decreases lipid deposition.[163] Bontempo and Rapp also analyzed the interactions between proteins and lipid on the surface of hydrophilic and rigid gas-permeable contact lenses *in vitro*.[146, 164] They reported specific interactions that occur on a hydrophilic contact lens surface when lipids and proteins are present concurrently. When a group IV lens undergoes protein deposition, the surface of the lens becomes less hydrophilic and therefore attracts increased lipids. For group II lenses, the proteins compete with more polar lipid deposited on the lens surface and displace them.[146] When rigid gas permeable lenses were examined for lipid and protein interactions on the lens surface, different interactions were found. The surface of an RGP lens is hydrophobic and thus attracts more lipids than proteins. The polarity of some lipid molecules allow for binding with the matrix and attraction toward the aqueous. When lipids bind to the contact lens, the surface becomes less hydrophobic and this allows for subsequent protein deposition.[164] In their final experiment, Bontempo and Rapp continued their protein and lipid interaction research by studying these interactions on group I and group IV lenses *in vivo*.[144] They found that lysozyme was preferentially deposited on group IV lenses due to the available negative charges attracting the strongly positively charged protein. Group IV lenses showed deposition for both protein and lipids, but the specific deposition composition depended on the individual.[144]

Some of the more recent work on conventional hydrogel deposition with lipid was undertaken by Tighe and colleagues.[145, 167-171] In the first of these, an *in vivo* study was conducted to evaluate the deposition of protein and lipid on FDA group II

lenses worn for various lengths of time.[170] This was the first work to demonstrate that degree of deposition was influenced by frequency of replacement, with significantly increased deposition being noted for lenses worn for three months as opposed to one month. Overall lipid deposition increased with longer replacement schedules and 44% less lipid was detected for the shorter replacement time, with individual lipid deposition being shown to vary greatly.[170] In a subsequent study,[167] protein deposition was shown to be related to the degree of ionicity of the contact lens material, being greater in FDA group IV materials, whereas lipid deposition was strongly related to the monomeric composition, with increased lipid deposition being encountered in FDA group II materials, particularly those containing N-vinyl pyrrolidone (NVP). Group II lenses containing polyvinyl alcohol (PVA) exhibited much less lipid deposition. Lipid deposition was also found to be dependent on the individual.[167]

Tighe and co-workers also examined both the effects of lens material and individual subject differences in lens spoliation. [171] This controlled clinical study involved clinical and analytical techniques to analyze the deposition of tear film components on group II and IV lenses. Lipid analysis using fluorescence spectrophotofluorimetry determined that contact lenses containing NVP have the highest lipid deposition compared to all other lens materials and that lipid deposition is greatly affected by patient-to-patient variations.[171] In a further study,[145] the progressive deposition of lipids was examined over a one-month period in both group II and group IV lenses. Lipid deposition was found to be a

cumulative process that does not plateau in a similar manner to that found in protein deposition on FDA group IV lenses. Once again, significant differences in individual lipid deposition were observed.[145] A related study by Tighe, Maissa and colleagues[168] found corroborating evidence that increased lipid deposition was detected on contact lenses that contained NVP and that lipid deposition was found to slowly imbed itself into the polymer matrix.[168]

An overall review of the studies reported by Hart, Rapp, Tighe and colleagues provide us with highly relevant information concerning the deposition of lipid into and onto hydrogel lenses. Their work shows that lipid deposition is more prominent on relatively hydrophobic substrates such as FDA group II materials, particularly those containing NVP, that large inter-subject variations in lipid deposition commonly occur and that the deposition appears to be cumulative, with no plateau occurring. In addition, surfactant cleaning is required to adequately remove lipid and that cleaners vary in their ability to remove these lipid deposits.

1.4.3 SILICONE HYDROGEL CONTACT LENSES: DEVELOPMENT AND PROPERTIES

In the past 30 years, contact lens wear has increased from 10 million to over 100 million wearers.[172] The reason for this drastic increase is due to patient's desire for safe, convenient, long term vision correction options to spectacles. Some patients turn toward laser refractive surgery, in particular LASIK (Laser-Assisted In Situ Keratomileusis), but many would rather wear a safe and comfortable contact

lens. This has resulted in the contact lens industry developing a new range of contact lens materials based upon silicone, which are termed “silicone hydrogel” (SH) contact lenses.[173]

Silicone hydrogel contact lens materials utilise silicone groups, from silicone rubber, combined with conventional hydrogel monomers. The silicone addition to the lens significantly increases the material’s oxygen transmission, whereas the hydrogel component allows for fluid transport and lens movement.[174]

From a historical perspective, water-containing hydrogel materials intended for extended wear (EW) were initially developed in the late 1970’s. Those lenses were commercially unsuccessful due to their inability to supply adequate oxygen to the cornea. Chronic deficiencies in oxygen can lead to permanent corneal damage due to the development of a number of hypoxic complications, including epithelial microcysts, epithelial thinning, loss of hemidesmosomes, changes in epithelial cell size and slower cell development.[175] During the same time frame, silicone-based silicone-elastomeric materials were introduced as contact lenses, but were only used for special therapeutic cases or as lenses for paediatric aphakia following cataract surgery.[176] These types of lenses had increased oxygen transmission compared with conventional hydrogel materials, which was highly beneficial to the cornea, but it was found that such lens materials rapidly deposited lipid from the tears and bound to the cornea during overnight wear, which severely limited their success.[177, 178]

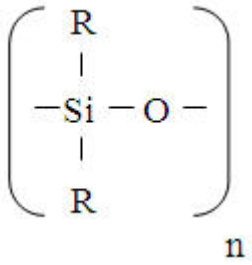
Polymers are based on the ability of atoms to bond together to form a long complex stable structure. Carbon's ability to bond with other carbon atoms, as well as various other atoms, including oxygen, hydrogen, nitrogen, and chlorine is the basis for polymer structure and function. Silicon is placed directly below carbon on the periodic table of elements and therefore behaves very similarly in its ability to bond with oxygen and hydrogen. Silicone-based polymers, siloxane polymers or silicones are ideal for contact lenses, as silicon-oxygen bonds are longer, flatter, and require less energy to rotate than carbon-oxygen or carbon-carbon bonds, thus allowing a contact lens based on silicone to be more flexible and less affected by temperature, but they are very hydrophobic. [179-181]

The contact lens industry has strived for decades to develop materials with the comfort and clinical performance of hydrogel lenses, and the oxygen transmission performance of silicone-elastomers. Through considerable financial investment, the release of such a group of materials – termed "silicone hydrogels" (SH) – became a reality in 1999.[179] Currently there are eleven silicone hydrogel contact lenses available. All lens materials are unique in polymer structure, surface treatment, modulus, oxygen transmissibility, patient fit, comfort, modality and deposition. The unique characteristics of each lens are outlined in Table 1-5.

The increased oxygen transport property of siloxane-based lens materials relates to the fact that oxygen is far more soluble in silicone rubber than it is in water or in polymethylmethacrylate (PMMA) contact lenses. This is because of the silicon-oxygen and silicon-carbon bonds that help make up the basis of silicone

rubber.[179] The siloxane groups incorporated into these contact lenses have the molecular structure displayed in Figure 1-8. [174] In conventional soft contact lens materials, oxygen dissolves in the water phase and is transported via the water components. In such materials, increased oxygen transmission is obtained by increasing the water content. However, the oxygen transport characteristics of water are significantly inferior to those seen in silicone.[174]

FIGURE 1-8: THE SILOXANE GROUP ARRANGEMENT WHEN FOUND INCORPORATED INTO A CONTACT LENS.



Si = the silicon, O= oxygen, R = the linking groups.[182]

TABLE 1-3: MATERIAL SPECIFICS OF THE ELEVEN COMMERCIALLY AVAILABLE SILICONE HYDROGEL CONTACT LENSES

Trade name	USAN	FDA category	Manufacturer	Modulus (MPa)	Centre thickness (μm)	Water content (%)	Oxygen transmissibility (DK/t)	Principal monomers
ACUVUE® ADVANCE®	galyfilcon A	I	Johnson & Johnson	0.43	70	47	86	mPDMS, DMA, HEMA, EGDMA siloxane macromer, PVP
ACUVUE® OASYS™	senofilcon A	I	Johnson & Johnson	0.72	70	38	147	mPDMS, DMA, HEMA, TEGDMA siloxane macromer, PVP
Focus® NIGHT & DAY®	lotrafilcon A	I	CIBA Vision	1.50	80	24	175	DMA, TRIS, siloxane macromer
AIR OPTIX™ AQUA	lotrafilcon B	I	CIBA Vision	1.22	80	33	138	DMA, TRIS, siloxane macromer
PureVision®	balafilcon A	III	Bausch & Lomb	1.06	90	36	101	NVP, TPVC, NVA, PBVC
Biofinity®	comfilcon A	I	Cooper Vision	0.75	80	48	160	M3U, FM0411M, HOB, IBM, NVP, TAIC, VMA
AVAIRA®	enfilcon A	I	Cooper Vision	0.50	80	46	125	M3U, BHPEA, MMA, POE, TREGDMA, VMA
PremiO	asmofilcon A	I	Menicon	0.91	80	40	161	Silicone methacrylates, silicone acrylates, DMA, pyrrolidone derivative
ACUVUE® TruEye™ (Canada/UK)	narafilcon A	I	Johnson & Johnson	0.66	85	46	118	Hydroxy-functionalized mPDMS, DMA, HEMA, TEGDMA, PVP
ACUVUE® TruEye™ (US)	narafilcon B	I	Johnson & Johnson	0.71	85	48	65	Hydroxy-functionalized mPDMS, DMA, HEMA, TEGDMA, PVP
Clariti™	filcon II 3	II	Sauflon	0.50	70	58	86	Alkyl methacrylates, silicone acrylates, siloxane monomers, NVP

BHPEA (2-(4-benzoyl-3-hydroxyphenoxy)ethyl acrylate); 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)])); MA (methacrylic acid); MMA (methyl methacrylate); mPDMS (monofunctional polydimethylsiloxane); NVA *N*-vinyl aminobutyric acid); NVP (N-vinyl pyrrolidone); PBVC (poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate]); PC (phosphorylcholine); POE 2-(2-propenyloxy)ethanol); PVP (poly(vinylpyrrolidone)); TAIC (1,3,5-triprop-2-enyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione); TEGDMA (tetraethyleneglycol dimethacrylate); TPVC (tris-(trimethylsiloxysilyl) propylvinyl carbamate); TREGDMA (triethylene glycol dimethacrylate); TRIS (trimethylsiloxy silane); VMA N-Vinyl-N-methylacetamide.

The first generation silicone hydrogels contact lens materials, which entered the market over ten years ago, were CibaVision's Night & Day (lotrafilcon A) and Bausch & Lomb's PureVision (balafilcon A) lenses. The Night & Day (ND) lens and CibaVision's newer Air Optix (lotrafilcon B) (AOp) lenses have a biphasic, inter-penetrating network-like or two-channeled molecular structure, where the fluorosiloxane phase (silicone phase) facilitates the majority of oxygen transmission and storage and the hydrogel phase transmits water and a small amount of oxygen for lens movement.[183, 184] These two phases work together for smooth transportation of oxygen and water. The exact materials used for this lens are a fluoroether macromer co-polymerized with the monomers trimethylsiloxy siloxane (TRIS) and N,N-dimethyl acrylamide (DMA).[184] PureVision (PV) lenses are a homogenous combination of the silicone-containing monomer polydimethylsiloxane (a vinyl carbamate derivative of TRIS) co-polymerized with the hydrophobic hydrogen monomer N-vinyl pyrrolidone (NVP).[180, 184-186]

As mentioned above, initial attempts to use silicone within hydrogel lenses in the silicone elastomers released in the 1970's and early 1980's failed due to lipid deposition, increased lens binding to the cornea and decreased in-eye wettability of the lens, due to the exposure of hydrophobic silicone on the surface of the lens material.[174] To minimize this problem, a process to convert the hydrophobic surface to a more hydrophilic lens surface is required.[187] Other factors also need to be taken into account when developing a successful surface treatment. Ideally, the treatment needs to maintain a stable tear film layer, provide low bacterial adherence, minimise deposition of substances from the tears, and be non-irritating.[188]

The ND and PV lenses have different methods of creating this surface treatment. The ND lenses are permanently surfaced in a gas plasma reactive chamber to give the lens a thin, high refractive index, homogenous hydrophilic surface. In contrast, the PV lenses are also treated in a gas plasma reactive chamber, but this chamber alters the silicone to give the surface of the lens hydrophilic glassy islands to mask the underlying relatively hydrophobic material.[174] The process involved in the gas plasma reactive chamber includes many complex steps, including etching, ablation, oxidation, and polymerization. The steps are controlled by several factors, and the success of the coating depends heavily on controlling the specific parameters required.[182] Both the ND and PV lens surface treatments are a fundamental part of the lens and are not just surface modifications that can easily be removed during the cleaning and disinfection process.[189]

The second generation silicone hydrogels were the two lenses introduced by Johnson & Johnson: Acuvue Advance (galyfilcon A) and Acuvue OASYS (senofilcon A). These two lens materials are modified from the Tanaka monomer and have incorporated siloxy macromers, HEMA and DMA. One important difference between these lenses and the others described above is that Acuvue Advance (AA) and Acuvue OASYS (AvO) have a proprietary internal wetting agent (HydraClear™), which is based upon polyvinyl pyrrolidone (PVP) and provides increased lens wettability.[190] The other unique property of these two Johnson & Johnson contact lens materials is that they both have Class 1 UV blocking capabilities.[190-192]

The third generation silicone hydrogels, Biofinity (comfilcon A) and Avaira (enfilcon A), were released by CooperVision and are not based on the TRIS molecule, require no surface treatments or wetting agents, and contains a unique mix of polymers. These two materials, Biofinity (BIO) and Avaira (AVA), are unique materials as they defy some of the oxygen permeability and wettability relationships which were previously defined.[193]

The latest silicone hydrogels to entire the world market are the Johnson & Johnson lenses called Acuvue TruEye (narafilcon A and B), the Menicon lens PremiO (asmofilcon A), and the Sauflon material Clariti (filcon II 3). Acuvue TruEye (AvT) is the first daily disposable silicone hydrogel contact lens material. It has incorporated siloxy macromers, HEMA and DMA and has an improved formulation of the Hydraclear internal wetting agent.[194] TruEye also has the highest possible UV protection provided by the other two Johnson & Johnson lens materials.[195] The

Menicon PremiO (PrO) lens polymerizes hydrophilic monomers and siloxane components together using a patented system called MeniSilk.[196] The material then undergoes a surface modification using the Nanoglass technology which combines both the plasma oxidation and plasma coating process to provide a smooth surface with a low contact angle.[196] Lastly, the Sauflon Clariti (Clr) lens is considered to be inherently wettable with no surface modifications necessary and is composed of acrylates, siloxane monomers and NVP.[197]

1.4.4 LIPID DEPOSITION ON SILICONE HYDROGEL CONTACT LENS MATERIALS

Since the release of silicone hydrogels onto the market over 10 years ago, there has only been a handful of papers examining their *in vitro* and *ex vivo* lipid deposition. For this reason, each paper published will be discussed below.

The first studies conducted examining lipid deposition on silicone hydrogel lens materials were that of Jones and Senchyna,[198] who investigated the deposition of both protein and lipid on balafilcon A and lotrafilcon A. Their work clearly showed that protein deposition on silicone hydrogel materials was significantly less (< 15 μg/lens) than found on Group IV conventional hydrogels; however the protein was more denatured. In contrast, the more hydrophobic surfaces of the siloxane-based lenses resulted in substantially greater amounts of lipid deposition being seen (up to 600 μg/lens).[198]

Subsequent studies from other research laboratories have also found an increase in lipid deposition on silicone hydrogels, but not to the same extent that Jones and Senchyna originally reported. In 2006, Maziarz *et al*. analyzed non-polar lipids deposited on *ex vivo* worn silicone hydrogel contact lens materials and analyzed them using two different HPLC protocols.[199] Cholesterol, oleic acid, and oleic acid methyl ester were the three lipids examined for their deposition on balafilcon A, lotrafilcon A, and galyfilcon A contact lens materials worn on a daily wear and continuous wear basis (balafilcon A only).[199] The overall order of deposition was as follows: balafilcon > galyfilcon > lotrafilcon A, with cholesterol consistently depositing in the highest quantity out of the three lipids tested (~20 µg/lens). Oleic acid and its methyl ester only deposited occasionally and often the deposition was lower than the level of detection.[199]

The findings of greater lipid deposits on silicone hydrogels were also obtained during a clinical study conducted by Cheung *et al*. in Hong Kong in 2007.[200] Participants were fitted with galyfilcon A in one eye and etafilcon A in the contralateral eye and after two weeks of wear time, significant increases in grade 3 and 4 lipid deposits (assessed via visual recognition) on galyfilcon were identified. The lipid deposits either had the appearance of lens calculi or hazy films. Other than deposits, no differences were found in lens fitting, vision, staining, subjective comfort, or tear film thinning. No biochemical analysis was completed on these lens samples and after the final follow-up appointment only half of the participants preferred the silicone hydrogel.[200]

The effect of *in vitro* lipid deposition on the wettability of silicone hydrogel and conventional hydrogel contact lens materials were examined by Lorentz *et al*. in 2007.[201] This study involved the incubation of various contact lens materials in saline, and two different concentrations of lipid incubation solution for both 2 and 5 days. The lenses were then removed from their solution and advancing contact angles were assessed using the sessile drop technique. It was determined that initial lipid deposition may increase the wettability of certain contact lens materials, especially for surface treated silicone hydrogels and conventional hydrogels. No differences were found in the non-surface treated silicone hydrogels.[201]

In 2008, Carney *et al*. conducted a large *in vitro* study examining the kinetic uptake of fluorescently-tagged cholesterol and phosphatidylethanolamine, in a single lipid incubation solution, with a range of both silicone hydrogel and conventional hydrogel lens materials.[202] Their findings confirmed that there is an increase in lipid uptake on silicone hydrogel lenses, but they also found differences in deposition within silicone hydrogels, with lotrafilcon A and B depositing similar amounts of lipid to conventional materials. This publication was one of the first to document the kinetic uptake over a 20 day period, characterize differences in a large number of lens materials, and see differences in polar and non-polar lipid deposition. Overall, Carney found that balafilcon, senofilcon, and galyfilcon silicone hydrogels deposited the most lipid and that deposition peaked at just over 20 μg/lens for cholesterol and ~5.0 μg/lens of phosphatidyl-ethanolamine.[202]

In vitro lipid deposition studies on silicone hydrogels were also conducted by Iwata *et al*. in 2008.[203] The research conducted by this laboratory was aimed at optimizing a gas chromatography mass spectrometry (GC-MS) method to analyze wax, squalene, cholesterol and their esters on silicone hydrogels. Six different lenses were extracted with (70:30) chloroform: methanol following a 60 hour *in vitro* incubation in an artificial tear solution containing 3 or 30 μg/mL of each of the lipids of interest and a range of salts and proteins. The GC-MS method examined was found to be sensitive, reliable and was able to recover at least 80% of the lipid deposited, depending on the mass of lipid originally absorbed. Overall the order of deposition was similar to previous studies, finding that lotrafilcon A, B and etafilcon A are the lowest lipid depositors (1 μg/lens) and galyfilcon A and balafilcon A are the highest (~8 μg/lens). This paper was the first to examine, utilize and quantify squalene, a lipid secreted by the sebaceous glands, in addition to the usual cholesterols.

In the past few years, Willcox and co-workers from Australia have studied and published several papers detailing various aspects of lipid deposition.[204-206] The first paper, published in 2009,[205] quantified cholesterol and total protein, on *ex vivo* silicone hydrogel lenses cleaned with four different care regimes. Cholesterol was extracted from the various lens materials using (1:1) chloroform: methanol and analyzed using TLC and electron ionization MS. Overall, cholesterol deposition was highest on balafilcon A materials (4-8 μg/lens) and lowest on lotrafilcon B materials (0.1-0.5 μg/lens).[205] When the care regimes were examined, it was found that the

different cleaning solutions were more efficient at cleaning different contact lens materials. Opti-Free RepleniSH and AQuify were found to be the most efficient at removing lipid from most lens materials, and ClearCare was the least efficacious. This study was one of the first to outline the variation in cholesterol deposition with the use of different care regimes and lens materials.[205]

The second paper published by the Australian group examined human-worn balafilcon A and senofilcon A lens materials, their phospholipid and cholesterol deposition, and the differences when cleaned with three different care regimes.[204] The levels of contact lens deposition were then compared to the concentration of cholesterol and phospholipids in tears and both tear and contact lens deposition samples were analyzed using various MS techniques. In total, 23 different species of sphingomyelin and phosphatidylcholine were identified in tears and contact lens depositions.[204] The pattern of these polar lipids were similar, however differences did exist between the different lenses and care solutions used. Between the two lens materials tested, senofilcon A deposited significantly more lipid and cholesterol was deposited in the highest mass.[204]

The most recent publication from Willcox *et al*. explored the relationship between bacterial adhesion and cholesterol deposition on two silicone hydrogel contact lens materials, senofilcon A and balafilcon A.[206] It was found that balafilcon A lenses worn for one month deposited more cholesterol than senofilcon A worn for two weeks and that bacterial adhesion onto worn balafilcon A lenses was reduced compared to unworn lenses.[206] In the end, *Pseudomonas aeruginosa* and

Staphylococcus aureus adhesion was not influenced by the presence of cholesterol. This was the first paper to examine the interaction of lipid and bacterial adhesion.[206]

Mirroring some of the silicone hydrogel work from Australia, the Nichols group in the United States have also been examining both *ex vivo* and *in vitro* lipid deposition.[207, 208] Their first study focussed on quantifying cholesteryl esters and cholesterol from human worn and *in vitro* incubated lotrafilcon B and galyfilcon A lens materials.[208] For the *in vitro* portion, the two lens materials were incubated in a cholesteryl oleate incubation solution with a range of concentrations for three days and for the *ex vivo* portion, participants wore each lens material for about 7 days, at least 10 hours each day.[208] Following incubation or wear time, the lenses were extracted with (2:1) chloroform: methanol and the cholesterol content was assessed using an enzymatic cholesterol esterase assay. This study found that galyfilcon deposited significantly more cholesterol and its esters on both the *in vitro* and *ex vivo* lenses when compared with lotrafilcon B. The benefits of a two-step extraction method and individual differences in cholesterol deposition between individuals were also revealed.[208]

The second study conducted by the Nichols lab was published in 2010 and analysed *in vitro* lipid deposition on contact lens materials.[207] This study involved the *in vitro* incubation of eight silicone hydrogel materials and one conventional hydrogel lens material, in two different single lipid incubation solutions for 1 and 14 days. The two lipids examined were cholesteryl oleate and phosphatidylcholine and each was quantified individually by using their corresponding specific assays.

Overall, most of the contact lens materials deposited the same or a larger mass lipid after 14 days of incubation, with the phosphatidylcholine deposition ranging from 0.54 to 5.77 μg/lens throughout the entire experiment.[207] Similar trends of equal or greater masses depositing after 14 days of incubation were also seen with cholesteryl oleate, however a significant decrease in deposited cholesteryl oleate was found with etafilcon A after 14 days. Generally, cholesteryl oleate deposition ranged from 0.14 to 6.84 μg/lens.[207] This study showed the marked variability in deposited lipids among the different lens materials.

Also in 2010, the details of a clinical study was published where the authors had extracted and quantified total lipid, cholesterol and their esters, and phospholipid levels on three silicone hydrogel lenses: senofilcon A, galyfilcon A and asmofilcon A following two weeks of wear.[209] Total lipid, as quantified from the sulfo-phospho-vanillin reaction, was on average 32.9 to 42.1 μg/lens, cholesterol and cholesteryl ester deposition was 26.2 to 31.1 μg/lens, and phospholipids deposition ranged from 1.1 μg/lens (galyfilcon A) to 7.0 μg/lens (asmofilcon A).[209] No significant differences between lens materials for total lipid or cholesterols were found, however the differences in phospholipid deposition between lens materials were statistically significant.[209] In this study, the multipurpose solutions utilized by the participants were not listed or examined and differences in comfort were not explored.

Most recently, a paper published by Heynen *et al*. from the Centre for Contact Lens Research in Canada quantified the amount of non-polar lipids from senofilcon

A lenses that were either cleaned in Opti-Free RepleniSH or ClearCare following cross-over study.[210] After each 2 weeks of wear, all lenses were extracted and several non-polar lipids were quantified using normal phase HPLC. Out of the five lipids that were examined, two of them were below the limit of quantification (triolein and oleic acid), or not detected at all (oleic acid methyl ester). Cholesteryl oleate and cholesterol were found to be the most prevalent lipid deposited, with their combined mass on average being over 20 μg/lens.[210] Differences in the contact lens cleaning solutions utilized were also found, with lenses cleaned with Opti-Free RepleniSH depositing significantly less cholesteryl oleate and total lipid.[210]

Many of the papers discussed to this point have examined *in vitro* or *ex vivo* lipid deposition and the factors that may affect the deposition profile, such as lens material, care regime, or analysis technique. However, an exploratory paper examining the possible use of phospholipids as a wetting agent to aid in contact lens comfort was published in April 2011.[211] The authors examined the uptake and release of phosphatidylcholine on a CIBA Vision silicone hydrogel lens using radiochemical analysis and found that the uptake of phosphatidylcholine did not alter the lens wettability or optical clarity of the lens. They also found that the rate of phospholipid release was faster when soaked in an artificial tear solution, as opposed to just water.[211] This study introduces the concept that phospholipids, due to their structure, may be utilized to alter the surface of silicone hydrogel lenses to make them more wettable and ultimately more comfortable. More work is required to test and realize the full extent of this technique.

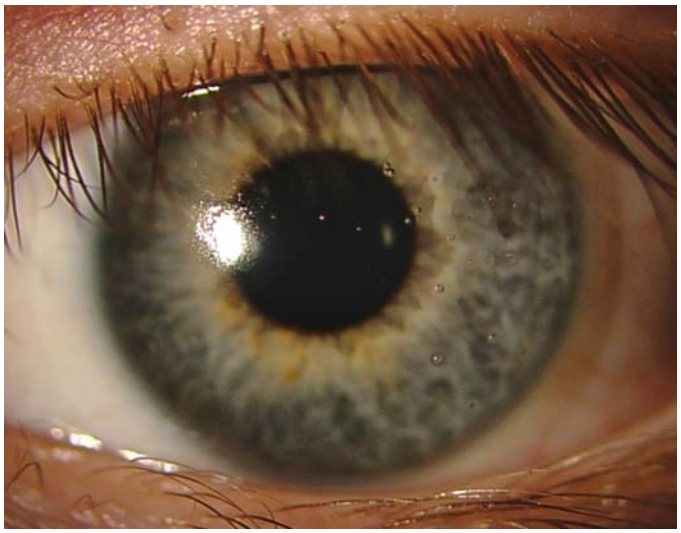
The following section was published as follows:

1.4.5 THE FUTURE OF CONTACT LENS LIPID DEPOSITION RESEARCH

Out of all the lipid deposition research conducted to date, what is unequivocal is that certain patients, when refitted from conventional HEMA-based materials into silicone hydrogels, exhibit clinically significant deposition on their silicone hydrogels that may not have been problematic with their HEMA-based materials (Figure 1-9).[200, 212] One study reported this to occur in approximately 15% of silicone hydrogel wearers who use their care products using a "no-rub" regime and do not rub and rinse their lenses at the of the day.[213] When patients were advised to rub their lenses prior to disinfection, this level of clinically relevant deposition reduced to a negligible amount. As described above, the major type of deposition on silicone hydrogels is lipids, with some denatured proteins, both of which require a physical rub to maximize their removal from the surface of lenses. Based upon the clinical data thus far, it would appear that silicone hydrogel wearers would benefit from being advised to use their care regimens with both a rub and rinse step being instigated prior to overnight soaking.[213] Other methods to help minimize such deposition[212] include replacing the lenses frequently, as lipids do progressively accumulate over time,[145, 170] using a dedicated surfactant cleaner if merely rubbing with a multipurpose system fails, and treating any co-existing blepharitis or

meibomian gland dysfunction in an attempt to produce a more stable, healthy lipid layer to the tear film.[214-218]

FIGURE 1-9: LIPID FILM AND LENS CALCULI ON A SILICONE HYDROGEL LENS AFTER 3 WEEKS OF WEAR. THE PATIENT HAD PREVIOUSLY WORN AN FDA GROUP IV LENS ON A FOUR-WEEKLY REPLACEMENT PERIOD WITH NO SUCH DEPOSITION BEING SEEN.



Reproduced with permission from: Lorentz H, Jones L. Lipid deposition on hydrogel contact lenses: how history can help us today. Optom Vis Sci 2007;84:286-95. ©The American Academy of Optometry 2007.

Ultimately, the degree of deposition remains irrelevant unless it results in symptoms or clinically significant signs, and the effects that lipid deposition has on either of these is not well documented. In fact, relatively few studies have directly linked deposition with alterations in symptoms and signs, and most of these have looked at deposition overall rather than the specific impact of lipid deposition alone. It is known that deposition of tear film components can reduce lens comfort,[170, 219-221] probably due to reduced lens wettability.[222] As deposition occurs on the lens

surface, the contact lens becomes progressively dewetted, resulting in poor wettability and subsequent sensations of dryness and discomfort. Poor vision is another negative affect of deposition.[223, 224] These symptoms can lead to discontinuation of lens wear[219, 221] and the more uncomfortable and irritating a contact lens becomes, the more likely the individual will remove the lens.[221] Whether these symptoms are due to lipid deposition, protein deposition or a combination of both remains to be confirmed.

On reviewing the literature to-date, much work remains to be undertaken to further our understanding of the processes involved in lipid deposition, particularly on silicone hydrogels. Many of the studies thus far have used *in vitro* methodologies, which fail to take into account lens surface drying between blinks, which will result in increased hydrophobicity and enhance deposition, and the constant replenishment of the lipid within the surrounding fluid. Such studies need to be complemented and confirmed by *ex vivo* studies to ensure that the results are comparable to that found clinically. Other topics that have been inadequately examined are the degree to which lipid penetrates into the matrix of lens materials, the influence of various care regimens, the interaction between lipids and other constituents of the tear film, the kinetics of lipid deposition and the arrangement of lipid types on the surface. These are all subjects that require much greater understanding if our ability to further the development of lens materials, particularly siloxane-based materials, is to progress. What is clear from this literature review is that there are many factors that can affect the deposition

process and dictate the ultimate amount of lipid on the contact lens, with material composition, replacement interval, care regimen and individual patient variability all playing significant roles. The future for lipid research on both the tear film and its relationship to contact lens deposition remains bright, but novel methodologies to examine the lipids involved in contact lens deposition and dry eye require significant intellectual input to unravel these complex interactions.

1.5 LIPID COLLECTION METHODS

To study the lipid from the tear film, meibomian glands or that which is deposited on contact lenses requires initial collection and then analysis of the lipid. When analyzing lipid on contact lenses, the lenses are exposed to either an *in vitro* artificial incubation solution[162, 163, 201, 203, 207, 225] or removed from the eye after a pre-designated period of time.[144, 165, 204, 205, 210] The lenses are then exposed to an extraction solvent, which is commonly based on methanol and chloroform in various ratios,[163, 203, 205, 207, 210] and the extract is then analysed by a number of various analytical methods, as described below.

There are different procedures that are commonly used to collect lipid from various areas in the eye. The methods used to obtain meibomian gland secretions and samples from tears will be briefly discussed in the following section of this thesis. However, despite the method of collection it has been found that proper storage and handling is imperative to ensure that the lipids remain free of

contamination and in their initial state before analysis. Lipids should be stored in an oxygen-free environment by drying the sample under nitrogen or argon in a plastic-free container and placing it dry and dark location at -80°C. [55, 226] For lipid, only glass, stainless steel and Teflon® are recommended storage containers. [55, 226]

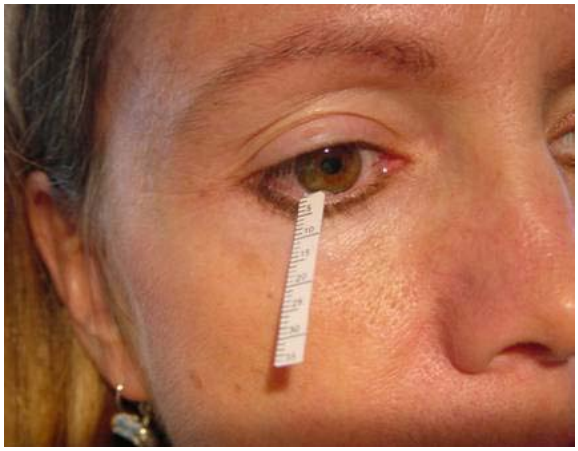
1.5.1 TEARS

There are two commonly used methods to collect tear film samples for the analysis of lipid content: Schirmer strips[41, 148] and microcapillary tubes. [91, 123, 227, 228] Schirmer strips are filter paper strips which are commonly used to help diagnose dry eye syndrome, but can also be used to collect tear film samples. Schirmer strips are positioned to contact only the bulbar conjunctiva of the eye and to absorb tear fluid (Figure 1-10).[148] This procedure is usually completed without an anaesthetic and is fairly uncomfortable for the participant. Following collection, the tear film lipids can then be extracted from the strips and analyzed. The main disadvantages of this technique is that the strips can become contaminated with cellular lipids during collection.[67]

The most popular method to collect lipid tear film samples involves using microcapillary tubes. These are tiny glass tubes that draw in fluid via capillary action when the tube is placed in contact with the tear film. The microcapillary tube is gently placed in the eye to collect tears from the lower tear pool that forms above the lower lid (Figure 1-11).[91] In some cases, experimenters purposely collect

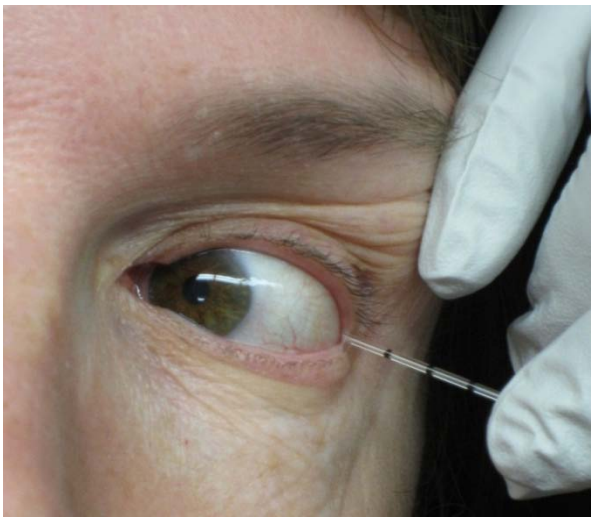
stimulated tears[91], but usually un-stimulated tears are preferred.[227] Once a tear film sample is collected in the microcapillary tube, the fluid is then removed, extracted and analyzed. The popularity of this method can be seen in the number of studies that have used this technique for the collection and analysis of lipids in the tear film.[91, 123, 227, 228]

FIGURE 1-10: TEAR FILM COLLECTION USING A SCHIRMER STRIP



Thank you to Sruthi Srinivasan for the use of this picture

FIGURE 1-11: TEAR FILM COLLECTION USING A GLASS MICROCAPILLARY TUBE.



Thank you to Sruthi Srinivasan for the use of this picture

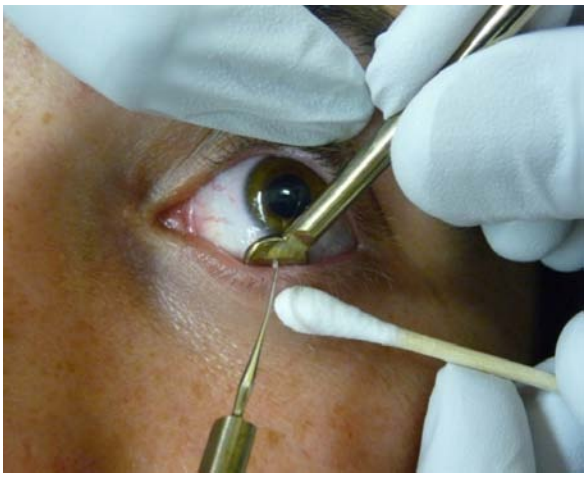
1.5.2 MEIBOMIAN GLAND FLUID

The principal method used to collect lipid from the meibomian glands involves wiping the lid clean with a sterile swap, compressing the eyelid to gently squeeze out the lipids, and collecting the lipid.[34, 39, 229] The lid can be compressed between a lid conformer and a swab (Figure 1-12) [34, 39, 42, 44, 229, 230] or between the clinician's fingers,[11, 51, 128, 231] with or without the use of an anaesthetic. The meibomian gland secretions can then be collected using a spatula[34, 39, 42, 44, 229, 230] or a curette.[51, 231]

Meibomian gland lipid collection at the orifices using microcapillary tubes has also been utilized.[34] This method is not as invasive for the participant, however the disadvantages of this procedure include: smaller volumes being collected, the

samples quickly solidify at room temperature, and small volumes of tears may also be collected with the meibum thus contaminating the meibum sample.[29, 36, 54, 67]

FIGURE 1-12: "SQUEEZING" THE MEIBOMIAN GLANDS OF THE LOWER LID TO EXPRESS MEIBOMIAN GLAND FLUID, IN A PATIENT WITH FRANK MEIBOMIAN GLAND DYSFUNCTION



Thank you to Sruthi Srinivasan for the use of this picture

1.6 LIPID ANALYSIS METHODS

In the early days of research investigating lipid deposition on lens materials, qualitative techniques based on histochemical staining were used, primarily to determine the presence or absence of lipids only.[162] Light microscopy and electron microscopy were typically used in conjunction with these staining techniques, to determine differences in deposition patterns.[147, 148]

More recently, quantitative or semi-quantitative methods have been employed, typically based around the use of chromatographic methods. The three common chromatographic techniques used to analyze lipids are thin layer

chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC). HPLC and GC are now commonly linked with mass spectroscopy (MS) analysis to become a very sensitive and powerful method of qualitative and quantitative analysis. The methods described below are standard methods for the examination of lipid from the tear film, meibomian gland fluid or from contact lens depositions.

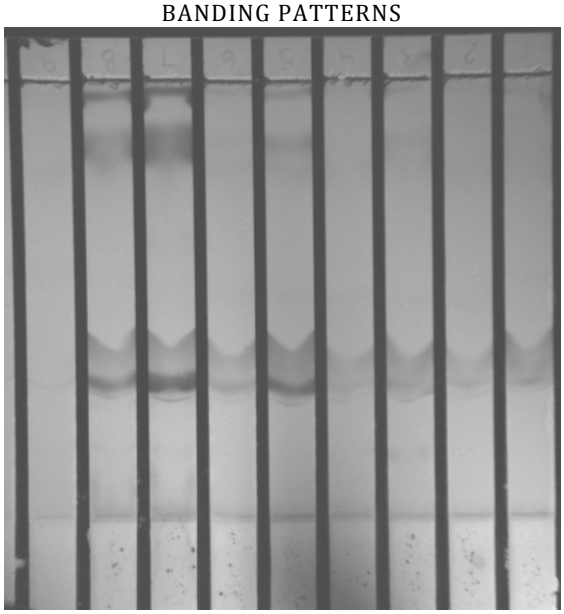
1.6.1 THIN LAYER CHROMATOGRAPHY (TLC)

In every TLC procedure, a plate made of glass, metal or plastic is coated with a thin layer of solid adsorbent material, usually alumina or silica.[232] The sample is applied to the bottom of the plate, which is then placed in an enclosed chamber with a shallow pool of solvent. The liquid in the chamber is known as the mobile phase and is drawn up the plate via capillary action.[232] The components in the sample solution separate on the plate according to their differing solubilities, polarity and their strength of adsorption.[232] If the sample solution contains many different types of substances to be separated, then more than one solvent can be used. The types of substances separated dictate the method that will be used to view the plate.[232] For ink separation, the bands can be seen by the naked eye. If the components separated are colourless, then the compounds can be viewed under UV light. In the case of lipids, the plate can be charred by a fine mist spray of sulphuric acid and is then baked. This blackens the resolved bands so they can be viewed without special equipment.[232] A TLC plate used to separate and identify lipids found on contact

lenses can be seen in Figure 1-13. If individual lipid species identification is required then the TLC lipid band is removed, further processing of the sample is required and then the sample can be analyzed using other techniques including HPLC, GC, HPLC-MS and GC-MS .[73]

TLC is a fundamental qualitative method to determine the classes of lipids which are present in a sample. It can be used for quantitative means with limited accuracy, however it often requires a fairly large volume of sample, which is not usually available in tears and meibum.[55] Another limitation of TLC lies in the techniques propensity to encourage sample degradation due to lengthy exposure to air during processing and band charring, which prevents further analysis.[67] Due to these restrictions, some of the earlier research was conducted with TLC[51, 148, 233] but now more powerful techniques are available.

FIGURE 1-13: A SAMPLE TLC PLATE CHARRED WITH SULPHURIC ACID TO VISUALIZE LIPID BANDING PATTERNS



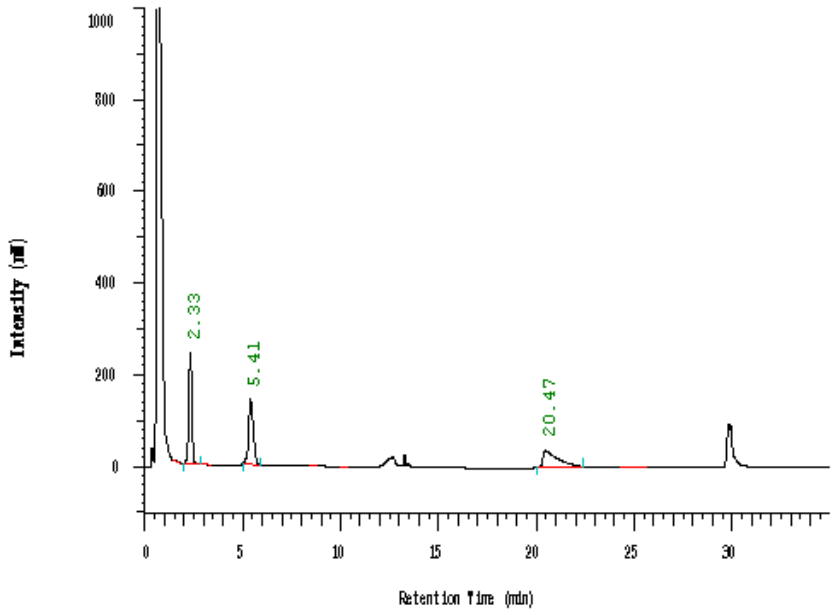
A sample TLC plate charred with sulphuric acid to visualize lipid banding patterns following lipid removed from an experiment in which 5 model lipids were deposited onto silicone hydrogel contact lenses using an *in vitro* model and were then extracted and separated using TLC. Each lane represents one extract from one contact lens. The various bands that are visible are due to the presence of one or more of the lipids extracted.

1.6.2 HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a significantly more technically advanced chromatography method of separation, which is relatively easy to use and is not limited by the volatility or stability of the sample compound.[232] The separation techniques involve mass transfer between the stationary and mobile phases. Like its name, HPLC uses a liquid mobile phase to separate the components of a substance. The first step to separate a mixture is to dissolve it in a solvent and then to force it through a chromatographic column under high pressure, where the mixture is separated into its individual components.[232]

The type of compounds being separated dictates the types of solvents, columns, and detectors used to analyze the sample. Frequently, different HPLC procedures are used to analyze polar and non-polar lipids.[166] Once separated, the lipids of interest are studied by using specific detectors such as UV absorption, fluorescence, infrared, flame ionization, radioactive or mass spectrometry. Due to the variety of solvents, columns, and detectors available, HPLC has proven to be a very powerful tool in lipid analysis which is seen in the number of studies that have used this technique.[11, 120, 165, 210, 234] An HPLC lipid chromatogram, used to identify and quantify lipid on contact lenses can be seen in Figure 1-14.

FIGURE 1-14: A TYPICAL REVERSE-PHASE LIPID CHROMATOGRAM ANALYZED USING UV-LC



A typical reverse-phase lipid chromatogram analyzed using UV-LC for an experiment in which 5 model lipids were deposited onto silicone hydrogel contact lenses using an *in vitro* model and were then extracted and separated using HPLC. Each annotated peak represents one specific lipid identified at a wavelength of 205 nm.

1.6.3 GAS CHROMATOGRAPHY (GC)

Gas chromatography (GC) induces separation of a compound using a gaseous mobile phase.[235] The main component of the GC system is the separation column. Since the sample is carried through the column within the gaseous phase, the sample must be volatile. Samples of low volatility can be separated at high temperatures that allow for a high vapour pressure.[235] However, samples separated at temperatures that are too high can cause unwanted decomposition of the sample and its components. Another limitation of GC relies on its lengthy preparations steps and the difficulty in analysis. The specific compound classes that can be analyzed via GC are dictated by their thermal stability.[235] Therefore, large polar molecules are not usually separated using GC. The separated compounds can be identified by their various retention times[235] and there have been many studies that have utilized GC techniques to analyze lipid. [27,44,38]

Just like HPLC, GC techniques can be linked to various types of detectors including mass spectrometers, infrared, UV absorption, and flame ionization. These detectors produce a chromatogram that is analyzed to identify each component. The type of detector used depends on the class of compound.[235]

1.6.4 CHROMATOGRAPHIC TECHNIQUE NOTES

Many studies have used TLC, HPLC, or GC or a combination of these to quantify lipid deposits from contact lenses, meibomian gland, and tear film samples. Often

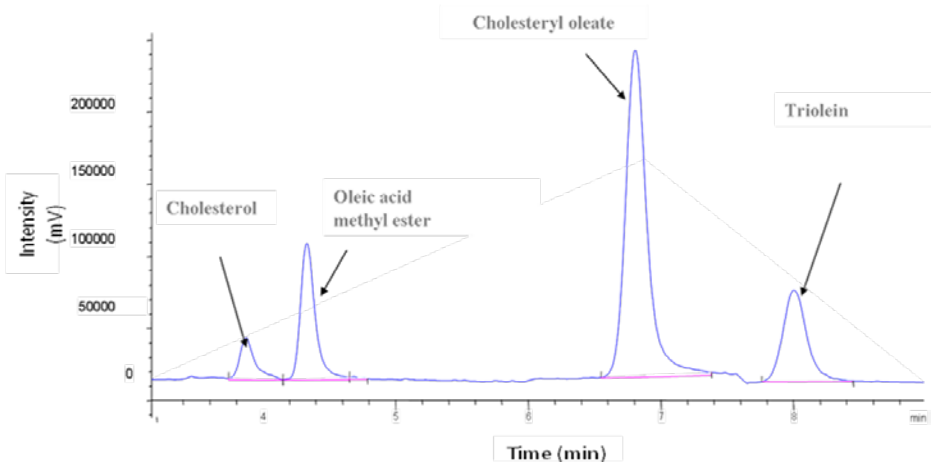
one chromatographic technique is not sufficient to analyze all lipid types found in the eye due to the wide range of polar and non-polar lipids. Each method has its own strengths with certain lipid types. TLC is usually used as a general separation technique where many different lipid types are separated from a complex unknown sample.[232] Following TLC separation, the broad lipid bands, that represent different groups of lipids, are removed and analyzed using other techniques like HPLC and GC. HPLC is often used for the separation of polar lipids, like cholesterols, and GC for the separation of non-polar lipids, like fatty acid methyl esters.[232] TLC has been used to quantify lipid content from extracted contact lens deposition,[144, 146, 148, 163-165, 233] from tear samples,[91, 148] and from meibomian gland secretions.[11, 42, 44, 46, 51, 121, 229, 236] The quantification of lipids within these samples allow for a comprehensive understanding of the lipoidal role in the eye and what and how external factors affect lipid content. HPLC has also been commonly used to analyze lipid content. Jones *et al.*[189, 198, 210] utilized HPLC to quantify *in vitro* and *in vivo* lipid content deposited on silicone hydrogel lenses. In contrast, HPLC has been used to analyze lipid content from contact lens materials,[120, 161, 165, 166, 210] tears[120, 234] and meibomian gland secretions.[2, 42, 57, 66, 73, 230, 231, 237] GC is most often used for meibomian gland secretions[2, 11, 42, 44, 46, 51, 230, 236, 237] and occasionally tears.[91] The majority of these studies have used at least two chromatographic techniques.

1.6.5 MASS SPECTROMETRY (MS)

Mass spectrometry is very commonly used as a detector due to its ability to reveal structural detail about the sample under investigation, its sensitivity, its efficiency, and its ability to quantify its components.[55, 67, 232] However, it is a costly technique.[232] The process of mass spectrometry involves bombarding the sample with high-energy electrons that creates ions that are separated in a magnetic or electric field according to their mass-to-charge ratio. The resulting output is a spectrum of peaks corresponding to the molecular fragments and ionized molecules.[232]

Specifically, mass spectrometry detection methods have been used to determine lipid content in meibomian gland secretions [40, 51, 57, 73, 238, 239] and contact lenses.[165, 203, 204] The common MS techniques utilized for lipids are atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI). The choice between these two methods usually resides with the type of lipid that will be analyzed: polar or nonpolar. APCI is usually utilized for nonpolar compounds and ESI has been found to function best for more polar species.[67] An example of a mass spectrometer chromatogram used to identify the lipids in tears can be seen in Figure 1-15. Mass spectrometry detection methods are often used in eye-related lipid research, but it is not the only detection method, as UV absorption and fluorescence are also used.[73]

FIGURE 1-15: A SAMPLE MASS SPECTROMETRY LIPID STANDARD CHROMATOGRAM.



A sample mass spectrometry lipid standard chromatogram. Four lipid standards at a concentration of 2 ppm were analyzed using LC MS in ESI SIM mode with a mobile phase of chloroform, methanol and 10mM NH_4OAc. Each annotated peak represents one specific lipid. Figure courtesy of Yu Gu.

1.6.6 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

Nuclear magnetic resonance is a technique that measures an atomic nucleus's quantum magnetic properties. This technique can be utilized for any nucleus that has a spin, with common examples being ^{13}C, ^{31}P, and ^{1}H, just to name a few.[240, 241] NMR is a non-destructive method of analyzing organic molecules like lipids and their molecular structure; however the technique struggles due to low sensitivity and therefore requires long processing time.[45, 110, 242, 243]

1.6.7 FLUORESCENT LIPIDS

Other techniques are available for the quantification of lipids taken from tissues and contact lens surfaces. One technique involves a fluorescence assay for contact lens deposits.[145, 167, 170] Fluorescence techniques can be used to analyze lipid

deposition due to the fluorescence signal emitted from lipids themselves. In this technique, lenses are placed in distilled water in a quartz cell. The sample is excited with an incident beam measured at a wavelength of 360 nm and the emission peak is monitored at a wavelength of 440 nm.[145, 167, 170] The height of the emission peak is correlated with the amount of lipid deposition on the lens. Lipid deposition on conventional hydrogel lenses were analyzed using this technique to discover the deposition patterns on group II and group IV lenses that were previously discussed. This method is accurate for determining relative total lipid content, but not applicable for individual lipid concentrations.[145, 167, 170]

A second fluorescence technique has been used in the past. This technique involves staining the lenses with Nile Red, a fluorescence probe. [244] The lenses are then mounted on silica plates and loaded into a fluorescence cell and imaged using customized equipment. From this technique, differences in lipid deposition between different contact lens materials could be seen. This method of quantification is an imaging technique, which is not applicable for individual lipoidal species quantification.[244]

A third fluorescence technique is available for *in vitro* examination of lipid or protein deposits on the surface and for examination of the deposition that occurs within the contact lens matrix. This technique utilizes proteins or lipids that have a fluorescent probe chemically attached to it and examination of the material either by a fluorescent plate reader or by confocal microscopy.[202, 245-248] This technique of utilizing lipids tagged with a fluorescent probe is more conducive for the

examination of lipid deposition on silicone hydrogel lens materials, as silicone hydrogels will interfere with the natural lipoidal signals. Therefore, the addition of a probe molecule increases the fluorescent signal so that it is seen over the lens background. This technique has been used to examine kinetic uptake of lipids onto lens materials and the depth of lipid penetration into the matrix.[202, 248] However, one limitation of this technique lies in the incorporation of a fluorescent probe that is a substantial size relative to the lipid or protein, possibly its own charge, and chemical characteristics, all which may interfere with the natural interactions of the lipid with its surroundings and the lens surface. [245]

1.6.8 RADIOCHEMICAL EXPERIMENTS

One of the techniques that can be used to analyze deposition in an *in vitro* model is radiolabeled lipids[225] or proteins.[139, 249-253] By inserting radiolabeled lipids such as ^{3}H-cholesteryl oleate and ^{14}C-dioleoyl phosphatidylcholine into complex artificial incubation solutions and incubating the lenses, Prager and colleagues[225] were able to quantitatively analyze the radiolabeled lipid using scintillation beta counting. Radiochemical experiments have the benefit that the radioactive "label" is thought not to change the lipid or protein in size, structure or function, which is different than other analysis techniques that use proteins or lipids that are labelled by adducted probes.

1.6.9 LIPID ANALYSIS NOTES

It is clear from reviewing the literature on lipid analysis that this is a very technically challenging area, whether the lipid under investigation is from the tear film, meibomian glands, or contact lens materials. Since the eye contains such a large range of lipid types, there is no one direct or correct method to analyze all the lipids present. Therefore, more research on methods of lipid analysis must be undertaken, particularly given the interest in these areas relating to the role of lipids in dry eye and the deposition of lipids on hydrophobic silicone hydrogel materials.

1.7 CONTACT LENS SURFACE CHARACTERIZATION

There are a whole new range of techniques that have been or could be used for surface characterization of contact lens materials. Many of these techniques have been extensively used in biomaterials research and have the ability to contribute vast amounts of information for the contact lens world.

1.7.1 CONTACT ANGLE AND WETTABILITY

The wettability of a contact lens is described as the ability of the tear film to cover the surface of a contact lens.[254] Conventional hydrogel contact lenses have relatively high water contents with a hydrophilic surface. Therefore, these lenses (when fresh out of the original packaging) have no issues with wettability. However,

once these lenses have been inserted into the eye, changes to the lens occur which can decrease their wettability. [255]

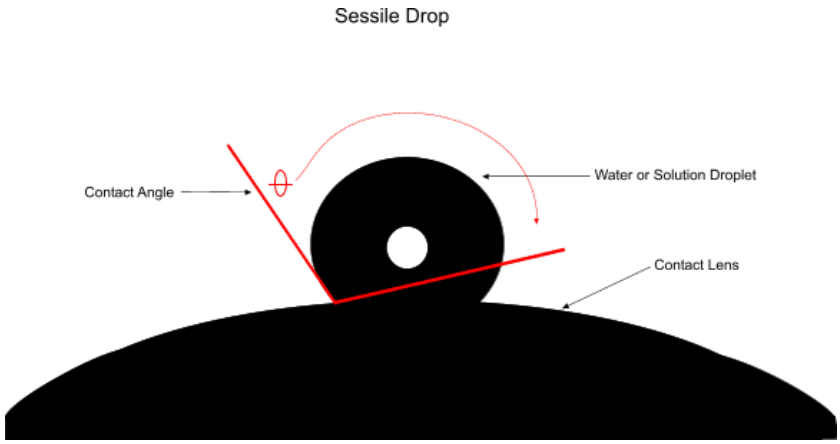
There are two factors that can lead to a lens becoming decreasingly wettable. When the lens is placed on the cornea, water from the lens will be lost to the tear film and into the environment. The amount of water lost will depend on the environmental conditions the lens is exposed to. This process is progressive and therefore occurs over an extended period of time. The second factor is a dynamic process that occurs during blinking. Every time a contact lens wearer blinks the surface of the contact lens and the tear film changes. When the contact lens is exposed to the tear film and other aqueous substances the lenses hydrophilic groups "flip" or re-orientate so that they are exposed at the surface. In contrast, when the lens is exposed to hydrophobic environments, such as air, the hydrophilic groups "flip" to expose the hydrophobic groups of the lens. This process is called chain rotation. This progressive evaporation of water contributes to the discomfort felt by lens wearers at the end of the day.[255]

Historically, wettability is measured by the contact angle that forms between the hydrogel lens material and a water interface. The three most common methods of measuring the contact angle of a contact lens material: sessile drop, captive bubble, and Wilhelmy plate.

In the sessile drop method, a contact lens has any excessive surface fluid removed using lens paper and is placed posterior side down on a convex shaped mantle. A 5 μl drop of water or saline is dispensed from a syringe on to the apex of

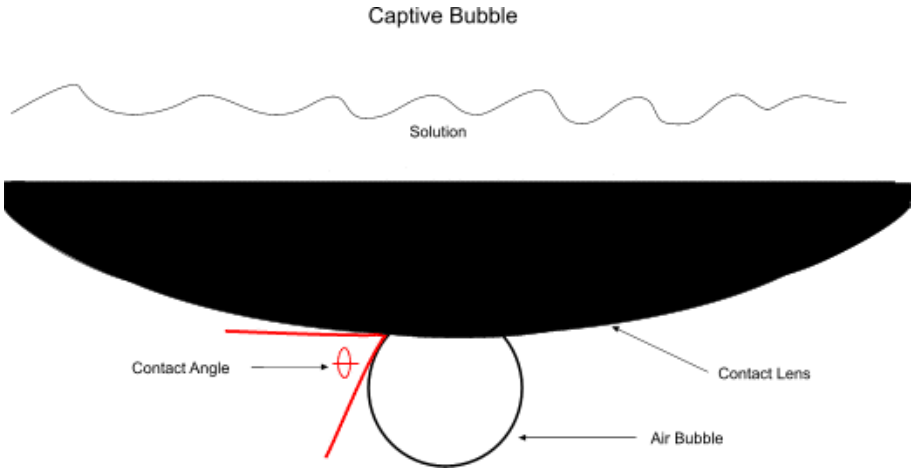
the contact lens. The drop is allowed to settle for 2-3 seconds and the contact angle can be measured between the drop of water and the contact lens surface, as seen in Figure 1-16. The sessile drop method measures the advancing contact angle.

FIGURE 1-16: THE SESSILE DROP METHOD USED TO MEASURE THE CONTACT ANGLE AND ULTIMATELY THE WETTABILITY OF A CONTACT LENS.



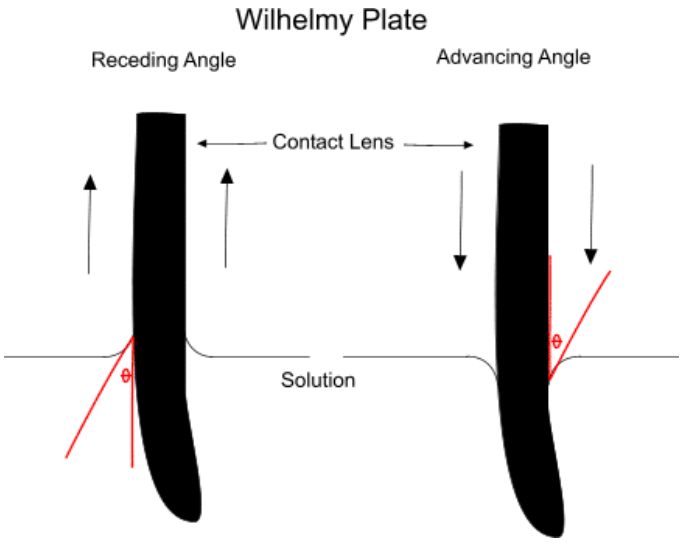
In the captive bubble method, the contact lens is immersed in a chamber that contains saline solution or water with the contact lens oriented anterior side down. An air bubble is placed on the apex of the contact lens surface using a syringe. The contact angle between the contact lens surface and the air bubble is then measured. This technique can be seen in Figure 1-17. [256,257] This technique is analogous to a receding contact angle.

FIGURE 1-17: THE CAPTIVE BUBBLE TECHNIQUE TO MEASURE THE CONTACT ANGLE AND ULTIMATELY THE WETTABILITY OF A CONTACT LENS.



The Wilhelmy plate method can be used to measure both the advancing and receding contact angles of the same contact lens. In this method, a contact lens is mechanically lowered into a beaker containing water or a saline solution. Advancing contact angles are measured as the contact lens is being lowered into the solution. Then, receding contact angles are measured as the contact lens is slowly being removed from the solution. The specific angles that are being measured in each case can be seen in Figure 1-18.[256,257]

FIGURE 1-18: THE WILHELMY PLATE TECHNIQUE TO MEASURE THE CONTACT ANGLE AND ULTIMATELY THE WETTABILITY OF A CONTACT LENS.



Each method of measuring contact angles has its advantages and disadvantages and each method delivers different final measurement values.[256, 258, 259] However the contact angle is measured, better contact lens material wettability corresponds with smaller contact angles. Therefore, the ideal contact angle is zero, which would denote a completely wettable contact lens. Unfortunately, the newer silicone hydrogel contact lens materials are more hydrophobic and thus have the distinct characteristic of being unwettable in nature. This is the main reason for surface treatments and internal wetting agents. [259]

Contact angle methods are based on the contact angle or Young-Dupree equation as seen below:[260]

EQUATION 1-1: YOUNG-DUPREE EQUATION

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cos \theta_e$$

Where:

γ_{SV} = solid/vapour interfacial tension, γ_{SL} = solid/liquid interfacial tension, γ_{LV} = liquid/vapour interfacial tension, $\cos \theta_e$ = equilibrium contact angle

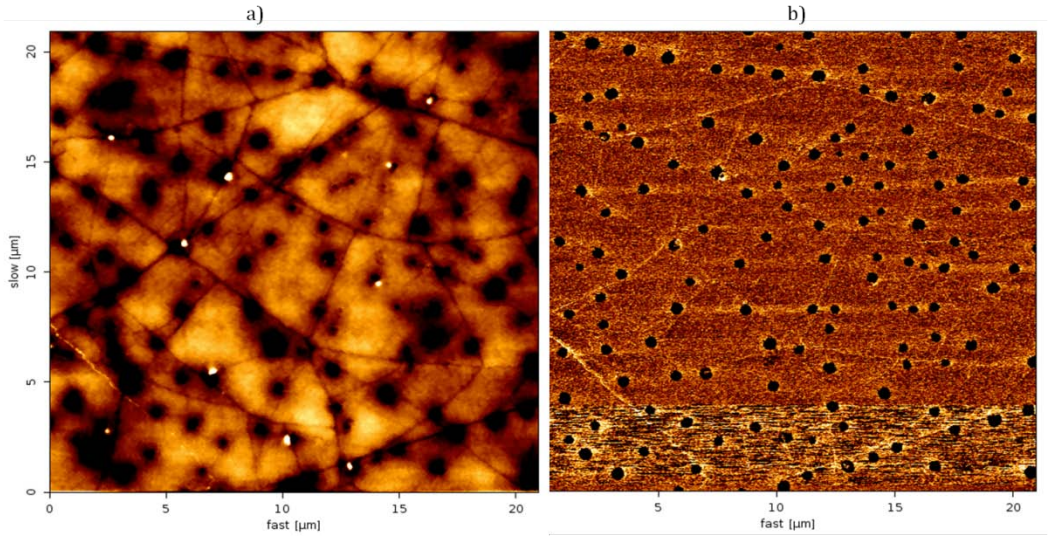
Overall, novel silicone hydrogel materials are, as described previously, less wettable than conventional hydrogels and thus the issue of contact lens wettability has become a topic of great interest again.[201, 258, 261-268]

1.7.2 MICROSCOPY

Microscopy is a powerful technique to help characterize the surface morphology and the environmental interactions of biomaterials.[269-271] There are many different types of microscopy which have aided in our understanding of the materials surfaces, such as: scanning election microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), and confocal scanning microscopy. [269, 272] Microscopy techniques such as TEM and SEM have the advantage of creating very high resolution images, however sample preparation is extensive and destructive, and these processes can alter the surface characteristics of the material. [269]

Atomic force microscopy has become increasingly utilized for biomaterials and contact lens surface characterization (Figure 1-19).[273-281] In AFM, the surface is "felt" by a probe which measures the interactions, either repulsive or attractive, and converts it into a high resolution image.[282] AFM has the advantage over other microscopic techniques in that the sample does not have to be processed prior to analysis and it can be imaged in an aqueous environment.[282] To obtain high quality images, the researcher must carefully choose the AFM tip, AFM settings and the optimal mode to image in.[282] One of the main disadvantages of AFM is the length of time it takes to image the sample.[269]

FIGURE 1-19: ATOMIC FORCE MICROSCOPY IMAGES TAKEN FROM BALAFILCON A SHOWING THE SILICATE GLASSY ISLANDS AND POROUS NATURE OF THE MATERIAL. A) IMAGE TAKEN IN CONTACT MODE B) IMAGE TAKEN IN PHASE CONTRAST MODE. 20X 20μM



Thank you to Zoya Leonenko, Liz Drolle and Sarah Hagedorn for the use of this picture.

Confocal microscopy is a powerful technique that is able to visually section a sample so that different portions of the sample are imaged individually through the z-plane.[269] This technology requires no special sample preparation and therefore biomaterials can be imaged and analyzed without the need for freezing, embedding or physical sectioning. Fluorescent confocal microscopy has been used in hydrogel and biomaterial research, very successfully.[202, 245-248, 283-285]

1.7.3 SPECTROSCOPY

Spectroscopy techniques have become valuable tools for the analysis and characterization of biomaterials. Not only can spectroscopy give information regarding the chemical structure and morphology of a biomaterial surface, but also the constituent elements.[269, 286] In addition to that, some of the methods can be performed in real time with very little sample preparation and processing. The main types of spectroscopy being used for biomaterial analysis are X-ray photoelectron spectroscopy (XPS),[270, 287-292] secondary ion mass spectrometry (SIMS),[289, 293, 294] attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR),[291, 292, 295] and surface matrix-assisted laser desorption ionization mass spectrometry (Surface MALDI).[288, 296-299] Each of these methods has their own limitations, advantages and unique abilities for biomaterial and contact lens research.

2 OBJECTIVES AND IMPORTANCE

To date, there has been very little research analyzing the factors that affect lipid deposition on silicone hydrogel contact lens materials and comparing them to conventional hydrogel deposition. Much of the recent research has been spent simply assessing the quantity of lipid depositing during *in vitro* and *ex vivo* experiments, however very little work has been done trying to characterize the types of interactions that affect deposition in both laboratory-based and human contact lens studies. Lipid deposition is a multifactorial phenomenon and to simply assess *ex vivo* lenses, without being able to control all of the variables could lead to incorrect assumptions. However, by using an *in vitro* model and building it up one step at a time, the individual variables can be assessed and catalogued for future more complex model analysis.

The main research questions examined throughout this thesis are: What are the variables that influence lipid deposition during human contact lens wear and how can those variables be incorporated into a physiologically relevant laboratory-based *in vitro* model of lipid deposition?

With that research question in mind, the overall objectives of this thesis were to develop an *in vitro* model of lipid deposition by systematically analysing some of the factors that may influence carbon-14 cholesterol and phosphatidylcholine deposition such as: the artificial tear solution composition used for incubation, the extraction solvent system, the cleaning regimes used to clean the contact lenses, the

effect of incubation time, and the effect of intermittent air exposure has on lipid deposition. By conducting these individual experiments it is the hope that a better model for lipid deposition can be obtained and that more knowledge will be gained regarding the factors that influence both *in vitro* and *ex vivo* contact lens lipid deposition.

In the following chapter of this thesis, the development of a complex and stable artificial tear solution optimized specifically for in-vial incubations of contact lenses is described. The development of this artificial tear solution is the first step in developing an *in vitro* model for the deposition of biomolecules.

3 CONTACT LENS PHYSICAL PROPERTIES AND LIPID DEPOSITION IN A CHARACTERIZED ARTIFICIAL TEAR SOLUTION

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	Concept / Design	Acquisition of data	Analysis	Write-up / publication
Lorentz	Y	Y	Y	Y
Heynen	Y	Y	Y	Y
Kay	-	Y	Y	-
Dominici	-	Y	Y	Y
Khan	-	Y	-	-
Ng	-	Y	-	-
Jones	Y	-	-	Y

Tables: 8

Figures: 6

3.1 OVERVIEW

Purpose: To characterize solution properties of a physiologically relevant artificial tear solution (ATS) that contains a range of tear film components within a complex salt solution, measure contact lens parameters and lipid deposition after incubation in 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 ISO 18369-2; the diameter, centre 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

centre thickness, diameter and calculated base curve measurements showed no changes beyond what is 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 the 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.

3.2 INTRODUCTION

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 temporary biomaterial that is exposed to a very complex 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, humidity, occupation, systemic diseases, medications, alcohol consumption and diet.[6-9]

The composition of the human tear film is complex, and is believed to contain several layers, including a glycocalyx 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 favoured, 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 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 material 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. In addition to this, many of the *in vitro* deposition models that have been used to-date do not address several important factors affecting deposition on human-worn contact lenses, including the complexity of the tear film, the inter-blink period where the contact lens is directly exposed to air, and the sheer force that occurs during blinking. All of these factors can greatly contribute to the deposition and wettability of a contact lens material.[32-34]

More recently, researchers have started to increase the complexity of the artificial tear solutions used to mimic the tear film. Mirejovsky *et al*., was the first to

report on the use of a complex artificial tear film that contained a range of salts, proteins, and lipids.[35] 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.[32] In this paper, 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 maintain its properties and lens parameters 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.

3.3 METHODS

3.3.1 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.

3.3.2 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 3-1. These specific salts and their relative concentrations are based on literature values.[35-38] 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, a preservative and antimicrobial agent, was added to the system. The use of ProClin 300 allows for incubation at 37^{o}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.[39] However, if the solution was to be used immediately then purging with nitrogen gas equilibrated the solution to the desired pH much faster.

TABLE 3-1: 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		

3.3.3 CONCENTRATED LIPID STOCK SOLUTION

The next step in the ATS preparation was to make a concentrated lipid stock. Here, a 2000X 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: 1ether 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®, wrapped in foil and stored at -20°C until required. Table 3-2 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, 35, 40-42]

TABLE 3-2: MOLECULAR AND EXPERIMENTAL DETAILS OF THE SPECIFIC LIPIDS USED FOR ALL LIPID DOPING SOLUTIONS[28, 35, 40-42]

	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

3.3.4 LIPID ARTIFICIAL TEAR SOLUTION

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

3.3.5 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 3-3 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.[35, 43-50] All proteins and mucin were purchased from Sigma (Oakville, ON). 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 minutes, to prevent destruction of the proteins.[51]

TABLE 3-3: PROTEIN AND MUCIN CONCENTRATIONS 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 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

3.3.6 SOLUTION PROPERTIES

3.3.6.1 PH AND OSMOLALITY

In order to test the consistency of the ATS's pH and osmolality during *in vitro* incubations, a 28 day 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).

3.3.6.2 SURFACE TENSION AND HOMOGENEITY OF ATS

In order 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. The surface tension was measured using the Wilhelmy Balance using a platinum ring and the homogeneity of the solution was tested by staining liposomes in the ATS with Nile Red. In order 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.[35] Then 20 µL of the Nile red test solution was then pipetted onto a slide (prewashed with Methanol), and a cover slip was placed on top. The sample was then examined and photographed on the microscope at 10x and 40x 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.

3.3.7 LENS PARAMETERS

Five contact lens materials were tested in triplicate including: Acuvue® 2 [etafilcon A; Vistakon], Proclear® [omafilcon A; CooperVision], Acuvue® OASYS™ [senofilcon A; Vistakon], Biofinity® [comfilcon A; CooperVision], PureVision™

[balafilcon A; Bausch & Lomb]. The material characteristics of all contact lens materials can be found in Tables 3-4 and 3-5. All lens materials tested had a spherical power of -3.00D and had an approximate base curve of 8.6 ± 0.2 mm. The individual lenses were measured at three times: out of the blister pack, after 40 hours of soaking in CSS, and after 2 weeks' incubation at 37°C in the ATS previously described. The centre 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 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.

TABLE 3-4: CONVENTIONAL HYDROGEL CONTACT LENS MATERIAL CHARACTERISTICS UTILIZED

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
Dk/t (x10^{-9})	31.0	52.3
Water Content	58%	62%
FDA Class	Group IV	Group II

Dk/t: oxygen transmissibility; USAN: United States adopted name;
HEMA (poly-2-hydroxyethyl methacrylate); MA (methacrylic acid); PhC (phosphorylcholine)

TABLE 3-5: SILICONE HYDROGEL CONTACT LENS MATERIAL CHARACTERISTICS UTILIZED

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
Dk/t (x10^{-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

Dk/t: oxygen transmissibility; 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)

3.3.8 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 the flowchart, Figure 3-1. 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). In order to facilitate sensitive quantification of lipid deposition, both ATS solutions were prepared by adding a small aliquot of one of two radiolabelled lipids (Table 3-6); ^{14}C-cholesterol or ^{14}C-phosphatidylcholine. The radiolabeled cholesterol and phosphatidylcholine was added to the ATS at a concentration of 3% and 8.5% of the total individual lipid concentration respectively. 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 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.

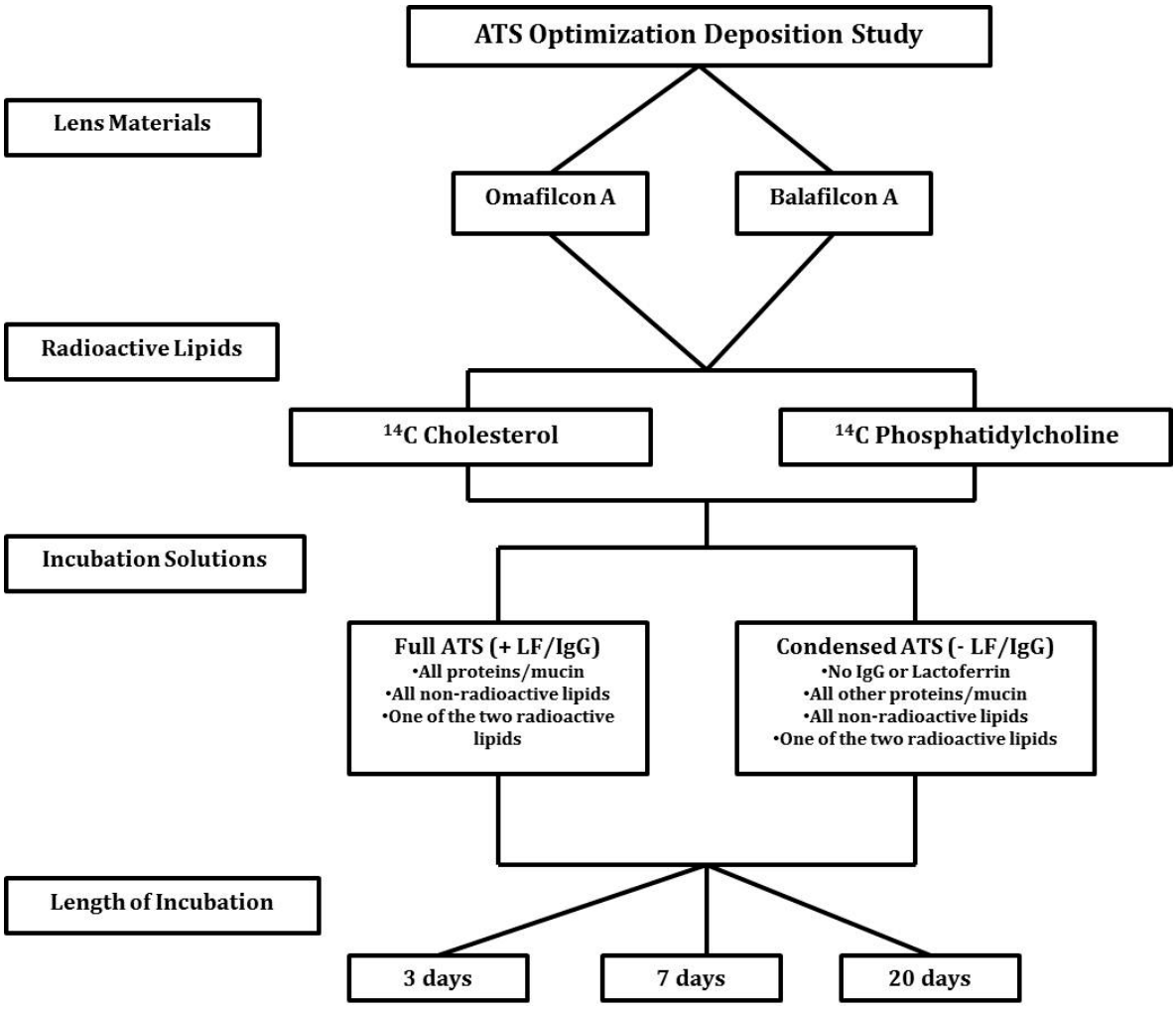
The extract vials were dried completely using nitrogen evaporation at 37°C. All samples were re-suspended in 1 mL of chloroform, sonicated for one minute, and 10 mL of Ultima Gold F scintillation cocktail (Perkin-Elmer) was added. The vials were submitted for liquid scintillation beta counting to determine the mass of radioactive lipid deposited. In the experiment, the radioactive lipid was used as a probe and the ratio of radioactive lipid to non-radioactive lipid in the incubating ATS was kept constant. Therefore, quantification of the total amount of cholesterol

and phosphatidylcholine deposited was extrapolated and calculated using standard radioactive lipid calibration curves.

TABLE 3-6: RADIOACTIVE LIPID CHARACTERISTICS

	Cholesterol [C]	**L-α-DiPalmitoyl-Phosphatidylcholine [PC]**
Radiolabel and Position	4-14C	DiPalmitoyl-1-14C
Molecular Weight (g/mol)	386.7	734.0
Specific Activity (mCi/mmol)	49.78	114
Supplier	Perkin-Elmer	Perkin-Elmer

FIGURE 3-1: OPTIMIZED ATS LIPID DEPOSITION STUDY OUTLINE



3.3.9 STATISTICAL ANALYSIS

Statistical analysis was completed using Statistica 9 software. Lens parameter statistical analysis was completed using a paired t-test and lipid deposition data was analyzed using repeated measures ANOVA. $p<0.05$ was considered statistically significant.

3.4 RESULTS

3.4.1 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.

3.4.2 SURFACE TENSION AND HOMOGENEITY OF ATS

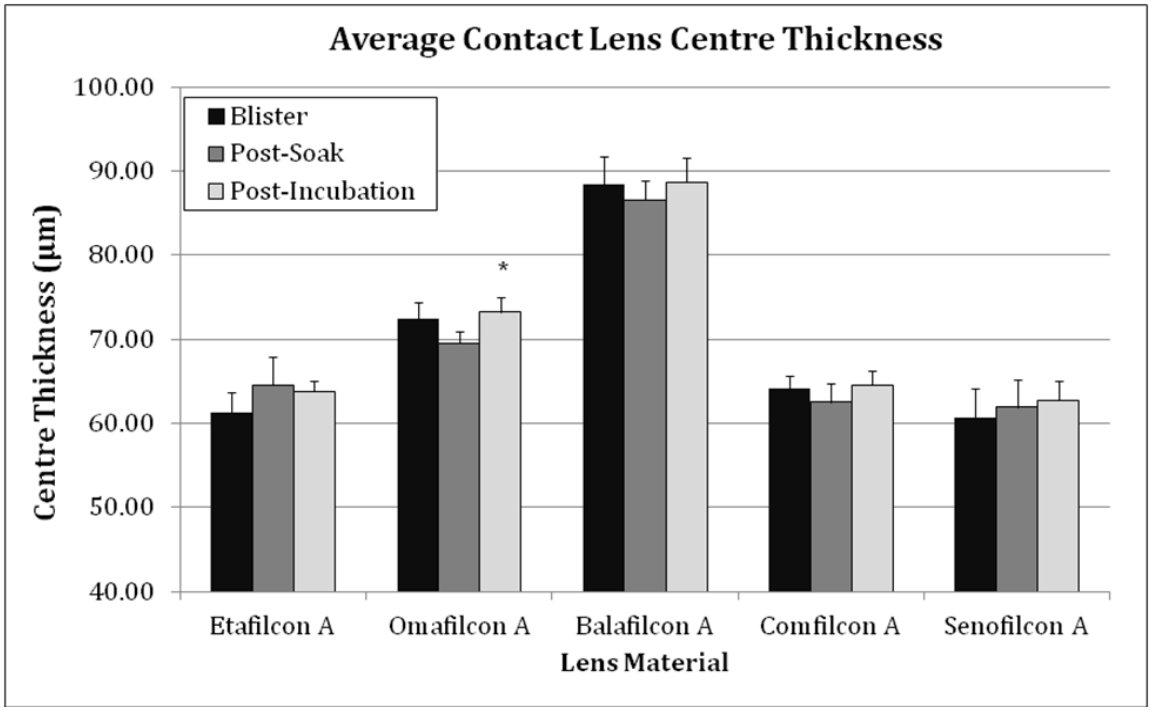
After the complex salt solution and ATS preparation was complete, several aliquots of each solution were stained with Nile Red examined microscopically at 200x –400x and photographed. Following a three week in-vial incubation, ATS aliquots were once again stained and photographed. Following staining 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 6-20 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.

3.4.3 LENS PARAMETERS

The centre 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 in Figure 3-2. 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 centre thickness following two week incubation in ATS. These changes in centre thickness would not correlate to any significant clinically relevant changes *in vivo.*

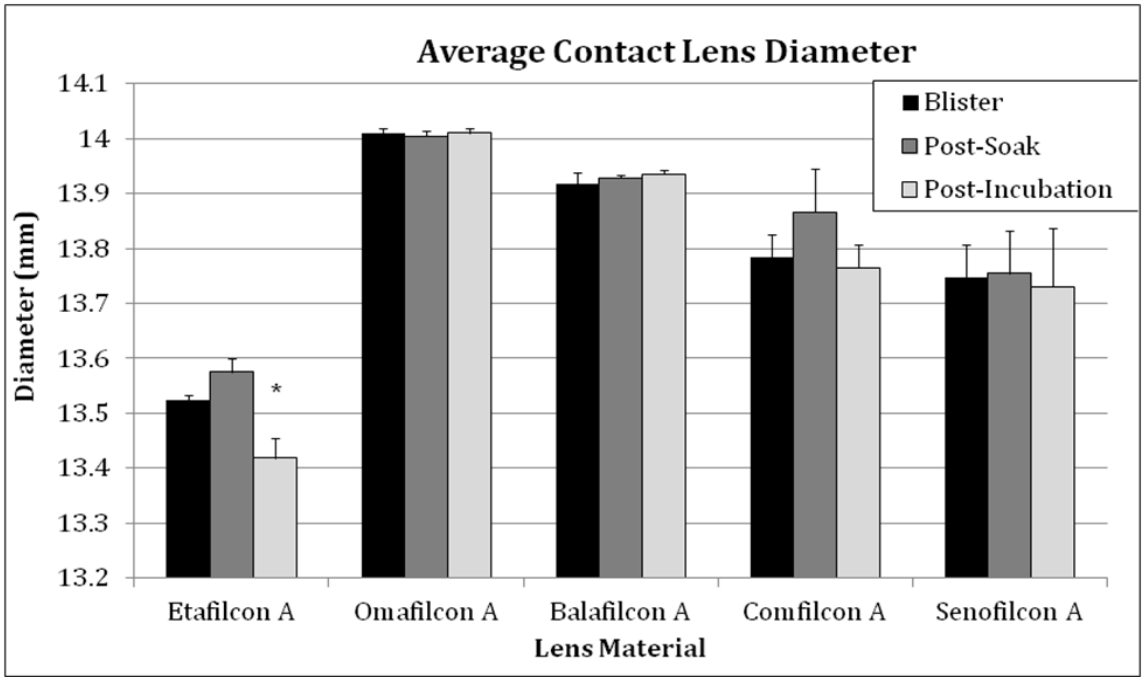
FIGURE 3-2: AVERAGE CENTRE THICKNESS AS MEASURED DIRECTLY FROM THE BLISTER PACK, AFTER A SALINE SOAK, AND FOLLOWING 14 DAY INCUBATION



* denotes statistically significantly different from blister, $p<0.05$

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 3-3. 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 significant clinically relevant changes *in vivo.*

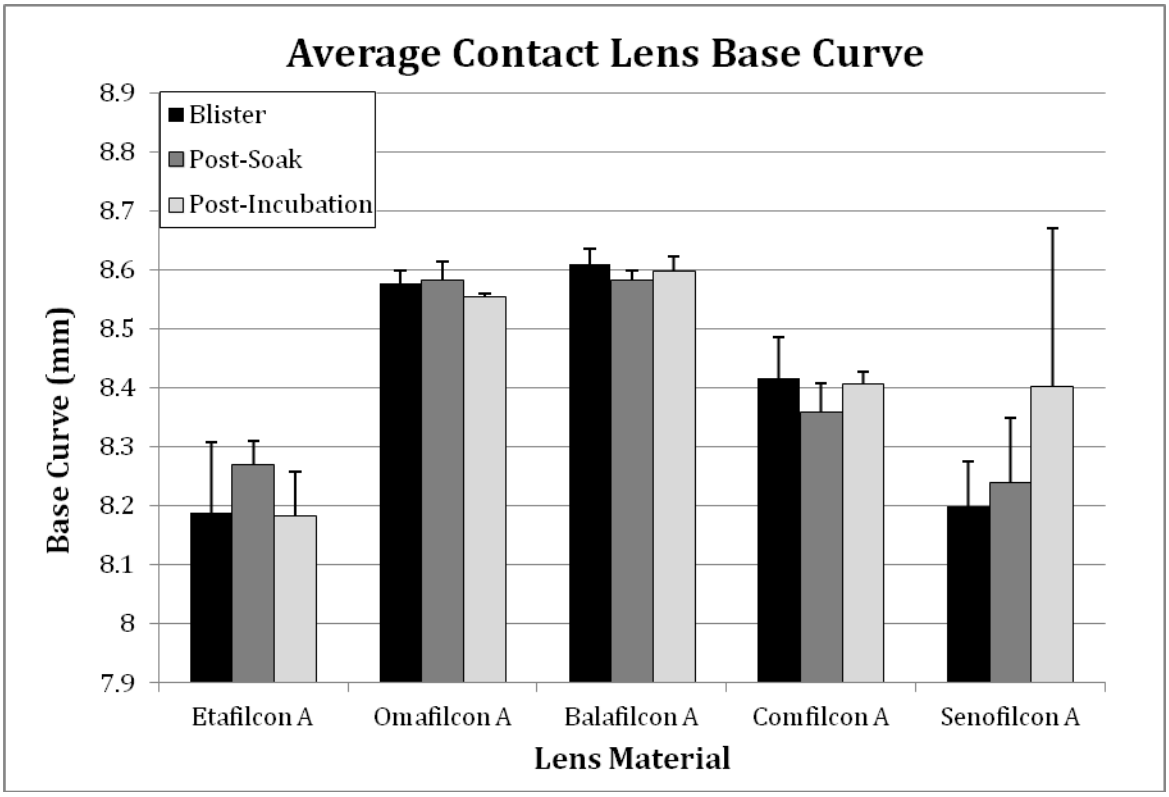
FIGURE 3-3: AVERAGE LENS DIAMETER AS MEASURED DIRECTLY FROM THE BLISTER PACK, AFTER A SALINE SOAK, AND FOLLOWING 14 DAY INCUBATION



* denotes statistically significantly different from blister, $p<0.05$

Average base curve results for each contact lens material after each lens treatment are displayed in Figure 3-4. No statistically significant differences were seen when comparing the blister pack measurements to the post-incubation in ATS measurements for any lens material.

FIGURE 3-4: AVERAGE BASE CURVE AS MEASURED DIRECTLY FROM THE BLISTER PACK, AFTER A SALINE SOAK, AND FOLLOWING 14 DAY INCUBATION



3.4.4 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 3-5 and Figure 3-6. 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 $p \leq 0.001$. 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, $p\leq 0.008$. 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 3-7 and Table 3-8.

FIGURE 3-5: CHOLESTEROL KINETIC UPTAKE DEPOSITION

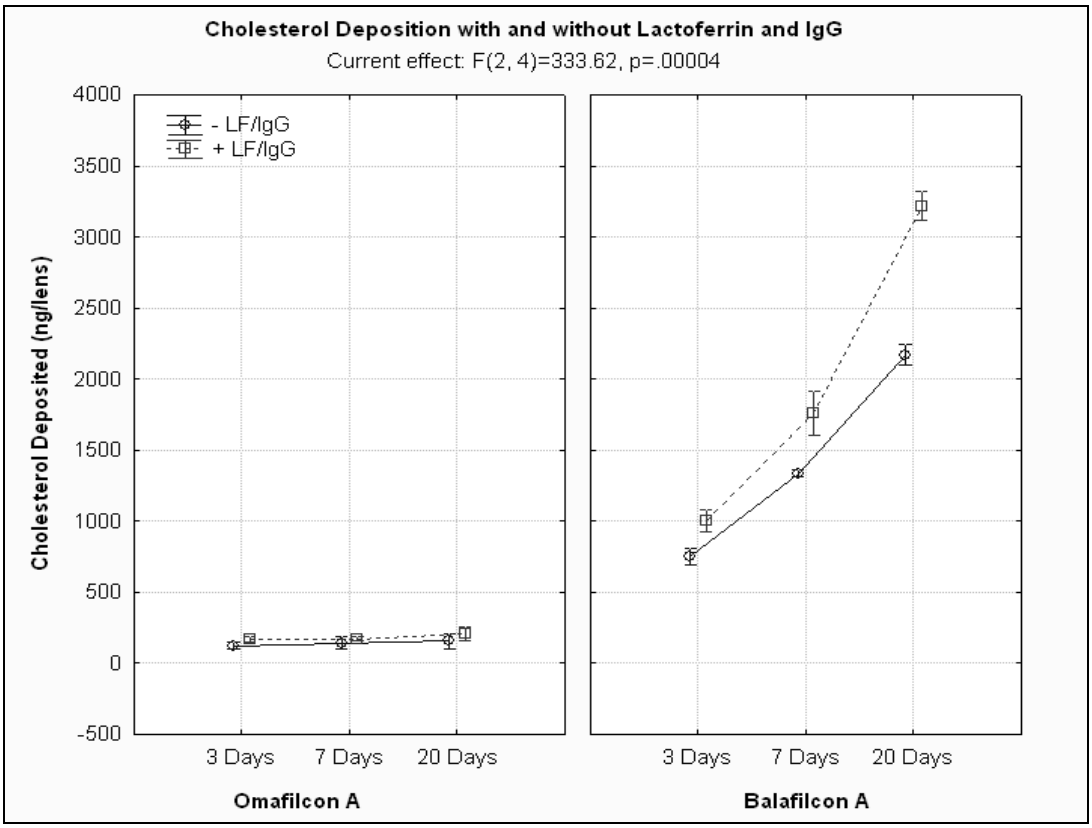


TABLE 3-7: CHOLESTEROL REPEATED MEASURES ANOVA RESULTS

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

FIGURE 3-6: PHOSPHATIDYLCHOLINE KINETIC UPTAKE DEPOSITION

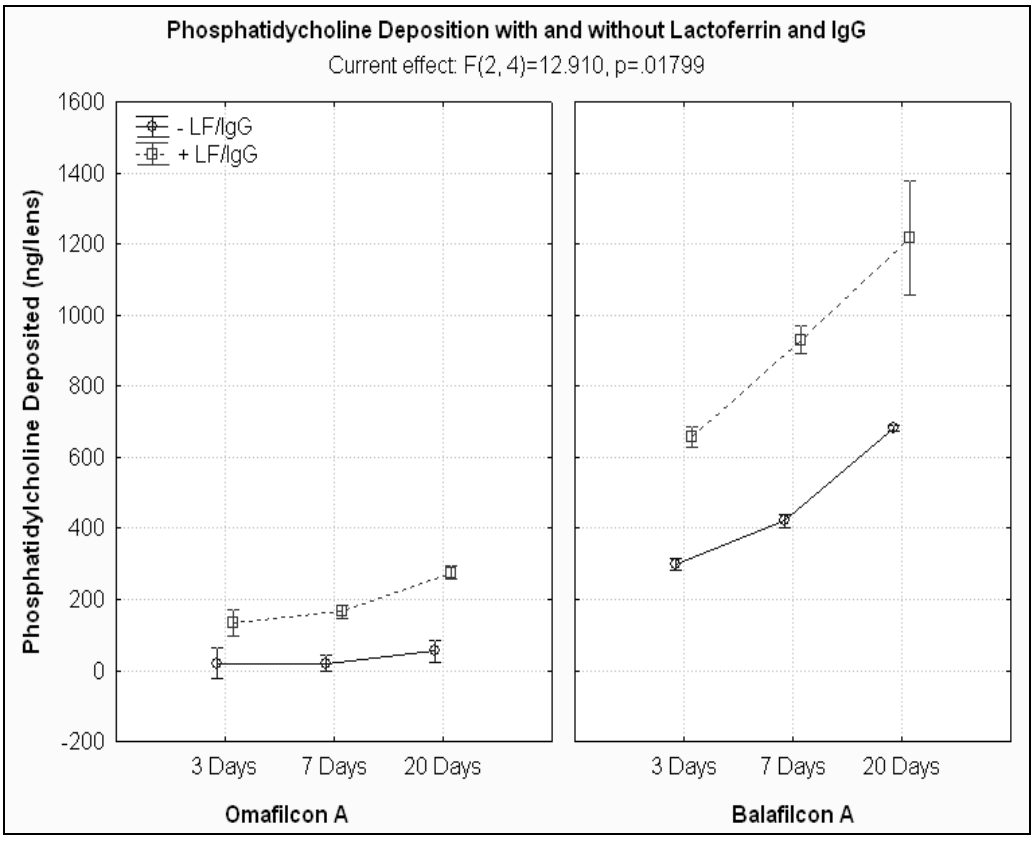


TABLE 3-8: PHOSPHATIDYLCHOLINE REPEATED MEASURES ANOVA RESULTS

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

3.5 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 have 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 last 25 years and are continually being utilized. In the mid-80's, Castillo *et al*. utilized 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.[52] Garrett *et al*.[24] and several studies from Jones *et al*.[18, 29, 30] utilized 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*. published a similar paper examining the uptake of cholesterol oleate and phosphatidylcholine separately in an undisclosed buffer solution.[31] In most of these publications, a phosphate buffered 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*.[53] and Bohnert *et al*.[54] 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.[55] In each of these three examples, lipids and protein components were not mixed together within the ATS and there was no incorporation of mucin.

Recent work from our laboratory[32] and past work from Bontempo and Rapp[23, 56] 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 behaviours. 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.

Papers have been published introducing more complex *in vitro* artificial tear solutions. The first of these papers was by Mirejovsky *et al*. in 1991, where lipids, proteins, mucin, and a variety of salts were all incorporated to form a complex tear solution.[35] Mirejovsky's ATS contains a range of different proteins, lipids from different classification groups, and a non-physiological 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, 46] Bontempo and Rapp,[56, 57] and Iwata *et al*.[58] Prager and Quintana's solution has the same protein portion as the Mirejovsky ATS and the lipid portion is similar, but instead of using a specialized blend of salts, Prager and Quintana use a Hank's Balanced Salt solution as their saline base.[25, 46] The Bontempo and Rapp 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.[56, 57] The most

recent solution of note is the one utilized by Iwata *et al*.[58] This solution uses a mixture of four lipids, three proteins and a simplistic saline base.[58]

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 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.[59, 60] Therefore, the biophysical arrangement of the ATS doesn't 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 analogue 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 physiological buffers, glucose and urea, all within a stable system specially designed

for in-vial incubations. All of these previously published solutions are indeed a great improvement over the more simplistic solutions; however none of the papers have shown the stability of their solutions especially in terms of their pH and osmolality during contact lens incubations.

It is known that the pH and osmolality of a stable human tear film is 6.6-7.8[39] and 305 mmol/kg[61] respectively and that the surface tension of tears is 40-46 dynes/cm.[62] 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 physiological 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 change in pH easily, 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 biological 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 remain 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*., admit that due to the incorporation of chloroform in their incubation solution, the lens materials do indeed swell.[31] 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 prior to lens incubation. None of the other papers have reported measuring the diameter, centre thickness or base curve prior to 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,[63] contact lens materials are only allowed to change by ±0.20 mm in diameter and base curve and by approximately ±18 μm in centre 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, centre 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 centre 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.[63] 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, 58, 64] Cholesterol and phosphatidylcholine were chosen for examination using a radiochemical experiment. Radiochemical experiments have been widely used in biomaterials research[65-70] including contact lens research, especially protein deposition research.[18, 24, 25, 29, 30, 71] 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[59, 64, 72-74] and phosphatidylcholine was chosen as a polar lipid species, due to its presence in the tear film.[11, 75, 76]

The results of the deposition experiment clearly showed that lipid deposition, especially on balafilcon A lenses tend to continually deposit without plateau

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 had previously examined the impact that ATS composition has on lipid and protein deposition for conventional hydrogel lenses,[56] 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[77] 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, 78, 79] 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 have 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 w*ork from Carney *et al*.,[28] Iwata *et al*.,[58] and Pucker *et al*.,[31] all cited higher deposition values than the work presented here. However, these other *in vitro* studies had one 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, 58] 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*.[59] quantified 4.1-8.2 µg/lens after 30 days of wear (depending on the cleaning solution used) and Saville *et al*.[80] found 3.9 µg/lens after 30 nights of wear. Saville 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.[80] 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 conducive to 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.

3.6 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.

3.7 ACKNOWLEDGEMENTS

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In this chapter, the efficiency of removal of deposited cholesterol and phosphatidylcholine from both conventional and silicone hydrogel contact lens materials is examined using radiochemical experiments and various extraction solvent protocols, when necessary. Optimized extraction efficiencies of 85% or greater were desired.

4 EXTRACTION EFFICIENCY OF *IN VITRO* ADSORBED LIPIDS DETERMINED BY RADIOCHEMICAL METHODS

Holly Lorentz, Miriam Heynen, Lise Kay, Hendrik Walther, Lyndon Jones

	Concept / Design	**Acquisition of data**	**Analysis**	**Write-up / publication**
Lorentz	Y	Y	Y	Y
Heynen	Y	Y	Y	Y
Kay	-	Y	Y	-
Walther	-	Y	-	-
Jones	Y	-	-	Y

Tables: 7

Equations: 2

4.1 OVERVIEW

Purpose: To examine and optimize the ability of several extraction systems to remove deposited cholesterol and phosphatidylcholine from various contact lens materials using ^{14}C-labeled model lipids.

Methods: A previously optimized complex artificial tear solution (ATS) that contained a mixture of proteins, lipids and one of either ^{14}C-cholesterol or ^{14}C-phosphatidylcholine was used to incubate six different contact lens materials (etafilcon A, lotrafilcon B, omafilcon A, senofilcon A, comfilcon A, and balafilcon A) for seven days. Following incubation, all lenses were rinsed in saline and then three extractions of 3 hrs at 37˚C in 2 mL of (2:1) chloroform: methanol were completed. All samples including rinses, contact lens incubated ATS, lens extracts, and standards were processed and quantified using standard calibration curves to calculate masses deposited and extraction efficiencies. If the extraction efficiency was calculated to be less than 85% then alternate extraction solvent systems were tested.

Results: Extraction efficiencies for deposited cholesterol were calculated to be >90% with the extraction protocol of 3 hrs at 37˚C in 2 mL of (2:1) chloroform: methanol. The extraction efficiency of phosphatidylcholine with (2:1) chloroform: methanol was >98% for four of the six contact lens materials tested. Balafilcon A and lotrafilcon B lens materials were re-tested with three different extraction procedures in an attempt to increase extraction efficiency beyond 85%. The (60:50:1:4) chloroform: methanol: acetic acid: water extraction procedure removed

over 20% more phosphatidylcholine when compared with the original procedure. Balafilcon A was the highest depositor of cholesterol, lotrafilcon B was the highest depositor of phosphatidylcholine, and etafilcon A was the lowest depositor for both model lipids tested.

Conclusions: An efficient extraction protocol for deposited cholesterol and phosphatidylcholine on two conventional hydrogel and four silicone hydrogel contact lens materials has been optimized based on chloroform and methanol. Confirming previous studies, conventional hydrogel contact lens materials deposited significantly less lipid than silicone hydrogel lenses; following one week of incubation in an artificial tear solution.

4.2 INTRODUCTION

To-date, most contact lens deposition studies have investigated protein deposition,[1-11] with very little published data on lipid deposition, particularly silicone hydrogel lipid deposition.[12-16] However, lipid deposition on silicone hydrogel lens materials is currently a "hot" topic at contact lens conferences around the world, with reports suggesting that these relatively hydrophobic materials tend to deposit more lipid than conventional hydrogels.[15, 17, 18] Lipid deposits may result in decreased comfort, visual acuity, and wettability over time[19] for the lens wearer and induce chemical changes in the contact lens itself.[20]

As described above, data characterizing lipid deposition on silicone hydrogel contact lens materials *in vitro* or *ex vivo* is sparse. *In vitro* models exploring contact lens deposits from tear film components can be utilized to assess tear film interactions, kinetics of deposition, confirmation of the deposited species, penetration of deposits into the lens matrix, and the general factors that affect deposition. One of the techniques that can be used to analyze deposition in an *in vitro* model is one in which radiolabeled lipids[21] or proteins [2, 9, 22-25] are used as reporter molecules to quantify the degree of deposition. Radiochemical experiments have the benefit that the radioactive "label" is thought not to change the lipid or protein in size, structure or function, which is different to other analysis techniques that use proteins or lipids which have a conjugated probe molecule attached. Specifically, fluorescently labelled molecules are known to exhibit altered structures, masses, functions and interactions with their surroundings, as the fluorescent probe itself has its own structure, mass and active sites to react with surrounding materials.[26]

When building an *in vitro* model of lipid deposition on contact lenses based on radiochemical carbon-14 experiments, our laboratory has found (unpublished data) that extraction of the lipid from the contact lens is required for accurate quantification of the deposited species. This is mainly due to the inability of the scintillation cocktail to penetrate the lens matrix and quantify the radioactivity, in addition to the lens polymer masking the scintillation signal. Since an extraction step is required, it is also imperative to ensure that all (or at least the majority) of

the deposited lipid is extracted from the lens material. Therefore, the extraction system used must be evaluated and optimized. Each individual lipid and contact lens material is unique; therefore to ensure optimal extraction efficiency it is imperative that every combination is tested independently. In many *in vitro* models and *ex vivo* experiments, the most desirable lipid extraction procedure is one that removes all the different lipid species present in the sample. However, in some cases a broad-spectrum extraction protocol is not required and therefore the optimal procedure may selectively extract one or more specific lipids. Whichever extraction profile is used, it should be tested for its reproducibility and efficiency.

An examination of the literature reveals that a wide range of extraction solvents and procedures have been used to remove deposited lipid from contact lenses. Common extraction solvents include chloroform,[14, 27] methanol,[28-30] toluene[31] and tetrahydrofuran,[13] just to name a few. Furthermore, each experiment describes variations in length of extraction time, presence or absence of agitation, and differences in temperature during the removal process. However, very few publications have examined the efficiency of the reported extraction protocol and there is clearly no widely accepted standard for lipid extraction.

Our laboratory is developing an *in vitro* model of lipid deposition for conventional hydrogel and silicone hydrogel contact lens materials using an optimized artificial tear solution that is based on a stable and complex salt solution.[32] Using this model and with the addition of lipids radiolabeled with ^{14}C, the extraction efficiency of one or many different extraction solvent systems can be

evaluated using several different lipids and contact lens materials. In the experiment described below, six different contact lens materials were incubated in an artificial tear solution (ATS) complete with lipids, proteins, mucin and one of two different ^{14}C radiolabeled lipids. Following incubation, all lens materials were extracted and all other experimental sources of lipid were processed alongside standard samples, such that extraction efficiency could be calculated.

4.3 METHODS

4.3.1 ARTIFICIAL TEAR SOLUTION (ATS)

The details of preparing a non-radioactive ATS have been previously reported[32] and expanded on in Chapter 3, and thus only a summarized version will be given here, along with the adjustments required for the preparation of a radioactive ATS. The individual components of the ATS and their concentrations can be found in Table 4-1. Preparation of a radioactive ATS required four main steps. To begin, the complex salt solution was made by dissolving all of the salt components, in the order that they appear in Table 4-1, together into MilliQ water. Next, a concentrated lipid stock solution was made by dissolving the pure lipids together, in their required ratios, into (1:1) hexane and ether. This solution was stored at -20˚C until required for future use. Once the concentrated lipid stock was made, it was then used to make the lipid solution, by adding the required aliquot of lipid stock solution into the complex salt solution. The lipid solution was then placed into an ultrasonic bath set at 37˚C and purged with nitrogen to evaporate the stock solvent.

This helps to fully incorporate the lipid. The desired radioactive lipid was then added to the lipid solution and it was sonicated for 15 minutes. The radiolabeled cholesterol and phosphatidylcholine was added to the ATS at a concentration of 3% and 8.5% of the total individual lipid concentration respectively. The required proteins and mucin were then dissolved into the radioactive lipid tear solution and sonicated for an additional five minutes. The details of the radioactive lipids tested can be found in Table 4-2.

TABLE 4-1: ARTIFICIAL TEAR SOLUTION COMPONENTS

Salt component	**mM**	**Lipid Component**	**Concentration (mg/mL)**
Sodium chloride	90.0	Cholesterol	0.0018
Potassium chloride	16.0	Cholesteryl oleate	0.024
Sodium citrate	1.5	Oleic acid	0.0018
Glucose	0.2	Oleic acid methyl ester	0.012
Urea	1.2	Phosphatidylcholine	0.0005
Calcium chloride	0.5	Triolein	0.016
Sodium carbonate	12.0		
Potassium hydrogen carbonate	3.0	**Protein Component**	**Concentration (mg/mL)**
Sodium phosphate dibasic	24.0	Bovine Albumin	0.20
Hydrochloric acid (10 molar)	26.0	Hen Egg Lysozyme	1.90
ProClin 300	0.2 µL/ 1L	Bovine Mucin	0.15

TABLE 4-2: RADIOACTIVE LIPID CHARACTERISTICS

	Cholesterol [C]	L-α-DiPalmitoyl-Phosphatidylcholine [PC]
Radiolabel and Position	4-14C	DiPalmitoyl-1-14C
Molecular Weight (g/mol)	386.7	734.0
Specific Activity (mCi/mmol)	49.78	114
Supplier	Perkin-Elmer	Perkin-Elmer

4.3.2 INCUBATION VIAL PREPARATION

Borosilicate glass vials were used for all contact lens incubations and were pre-treated with non-radioactive ATS for four to seven days at 37˚C with constant shaking, to saturate the inside surface with lipid and protein prior to the radioactive ATS incubation. Previous studies in our laboratory (unpublished) have found that all vial materials readily bind some of the ATS components, therefore changing the concentration available to deposit onto the contact lens materials. Following pretreatment, the vials were then emptied, rinsed with saline, and radioactive ATS (with either ^{14}C-cholesterol or ^{14}C- phosphatidylcholine) was added.

4.3.3 CONTACT LENS MATERIALS

Six contact lens materials were tested in triplicate, including: Acuvue® 2 [etafilcon A; Vistakon], Proclear® [omafilcon A; CooperVision], Acuvue® OASYS™ [senofilcon A; Vistakon], Air Optix™ [lotrafilcon B; Ciba Vision], Biofinity® [comfilcon

A; CooperVision], PureVision™ [balafilcon A; Bausch & Lomb]. The material characteristics of all contact lens materials can be found in Tables 4-3 and 4-4.

TABLE 4-3: CONVENTIONAL HYDROGEL CONTACT LENS MATERIAL CHARACTERISTICS

Material Type	**Conventional Hydrogel**	
USAN	**Etafilcon A**	**Omafilcon A**
Proprietary name	Acuvue®2	Proclear®
Manufacturer	Johnson & Johnson	CooperVision
Monomers	HEMA, MA	HEMA, PhC
Surface Modification	None	None
Oxygen Transmissibility ($x10^{-9}$)	31.0	52.3
Water Content	58%	62%
FDA Class	Group IV	Group II

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

TABLE 4-4: SILICONE HYDROGEL CONTACT LENS MATERIAL CHARACTERISTICS

Material Type	**Silicone Hydrogel**			
USAN	**Senofilcon A**	**Lotrafilcon B**	**Comfilcon A**	**Balafilcon A**
Proprietary name	Acuvue® OASYS™	Air Optix™	Biofinity®	PureVision™
Manufacturer	Johnson & Johnson	CIBA Vision	CooperVision	Bausch & Lomb
Monomers	mPDMS, DMA, HEMA, siloxane macromer, EGDMA, PVP	DMA, TRIS, Siloxane macromer	M3U, FM0411M, HOB, IBM, NVP, TAIC, VMA	NVP, TPVC, NVA, PBVC
Surface Modification	PVP as an internal wetting agent	High refractive index plasma coating	None	Plasma oxidation
Dk/t (x10^{-9})	147	138	160	110
Water Content	38%	33%	48%	36%
FDA Class	Group I	Group I	Group I	Group III

Dk/t: oxygen transmissibility; 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); TRIS (trimethylsiloxy silane); VMA (N-Vinyl-N-methylacetamide)

4.3.4 EXPERIMENTAL DESIGN

Each of the six different contact lens materials were removed from their blister pack solutions and placed in 12-well plates, with 5 mL of the complex salt solution. They were soaked for 24 hours, while being gently rotated at 60 rpm, to remove any residual blister pack solution. After soaking, each contact lens was rinsed twice in complex salt solution, blotted on lens paper and placed in individual borosilicate glass incubation vials containing radioactive ATS that had one of the carbon-14 lipids described in Table 4-2. The vials were capped, sealed with Parafilm®, and incubated at 37˚C and 60 rpm for seven days. Four replicates of each lens material with each of the radioactive lipids were tested.

At the end of the seven days, each lens was rinsed in two successive vials, each containing 2 mL of complex salt solution, to remove loosely bound incubation solution. The lenses were then placed in 20 mL glass scintillation vials with 2 mL of (2:1) chloroform: methanol (v/v) extraction solution and were incubated for three hours each at 37°C while shaking. Each lens was extracted in this way three times and each individual extract was processed separately.

All vials, including unused ATS control solutions with no lenses, contact lens lipid extracts, used ATS, and the rinses were all dried down completely using

nitrogen evaporation and heat up to 40˚C. All samples were resuspended in chloroform, sonicated for one minute, and Ultima Gold F scintillation fluor was added. The samples were counted for their radioactive signal using the LS6500 Beckman Coulter liquid scintillation beta counter (Beckman Coulter, Mississauga, ON). In the experiment, the radioactive lipid was used as a probe and the ratio of radioactive lipid to non-radioactive lipid in the incubating ATS was kept constant. Therefore, quantification of the total amount of cholesterol and phosphatidylcholine deposited was extrapolated and calculated using standard radioactive lipid calibration curves.

By processing all used ATS solutions, lens rinses, and contact lens extracts in this experiment, the radioactivity and mass of lipid in each sample could be calculated. The extraction efficiency could then be calculated by comparing to controls the solutions, as shown in Equation 4-1 below. The % recovery of lipid was calculated using Equation 4-2.

EQUATION 4-1: EXTRACTION EFFICIENCY FORMULA

$$Extraction\ efficiency\ =\ 100\,x\frac{Extracts\ (1+2+3)}{Control\ ATS-((Rinses\ (1+2)+Spent\ ATS))}$$

EQUATION 4-2: % LIPID RECOVERED FORMULA

$$\%\ of\ Lipid\ Recovered\ =\ 100\,x\frac{(Extracts\ (1+2+3)\ +\ Rinses\ (1+2)\ +\ Spent\ ATS)}{Control\ ATS}$$

ATS and radioactive standards were prepared and processed alongside the experimental samples and the lipid that was unaccounted in the entire system was then assumed to be left un-extracted in the lens materials. If the extraction efficiency calculated for any lens material was less than 85%, then the (2:1) chloroform: methanol extraction procedure utilized was deemed unacceptable, and another extraction procedure was tested. The alternate extraction procedures that were tested were based on toluene, hexane, and other combinations of chloroform and methanol.

4.3.5 STATISTICAL ANALYSIS

Statistical analysis was completed using Statistica 9 and t-tests where applicable.

4.4 RESULTS

4.4.1 CHOLESTEROL

The results of the extraction efficiency experiment for cholesterol (C) are found in Table 4-5. From these results it can be seen that the largest amount of cholesterol is extracted from the contact lens materials during the first extraction using 2 mL of (2:1) chloroform: methanol, while shaking for 3 hours at 37˚C. On average, more than 90% of the total C extracted occurred with the first extraction, less than 10% was extracted in the second, and less than 1% was extracted in the

third and final extraction procedure. Cholesterol deposition after one week of incubation in a complex ATS was approximately 1650-2350 ng/lens on the silicone hydrogel materials and <150 ng/lens was deposited on the conventional HEMA materials. Balafilcon A contact lens materials deposited the most cholesterol, with 2335 ng/lens and etafilcon A was the lowest depositor, with 73 ng/lens.

After the lenses were removed from their radioactive incubation ATS, they were rinsed twice in 2 mL of saline. These two rinses together contained a total of 400–700 ng/lens of cholesterol and over 80% of this cholesterol was "washed off" during the first rinse. The mass of cholesterol removed in the rinses is the equivalent of 200–400 μL of the lens liquid envelope that was rinsed away. Omafilcon A lenses had the most cholesterol rinsed away per lens and etafilcon A had the least.

The extraction efficiency for each lens material was calculated independently and the overall percent recovery of the cholesterol was also calculated. The percent recovery of cholesterol from the entire experiment was 98.8% ± 1.62 and the extraction efficiency calculated was over 90% for all lens materials. Since the extraction efficiency for all of the contact lens materials was over 85%, no further optimization was required for cholesterol.

TABLE 4-5: CHOLESTEROL EXTRACTION EFFICIENCY RESULTS

	Conventional Hydrogels		Silicone Hydrogels			
	Etafilcon A	**Omafilcon A**	**Senofilcon A**	**Lotrafilcon B**	**Comfilcon A**	**Balafilcon A**
Total C extracted (ng/lens) ± STD	73.04 ± 8.89	130.79 ± 7.24	2190.12 ± 59.99	1678.79 ± 63.64	2122.22 ± 50.76	2335.10 ± 55.18
Average C in 1st extract (ng/lens) ± STD	72.56 ± 7.99	130.34 ± 7.50	2054.21 ± 55.83	1575.55 ± 63.12	2014.65 ± 71.52	2180.14 ± 51.57
% of total C extracted in 1st extract ± SD	99.44% ± 1.12	99.65% ± 0.71	93.8% ± 0.53	93.85% ± 0.41	95.37% ± 1.59	93.37% ± 0.59
% of total C extracted in 2nd extract ± SD	0.56 % ± 1.12	0.35 % ± 0.71	6.36 % ± 0.59	4.55 % ± 1.52	6.11 % ± 0.45	6.08 % ± 0.44
% of total C extracted in 3nd extract ± SD	0.00 % ± 0.00	0.00% ± 0.00	0.27 % ± 0.09	0.08 % ± 0.09	0.05 % ± 0.06	0.13 % ± 0.12
Extraction Efficiency ± STD	100 % ± 0.00	94.51% ± 3.91	100 % ± 0.00	91.75 % ± 3.45	90.38 % ± 2.15	96.48 % ± 2.27

* Extraction efficiency was calculated according to Equation 1. n=4

4.4.2 PHOSPHATIDYLCHOLINE

The results of the initial extraction efficiency experiment for phosphatidylcholine (PC) are found in Table 4-6. This table shows that the first of three extractions using 2 mL of (2:1) chloroform: methanol while shaking for 3 hours at 37˚C extracted the largest amount of PC of the three extractions. On average, more than 89% of the total PC extracted occurred with the first extraction, less than 7% was extracted in the second, and less than 1% was extracted in the third extraction. Silicone hydrogel contact lens materials deposited 600-800 ng/lens of PC after one week of incubation in the complex ATS and the conventional HEMA contact lenses deposited approximately 80 ng/lens. Lotrafilcon B contact lens materials deposited the most PC, with 774 ng/lens and etafilcon A deposited the least PC, with 57 ng/lens.

All contact lenses were rinsed twice in 2 mL of complex salt solution following removal from their incubation in radioactive ATS. The rinses held a total of 100–250 ng/lens of PC which represents a liquid envelop of 200–500 μL/lens. Over 80% of the PC was "washed off" in the first rinse alone. Omafilcon A lenses had the most PC rinsed away per lens and etafilcon A had the least.

The extraction efficiency for each lens material was calculated independently and the overall percent recovery of the PC was also calculated, using Equation 1. The percent recovery of PC from the entire experiment was 94.2% ± 9.8 and the extraction efficiency calculated was over, if not close to 100% for four of the materials tested (etafilcon A, omafilcon A, comfilcon A and senofilcon A). Due to the

low extraction efficiency for balafilcon A and lotrafilcon B, a secondary experiment was conducted on the extraction efficiency of phosphatidylcholine for these two lens materials.

TABLE 4-6: EXTRACTION EFFICIENCY RESULTS FOR PHOSPHATIDYLCHOLINE

	Conventional Hydrogel		**Silicone Hydrogel**			
	Etafilcon A	**Omafilcon A**	**Balafilcon A**	**Comfilcon A**	**Lotrafilcon B**	**Senofilcon A**
Total PC extracted (ng/lens) ± STD	56.61 ± 7.50	64.33 ± 15.10	699.81 ± 34.42	644.64 ± 21.50	774.15 ± 20.12	608.11 ± 24.04
Average PC in 1st extract (ng/lens) ± STD	42.75 ± 6.63	49.57 ± 13.61	580.33 ± 29.34	528.17 ± 18.14	664.39 ± 17.58	470.12 ± 19.52
% of total C extracted in 1st extract ± SD	89.70% ± 4.43	91.79 % ± 4.95	93.93 % ± 0.65	92.52% ± 2.44	93.74 % ± 0.32	93.30% ± 1.01
% of total PC extracted in 2nd extract ± SD	9.46 % ± 4.68	7.23 % ± 4.42	5.70 % ± 0.64	6.87 % ± 2.46	6.05 % ± 0.29	6.16 % ± 0.89
% of total PC extracted in 3nd extract ± SD	0.84 % ± 1.00	0.98 % ± 0.87	0.38 % ± 0.25	0.61 % ± 0.16	0.48% ± 0.16	0.54 % ± 0.17
Extraction Efficiency ± STD	100 % ± 0.00	100 % ± 0.00	75.00% ± 15.11	100% ± 0.00	85.42 % ± 6.08	100% ± 0.00

* Extraction efficiency was calculated according to Equation 1. n=4

The results of the secondary PC extraction efficiency experiment for balafilcon A and lotrafilcon B are seen in Table 4-7. The different extraction procedures tested were: the original (2:1) chloroform: methanol "2CM" and three new extraction methods including: (1:1) chloroform: methanol[33] "CM", (5:1) toluene: isopropanol[31] "TI", and (60:50:1:4) chloroform: methanol: acetic acid: water[34] "CMAW". The CMAW extraction method extracted the most PC for balafilcon A lenses however, no statistically significant difference was found when compared with the 2CM method ($p > 0.05$). A statistically significant increase in PC was extracted using CMAW over 2CM for lotrafilcon B ($p < 0.001$). Generally, the CM extraction method was found to extract the least amount of PC for both lens materials tested. The calculated extraction efficiency for lotrafilcon B and balafilcon A with the CMAW extraction method tested close to 100% for both lens materials, therefore no further optimization is required.

TABLE 4-7: COMPARISON OF THREE DIFFERENT PHOSPHATIDYLCHOLINE EXTRACTION METHODS AGAINST THE 2:1 CHLOROFORM: METHANOL METHOD PREVIOUSLY TESTED

Extraction Method		Lotrafilcon B	p value	Balafilcon A	p value
2 CM	**ng/lens**	562.37 ± 8.93	-	765.92 ± 48.76	-
CM	**ng/lens**	534.97 ± 33.72	0.245	668.70 ± 4.08	***0.026***
	% change from 2CM	-4.87%		-12.69%	
TI	**ng/lens**	599.14 ± 46.01	0.246	729.49 ± 16.34	0.287
	% change from 2CM	+6.54%		-4.76%	
CMAW	**ng/lens**	686.90 ± 12.60	***<0.001***	806.48 ± 19.03	0.251
	% change from 2CM	+28.40%		+20.60%	

n=3, p values of < 0.05 are considered to be statistically significant
2CM = (2:1) chloroform: methanol, CM = (1:1) chloroform: methanol, TI = (5:1) toluene: isopropanol, CMAW = (60:50:1:4) chloroform: methanol: acetic acid: water
2CM, CM, CMAW extraction methods = 2 extractions for 3 hrs at 37˚C with shaking
TI extraction method = 2 extraction for 30 minutes at 37˚C with sonication

4.5 DISCUSSION

There are many different extraction protocols that are currently used to extract lipids from conventional hydrogel contact lenses and/or silicone hydrogel contact lens materials. Some of the common extraction solvent systems include chloroform: methanol,[14, 27, 33, 35] methanol,[28-30] toluene: isopropanol,[31] hexane[36] and tetrahydrofuran.[13] However, upon exploration of the literature, more than just the extraction solvents varied between experiments. The volume of solvent, the length of time the contact lens remains within the solvent, the number of extractions conducted, the temperature at which the extraction takes place, the level of agitation during extraction and the specific lipids being extracted are some of the other parameters that vary widely depending on the research group. With such a wide

variety of published protocols, it is impossible to know which is the most efficient procedure to maximally extract lipid from the contact lens materials.

Most publications do not mention the efficiency of their utilized extraction procedure and the few that do, either do not give a detailed description of how the results were obtained or the deposition methods used for the extraction efficiency portion were not the same methods of deposition as the experimental portion. Determining the efficiency of the extraction method is critical, especially when the method is eventually to be used for human worn contact lens extractions. Much of the *ex vivo* contact lens lipid deposition literature specifies the mass of lipids quantified from the lens. However, without a proper extraction efficiency experiment these quoted values may not be representative of the deposited lipid masses.

In the mid to late 1990's, Bontempo and Rapp completed a series of experiments examining lipid and protein deposition on conventional hydrogel contact lens materials.[28-30, 37] Their original experiment in 1994,[37] utilized a three step method for lipid extraction that involved various concentrations of methanol and rinses with chloroform: methanol (1:1). The experiment enabled a calculated extraction efficiency by processing unused and used samples of ATS and the lens extract samples via thin layer chromatography (TLC).[37] This *in vitro* study incubated lenses for 24 hours in an ATS containing four non-polar lipids. From this experiment, they determined that over 90% of the lipid was extracted. In future papers, the extraction procedure used was condensed to one extract with 50%

methanol,[29, 30] however the extraction efficiency that is quoted is the same as the multi-step extraction process.

More recently, two publications examining lipid deposition have tested and recorded extraction efficiency from silicone hydrogel contact lens materials.[18, 27] In both studies, the extraction efficiency experiment was completed by adsorbing known masses of lipid onto the lens, letting the samples dry and then extracting them back out with their specific extraction procedures. Iwata *et al*, adsorbed several known masses of several lipid types to one lens material, however later in the experiment several different lens materials are quantified.[27]

The radioactive *in vitro* lipid deposition model introduced in this paper is an ideal way to examine *in vitro* extraction efficiency. Since this model is currently being utilized in our laboratory for numerous *in vitro* deposition experiments, it was imperative to determine extraction efficiencies for each lens type in order to make meaningful comparisons. There are many variables in an *in vitro* model that can have an influence on the results, including manner of lipid deposition, materials examined and solution composition. Therefore, we sought to tightly control the system to accurately and consistently quantify lipid deposition.

It is widely known that there are large differences between conventional HEMA hydrogel and silicone hydrogel contact lens materials; however there are many differences within each of those classes of contact lenses as well. Conventional hydrogels are known to deposit protein and lysozyme in a fairly consistent manner and this level of deposition is broadly related to their FDA

classification, which is based on water content and ionicity. However, currently there is no recognized classification system for silicone hydrogel lenses. These materials vary widely in their characteristics due to the specific mix of polymers used and the presence or absence of a particular surface treatment. Therefore each silicone hydrogel lens is remarkably unique and for this reason each lens material being utilized needs to be tested individually.

In addition to the variation in lens materials, there are also variations in the types of lipids that are present in the tear film[38-41] and therefore variations in deposits onto contact lens surfaces.[13, 17, 30, 42] The characteristics of each lipid group also vary widely. Fatty acids, cholesterols and phospholipids, for example, all have different characteristics, bonds, and components and differences within each lipid family are prevalent as well. The function and behaviour of phospholipids themselves can vary greatly depending on the size and charge of the head group and the length, bonding and composition of the tails for that specific phospholipid. Therefore, completing an extraction efficiency experiment on one lipid species does not necessarily predict the extraction efficiency of other lipids, especially for lipids from other families.

This paper has introduced a systematic *in vitro* experimental process to examine the extraction efficiency for two lipid types and multiple lens materials. This experiment could easily be expanded to examine different incubation concentrations, lengths of incubations, *in vitro* models and could be transferred to the *ex vivo* situation.

As seen from this experiment, one individual extraction protocol does not always extract each lipid and lens material combination equally. Therefore, the specific extraction procedure utilized may depend on the experiment being conducted and whether a broad-spectrum extraction solution is desired or optimized extraction for a particular lipid and lens combination.

The value of rinsing lenses following removal from an incubation solution of an *in vitro* experiment was also demonstrated in this experiment. A relatively large mass of lipid was found to reside in a liquid envelope surrounding the lens and was not tightly bound or deposited to the lens. If this rinsing step had been eliminated then the quantified lipid would have been artificially high. This experiment is the first to report on the possible volume and mass of lipid that is removed in a simple rinse. Therefore, rinsing human-worn lenses prior to extraction and/or analysis as part of a clinical study should also be considered. Although, the tear envelope may not be as large in *ex vivo* lenses, there still may be a portion of tear film components that are present in this liquid phase surrounding the lens or loosely associated but not bound to the lens.

This optimized extraction protocol for deposited cholesterol was two extractions of 3 hrs at 37°C with shaking in 2 mL of chloroform: methanol (2:1). This procedure recovered over 90% of the cholesterol deposited during the incubation with the radioactive *in vitro* model. The same extraction procedure was optimized for the extraction of phosphatidylcholine from etafilcon A, omafilcon A, comfilcon A, and senofilcon A with extraction efficiencies calculated to be 100%. The optimized

extraction of phosphatidylcholine from balafilcon A and lotrafilcon B included two extractions of 3 hrs at 37˚C with shaking in 2 mL of chloroform: methanol: acetic acid: water (60:50:1:4), which recovered 100% of the phosphatidylcholine.

When the deposited mass of lipids quantified in this experiment is compared to other *in vitro* and *ex vivo* data, it can be seen that differences do exist. In this experiment, after 7 days of incubation in the ATS solution, the silicone hydrogel lenses deposited between 1.65-2.35 μg/lens of cholesterol and the conventional hydrogels deposited less than 0.15 μg/lens of cholesterol. Phosphatidylcholine deposition on silicone hydrogels was 0.60-0.80 μg/lens and the conventional HEMA contact lenses deposited approximately 0.08 μg/lens of phosphatidylcholine per lens. Much of the other *in vitro* lipid work completed recently have 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 w*ork from Carney *et al*.,[15] and Pucker *et al*.,[14] 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, different incubation volumes, different incubation times and replenishment of the ATS with fresh solution during incubation.[14, 15, 27] All of these factors may explain the higher deposition of cholesterol and phosphatidylcholine reported by others.

When the cholesterol and phosphatidylcholine deposition results found in this *in vitro* experiment are compared with recent *ex vivo* data, it is seen that the results tend to fall within the same range of deposition. However it is impossible to make direct comparisons as the wear time of the *ex vivo* lenses are much longer than the incubation time of this experiment, *ex vivo* lenses are cleaned nightly, and it would be incorrect to extrapolate the data.[18, 43] 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 quantified other lipids than those investigated in this experiment.

It is known that *in vitro* contact lens deposition models do not always mimic directly what occurs during human contact lens wear. Many times the masses deposited in an *in vitro* model are lower or higher than what is quantified *ex vivo*. There are many reasons for this, including: differences in ATS compositions and concentrations utilized, the simplicity of the model, the wear time or incubation time of the lens, differences in lens materials utilized, and an incomplete understanding of all the interactions present. The only way that *in vitro* models can become more physiologically relevant is to analyze all of the interactions that occur during human contact lens wear and then integrate them into newer *in vitro* models. Ultimately, *in vitro* models must be improved so that their results are more reliable, repeatable, and representative and only then will they provide a basis for research and development of new and existing products.

Finally, once again this experiment has supported other research that shows that conventional hydrogel contact lens materials deposit significantly less lipid

when compared to the silicone hydrogel lens materials. The presence or absence of a specific surface treatment did not alter this result.

4.6 CONCLUSION

In conclusion, an extraction protocol for deposited cholesterol and phosphatidylcholine on two conventional hydrogel and four silicone hydrogel contact lens materials has been optimized based on chloroform and methanol. An extraction efficiency of over 90% was achieved for both lipids and all contact lenses tested. Confirming previous studies, conventional hydrogel contact lens materials deposit significantly less lipid than silicone hydrogel lenses, following one week of incubation in an artificial tear solution.

4.7 ACKNOWLEDGEMENTS

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In the next chapter, the effects of the composition of the *in vitro* incubation solution utilized are examined. Three different lens materials and two specific radiolabelled lipids over two different incubation lengths are examined, to assess how the lipid deposition profiles are influenced by the surrounding tear film constituents. This experiment is designed to examine the competitive binding that occurs on a material due to the tear film composition.

5 THE IMPACT OF TEAR FILM COMPONENTS ON *IN VITRO* LIPID UPTAKE TO SILICONE HYDROGEL AND HYDROGEL CONTACT LENS MATERIALS

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	Concept / Design	Acquisition of data	Analysis	Write-up / publication
Lorentz	Y	Y	Y	Y
Heynen	Y	Y	Y	Y
Hagedorn	-	Y	-	-
Trieu	-	Y	-	-
Jones	Y	-	-	Y

Tables: 9

Figures: 4

5.1 OVERVIEW

Purpose: To analyze the influence of various tear film components on *in vitro* deposition of two lipids (cholesterol and phosphatidylcholine) on silicone hydrogel (SH) and conventional hydrogel (CH) contact lens materials.

Methods: Etafilcon A, balafilcon A, and senofilcon A were incubated in four different incubation solutions for 3 or 14 days: an artificial tear solution (ATS) containing six common lipids and 5 proteins, a protein tear solution (PrTS) containing 5 proteins and the lipid of interest, a lipid tear solution (LTS), containing 6 lipids and no proteins, and a single lipid tear solution (SLTS), containing the lipid of interest only. Proteins and lipids were at physiological concentrations. Each incubation solution contained one of the two radiolabelled lipids: ^{14}C-cholesterol (C) or ^{14}C-phosphatidylcholine (PC). After soaking, lenses were removed from the incubation solution, the lipids were extracted then quantified using a LS6500 Beckman Coulter beta counter. Deposition of lipids on lenses was calculated using standard calibration curves.

Results: This experiment examined several different parameters influencing lipid deposition on contact lenses, including: lens material, length of incubation, and the composition of the incubation solution. Overall, lipid deposited differently on different lens materials ($p<0.0005$), with the order of deposition most commonly being balafilcon > senofilcon > etafilcon. Incubation solution had a large impact on how much lipid was deposited ($p<0.00001$), though cholesterol and

phosphatidylcholine demonstrated different deposition patterns. Lipid deposition after 14 days of incubation was consistently greater than after 3 days ($p<0.02$).

Conclusion: This *in vitro* study demonstrates that C and PC deposition are cumulative over time and that SH materials deposit more lipid than a CH FDA group IV material. It also clearly demonstrates that deposition of C and PC is influenced by the composition of the incubation solution and that *in vitro* models must use more physiologically relevant incubation solutions that mimic the natural tear film if *in vitro* data is to be extrapolated to the *in vivo* situation.

5.2 INTRODUCTION

In vitro models for examining deposition of tear film components onto contact lens materials have been used for many years.[1-10] These models have been used to examine the protein-lipid-mucin interactions that occur on the surface and in the tear film,[1-14] to examine the conformation of protein on the surface of a contact lens,[5, 15] to examine the wettability,[16] contact angle and hydrophobicity of a lens material,[16, 17] and to analyze the interaction between corneal cells and their interaction with contact lens materials and solutions,[18-20] to name just a few. Although *in vitro* models can never incorporate every element of human contact lens wear, they are indispensable for quickly characterizing and assessing new and commercially available materials and cleaning solutions.

The first *in vitro* models utilized were simple.[1-6, 21] These models included contact lens materials being incubated in a single lipid or protein saline solution for as little as 24 hours for an extended wear lens. Earlier analysis techniques[11, 22] have been updated or replaced with increasingly sensitive technology, including high performance liquid chromatography (HPLC), mass spectroscopy (MS), radiochemical assessments and matrix assisted laser desorption ionization mass spectroscopy (MALDI-MS). These techniques are just a few of the methods which are able to assess the interaction of various biological fluids with biomaterials, sometimes without extraction, and can often quantify components in the picogram range.

In the last 20 years, *in vitro* models of contact lens wear have started to become more complex. In the early 1990's, Mirejovsky spear-headed this by using a complex artificial tear solution which incorporated lipids, proteins, and mucin dissolved within a multi-component saline solution.[10] Since then, many researchers have begun adding more physiological chemicals to their *in vitro* contact lens interaction models. However, still more research needs to be completed to analyze the influence that different variables have on contact lens deposition, comfort, wettability, and vision.

Of interest is the composition of the tear film or the artificial tear solution used for *in vitro* studies. It is known that even though the general composition of lipids found in the human tear film and meibum are similar,[22-28] there are still quite a few individual differences between the specific lipids and their concentrations. [29-37]

Variations in tear film composition have been recorded between people, and an individual's lifestyle can contribute to the tear film's unique composition. A person's diet, work environment, medication, alcohol consumption, and any systemic disease can all greatly affect their tear film composition.[29-37] This poses unique challenges when trying to develop an *in vitro* model and an artificial tear solution (ATS) for that model. In addition, the cost of the components of the ATS may not justify their inclusion, if the results are not impeded by their absence. Therefore, some of the main questions that need to be answered before an *in vitro* contact lens deposition model incorporating an ATS is finalized are: How do the components of the ATS contribute to the deposition profile? How does altering the complexity of the solution change deposition?

In the mid to late 1990's, Bontempo and Rapp conducted a set of experiments analyzing lipid deposition and the effect of lipid and protein interactions on conventional hydrogel and rigid gas permeable (RGP) contact lens deposition.[11-14] Their research found that FDA group III contact lens materials deposit the least lipid and group II lenses, the high water non-ionic lenses, deposit the most lipid.[11] This finding led them to develop the "pull/push" theory to explain lipid deposition. [11, 38] In their theory, the "pull" represents the contact lens polymer material attracting the lipids into the matrix and away from the aqueous ATS and the "push" represents the water content of the lens encouraging the lipid to move into the matrix.[11, 38] Bontempo and Rapp also found that when both lipids and proteins are present in the ATS the deposition pattern was different than when the contact lenses were

exposed to proteins alone or lipids alone. For example, they found that protein deposition onto group IV contact lens material rendered the surface of the lens less hydrophilic, resulting in a subsequent increase in the deposition of lipids. In contrast, when non-polar lipids bound to the contact lens, the surface became more hydrophobic, resulting in decreased protein deposition.[12, 13] Differences in the deposition of specific lipids were also found, as more polar lipids deposited differently than non-polar lipids, depending on the complexity of the ATS.[13]

The competitive binding research completed by Bontempo and Rapp was a major contribution to the industry's knowledge of deposition and the relationship between lipids and protein in the tear film and on the surface of a contact lens. However, many years have passed and we now have new contact lens materials based on silicone. These materials behave very differently than conventional hydrogel contact lens materials and therefore more work needs to be completed to examine the competitive binding of tear film components on these materials. Even within the group of silicone hydrogel lenses, many differences exist. Some lenses are surface coated, some have internal wetting agents, some have no coatings or wetting agents, some are based on trimethylsiloxy siloxane TRIS derivatives, and some are a combination of new siloxane macromers. Due to the uniqueness of each of these new materials it cannot be assumed that they will behave as the conventional hydrogel lenses or the same as each other.

The purpose of this study was to examine the competitive binding of tear film components onto various contact lens materials by altering the composition of the

ATS being used. A radiochemical *in vitro* model and an ATS, previously optimized by our laboratory, will be used to examine cholesterol and phosphatidylcholine deposition specifically. In order to examine the role of different tear film components on deposition, four different tear solution compositions will be examined including: a protein + lipid ATS, protein only, lipid only and single lipid. One conventional and two silicone hydrogel lens materials will be examined.

5.3 METHODS

5.3.1 ARTIFICIAL TEAR SOLUTION (ATS)

Chapter 3 of this thesis has comprehensively outlined the protocol to prepare the ATS that will be utilized in this experiment and therefore only a brief overview will be given here. Table 5-1 displays each of the components of the ATS and the final concentrations that were utilized. Preparing an ATS solution involves several steps. First, all of the salt components were dissolved into the desired volume of MilliQ water to prepare the complex saline solution (CSS). To avoid precipitation of some of the components, all of the ingredients were dissolved in the order that they are listed in Table 5-1. Before a lipid tear solution could be made, all of the pure lipids required for the specific experiment were first dissolved into a (1:1) hexane: ether solution at high concentrations. This lipid stock solution was prepared previously and stored at -20^{o}C until needed. Once the lipid stock was prepared and was equilibrated to room temperature, it was utilized to make a lipid tear solution by dispensing an aliquot of the stock into the required volume of saline. In order to evaporate the hexane: ether and to ensure that the lipids were fully incorporated,

the lipid solution was sonicated at 37°C while being purged with nitrogen gas. Once the odour of solvent had dissipated, the radioactive lipid was then dispensed into the solution and further sonicated for 15 minutes. The radiolabeled cholesterol and phosphatidylcholine was added to the ATS at a concentration of 3% and 8.5% of the total individual lipid concentration respectively. The lipid portion of the ATS was now complete and the proteins and mucin were then dissolved into the solution to create the full ATS. In this experiment, a number of different tear incubation solutions were utilized and therefore not all of these steps were required, therefore the individual steps utilized were adjusted according to the desired incubation solution. The radioactive lipids details can be found in Table 5-2.

TABLE 5-1: ARTIFICIAL TEAR SOLUTION COMPONENTS

Salt component	**mM**	**Lipid Component**	**Concentration (mg/mL)**
Sodium chloride	90.0	Cholesterol	0.0018
Potassium chloride	16.0	Cholesteryl oleate	0.024
Sodium citrate	1.5	Oleic acid	0.0018
Glucose	0.2	Oleic acid methyl ester	0.012
Urea	1.2	Phosphatidylcholine	0.0005
Calcium chloride	0.5	Triolein	0.016
Sodium carbonate	12.0	**Protein Component**	**Concentration (mg/mL)**
Potassium hydrogen carbonate	3.0	Bovine Albumin	0.20
Sodium phosphate dibasic	24.0	Hen Egg Lysozyme	1.90
Hydrochloric acid (10 molar)	26.0	Bovine Mucin	0.15
		Lactoferrin	1.90
ProClin 300	0.2 µL/ 1L	Immunoglobulin G	0.02

TABLE 5-2: RADIOACTIVE LIPID CHARACTERISTICS

	Cholesterol [C]	**L-α-DiPalmitoyl-Phosphatidylcholine [PC]**
Lipid type	Sterol	Phospholipid
Molecular Formula	$C_{27}H_{46}O$	$C_{40}H_{80}NO_8P$
Molecular Weight (g/mol)	386.7	734.0
Position of Radiolabel	4-^{14}C	DiPalmitoyl-1-^{14}C
Specific Activity (mCi/mmol)	49.78	114
Polarity of Molecule	Non-polar	Polar
Charge of Lipid	Neutral	Zwitterionic head group
Manufacturer	Perkin-Elmer	Perkin-Elmer

5.3.2 CONTACT LENS MATERIALS

Three contact lens materials were tested (n=4) including: Acuvue® 2 [etafilcon A; Vistakon], Acuvue® OASYS™ [senofilcon A; Vistakon], and PureVision™ [balafilcon A; Bausch & Lomb]. The material characteristics of all contact lens materials can be found in Table 5-3.

TABLE 5-3: CONTACT LENS MATERIAL CHARACTERISTICS

Material Type	**Conventional Hydrogel**	**Silicone Hydrogel**	
USAN	**Etafilcon A**	**Balafilcon A**	**Senofilcon A**
Proprietary name	Acuvue®2	PureVision™	Acuvue® OASYS™
Manufacturer	Johnson & Johnson	Bausch & Lomb	Johnson & Johnson
Monomers	HEMA, MA	NVP, TPVC, NVA, PBVC	mPDMS, DMA, HEMA, siloxane macromer, EGDMA, PVP
Surface Modification	None	Plasma oxidation	PVP as an internal wetting agent
Oxygen Transmissibility ($x10^{-9}$)	31	110	147
Water Content	58%	36%	38%
FDA Class	Group IV	Group III	Group I

USAN: United States adopted name; DMA (N,N-dimethylacrylamide); EGDMA (ethyleneglycol dimethacrylate); HEMA (poly-2-hydroxyethyl methacrylate); MA (methacrylic acid); mPDMS (monofunctional polydimethylsiloxane); NVA (*N*-vinyl aminobutyric acid); NVP (N-vinyl pyrrolidone); PBVC (poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate]); PVP (poly(vinylpyrrolidone)); TPVC (tris-(trimethylsiloxysilyl) propylvinyl carbamate)

5.3.3 EXPERIMENTAL DESIGN

A flow chart outlining the experimental setup is shown in Figure 5-1. 6 mL glass incubation vials (Wheaton, VWR, Mississauga) were all incubated in non-radioactive ATS for at least 4 days at 37°C before lens incubation. This process allows lipid and protein to coat the interior vial surface of the vial. Previous work in our laboratory has found that pre-treatment of the vials is a necessary step to

ensure that the contact lens incubation solution components do not preferentially adsorb to the vial and are therefore available to deposit onto the lens. This experiment utilizes several different solution formulations for the incubation of lenses. The vial pre-treatment solution that was used was matched with the specific incubation solution that was to be used. Following pretreatment, the vial's solution was removed, saline was used to rinse the vial, and 6.0 mL of the prepared radioactive solution was inserted.

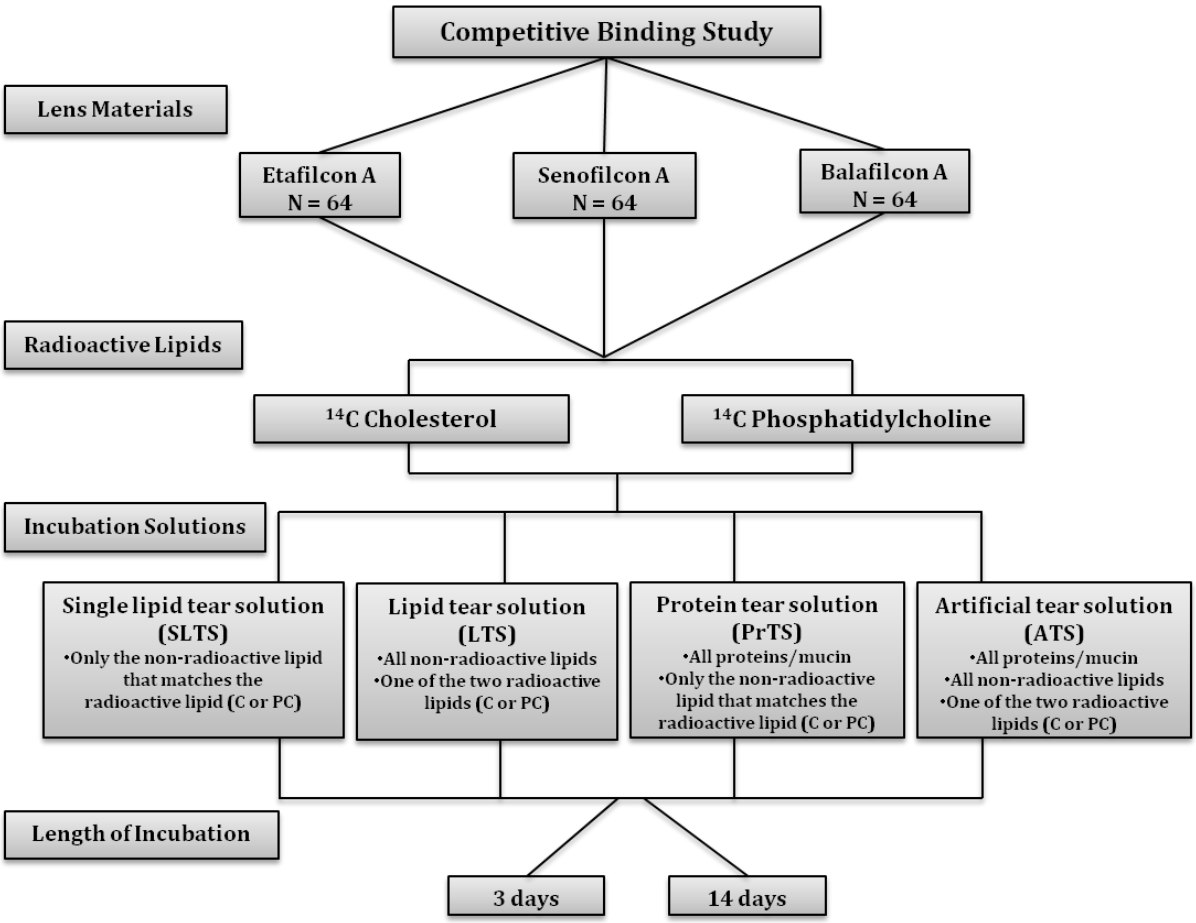
All contact lens materials were removed from their packaging solutions and then soaked in 12-well plates filled with CSS for at least 24 hours, to remove components of the individual blister pack solutions. After each lens was soaked, they were rinsed twice in CSS, blotted on lens paper and placed into their incubation vials that had been filled with one of four different incubation solution compositions: an ATS containing the full complement of lipids and proteins (ATS), a lipid only solution (LTS), a protein only solution (PrTS), and a single lipid solution (SLTS). Each of these solutions was tested with both radioactive lipids independently. The details of each of these solutions are outlined in Figure 5-1. The vials were capped, sealed with Parafilm®, and incubated at 37°C with shaking for 3 or 14 days. Four replicates of each lens material with each of the radioactive solutions were tested.

At the end of incubation times (3 or 14 days), each lens was rinsed twice in CSS, to remove loosely bound lipid. For extractions with the ^{14}C-cholesterol, the lenses were placed in 20 mL glass scintillation vials with 2 mL of 2:1 chloroform:

methanol extraction solution and incubated for three hours each at 37°C while shaking. Each lens was extracted twice and the extracts were pooled together for processing. For extractions with the ^{14}C-phosphatidylcholine, the lenses were placed in 20 mL glass scintillation vials with 2 mL of 60:50:1:4 chloroform: methanol: acetic acid: water extraction solution and were incubated for three hours each at 37°C while shaking. Once again, two extractions were completed and pooled together.

The contact lens extracts were then dried down using nitrogen evaporation, re-suspended in 1 mL of chloroform, sonicated for one minute, and 10 mL of Ultima Gold F scintillation fluor was added. The lens extract samples and prepared standard radioactive counts were counted for their radioactive signals using a Beckman-Coulter L6500 liquid scintillation counter. In the experiment, the radioactive lipid was used as a probe and the ratio of radioactive lipid to non-radioactive lipid in the incubating ATS was kept constant. Therefore, quantification of the total amount of cholesterol and phosphatidylcholine deposited was extrapolated and calculated using standard radioactive lipid calibration curves.

FIGURE 5-1: EXPERIMENTAL DETAILS AND DESIGN



5.3.4 STATISTICAL ANALYSIS

Statistical analysis was performed with Statistica 9, using repeated measures ANOVA and Tukey post-hoc analysis when required.

5.4 RESULTS

5.4.1 CHOLESTEROL

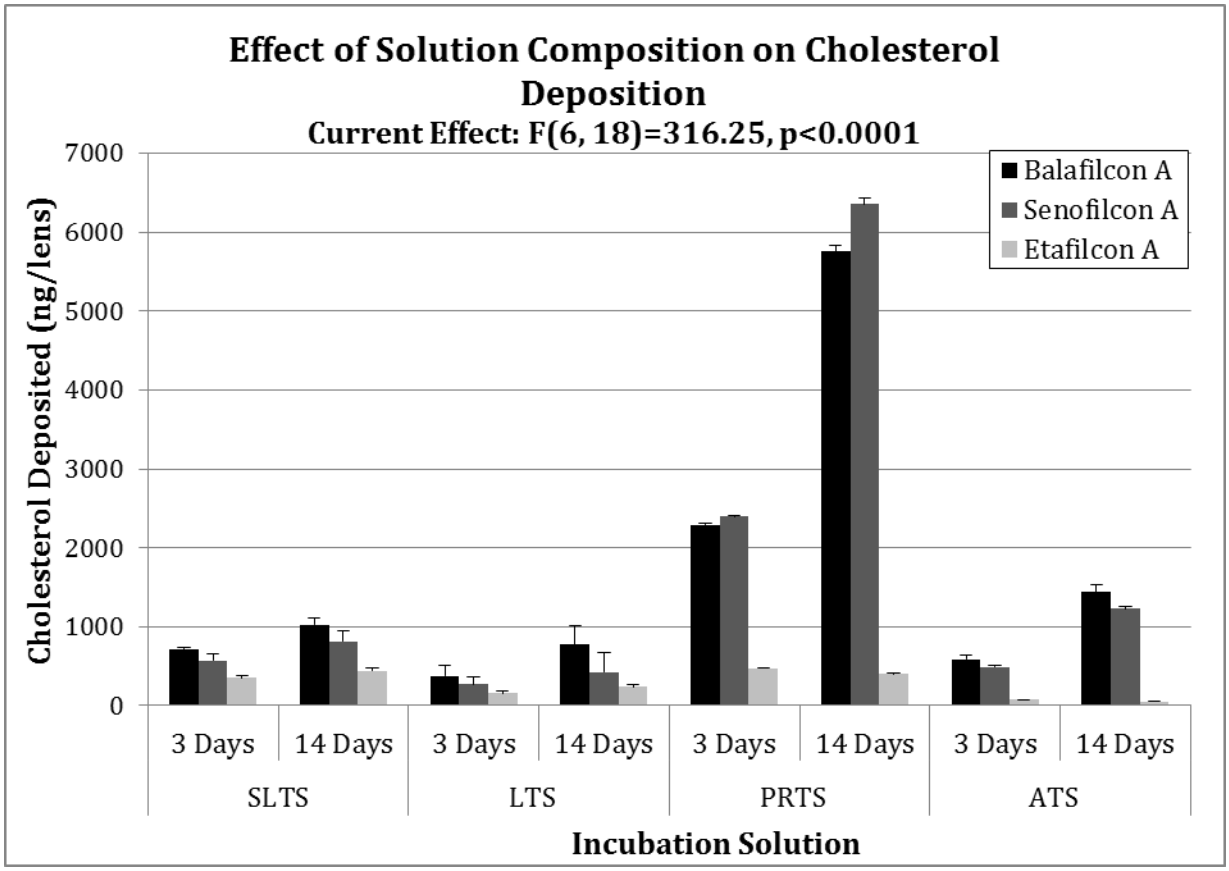
The results for cholesterol deposition on each of the three contact lens materials and for each of the four incubation solutions tested are found in Figure 5-2 and Table 5-4. Overall, after 14 days, balafilcon A and senofilcon A lenses deposited the most cholesterol when incubated in the PrTS (5754.73 ± 245.23 ng/lens and 6353.08 ± 255.19 ng/lens respectively), and the least in the lipid only LTS solution (770.19 ± 68.98 ng/lens and 423.73 ± 84.57 ng/lens respectively). Etafilcon A deposited the least amount of cholesterol for all incubation solutions.

TABLE 5-4: CHOLESTEROL DEPOSITION WITH VARIOUS LEVELS OF COMPLEX ARTIFICIAL TEAR SOLUTIONS

		Lens Material		
Solution	**Time**	**Balafilcon A**	**Senofilcon A**	**Etafilcon A**
SLTS	**3 Days**	716.71 ± 25.06	567.55 ± 86.32	354.28 ± 27.68
	14 Days	1027.30 ± 79.21	813.72 ± 128.82	439.06 ± 38.10
LTS	**3 Days**	378.21 ± 12.79	272.45 ± 8.77	155.98 ± 8.94
	14 Days	770.19 ± 68.98	423.73 ± 84.57	235.58 ± 11.53
PrTS	**3 Days**	2290.74 ± 124.61	2392.46 ± 88.59	477.17 ± 31.61
	14 Days	5754.73 ± 245.23	6353.08 ± 255.19	401.85 ± 24.59
ATS	**3 Days**	585.97 ± 51.33	491.05 ± 20.24	73.37 ± 4.70
	14 Days	1440.59 ± 97.21	1229.32 ± 21.84	56.02 ± 2.56

PrTS = protein tear solution, SLTS = single lipid tear solution, LTS = lipid tear solution, ATS = artificial tear solution

FIGURE 5-2: EFFECT OF SOLUTION COMPOSITION ON CHOLESTEROL DEPOSITION



PrTS = protein tear solution, SLTS = single lipid tear solution, LTS = lipid tear solution, ATS = artificial tear solution

The results of the repeated measures ANOVA are seen in Table 5-5. The repeated measures ANOVA results found statisticaly significant differences between all of the variables tested (lens materials, incubation time, and incubation solution) and the interactions between these variables as well.

TABLE 5-5: REPEATED MEASURES ANOVA RESULTS FOR CHOLESTEROL DEPOSITION

	SS	DF	MSq	F	p
Lens Material	37219611	2	18609805	1834.10	***<0.00001***
Incubation Time	17303371	1	17303371	1726.34	***0.000031***
Incubation Solution	103849125	3	34616375	3001.30	***<0.00001***
Lens*Time	8291614	2	4145807	545.20	***<0.00001***
Lens*Solution	44250798	6	7375133	911.62	***<0.00001***
Time*Solution	20892548	3	6964183	640.64	***<0.00001***
Lens*Time*Solution	12140883	6	2023480	316.25	***<0.00001***
Error	115171	18	6398		

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

When examining the specific solutions in which the lenses were incubated, it was found that cholesterol deposition on balafilcon A and senofilcon A followed the order of PrTS > SLTS > ATS > LTS after 3 days and PrTS > ATS > SLTS > LTS after 14 days of incubation. However, etafilcon A deposited the most cholesterol when incubated in the PrTS > SLTS > LTS > ATS after 3 days and SLTS > PrTS > LTS > ATS 14 days. Etafilcon A cholesterol deposition was lower and more variable between the time points and solutions. The orders listed above represent the ranking for the overall deposition amounts. However when the statistical differences between the

individual solutions are examined using a Tukey Post-hoc analysis (Table 5-6), it is found that not all solution comparisons were statistically different.

TABLE 5-6: SOLUTION COMPARISONS FROM TUKEY POST-HOC ANALYSIS FOR CHOLESTEROL DEPOSITION

	3 Day			14 Day		
	Balafilcon A	**Senofilcon A**	**Etafilcon A**	**Balafilcon A**	**Senofilcon A**	**Etafilcon A**
SLTS vs LTS	***0.0018***	***0.0075***	0.1787	***0.0274***	***0.0005***	0.1535
SLTS vs PrTS	***0.0002***	***0.0002***	0.8403	***0.0002***	***0.0002***	1.0000
SLTS vs ATS	0.7719	0.9981	***0.0122***	***0.0003***	***0.0003***	***0.0005***
LTS vs PrTS	***0.0002***	***0.0002***	***0.0031***	***0.0002***	***0.0002***	0.4120
LTS vs ATS	0.1351	0.0967	0.9953	***0.0002***	***0.0002***	0.2985
PrTS vs ATS	***0.0002***	***0.0002***	***0.0004***	***0.0002***	***0.0002***	***0.0014***

PrTS = protein tear solution, SLTS = single lipid tear solution, LTS = lipid tear solution, ATS = artificial tear solution

5.4.2 PHOSPHATIDYLCHOLINE

The results for phosphatidylcholine (PC) deposition on each of the three contact lens materials and for each of the four incubation solutions tested are found in Figure 5-3 and Table 5-7. Overall, after 14 days, balafilcon A and senofilcon A lenses deposited the most PC when incubated in the SLTS (581.39 ± 85.32 ng/lens and 366.95 ± 62.17 ng/lens respectively), and the least in the lipid only LTS solution

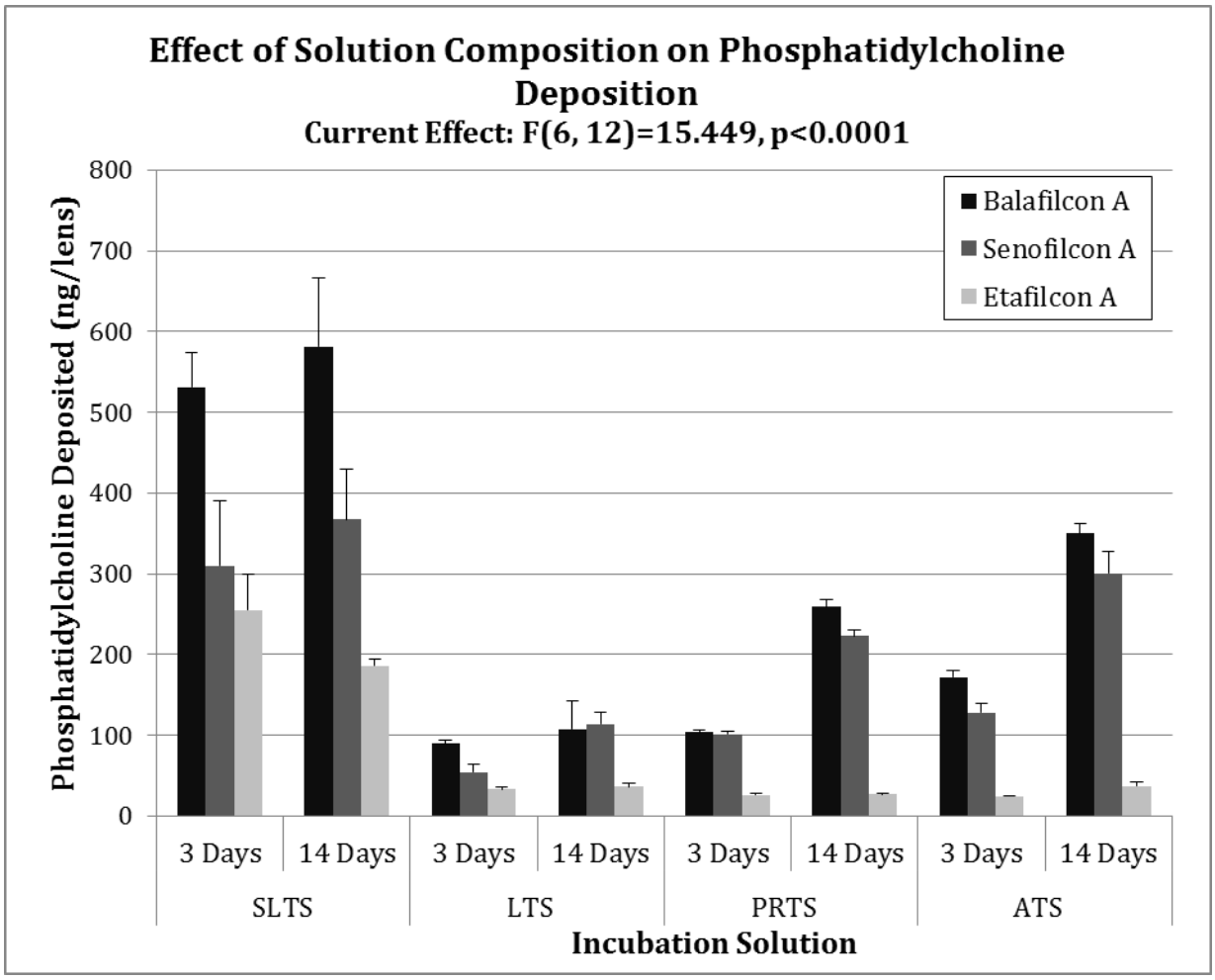
(107.89 ± 34.75 ng/lens and 114.25 ± 15.03 ng/lens respectively). Etafilcon A deposited the least amount of PC for all incubation solutions.

TABLE 5-7: PHOSPHATIDYLCHOLINE DEPOSITION WITH VARIOUS LEVELS OF COMPLEX ARTIFICIAL TEAR SOLUTIONS

		Lens Material		
Solution	**Time**	**Balafilcon A**	**Senofilcon A**	**Etafilcon A**
SLTS	**3 Days**	531.12 ± 42.98	309.68 ± 81.44	254.90 ± 44.75
	14 Days	581.39 ± 85.32	366.95 ± 62.17	186.13 ± 8.86
LTS	**3 Days**	89.87 ± 3.89	54.62 ± 9.94	33.54 ± 2.32
	14 Days	107.89 ± 34.75	114.25 ± 15.03	36.24 ± 3.74
PrTS	**3 Days**	103.75 ± 2.91	101.84 ± 3.89	25.84 ± 2.18
	14 Days	258.82 ± 9.56	222.80 ± 8.06	26.75 ± 1.81
ATS	**3 Days**	172.33 ± 8.44	127.91 ± 11.50	24.44 ± 1.04
	14 Days	350.12 ± 11.48	300.31 ± 27.53	37.49 ± 4.09

PrTS = protein tear solution, SLTS = single lipid tear solution, LTS = lipid tear solution, ATS = artificial tear solution

FIGURE 5-3: EFFECT OF SOLUTION COMPOSITION ON PHOSPHATIDYLCHOLINE DEPOSITION



PrTS = protein tear solution, SLTS = single lipid tear solution, LTS = lipid tear solution, ATS = artificial tear solution

The results of the repeated measures ANOVA are seen in Table 5-8. The repeated measures ANOVA results found statisticaly significant differences between all of the variables tested (lens materials, incubation time, and incubation solution) and the interactions between these variables as well.

TABLE 5-8: REPEATED MEASURES ANOVA RESULTS FOR PHOSPHATIDYLCHOLINE DEPOSITION

	SS	DF	MSq	F	p
Lens Material	460563	2	230282	107.732	***0.00032***
Incubation Time	68119	1	68119	64.840	***0.01508***
Incubation Solution	980088	3	326696	3752.869	***<0.00001***
Lens*Time	52333	2	26166	147.983	***0.00018***
Lens*Solution	146434	6	24406	9.952	***0.00045***
Time*Solution	41209	3	13736	10.554	***0.00830***
Lens*Time*Solution	15374	6	2562	15.449	***0.00005***
Error	1990	12	166		

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

When examining the specific solutions in which the lenses were incubated, it was found that PC deposition on balafilcon A and senofilcon A followed the order of SLTS > ATS > PrTS > LTS after 3 days and 14 days of incubation. However, etafilcon A deposited the most PC when incubated in the after 3 days SLTS > LTS > PrTS > ATS and SLTS > ATS > LTS > PrTS 14 days. Etafilcon A PC deposition was lower and more variable between the time points and solutions. The orders listed above represent the ranking for the overall deposition amounts. However when the statistical differences between the individual solutions are examined using a Tukey Post-hoc analysis (Table 5-9), it is found that not all solution comparisons are statistically different.

TABLE 5-9: SOLUTION COMPARISONS FROM TUKEY POST-HOC ANALYSIS FOR PHOSPHATIDYLCHOLINE DEPOSITION

	3 Day			14 Day		
	Balafilcon A	**Senofilcon A**	**Etafilcon A**	**Balafilcon A**	**Senofilcon A**	**Etafilcon A**
SLTS vs LTS	***0.0002***	***0.0002***	***0.0002***	***0.0002***	***0.0002***	***0.0002***
SLTS vs PrTS	***0.0002***	***0.0002***	***0.0002***	***0.0002***	***0.0002***	***0.0002***
SLTS vs ATS	***0.0002***	***0.0002***	***0.0002***	***0.0002***	***0.0034***	***0.0002***
LTS vs PrTS	0.9974	0.0836	1.0000	***0.0002***	***0.0002***	1.0000
LTS vs ATS	***0.0007***	***0.0017***	1.0000	***0.0002***	***0.0002***	1.0000
PrTS vs ATS	***0.0033***	0.5227	1.0000	***0.0005***	***0.0003***	1.0000

PrTS = protein tear solution, SLTS = single lipid tear solution, LTS = lipid tear solution, ATS = artificial tear solution

5.5 DISCUSSION

This study was designed to examine lipid binding to contact lenses in the presence or absence of other macromolecules. Specifically, the contact lens competitive binding profiles for cholesterol and phosphatidylcholine were examined by varying the components and complexity of the incubation solutions used, through use of a radiochemical carbon-14 *in vitro* model. Many other researchers are now beginning to incorporate a more complex artificial tear fluid into their *in vitro* models for material, deposition and solution testing. However, the question remains whether this complex and expensive artificial human tear analog

is really necessary and if it adds any pertinent information to the research conducted.

The results from this study show quite clearly that experiments performed with simple, moderately complex or complex incubation solutions will exhibit different deposition results. Cholesterol and phosphatidylcholine behaved differently in their deposition profiles between the four different incubation solutions and between silicone hydrogel and conventional hydrogel contact lens materials. For the two silicone hydrogel contact lens materials, cholesterol deposition was highest when the lenses were incubated in the PrTS, followed by the ATS then SLTS. The lowest deposition was found with the LTS incubation. This implies that the cholesterol is out-competed for binding sites when it is in the presence of other lipids, but when protein is present in the solution and most likely depositing on the material, protein deposition increases cholesterol deposition, as is seen with the deposition profile in the PrTS and ATS. This is likely occurring because protein deposition and denaturation is making the lens surface more hydrophobic during binding, thus providing additional binding sites for cholesterol. Protein denaturation is thought to be more prevalent on silicone hydrogels than on conventional hydrogel materials, where a much higher percentage of the protein remains in its native state.[5, 39, 40] This trend of increasing non-polar lipid deposition was also seen by Bontempo and Rapp in their solution composition studies with conventional hydrogel lenses.[12-14]

When examining the cholesterol deposition on etafilcon A, a different trend was seen. In this case, when incubating in the SLTS solution for 14 days, the greatest amount of lipid was deposited, followed closely by the PrTS. Incubation in the LTS deposited significantly less cholesterol and the ATS incubation deposited only minute amounts of cholesterol. However, the only statistical differences in cholesterol deposition on etafilcon A were found between the SLTS solution and the other three. Therefore, these results imply that cholesterol is easily out competed for the hydrophobic binding sites by other non-polar lipids on this material and that protein deposition does more to encourage deposition than other lipids.

When examining the phosphatidylcholine deposition a different trend of deposition was seen. The highest amount of PC was deposited on all materials using the SLTS solution, followed by ATS, PrTS and the LTS deposited the least amount of PC. This order of deposition was statistically significant for both silicone hydrogel materials, but this was not the case for etafilcon A. For the two silicone hydrogel lens materials, phosphatidylcholine was deposited in the highest masses when it did not have to compete with any other tear film constituents, proteins or lipids alike. PC is in fairly low concentration in the tear film and does not have a strong attraction toward these hydrophobic materials, and thus is easily out competed by more prevalent, hydrophobic and attractive lipids and proteins. This is evident when incubating in the LTS, as the other lipids available in the solution restrict the deposition of PC. When PC is surrounded with proteins, as is the case when incubating in the PrTS and ATS, PC deposition decreases when compared to SLTS

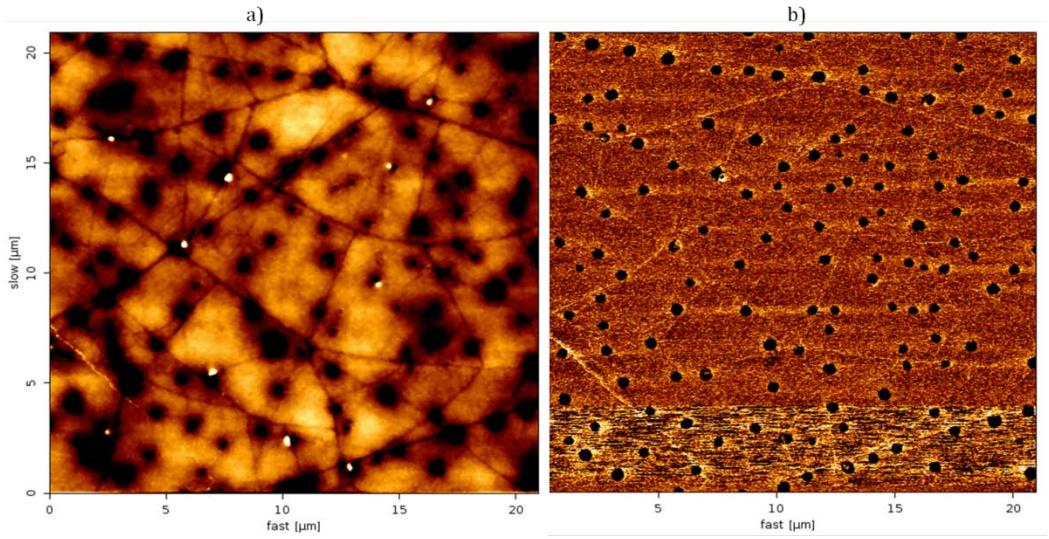
deposition levels, but increase significantly when compared to the LTS. This shows that protein deposition alters the lens surface chemistry so that it is less hydrophilic and thus creates more sites for PC to bind.

Etafilcon A results show a similar trend, with higher deposition of PC occurring when it is the only lipid present, however no significant differences were seen in deposition masses for the other three solutions, $p>0.05$. Therefore, this results shows that phosphatidylcholine has little affinity for etafilcon A, especially with other tear film components present.

When the overall deposition relating to lens material is analyzed, it is seen that balafilcon A usually accumulates the most lipid for both cholesterol and PC; however senofilcon A deposited the most cholesterol when incubating in the PrTS. Balafilcon A's propensity to deposit higher masses of lipid has also been found by other researchers, and has been attributed to both its polymer composition, polymeric structure and its plasma oxidation process.[40-42] As seen in Table 5-3, one of the monomeric constituents of balafilcon A is N-vinyl pyrrolidone (NVP),[43-46] a monomer known to be lipophilic and a cause of increased lipid deposition for FDA Group II conventional hydrogel lens materials[12, 13, 47, 48] which incorporate it. In addition to NVP, the incorporation of silicone, a very hydrophobic molecule also increases the lens' lipophilic nature. In order to mask the hydrophobic matrix of balafilcon A, the lens is subjected to a plasma oxidation process which converts the surface into silicate.[49] However, this does not create a continuous silicate surface but creates silicate "glassy" islands across the entire surface of the lens, Figure 5-

4a.[49] Therefore, there are portions of the polymer surface that have not been converted into silicate and are therefore still relatively hydrophobic. Finally, the balafilcon A material also contains a vast amount of pores, Figure 5-4b.[44, 50-53] These pores are classified as macropores and are much larger in size than what is common in other silicone hydrogels as network pores.[44, 51, 52] These macropores are thought to be continuous from the anterior to the posterior surface of the lens material and therefore are another area for lipid to deposit.[44, 51, 52] For a lipid such as cholesterol, which is non-polar, balafilcon A still provides many available hydrophobic sites and thus results in higher levels of cholesterol deposition. When analyzing phosphatidylcholine deposition, balafilcon A is also an ideal deposition matrix. Phosphatidylcholine is an amphiphilic molecule that contains both a hydrophilic "water loving" head group (in this case choline), and a hydrophobic "water hating" tail group which contains two fatty acid chains, one saturated and one unsaturated. This dual nature of PC allows it to then deposit not only on the hydrophobic portions of the surface, but the more hydrophilic silicate islands as well.

FIGURE 5-4: ATOMIC FORCE MICROSCOPY IMAGES TAKEN FROM BALAFILCON A. A) IMAGE TAKEN IN CONTACT MODE B) IMAGE TAKEN IN PHASE CONTRAST MODE. 20X 20µM



Senofilcon A, is a second generation silicone hydrogel lens and unlike balafilcon A, it does not have a silicate surface coating. However, it does have an internal wetting agent incorporated into its polymeric structure which is designed to act as an umbrella and potentially "hide" the hydrophobic silicone properties of the lens. This wetting agent is a high molecular weight molecule of poly vinyl pyrrolidone (PVP). [54] PVP is a polymer of the monomer NVP, which is known to be lipophilic in nature (as discussed with balafilcon A), and thus senofilcon A also deposits increased amounts of cholesterol and phosphatidylcholine, when compared with a conventional hydrogel such as etafilcon A.

Etafilcon A, is a FDA Group IV conventional hydrogel material composed of poly-2-hydroxyethyl methacrylate (HEMA) and methacrylic acid (MA). Conventional hydrogel materials based on HEMA have a long history of proving to be relatively

low lipid depositors, especially when compared with silicone hydrogels. However, ionic materials such etafilcon A tend to deposit large amounts of proteins, specifically lysozyme. This is because the MA in etafilcon A gives the lens material a net negative charge and therefore attracts positively-charged lysozyme through electrostatic interactions.[3, 55, 56] These electrostatic interactions cause heavy deposits of lysozyme onto these materials, thus allowing little lipid to deposit in comparison.

Much of the other *in vitro* lipid work completed recently has quantified higher masses of cholesterol and phospholipids (either phosphatidylcholine or phosphatidylethanolamine) depositing on these materials. *In vitro w*ork from Carney *et al*.,[1] and Pucker *et al*.,[6] 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 different incubation solutions, higher concentrations of lipids in the ATS, different incubation volumes, different incubation times and replenishment of the ATS with fresh solution during incubation.[1, 6, 9] All of these factors may explain the higher deposition of cholesterol and phosphatidylcholine

When the cholesterol and phosphatidylcholine deposition results found in this *in vitro* experiment are compared with recent *ex vivo* data, it is seen that the results tend to fall within the same range of deposition. However direct comparisons should not be made as the wear time of the *ex vivo* lenses are much longer than the incubation time of this experiment, *ex vivo* lenses are cleaned nightly, and it would

be incorrect to extrapolate the data.[42, 57] 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, and some of them examined different lipids than those quantified in this experiment.

The results from this experiment show that the composition of the incubation solution, the lipids under examination, length of incubation and the lenses utilized will all have an influence on the overall deposition profile. The interactions between the components of the tear film and the contact lens surface will dictate the deposition that occurs. If *in vitro* models are really meant to mimic *in vivo* conditions than it is imperative that more complex models are utilized and that every attempt is made to make the *in vitro* conditions as similar as possible to human contact lens wear. It is only by completing these types of experiments that we can improve an *in vitro* model's usefulness and systematically explore the relationships that are occurring during human contact lens wear and then test and incorporate them into the *in vitro* models. In the end, *in vitro* models need to become more physiologically relevant so that their use can be validated and provide a solid foundation for further research and development of new and existing products.

5.6 CONCLUSION

This *in vitro* study demonstrates that cholesterol and phosphatidylcholine deposition is cumulative over time and that SH materials deposit more lipid than a CH FDA group IV material. It also clearly demonstrates that deposition of cholesterol

and phosphatidylcholine is influenced by the composition of the incubation medium. Specifically, cholesterol exhibited significant increases in deposition with protein rich incubation solutions, however significant competition with other lipids decreased deposition in the lipid rich LTS and ATS solutions. Phosphatidylcholine deposited extremely well when it was the only component in the incubation solution, only moderately well with a protein rich ATS and ATS solutions and very poorly when it competes with other lipids in the LTS.

These results prove that *in vitro* models must use more physiologically relevant incubation solutions that mimic the natural tear film if *in vitro* data is to be extrapolated to the in vivo situation.

In the next chapter, the efficiency of hydrogen peroxide cleaning systems to remove bound cholesterol and phosphatidylcholine from three different contact lens materials was examined. Lenses were incubated in an artificial tear solution for eight hours and were then cleaned in one of two commercially available hydrogen peroxide cleaning systems. This cycling was completed for seven days and following the last cycle the lenses were extracted, analyzed and compared to control lenses.

6 USING AN *IN VITRO* MODEL OF LIPID DEPOSITION TO ASSESS THE EFFICIENCY OF HYDROGEN PEROXIDE SOLUTIONS TO REMOVE LIPID FROM VARIOUS CONTACT LENS MATERIALS

Holly Lorentz, Miriam Heynen, Helen Tran, Lyndon Jones

	Concept / Design	Acquisition of data	Analysis	Write-up / publication
Lorentz	Y	Y	Y	Y
Heynen	Y	Y	Y	Y
Tran	-	Y	Y	-
Jones	Y	-	-	Y

Figures: 3

Tables: 10

6.1 OVERVIEW

Purpose: To test the ability of two commercially available hydrogen peroxide disinfection solutions to remove lipid from conventional and silicone hydrogel contact lens materials incubated in an artificial tear solution (ATS) using radiochemical experiments.

Methods: Etafilcon A, senofilcon A and balafilcon A contact lens materials were incubated in an ATS solution containing a mixture of lipids, proteins, mucin and either ^{14}C-cholesterol or ^{14}C-phosphatidylcholine for 8 hours. Following incubation, the lenses were removed, rinsed, and placed for 16 hours in either ClearCare®, AOSept® or stored without solution (control). This process was repeated every day for one week until completion. The lenses were extracted with a previously optimized extraction protocol, evaporated, re-suspended, fluor added and counted for their radioactive signals. Masses of lipids deposited were calculated based on standard calibration curves, the disinfection solutions were compared and repeated measures ANOVA and post-hoc statistical analysis was completed using Statistica 9.

Results: The results of this experiment found that daily disinfection with hydrogen peroxide solutions reduced the amount of cholesterol and phosphatidylcholine deposited on the three contact lens materials examined, however in many cases the reduction in deposition was less than 15% when compared to the control. Disinfection with ClearCare resulted in the least deposited cholesterol and phosphatidylcholine for all materials, however not all of the comparisons were statistically significant.

Conclusions: Overall, the ClearCare hydrogen peroxide disinfection solution removed the most lipid from lenses when compared to AOSept or the control, for both lipids and all lens materials. However, the differences found were quite small at times and whether these differences are clinically significant are yet to be determined.

6.2 INTRODUCTION

In the mid-1990's, hydrogen peroxide and chlorine-based contact lens cleaning systems had a predominant stance in the market, comprising almost half of all cleaning regimes prescribed.[1, 2] Since then, the two-step hydrogen peroxide, requiring a second neutralization step, and chlorine-based systems have become basically obsolete and the one-step hydrogen peroxide systems have experienced a drastic drop in use due to the growing popularity of multipurpose solutions.[1, 2] Multipurpose solutions now account for over 85%[1, 2] of the market for contact lens cleaning and disinfection solutions and for many years have been the preference of patients due to their simplicity, ease of use, and speed of use. However, in recent years there have been two worldwide recalls of multipurpose solutions due to microbial keratitis outbreaks[3-5] and practitioners have seen an increased prevalence of corneal staining with the use of polyhexamethyl biguanide (PHMB)[6-8] containing multipurpose solutions in combination of certain lenses. Due to these factors, there has been a resurgence of interest in hydrogen peroxide systems, which had been a "gold standard" for years due to their disinfection power.[9, 10]

Historically, hydrogen peroxide-based systems have been very simplistic in their composition, with mainly buffers plus 3% hydrogen peroxide. However, over the past decade manufacturers have begun adding surfactants and various wetting agents to their hydrogen peroxide solutions. Of particular note are the surfactants, which are designed to aid in removal of debris, deposits and microorganisms[11-13] as well as enhance the wettability of the contact lens, specifically silicone hydrogel contact lenses.[14, 15] However, surfactants are known to vary in their ability to remove particular deposits, especially lipids.[16, 17]

Yearly, more and more practitioners are fitting their patients with silicone hydrogel lens materials. [18] These materials are superior to the conventional hydrogel materials in their marked increase in oxygen transmissibility; [19-22] however the incorporation of silicone into these materials results in a more hydrophobic or lipid-loving surface. [23-28] With lipid deposition-prone lenses becoming more prevalent in the market and contact lens cleaning solutions not necessarily designed to remove lipid, there is concern about the amount of lipid accumulating on the surface and in the matrix of lens materials, and its effects on comfort, vision, and wettability for the contact lens wearers. Specifically, the efficiency of hydrogen peroxide-based solutions to remove lipid from silicone hydrogel contact lenses has been questioned and of particular note is whether the addition of a surfactant into a hydrogen peroxide solution truly aids in lipid removal.

In vitro models of tear film deposition on contact lenses can be utilized to evaluate the cleaning efficiency of new experimental formulations and commercially available contact lens cleaning and disinfection solutions. The value of assessing contact lenses and contact lens cleaning and disinfection solutions via an *in vitro* model lay in its ability to control many experimental variables, eliminate the need for participant recruitment and remuneration, and the ability to test many conditions simultaneously, for example many different incubation periods. The other main advantage of using *in vitro* models for contact lens deposition and the effectiveness of contact lens disinfection solutions is that control "uncleaned" contact lenses deposited with tear film components can be prepared and then directly compared to the cleaned and disinfected lenses, allowing a true assessment of the efficiency of the disinfection solution.

The purpose of this experiment was to use the radioactive *in vitro* model of lipid deposition previously developed by our laboratory to compare cleaning efficacies of two hydrogen peroxide disinfection solutions on various lens materials, relative to uncleaned lenses.

6.3 METHODS

6.3.1 ARTIFICIAL TEAR SOLUTION

An abridged version of the artificial tear solution (ATS) preparation protocol will be described in this section, as the in-depth protocol has been previously

discussed[29] in Chapter 3. Table 6-1 and 6-2 lists all of the non-radioactive and radioactive ATS components and their concentrations. In general, the ATS preparation involves several steps beginning with the making of the salt solution, which acts as the base of the ATS. All components of the salt solution were dissolved into MilliQ water in the order listed in Table 6-1. Next, a hexane: ethyl ether (1:1) solution was used to dissolve pure lipids together in order to prepare a concentrated lipid stock solution. Larger volumes of this solution were made and frozen for future experiments. An aliquot of the lipid stock solution was then pipetted into the desired volume of salt solution. In order to evaporate off the hexane: ether and to create a solution of lipid homogeneity, the lipid salt solution, was purged with nitrogen while sonicating at 37°C, until the odour of the solvent had dissipated. Then the radioactive lipid was added into the solution and further sonicated for 15 minutes. The radiolabeled cholesterol and phosphatidylcholine was added to the ATS at a concentration of 3% and 8.5% of the total individual lipid concentration respectively. Once the lipid solution was complete, the ATS was finished by dissolving the proteins and mucin into the solution and sonicating the final solution for one to two minutes.

TABLE 6-1: ARTIFICIAL TEAR SOLUTION COMPONENTS

Salt component	mM	Lipid Component	Concentration (mg/mL)
Sodium chloride	90.0	Cholesterol	0.0036
Potassium chloride	16.0	Cholesteryl oleate	0.048
Sodium citrate	1.5	Oleic acid	0.0036
Glucose	0.2	Oleic acid methyl ester	0.024
Urea	1.2	Phosphatidylcholine	0.001
Calcium chloride	0.5	Triolein	0.032
Sodium carbonate	12.0	**Protein Component**	**Concentration (mg/mL)**
Potassium hydrogen carbonate	3.0	Bovine Albumin	0.20
Sodium phosphate dibasic	24.0	Hen Egg Lysozyme	1.90
Hydrochloric acid (10 molar)	26.0	Bovine Mucin	0.15
		Bovine Immunoglobulin G	0.02
ProClin 300	0.2 µL/ 1L	Bovine Lactoferrin	0.90

TABLE 6-2: RADIOACTIVE LIPID CHARACTERISTICS

	Cholesterol [C]	L-α-DiPalmitoyl-Phosphatidylcholine [PC]
Radiolabel and Position	4-14C	DiPalmitoyl-1-14C
Molecular Weight (g/mol)	386.7	734.0
Specific Activity (mCi/mmol)	49.78	114
Supplier	Perkin-Elmer	Perkin-Elmer

6.3.2 CONTACT LENS MATERIALS AND DISINFECTION SOLUTIONS

Three contact lens materials were tested: Acuvue® 2 [etafilcon A; Vistakon (ETA)], Acuvue® OASYS™ [senofilcon A; Vistakon (SEN)], and PureVision™ [balafilcon A; Bausch & Lomb (BAL)](Table 6-3). The disinfection solutions tested were: ClearCare® and AOSept®, both manufactured by CIBA Vision (Table 6-4).

TABLE 6-3: CONTACT LENS MATERIAL CHARACTERISTICS

Material Type	**Conventional Hydrogel**	**Silicone Hydrogel**	
USAN	**Etafilcon A**	**Balafilcon A**	**Senofilcon A**
Proprietary name	Acuvue®2	PureVision™	Acuvue® OASYS™
Manufacturer	Johnson & Johnson	Bausch & Lomb	Johnson & Johnson
Monomers	HEMA, MA	NVP, TPVC, NVA, PBVC	mPDMS, DMA, HEMA, siloxane macromer, EGDMA, PVP
Surface Modification	None	Plasma oxidation	PVP as an internal wetting agent
Dk/t (x10^{-9})	31	110	147
Water Content	58%	36%	38%
FDA Class	Group IV	Group III	Group I

Dk/t: oxygen transmissibilityUSAN: United States adopted name; DMA (N,N-dimethylacrylamide); EGDMA (ethyleneglycol dimethacrylate); HEMA (poly-2-hydroxyethyl methacrylate); MA (methacrylic acid); mPDMS (monofunctional polydimethylsiloxane); NVA (*N*-vinyl aminobutyric acid); NVP (N-vinyl pyrrolidone); PBVC (poly[dimethysiloxy] di [silylbutanol] bis[vinyl carbamate]); PVP (poly(vinylpyrrolidone)); TPVC (tris-(trimethylsiloxysilyl) propylvinyl carbamate)

TABLE 6-4: HYDROGEN PEROXIDE SOLUTION PROPERTIES

Solution	ClearCare®	AOSept®
Manufacturer	CIBA Vision	CIBA Vision
Disinfectant	3% hydrogen peroxide	3% hydrogen peroxide
Surfactant	Pluronic 17R4	none
Other Components	Phosphonic acid, sodium chloride, phosphate buffer system	Phosphonic acid, sodium chloride, phosphate buffer system
Neutralization Method	Platinum Disc	Platinum Disc

6.3.3 INCUBATION VIAL PREPARATION

All 6 mL borosilicate glass incubation vials were pre-conditioned with non-radioactive ATS with shaking at 37°C for at least four days. This pre-conditioning allows the vial surface to become coated with lipid and protein prior to contact lens incubation, thereby allowing the full concentration of lipid and protein in the radioactive ATS incubation solution to be available. Following pre-conditioning, the vials were emptied, rinsed out with saline, and the fresh radioactive contact lens incubation solution was then be dispensed into the vials.

6.3.4 EXPERIMENTAL DESIGN

A flow chart explaining the seven day experimental design can be found in Figure 6-1. On the first morning, all contact lenses were removed from their blister pack solution and placed directly into 1.5 mL of the incubation ATS containing of

one of the two radiolabeled lipids: ^{14}C-cholesterol or ^{14}C-phosphatidylcholine. Each lens material was incubated in the radioactive ATS at 37^{o}C with gentle shaking for the eight hours, which is intended to represent a normal time for a patient wearing a contact lens. After the 8 hours of incubation, all lens materials were removed from the incubation solution, rinsed in three successive 250mL beakers of complex salt solution (CSS), and gently dabbed on lens paper to remove excess solution. The rinsed lenses were then placed into the appropriately marked left/right domed lens holder (2 lenses per holder, 3 holders per solution; n=6) containing 10 mL of either ClearCare (CC) or AOSept (AO). The lens holder was then inserted into the disinfection solution and the lens case was tightened. The lens cases were kept at room temperature for 16 hours (overnight). A set of control lenses were also processed alongside disinfected lenses. These controls (CNRL) represented the total deposition of lipid on lenses that were not disinfected daily. The control lenses were rinsed using the same protocol, but instead of being placed in disinfection solution, lenses were returned to their original incubation vials, which had been rinsed and emptied of the incubation ATS. The lenses, in their emptied vials, were then stored at 4°C overnight. This was 1 cycle.

The next morning, following the disinfection cycle, the lenses were removed from the disinfection solutions and rinsed as previously described and placed back into the original incubation vials containing fresh radioactive ATS to start the next cycle. The fresh radioactive ATS was directly added to the control vials and all of the lenses were incubated at 37^{o}C for another 8 hrs. The empty contact lens holders and lens cases were cleaned by rinsing them with hot running tap water followed by

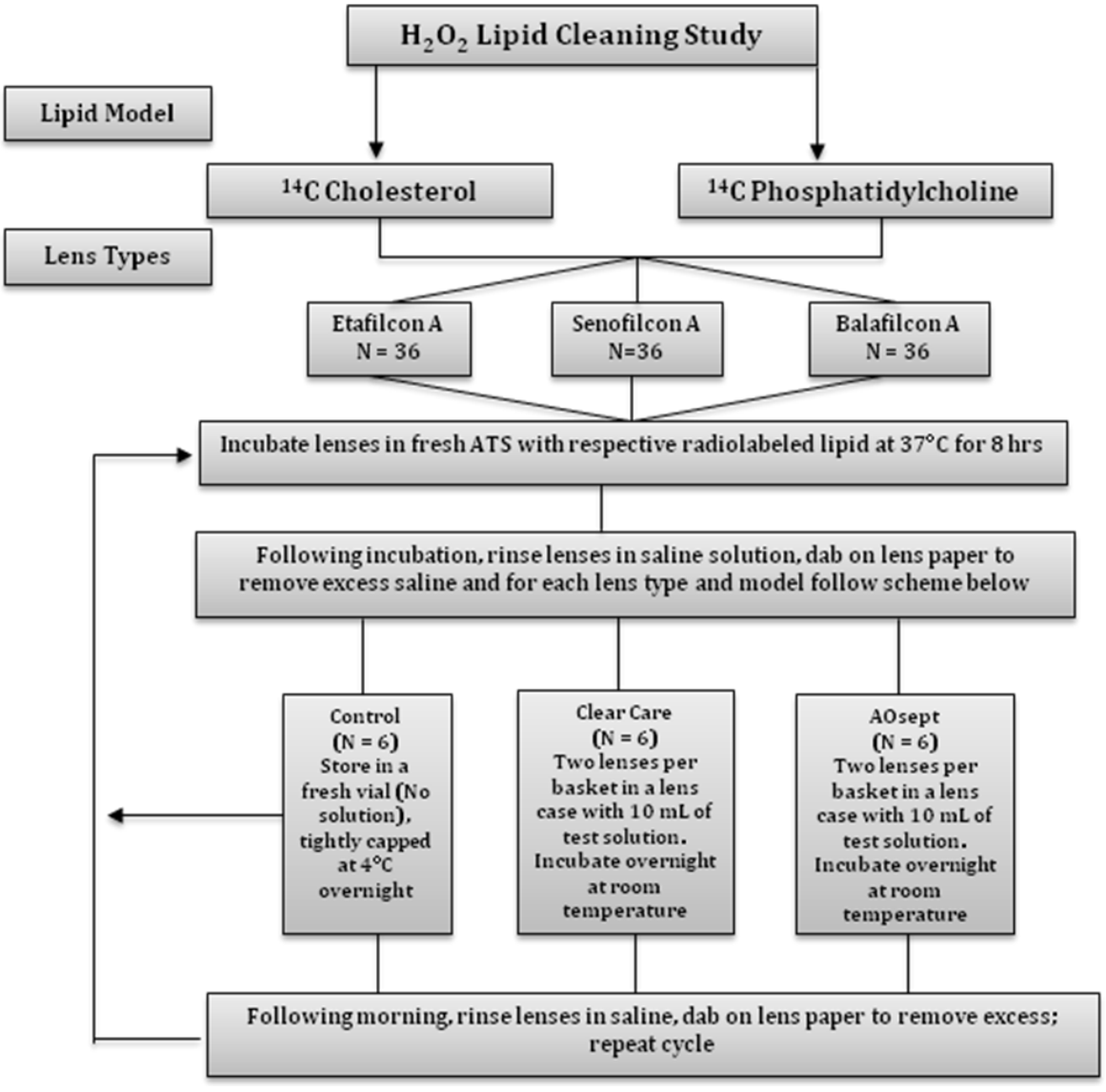
rinsing them in reverse osmosis water and letting them air-dry prior to the next disinfection cycle. No harsh chemicals or mechanical treatments were used to the clean lens holders and lens cases. Alternating cycles of lens incubation in the ATS and disinfection were carried out throughout the study period (7 days).

At the end of the 7-day cycle (disinfection period), lenses were removed and rinsed by dipping into three successive 250 mL beakers containing CSS and dabbed on lens paper to remove excess liquid. The ^{14}C-cholesterol incubated lenses were placed into 20 mL glass scintillation vials with 2 mL of (2:1) chloroform: methanol (v/v) extraction solution and were incubated for three hours each at 37°C with shaking. Each ^{14}C-cholesterol incubated lens was extracted in this way two separate times and both extracts were pooled in the same vial. The ^{14}C-phosphatidylcholine incubated lenses were placed into 20 mL glass scintillation vials with 2 mL of (60:50:1:4) chloroform: methanol : acetic acid : water (v/v/v/v) extraction solution and were incubated for three hours at 37°C with shaking. Each ^{14}C-phosphatidylcholine incubated lens was extracted in this way on two separate occasions and both extracts were pooled together.

The extract vials were then dried down completely using nitrogen evaporation and heat up to 37^{o}C. All contact lens extracts were re-suspended in 1 mL of chloroform, sonicated for one minute, and 10 mL of Ultima Gold F scintillation cocktail was added. The vials were then submitted for beta counting using a Beckman-Coulter L6500 liquid scintillation counter. In the experiment, the radioactive lipid was used as a probe and the ratio of radioactive lipid to non-

radioactive lipid in the incubating ATS was kept constant. Therefore, quantification of the total amount of cholesterol and phosphatidylcholine deposited was extrapolated and calculated using standard radioactive lipid calibration curves.

FIGURE 6-1: FLOW CHART OF EXPERIMENTAL DESIGN



6.3.5 STATISTICAL ANALYSIS

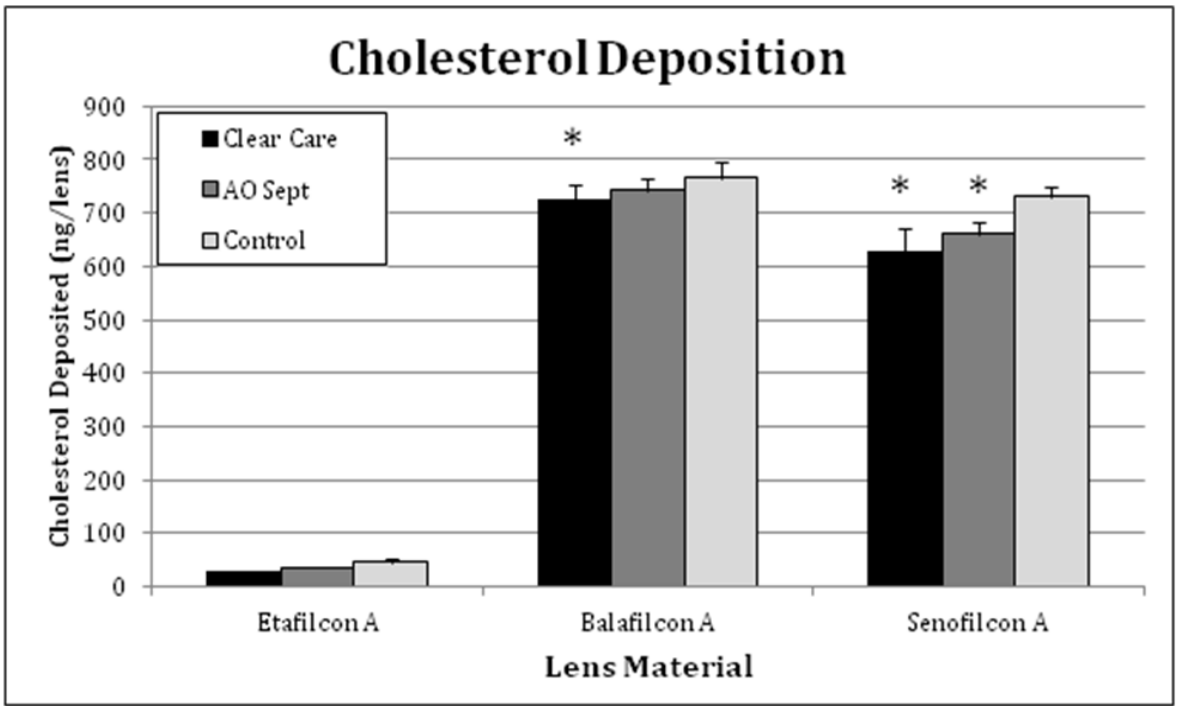
Statistical analysis was performed with Statistica 9, using repeated measures ANOVA and Tukey post-hoc analysis when required.

6.4 RESULTS

6.4.1 CHOLESTEROL

The results for cholesterol deposition on each of the three contact lens materials and for disinfection solutions tested are found in Figure 6-2 and Table 6-5. Balafilcon A lenses deposited the most cholesterol, followed by senofilcon A and lastly etafilcon A, which had very little cholesterol deposition. For all three lens materials, the control lenses deposited the most cholesterol and all of the lens materials that were disinfected with ClearCare had the least amount of cholesterol deposited. Etafilcon A lenses disinfected with ClearCare were found to have 40% less cholesterol deposited when compared with the controls. In contrast, balafilcon A and senofilcon A had 6% and 14% less cholesterol, respectively. Etafilcon A lenses disinfected with AOSept had 23% less deposited cholesterol when compared with the control. Balafilcon A had 3% less and senofilcon A had 10% less cholesterol deposited on the lenses when compared to the control lenses.

FIGURE 6-2: CHOLESTEROL DEPOSITION



* denotes statistical difference from corresponding lens control

TABLE 6-5: CHOLESTEROL DEPOSITION (NG/LENS)

		Disinfection Solution		
		ClearCare	**AOSept**	**Control**
Etafilcon A	**Avg C (ng/lens ± SD)**	27.99 ± 3.21	35.96 ± 2.82	46.76 ± 6.08
	Avg mass removed (ng/lens)	18.77	10.80	-
	Avg % C removed	40%	23%	-
Balafilcon A	**Avg C (ng/lens ± SD)**	724.27 ± 28.28	743.13 ± 21.05	766.10 ± 30.76
	Avg mass removed (ng/lens)	41.83	22.97	-
	Avg % C removed	6%	3%	-
Senofilcon A	**Avg C (ng/lens ± SD)**	630.19 ± 42.13	661.22 ± 20.91	730.97 ± 17.93
	Avg mass removed (ng/lens)	100.78	69.75	-
	Avg % C removed	14%	10%	-

The results of the repeated measures ANOVA are seen in Table 6-6 and the results of the Tukey post-hoc comparison analyzing the individual variables can be seen in Table 6-7. The repeated measures ANOVA results found statisticaly significant differences between the disinfection solutions used and between the lens materials tested, and the interaction between the two variables. As for the post-hoc results, balafilcon A and senofilcon A materials disinfected with ClearCare had statistically less cholesterol deposited when compared with the control ($p<0.05$), however etafilcon a had no statistical differences ($p=0.88$). Etafilcon A and balafilcon A lens materials had no statisical difference in cholesterol deposition ($p>0.05$) when disinfected with AOSept, however senofilcon A did have statsitically less cholesterol ($p=0.002$). When the two hydrogen peroxide solutions were

compared for each lens material, no statistically significant differences were seen ($p>0.45$).

TABLE 6-6: REPEATED MEASURES ANOVA FOR CHOLESTEROL DEPOSITION

	SS	DF	MSq	F	p
Solution	21646	2	10823	15.25	***0.0019***
Lens Material	4524876	2	2262438	3431.98	***<0.0001***
Solution * Lens Material	9272	4	2318	6.31	***0.0030***
Error	5878	16	367		

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

TABLE 6-7: TUKEY POST-HOC ANALYSIS COMPARING THE VARIOUS DISINFECTION ON CHOLESTEROL DEPOSITION

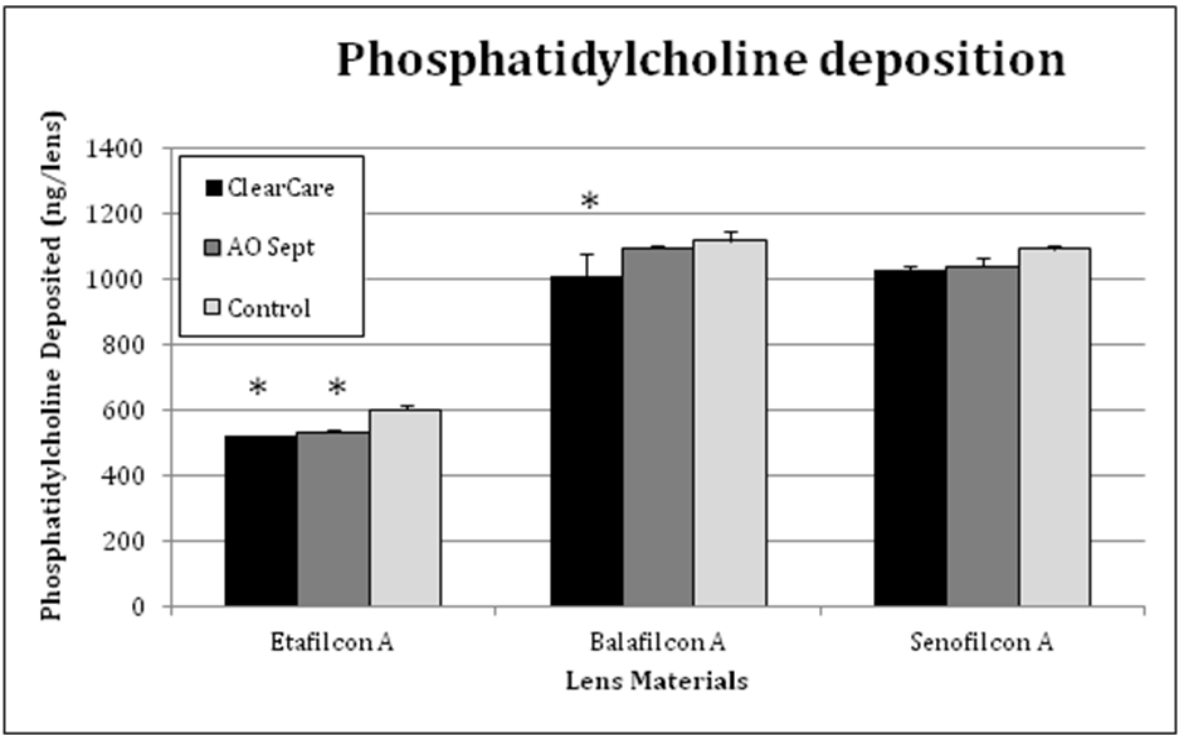
Solution	Lens	CC ETA	CC BAL	CC SEN	AO ETA	AO BAL	AO SEN	CNRL ETA	CNRL BAL
CC	**BAL**	***0.0002***							
CC	**SEN**	***0.0002***	***0.0002***						
AO	**ETA**	0.9991	***0.0002***	***0.0002***					
AO	**BAL**	***0.0002***	0.8284	***0.0002***	***0.0002***				
AO	**SEN**	***0.0002***	***0.0080***	0.1995	***0.0002***	***0.0006***			
CNRL	**ETA**	0.8813	***0.0002***	***0.0002***	0.9960	***0.0002***	***0.0002***		
CNRL	**BAL**	***0.0002***	***0.0493***	***0.0002***	***0.0002***	0.5403	***0.0002***	***0.0002***	
CNRL	**SEN**	***0.0002***	0.9896	***0.0002***	***0.0002***	0.9991	***0.0015***	***0.0002***	0.2348

CC= ClearCare, AO = AOSept, CNRL = control uncleaned lenses,
BAL = balafilcon A, ETA = etafilcon A, SEN = senofilcon A

6.4.2 PHOSPHATIDYLCHOLINE

The results for phosphatidylcholine deposition on each of the three contact lens materials and the two disinfection solutions tested are found in Figure 6-3 and Table 6-8. Balafilcon A and senofilcon A lenses deposited very similar quantities of phosphatidylcholine, and etafilcon A deposited very little phosphatidylcholine when compared with the two silicone hydrogel lens materials. For all three lens materials, the control lenses deposited the most phosphatidylcholine and all lens materials that were disinfected with ClearCare had the least amount of phosphatidylcholine deposited. Etafilcon A lenses disinfected with ClearCare were found to have 13% less phosphatidylcholine deposited when compared with the controls, but balafilcon A and senofilcon A only had 9% and 6% less phosphatidylcholine, respectively. Etafilcon A lenses disinfected with AOSept had 11% less deposited phosphatidylcholine when compared with the control. Balafilcon A had 2% less and senofilcon A had 5% less phosphatidylcholine deposited on the lenses when compared to the control lenses.

FIGURE 6-3: PHOSPHATIDYLCHOLINE DEPOSITION



* denotes statistical difference from corresponding lens control

TABLE 6-8: PHOSPHATIDYLCHOLINE DEPOSITION (NG/LENS)

		Disinfection Solution		
		ClearCare	**AOSept**	**Control**
Etafilcon A	**Avg PC (ng/lens ± SD)**	519.67 ± 4.05	532.90 ± 3.79	599.89 ± 12.21
	Avg mass removed (ng/lens)	80.22	66.99	-
	Avg % PC removed	13%	11%	-
Balafilcon A	**Avg PC (ng/lens ± SD)**	1009.27 ± 64.77	1092.28 ± 8.17	1113.96 ± 29.21
	Avg mass removed (ng/lens)	104.69	21.68	-
	Avg % PC removed	9%	2%	-
Senofilcon A	**Avg PC (ng/lens ± SD)**	1025.68 ± 11.42	1038.69 ± 25.34	1091.16 ± 11.74
	Avg mass removed (ng/lens)	65.48	52.47	-
	Avg % PC removed	6%	5%	-

The results of the repeated measures ANOVA are seen in Table 6-9 and the results of the Tukey post-hoc comparison analyzing the individual variables can be seen in Table 6-10. The repeated measures ANOVA results found statisticaly significant differences between the disinfection solutions used and between the lens materials tested, however the interaction between the two variables was not statisically significant (p=0.074). As for the post-hoc results, balafilcon A and etafilcon A disinfected with ClearCare had statistically less phosphatidylcholine deposited when compared with the control (p≤0.01), but senofilcon A did not show a significant difference in deposition (p=0.07). Only the etafilcon A lens materials had statisically less phosphatidylcholine deposited (p=0.04) when disinfected with AOSept and compared to the control. When the two hydrogen peroxide solutions were compared for each lens material, only balafilcon A lenses had statistical differences in deposition (p<0.01).

TABLE 6-9: REPEATED MEASURES ANOVA FOR PHOSPHATIDYLCHOLINE DEPOSITION

	SS	DF	MSq	F	P
Solution	0.05276	2	0.02638	62.81	***<0.0001***
Lens Material	2.59643	2	1.29821	3369.63	***<0.0001***
Solution * Lens Material	0.00934	4	0.00233	2.62	0.0740
Error	0.01425	16	0.00089		

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

TABLE 6-10: TUKEY POST-HOC ANALYSIS COMPARING THE VARIOUS DISINFECTION SOLUTIONS ON PHOSPHATIDYLCHOLINE DEPOSITION

Solution	Lens	CC ETA	CC BAL	CC SEN	AO ETA	AO BAL	AO SEN	CNRL ETA	CNRL BAL
CC	**BAL**	***0.0002***							
CC	**SEN**	***0.0002***	0.9942						
AO	**ETA**	0.9966	***0.0002***	***0.0002***					
AO	**BAL**	***0.0002***	***0.0095***	***0.0458***	***0.0002***				
AO	**SEN**	***0.0002***	0.9360	1.0000	***0.0002***	0.0969			
CNRL	**ETA**	***0.0100***	***0.0002***	***0.0002***	***0.0427***	***0.0002***	***0.0002***		
CNRL	**BAL**	***0.0002***	***0.0014***	***0.0062***	***0.0002***	0.9742	***0.0136***	***0.0002***	
CNRL	**SEN**	***0.0002***	***0.0143***	0.0679	***0.0002***	1.0000	0.1402	***0.0002***	0.9291

CC= ClearCare, AO = AOSept, CNRL = control uncleaned lenses
BAL = balafilcon A, ETA = etafilcon A, SEN = senofilcon A

6.5 DISCUSSION

This study was designed to examine the efficacy of lipid removal from contact lenses by two different hydrogen peroxide contact lens disinfection solutions, using our *in vitro* radiochemical lipid deposition model. The ideal contact lens cleaning and disinfection solution must fulfill many criteria. It must: rapidly disinfect a broad range of microorganisms, be safe for the ocular tissue, compatible with all lens materials, inexpensive and easy to use, condition the lens surface and reduce deposition of tear film components, including the removal of lipid deposition.[30] Little work has been published on lipid removal from contact lenses. In addition, new materials have been introduced into the market and the specific contact lens material characteristics may also play a role in altering lipid removal by cleaning

and disinfection solutions. For example, the currently available silicone hydrogel contact lens materials all have very different structures and polymers when compared to each other and the conventional hydrogel materials. Some of the materials are trimethylsiloxy silane (TRIS) derivatives, some are newer silicone macromers, some have solid surface treatments, some have leaky surface treatments, some have internal wetting agents, and some have no wetting agents (Table 6-3). With all of these variations, it is possible that a particular contact lens solution formulation may show enhanced efficacy when cleaning a particular lens material but not another.

From this experiment it was seen that the two CibaVision hydrogen peroxide systems tested, AOSept and ClearCare, vary in their ability to remove lipid from contact lens materials and that the process was governed both by the specific lipid and contact lens material under investigation. On average, silicone hydrogel lenses disinfected by ClearCare, had up to a 15% reduction in deposited cholesterol and phosphatidylcholine when compared to the control lenses. This was equivalent to about 110 ng of lipid per lens removed when compared to the controls. The conventional hydrogel, etafilcon A, did have a higher percentage removal of cholesterol when disinfected with ClearCare (40%); however the average mass of lipid removed was only 18.77 ng, which is a lower amount than what was removed from silicone hydrogels disinfected with the same solution. AOSept-disinfected silicone hydrogel lens materials showed a similar trend compared to ClearCare, except with a marginal loss in cleaning efficiency: silicone hydrogel cleaning

efficiency was on average <10% and on the conventional hydrogel was <23%. All lens materials disinfected with AOSept removed a maximum of 70 ng of lipid.

The trend showing a marginal increase in cleaning efficiency with ClearCare over AOSept, especially for cholesterol removal on senofilcon A lenses, is attributed to the addition of the surfactant, Pluronic 17R4. Even though, the statistical analysis resulted in some significant differences in deposition between the two different disinfection solutions and between the disinfection solutions and the control, these differences may not be clinically significant for a contact lens wearer. The statistical analysis does confirm however, that there was a high degree of repeatability in the *in vitro* model tested, as the six replicates of each variable completed had very small standard deviations. The model was also able detect small differences in contact lens lipid deposition when using different disinfection solutions.

The next stage of this research will be to compare the efficiency of hydrogen peroxide versus multipurpose contact lens cleaning solutions to remove deposited lipid from various contact lens materials and to compare the data to published clinical data. A few studies have recently examined the differences in lipid deposition when different contact lens cleaning solutions were utilized during a clinical *ex vivo* study. Zhao *et al*.[31] in 2009 published a clinical study examining cholesterol and protein deposition on various silicone hydrogel contact lens materials where the participants had utilized a number of different contact lens cleaning and disinfection solutions, including one hydrogen peroxide solution (ClearCare) and three multipurpose contact lens cleaning solutions. In this study

they found that the amount of cholesterol deposited was correlated to both the cleaning and disinfection solution utilized and the specific silicone hydrogel lens material that was worn.[31] They also found that the hydrogen peroxide solution tested was not the most efficient at removing cholesterol from human worn contact lenses.[31] In their study they found that balafilcon A lenses deposited 8.0±1.9 µg/lens and senofilcon A lenses deposited on average 1.2±0.4 µg/lens of cholesterol when disinfected with ClearCare and worn for 30 days. [31]

Another study was recently published by Heynen *et al*.[32] examining senofilcon A contact lens materials and their subsequent deposition of non-polar lipids following a clinical study where participants used either ClearCare or a 1-step multipurpose solution to clean their lenses. This study found that the overall lipid deposition and total cholesteryl oleate deposition were significantly less on the senofilcon A lenses disinfected with the multipurpose solution.[32] Average cholesterol deposition was similar between the two types of cleaning and disinfection solutions with deposition quantified to be 1.3 and 1.4 µg/lens. Once again, this study supports previous findings that lipid deposition is more efficiently removed from silicone hydrogel contact lens materials by a multipurpose solution.

When the cholesterol deposition results found in this *in vitro* experiment are compared with these recent *ex vivo* publications, it is seen that some differences do exist. However, at times it is difficult to make direct comparisons as the wear time of the *ex vivo* lenses are longer than the incubation time of this experiment.[31-33] To date no studies have examined the effect of hydrogen peroxide disinfection solution

on phosphatidylcholine deposition. One limitation and difference between the experimental design utilized here and average human contact lens wear, is that the lenses in this experiment were incubated for 8 hours in the ATS then removed and disinfected for 16 hours. In reality average human contact lens wear is closer to 16 hours, with disinfection for 8 hours. Therefore, *in vitro* deposition values similar to *ex vivo* reported masses may be possible by simply increasing the incubation time of the lenses and decreasing the disinfection time, to better mimic "normal" contact lens wearing cycles.

In vitro models can be utilized to evaluate the cleaning efficacy of new and/or commercially available contact lens cleaning and disinfection solutions and to evaluate the deposition profiles of new and/or commercially available contact lens materials. The radiochemical *in vitro* deposition model developed by our laboratory has shown to be a sensitive, repeatable and versatile model that can be used for the screening of new products. In addition to this, the model is easily altered to enable rapid investigations using a simplistic model or more physiologically relevant analysis using a multifaceted model that incorporates a complex artificial tear solution, and increased number of variables. The concentrations of the ATS tear film components can be easily altered to mimic tear film concentrations which are typical in conditions such as meibomian gland dysfunction or Sjogren's disease or to reach a desired mass of deposition. These conditions can all be analyzed while comparing to a control lens that has not been disinfected, so that an absolute reference is obtained. Of course, *in vitro* models will never be able to mimic all of the variables and complexity present in human contact lens wear; however they can aid

in the development of new products and provide valuable information on contact lens interactions with the surrounding tear film.

6.6 CONCLUSION

Overall, the ClearCare hydrogen peroxide solution which contained a surfactant removed the most lipids from lenses when compared to AOSept or the control, for both lipids and all lens materials. However, the differences found were quite small at times and whether these differences are clinically significant are yet to be determined.

The *in vitro* model of lipid deposition utilized in this experiment was found to be repeatable and sensitive enough to detect small differences in lipid deposition on various lens materials and with the use of different disinfection solutions.

6.7 ACKNOWLEDGEMENTS

This study was sponsored by ALCON Research Ltd.

In this chapter, the effects of intermittent air exposure on *in vitro* lipid deposition of several contact lens materials using a radiochemical experiment are analyzed with the use of a "model blink cell" designed and built in our laboratory.

7 THE IMPACT OF INTERMITTENT AIR EXPOSURE ON THE DEPOSITION OF LIPIDS ON SILICONE HYDROGEL AND CONVENTIONAL HYDROGEL CONTACT LENS MATERIALS

Holly Lorentz, Miriam Heynen, Warda Khan, Diana Trieu, Lyndon Jones

	Concept / Design	Acquisition of data	Analysis	Write-up / publication
Lorentz	Y	Y	Y	Y
Heynen	Y	Y	Y	Y
Khan	Y	Y	Y	-
Trieu	Y	Y	-	-
Jones	Y	-	-	Y

Tables: 7

Figures: 6

7.1 OVERVIEW

Purpose: To evaluate the influence of air exposure during *in vitro* deposition of two model lipids on silicone hydrogel (SH) and conventional hydrogel (CH) contact lens materials, via a custom-designed model blink cell (MBC).

Methods: Four SH (balafilcon A; lotrafilcon B; comfilcon A ; senofilcon A) and two CH (etafilcon A and omafilcon A) contact lens materials were mounted on six pistons and placed in a controlled atmosphere chamber at 35˚C with a relative humidity of 18%. The pistons were connected to a motor that cycled the contact lenses in and out of a custom-designed artificial tear solution (ATS) that closely mimicked the human tear film composition. Lenses were cycled for 10 hours; 2 seconds in the ATS then exposed to air for 5 seconds, which allowed the tear film to break over the surface of the contact lens. Control lenses were kept submerged for 10 hours. The incubation solution used was a complex ATS that contained six common tear film lipids, proteins, mucin, salts and a trace amount of one of the radioactive lipids; ^{14}C-cholesterol or ^{14}C-phosphatidylcholine. A longer term incubation was tested with lotrafilcon B and balafilcon A materials incubated in ^{14}C-cholesterol ATS. For this study, air exposed lenses were cycled for 14 hours then submerged for 10 hours. This was completed for 6 days and the control lenses were simply submerged for all 6 days. Following incubation, each lens was extracted twice in 2 mL of chloroform: methanol (2:1, v/v) for three hours, evaporated under nitrogen, re-suspended in chloroform and then Ultima Gold F scintillation cocktail. Extracts were counted in a LS6500 Beckman Coulter beta counter and raw data were translated into absolute

amounts (ng/lens) via extrapolation from standard curves run for each of the test lipids.

Results: For the two model lipids tested, SH lens materials deposited statistically more lipid than the CH lens materials, with balafilcon A depositing the most lipid. Air exposure significantly increased the amount of cholesterol that deposited on omafilcon A, balafilcon A, comfilcon A, and senofilcon A ($p \leq 0.03$). No change in deposition was seen for lotrafilcon B and etafilcon A ($p > 0.05$). The longer term incubation of lotrafilcon B and balafilcon A showed statistically significant increases in cholesterol deposition for both lens materials that were exposed to air ($p < 0.02$). All lenses exposed to air resulted in increased amounts of phosphatidylcholine deposited. These levels were statistically significantly higher ($p < 0.04$) for lotrafilcon B, senofilcon A, comfilcon A and omafilcon A, but not statistically significant ($p > 0.05$) for balafilcon A or etafilcon A.

Conclusion: This model has demonstrated that lipid deposition kinetics can be impacted by air exposure and that lipid deposition profiles are contact lens dependent. *In vitro* models must begin to use more physiologically relevant incubation solutions and conditions that mimic contact lens wear within the natural tear film if *in vitro* data is to be extrapolated to the *in vivo* situation. These methodologies will provide hitherto unavailable information on the way in which lipid interacts with silicone hydrogel materials and will be of interest to the contact lens industry, clinicians and other areas of biomaterial research.

7.2 INTRODUCTION

In vitro models in contact lens research have been used previously to examine lipid deposition, protein deposition, protein denaturation and interactions with tear film components. [1-14] Many of the *in vitro* models utilized are simple model systems where an individual lipid or protein was examined in isolation, without the incorporation of the other prevalent tear film components.[4, 5, 9, 10, 13, 14] These *in vitro* models are too simplistic and they ignore several key elements of human contact lens wear. If *in vitro* models are really meant to mimic human conditions, the models utilized have to become more physiologically relevant so that it is more conducive to the human contact lens wear experience. This, in turn, should provide better and more true-to-life data on contact lenses and tear film interactions.

Some of these key elements that were not addressed in early *in vitro* models included the complexity of the tear film, the exposure to air in-between blinks and the sheering force of the lid. Our laboratory, and others, have begun using a more complex tear film solution, which contains mixes of both lipids and proteins, in their *in vitro* models.[2, 3, 15-20] However, very few if any researchers have looked at the effect of air exposure or the lid effects on lipid or protein deposition.

The effect of lens drying in the inter-blink period is especially of interest in lipid deposition on silicone hydrogel contact lens materials, as these lenses are more hydrophobic and less wettable than conventional hydrogel materials. These lens properties are attributed to the silicone components of the contact lenses. Silicone

is known for its superior ability to carry oxygen, which is ideal for daily and especially extended wear contact lens materials, however silicone is known to be very hydrophobic in nature. This hydrophobicity makes the lens material more lipophilic and less wettable, which can lead to contact lens associated discomfort, increased lipid deposition and decreased visual acuity for the wearer. [21]

When a contact lens material is naturally hydrophobic and unwettable, the anterior tear film that covers the lens surface may not remain stable and intact for a long period of time. Therefore, the tear film may break, collapse and recede across the surface of the lens prior to the next blink. This breaking of the tear film encourages the lipid layer of the tear film to come into direct contact with the lens material itself, which could increase the degree of lipid deposition. This process could be exacerbated by the fact that silicone hydrogels, much like their conventional hydrogel predecessors, have very freely rotating polymer structures. This means, when the polymer is exposed directly to air it will rotate to expose its more hydrophobic backbone and "hide" the hydrophilic moieties toward the matrix. This may form an inward attraction for lipid to deposit during the inter-blink period.

When a blink occurs, the tear film will re-form over the anterior surface of the contact lens and the hydrophilic polymer moieties flip back to the surface to come in contact with the aqueous phase of the tear film. Deposited lipid on the lens surface may then be encouraged to move further into the lens matrix to avoid the aqueous phase of the tear film and the hydrophilic moieties of the lens polymer. This is a very

dynamic process that may have a huge impact on the quantity of lipid, types of lipid, state of the lipid, and the mechanism of how the deposition onto and into contact lens materials occurs.

In order to test this effect of air exposure on lipid deposition using an *in vitro* model, a novel device was built by our laboratory. This device, called a "model blink cell" (MBC), was designed to mimic the inter-blink drying time that occurs during contact lens wear. This MBC is placed into an atmospherically controlled chamber and mounted contact lenses are cycled in and out of an artificial tear solution (ATS). When the lenses are out of the ATS the artificial tear film will "break" over the surface of the lens and allow for drying, to mimic human contact lens wear. This was completed and tested against an *in vitro* model where the contact lenses remained submerged in the ATS for the entire incubation time, without cycling, similar to traditional *in vitro* vial incubations.

In this experiment, two different model lipids, ^{14}C-cholesterol and ^{14}C-phosphatidylcholine, were examined for their deposition onto conventional and silicone hydrogel contact lens materials using our specially designed model blink cell in either fully submerged or air exposure mode.

7.3 METHODS

7.3.1 ARTIFICIAL TEAR SOLUTION (ATS)

Chapter 3 of this thesis introduced the protocol to prepare an ATS solution specifically optimized for closed-system or in-vial incubations. However, this study involved an experiment that required incubation of contact lenses in an open-to-air system; therefore an ATS was developed for open-system incubations which were able to remain stable in pH and osmolality throughout the incubation period when exposed to air. This ATS is very similar in composition and preparation to the previously described system outlined in Table 3-1 and the changes from the original in-vial ATS components are italicized and remain limited to the complex salt solution.

Preparing a radioactive open-system ATS involves the same main steps in protocol. The preparation of the complex salt solution was the first step in preparing this ATS. The salt components were mixed into the required amount of MilliQ water in the concentrations and order that they appear in Table 7-1. The second step was to construct the lipid stock, which was a concentrated mix of pure lipids dissolved into hexane and ethyl ether in their desired ratios. The lipid stock can be prepared in advance and then stored at -20^{o}C prior to use. The third step in the ATS preparation was dissolving the lipid stock into complex salt solution, drive in nitrogen gas and sonicate for at least 30 minutes to complete the lipid tear solution. This process ensures that the lipid was well mixed into the saline solution and confirms that the hexane and ether were evaporated. The fourth step was to

incorporate the radioactive lipid, which was used as an experimental tracer. The radiolabeled cholesterol and phosphatidylcholine was added to the ATS at a concentration of 5.6% and 27% of the total individual lipid concentration respectively. Following an additional 15 minutes of sonication, the protein/mucin were then dissolved into the solution and sonicated for an additional 5 minutes. The radioactive lipid details can be found in Table 7-2.

TABLE 7-1: ARTIFICIAL TEAR SOLUTION COMPONENTS FOR THE OPEN-INCUBATION SYSTEM

Salt component	**mM**	**Lipid Component**	**Concentration (mg/mL)**
Sodium chloride	*110.0*	Cholesterol	0.0018
Potassium chloride	16.0	Cholesteryl oleate	0.024
Sodium citrate	1.5	Oleic acid	0.0018
Glucose	0.2	Oleic acid methyl ester	0.012
Urea	1.2	Phosphatidylcholine	0.0005
Calcium chloride	0.5	Triolein	0.016
Sodium carbonate	12.0		
Potassium hydrogen carbonate	3.0	**Protein Component**	**Concentration (mg/mL)**
Sodium phosphate monobasic	*26.0*	Bovine Albumin	0.20
Hydrochloric acid (10 M)	*2.33*	Hen Egg Lysozyme	1.90
ProClin 300	0.2 µL/ 1L	Bovine Mucin	0.15

TABLE 7-2: RADIOACTIVE LIPID CHARACTERISTICS

	Cholesterol [C]	L-α-DiPalmitoyl-Phosphatidylcholine [PC]
Radiolabel and Position	4-14C	DiPalmitoyl-1-14C
Molecular Weight (g/mol)	386.7	734.0
Specific Activity (mCi/mmol)	49.78	114
Supplier	Perkin-Elmer	Perkin-Elmer

7.3.2 CONTACT LENS MATERIALS

Six contact lens materials were tested in triplicate: Acuvue® 2 [etafilcon A; Vistakon], Proclear® [omafilcon A; CooperVision], Acuvue® OASYS™ [senofilcon A; Vistakon], Air Optix™ [lotrafilcon B; Ciba Vision], Biofinity® [comfilcon A; CooperVision], PureVision™ [balafilcon A; Bausch & Lomb]. The material characteristics of all contact lens materials can be found in Tables 7-3 and 7-4.

TABLE 7-3: CONVENTIONAL HYDROGEL CONTACT LENS MATERIAL CHARACTERISTICS

Material Type	**Conventional Hydrogel**	
USAN	**Etafilcon A**	**Omafilcon A**
Proprietary name	Acuvue®2	Proclear®
Manufacturer	Johnson & Johnson	CooperVision
Monomers	HEMA, MA	HEMA, PhC
Surface Modification	None	None
Oxygen Transmissibility ($x10^{-9}$)	31.0	52.3
Water Content	58%	62%
FDA Class	Group IV	Group II

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

TABLE 7-4: SILICONE HYDROGEL CONTACT LENS MATERIAL CHARACTERISTICS

Material Type	**Silicone Hydrogel**			
USAN	**Senofilcon A**	**Lotrafilcon B**	**Comfilcon A**	**Balafilcon A**
Proprietary name	Acuvue® OASYS™	Air Optix™	Biofinity®	PureVision™
Manufacturer	Johnson & Johnson	CIBA Vision	CooperVision	Bausch & Lomb
Monomers	mPDMS, DMA, HEMA, siloxane macromer, EGDMA, PVP	DMA, TRIS, Siloxane macromer	M3U, FM0411M, HOB, IBM, NVP, TAIC, VMA	NVP, TPVC, NVA, PBVC
Surface Modification	PVP as an internal wetting agent	High refractive index plasma coating	None	Plasma oxidation
Dk/t (x10^{-9})	147	138	160	110
Water Content	38%	33%	48%	36%
FDA Class	Group I	Group I	Group I	Group III

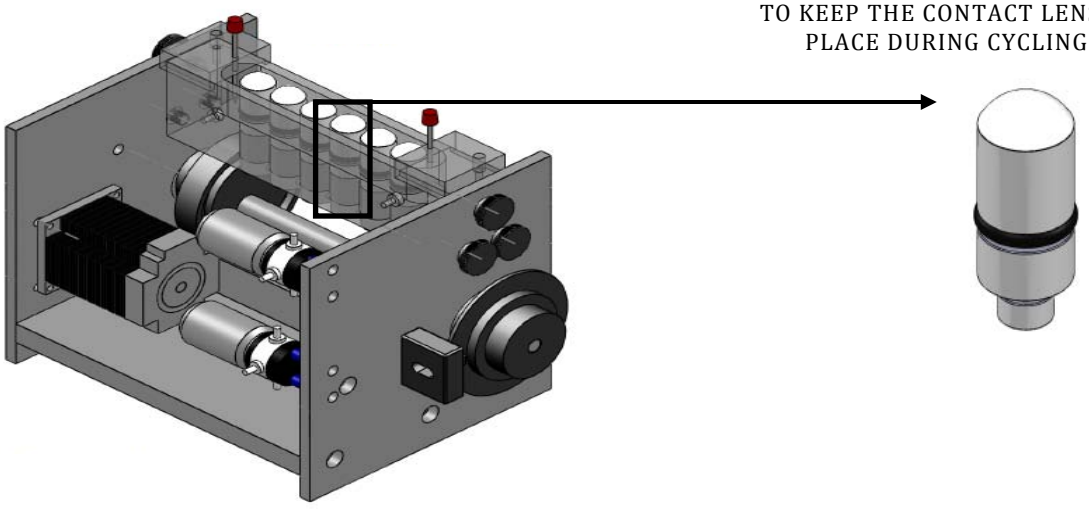
Dk/t: oxygen transmissibility; USAN: United States adopted name; DMA (N,N-dimethylacrylamide); EGDMA (ethyleneglycol dimethacrylate); FM0411M (2-ethyl [2-[(2-methylprop-2-enoyl)oxy]ethyl]carbamate); HEMA (poly-2-hydroxyethyl methacrylate); HOB ((2RS)-2-hydroxybutyl 2-methylprop-2-enoate); IBM (Isobornyl 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); TRIS (trimethylsiloxy silane); VMA (N-Vinyl-N-methylacetamide)

7.3.3 MODEL BLINK CELL

The "model blink cell" (MBC) is a device specially designed and built in our laboratory to mimic the inter-blink period during contact lens wear (Figures 7-2 and 7-3). The blink cell itself is composed of a trough with contact lenses mounted on six form-fitting Teflon® pistons (Figure 7-1) connected to a motor that cycles the pistons in and out of the artificial tear solution contained in the trough. The cycling of the pistons is programmable so that a desired blink frequency can be chosen. The model blink cell itself is contained within a temperature and humidity controlled chamber so that physiological parameters can be maintained during incubation.

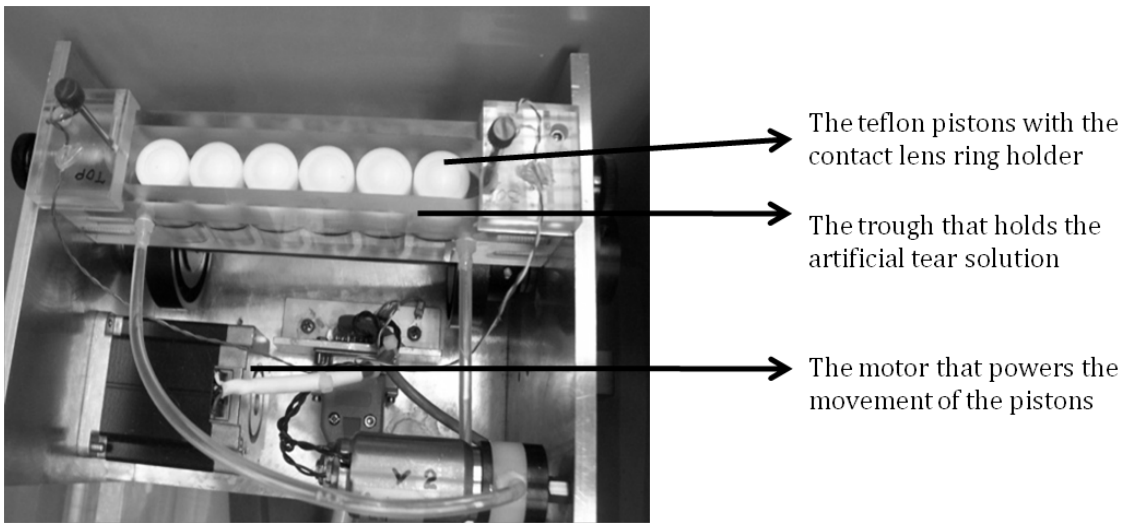
FIGURE 7-2: A DRAWING OF THE MODEL BLINK CELL REMOVED FROM THE ATMOSPHERIC CHAMBER

FIGURE 7-1: THE FORM-FITTING PISTON TO HOLD THE CONTACT LENSES. A CIRCULAR RING IS FITTED OVERTOP THE PISTON TO KEEP THE CONTACT LENS IN PLACE DURING CYCLING



CAD drawings above produced by SolidWorks

FIGURE 7-3: LABELED PHOTOGRAPH OF THE MODEL BLINK CELL



7.3.4 EXPERIMENTAL DESIGN

All contact lens materials were soaked in saline with agitation for 24 hours to remove the blister pack solution components. Following pre-soaking, the lenses were blotted on lens paper before mounting onto the pistons in the MBC.

Two identical model blink cell units were presoaked with ATS without radioactivity for at least 48 hours to reduce any non-specific binding to the MBC of lipid and proteins in subsequent incubations. When the contact lenses were ready for incubation, the ATS was replaced with fresh ATS containing a single radioactive lipid. Three replicates of two contact lens types were incubated in each unit. One unit cycled the lenses in and out of ATS (referred to as “air exposed”) and the lenses in the other unit remained submerged for the duration of the experiment (“submerged”). The blink rate was set such that the lenses were submerged for 2 seconds and then were out of solution and exposed to the atmosphere for 5 seconds.

The atmosphere in the chamber was set at 36˚C ± 1˚C and had a relative humidity of 16% ± 1%. Both MBC units were filled with 18 mL of ATS solution, containing a single tracer of radioactive (cholesterol or phosphatidylcholine). To compensate for solution evaporation, both units were refilled as needed, maintaining appropriate osmolality and final volume. Uniformity of the ATS between the units was achieved by pooling ATS from both units during the refill.

7.3.4.1 SHORT-TERM INCUBATION

The first experimental test mimicked wear of a daily disposable contact lens with one 10 hour incubation period. This short-term test was conducted with both radioactive lipids and all lens materials. Following the 10 hour incubation in either the submerged or air exposed model blink cell the lenses were removed and processed as described below.

7.3.4.2 LONG-TERM INCUBATION

The long-term incubation experiment was tested on balafilcon A and lotrafilcon B contact lens materials for six days to mimic extended wear conditions. Air exposed lenses in this experiment were cycled in and out of the solution for 14 hours (to mimic wear time) and were then submerged for the 10 hours (to mimic the overnight wear). This cycle was repeated for each of the 6 days. Submerged lenses were simply submerged for the entire 6 days. Fresh radioactive ATS replaced

the used solution every other day. Following the long-term incubations, the lenses were removed and processed as will be described next.

7.3.5 PROCESSING OF CONTACT LENSES

At the end of the incubation, the lenses were removed, rinsed in saline and extracted twice with 2 mL of chloroform: methanol (2:1, v/v) for three hours at 37^{o}C with constant shaking. The extracts were dried under nitrogen and re-suspended in 1 mL of chloroform, and 10 mL of Ultima Gold F scintillation cocktail then counted using the LS6500 Beckman Coulter beta counter. In the experiment, the radioactive lipid was used as a probe and the ratio of radioactive lipid to non-radioactive lipid in the incubating ATS was kept constant. Therefore, quantification of the total amount of cholesterol and phosphatidylcholine deposited was extrapolated and calculated using standard radioactive lipid calibration curves.

7.3.6 STATISTICAL ANALYSIS

Statistical analysis was performed using Statistica 9 and independent t-tests.

7.4 RESULTS

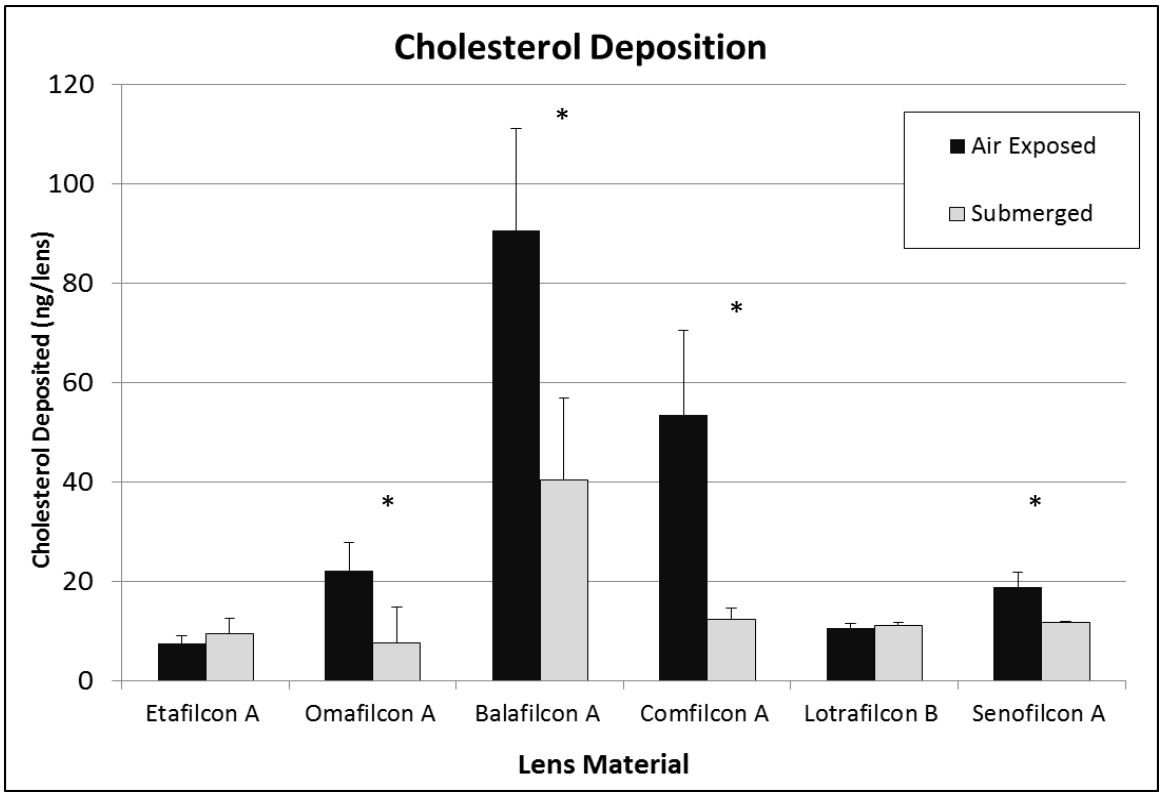
7.4.1 CHOLESTEROL

Exposure to air significantly increased ($p \leq 0.03$) the amount of cholesterol deposited on most lenses (omafilcon A; balafilcon A; comfilcon A; senofilcon A), with lotrafilcon B and etafilcon A depositing lower (but statistically identical) amounts (Table 7-5 and Figure 7-4). The order of cholesterol deposits in submerged lenses was omafilcon A = etafilcon A = lotrafilcon B = senofilcon A = comfilcon A <balafilcon A. Air exposure for cholesterol deposits occurred in the following order: etafilcon A < lotrafilcon B < senofilcon A < omafilcon A < comfilcon A < balafilcon A. As can be seen from Table 7-5, the air exposed lenses generally deposited 1.6-4.3x more cholesterol than the submerged lenses, except for etafilcon A and lotrafilcon B materials, which deposited equal or slightly less cholesterol.

TABLE 7-5: CHOLESTEROL UPTAKE AFTER 10 HOURS ON VARIOUS LENS MATERIALS (NG/LENS ± SD) SUBMERGED OR AIR EXPOSED. T-TESTS (N=3) WERE PERFORMED AND A P VALUE <0.05 WAS CONSIDERED SIGNIFICANTLY DIFFERENT.

Lens Material	Submerged (A) (ng/lens ± SD)	Air-Exposed (B) (ng/lens ± SD)	B ÷ A	p
Etafilcon A	9.5 ± 3.0	7.4 ± 1.7	0.78	0.349
Omafilcon A	7.7 ± 7.2	22.2 ± 5.7	2.88	***0.014***
Balafilcon A	40.3 ± 16.6	90.6 ± 20.6	2.25	***0.030***
Comfilcon A	12.4 ± 2.2	53.4 ± 17.0	4.31	***0.014***
Lotrafilcon B	11.1 ± 0.7	10.6 ± 0.9	0.95	0.487
Senofilcon A	11.7 ± 0.3	18.8 ± 3.1	1.61	***0.017***

FIGURE 7-4: CHOLESTEROL UPTAKE AFTER 10 HOURS ON VARIOUS LENS MATERIALS



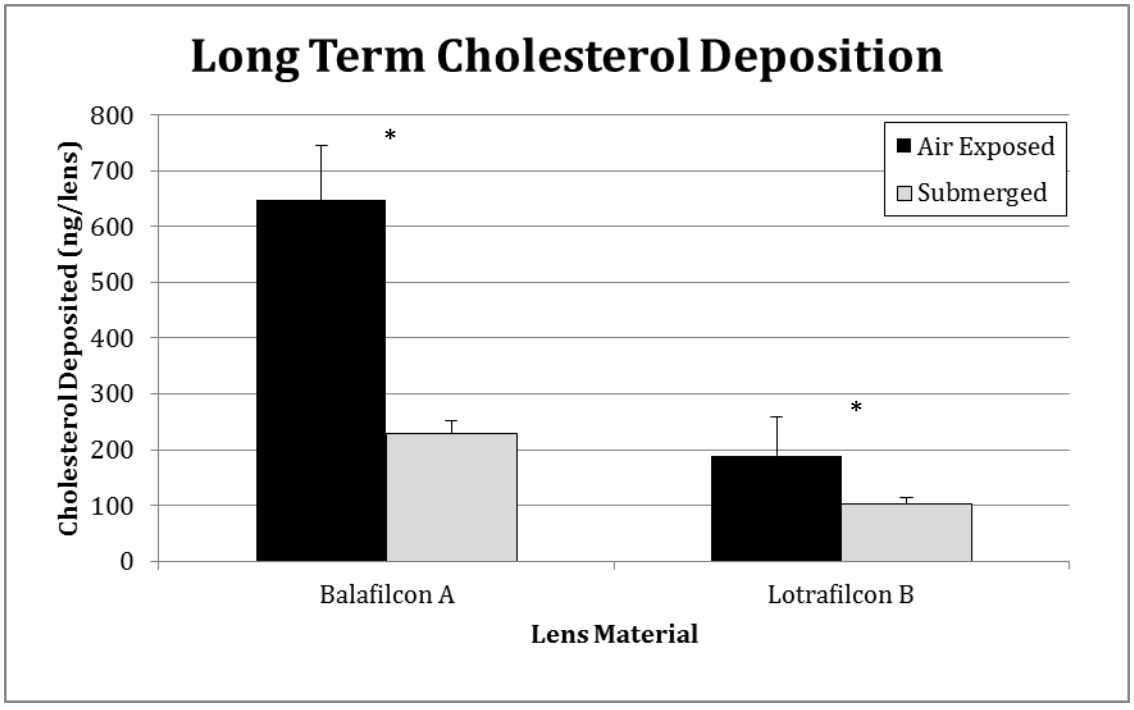
The longer term incubation was tested for both balafilcon A and lotrafilcon B lenses. These specific lenses were chosen as they are both approved for at least 6 days of continuous wear and they represented the two extremes of silicone hydrogel lens deposition in the short-term 10 hour experiment, both in quantity of cholesterol deposited and in the effect of air exposure. After 6 days of incubation in the MBC both lens material exhibited a statistically significant increase in cholesterol deposition in the air exposed lenses ($p<0.011$) and both materials had deposited significantly more cholesterol when compared with the 10 hour experiment (Table 7-6 and Figure 7-5). Once again, balafilcon A lenses deposited significantly more lipid than lotrafilcon B lenses and overall the air exposed lenses

deposited 2.8x more cholesterol on balafilcon A and 1.8X more cholesterol on lotrafilcon B materials.

TABLE 7-6: CHOLESTEROL UPTAKE AFTER 6 DAYS ON TWO CONTACT LENS MATERIALS (NG/LENS ± SD) SUBMERGED OR AIR EXPOSED. T-TESTS (N=3) WERE PERFORMED AND A P VALUE <0.05 WAS CONSIDERED SIGNIFICANTLY DIFFERENT.

Lens Material	Submerged (A) (ng/lens ± SD)	Air-Exposed (B) (ng/lens ± SD)	B ÷ A	p
Balafilcon A	227.71 ± 69.68	646.87 ± 98.75	2.84	*0.011*
Lotrafilcon B	102.78 ± 10.85	189.30 ± 23.93	1.84	*0.005*

FIGURE 7-5: CHOLESTEROL UPTAKE AFTER 6 DAYS ON TWO LENS MATERIALS



7.4.2 PHOSPHATIDYLCHOLINE

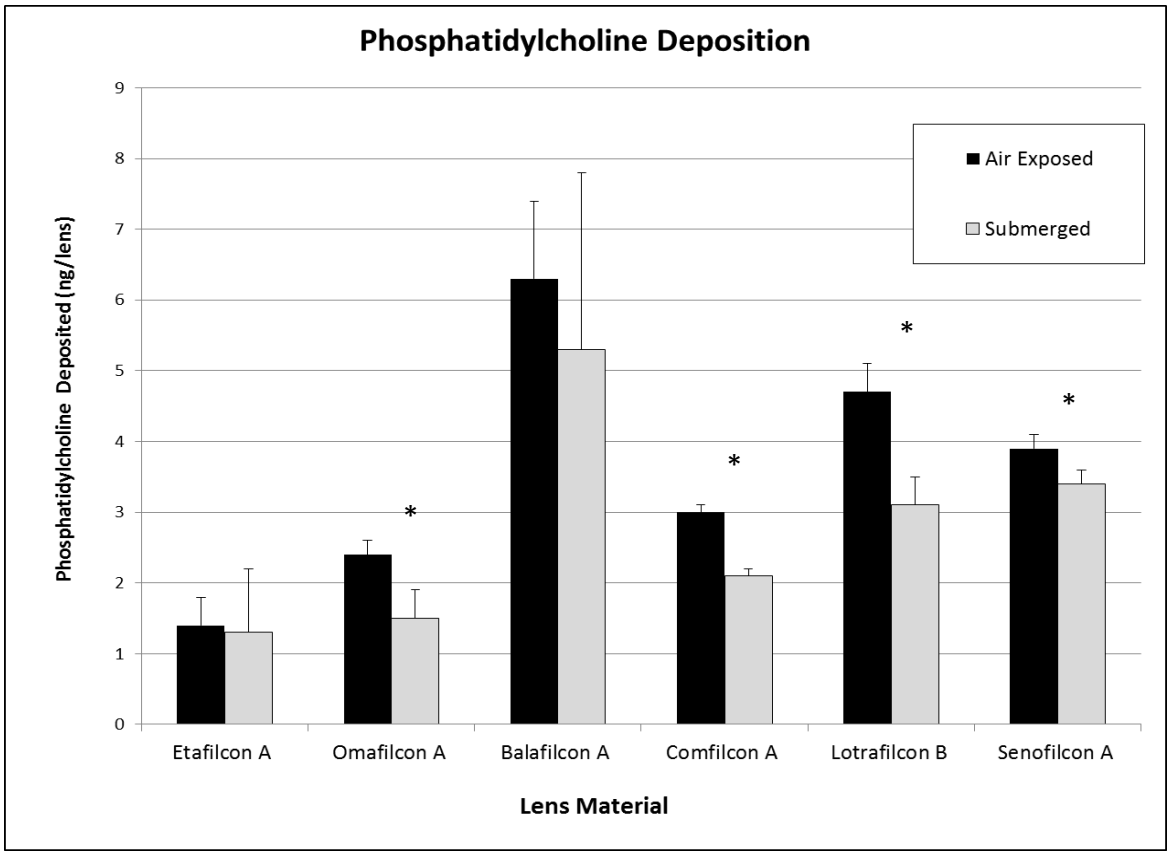
For all lenses, air exposure resulted in higher levels of phosphatidylcholine (PC) being deposited. These levels were statistically significantly higher ($p<0.04$) for the neutral charge FDA Group I (lotrafilcon B, senofilcon A, comfilcon A) and II lenses (omafilcon A) exposed to air. However, no differences in deposition were found ($p>0.05$) for the ionic FDA Group III (balafilcon A) or IV (etafilcon A) lens materials (Table 7-7 and Figure 7-6).

The masses deposited were all low, reflecting the low concentration of PC in the solution. The relative increase of PC on air-exposed lenses is smaller than the effect seen with cholesterol, in the range of 17% more for senofilcon A and up to 60% more for omafilcon A (Table 7-7). The order of deposition for submerged lenses was etafilcon A < omafilcon A < comfilcon A < lotrafilcon B < senofilcon A < balafilcon A. Exposing the lenses to air resulted in the following order of PC deposition: etafilcon A =omafilcon A < comfilcon A < senofilcon A = lotrafilcon B = balafilcon A.

TABLE 7-7: PHOSPHATIDYLCHOLINE UPTAKE AFTER 10 HOURS ON VARIOUS LENS MATERIALS (NG/LENS ± SD) SUBMERGED OR AIR EXPOSED. T-TESTS (N=3) WERE PERFORMED AND A P VALUE <0.05 WAS CONSIDERED SIGNIFICANTLY DIFFERENT.

Lens Material	Submerged (A) (ng/lens ± SD)	Air-Exposed (B) (ng/lens ± SD)	B ÷ A	p
Etafilcon A	1.3 ± 0.9	1.4 ± 0.4	1.08	0.831
Omafilcon A	1.5 ± 0.4	2.4 ± 0.2	1.60	***0.006***
Balafilcon A	5.3 ± 2.5	6.3 ± 1.1	1.19	0.579
Comfilcon A	2.1 ± 0.1	3.0 ± 0.1	1.43	***0.001***
Lotrafilcon B	3.1 ± 0.4	4.7 ± 0.4	1.52	***0.008***
Senofilcon A	3.4 ± 0.2	3.9 ± 0.2	1.15	***0.032***

FIGURE 7-6: PHOSPHATIDYLCHOLINE UPTAKE AFTER 10 HOURS ON VARIOUS LENS MATERIALS (NG/LENS ± SD)



7.5 DISCUSSION

Examining lipid deposition on contact lenses in an *in vitro* model system helps researchers and developers to gain an understanding of the processes and interactions that occur *in vivo*. Our laboratory has developed a blink-cell model to simulate the inter-blink tear film thinning and drying that occurs during the inter-blink period of contact lens wear. This model has demonstrated that a change in the kinetics of lipid deposition occurs when incubating contact lenses using this intermittent air-exposure method versus incubating lenses in a fully submerged aqueous environment.

This experiment specifically found that the effect of intermittent air exposure on lipid deposition is both lipid and lens material dependent. For example, etafilcon A lens materials did not differ from control values when this material was exposed to air during the blink cycle for either lipid examined. However, omafilcon A, senofilcon A and comfilcon A all deposited significantly more cholesterol and PC when incubated with intermittent air exposure. Balafilcon A, showed no statistical differences in deposition with PC but did with cholesterol. In contrast, lotrafilcon B had the opposite occurrence with the 10 hour incubation, but when lotrafilcon B was incubated to mimic 6 days of continuous wear, significantly higher cholesterol deposition was found.

Cholesterol deposition increased for all lens materials when intermittently exposed to air, with the exception of etafilcon A. Overall, PC deposition increased on all lens materials when exposed to air, however only four of the six materials had

statistically significant differences. Once again, etafilcon A was one of the statistically unaffected lenses. Etafilcon A is well known as a material which deposits little amounts of lipid but deposits large amounts of lysozyme, due to its hydrophilic hydroxyl moieties.[13] Therefore, it is not a surprise that its lipid deposition profile is not easily manipulated for either cholesterol or PC. The second lens which was found to have no statistical difference in PC deposition when exposed to air was balafilcon A, which may be due to larger standard deviations in the data. On average, exposure to air had a larger impact on cholesterol deposition when compared to PC deposition and lens materials deposited more cholesterol, on average, than PC.

The deposition of both cholesterol and PC in this experiment is lower than other *in vitro* and *ex vivo* experiments conducted.[4, 5, 16, 22, 23] There are several reasons for this: first, the experimental procedure tested for all lenses was the short term (10 hour) incubation, without replenishment of the solution, which is a shorter incubation time than most other *in vitro* experiments. The short time frame was used to mimic the wear time on a daily disposable lens and was utilized to determine the impact that air exposure has on lipid deposition. If statistical differences in deposition could be found after only 10 hours of incubation then it can be deemed a significant effect and longer incubation times would be expected to find greater masses of deposition and similar if not greater deposition effects with air exposure. Secondly, phosphatidylcholine deposition was very low, which is likely due to its low concentration in the ATS. Lastly, the model blink cell will favour anterior surface deposition and discourage deposition on the posterior surface.

This is because the lens needs to be clipped in place with a specially designed ring manufactured to fit on the model blink cell pistons. Due to its structure, this ring will reduce the flow of ATS beneath the lens and it will also reduce the anterior lens surface area exposed and available for deposition. All of these factors together explain the lower deposition found in this *in vitro* model. Although the tear film exchange on the posterior side of human-worn soft lenses is reduced when compared to rigid lenses, there is still much more movement and tear film exchange than in the model blink cell due to lens movement during blinking.

It is hypothesized that the exposure to air increases the lipid deposition on conventional and silicone hydrogel lens materials due to their polymer mobility and chain rotation, as mentioned previously. This chain rotation or hysteresis can be analyzed while measuring wettability through advancing and receding contact angle measurements such as sessile drop, captive bubble or Wilhelmy plate techniques, just to name a few.[24] In human contact lens wear, immediately following a blink, the lens is immersed in the tear film and lenses hydrophilic moieties face outward with the hydrophobic back bone situated within the matrix.[24] As the period between blinks grows longer, the tear film begins to thin and then break over the surface of the contact lens bringing the lipid layer and then air into direct contact with the lens material. When the lens is exposed to lipid and/or air the previous lens polymer arrangement becomes undesirable and the hydrophilic groups will rotate inward to expose the materials' hydrophobic backbone to the air.[24] This then encourages lipid to deposit and then the air exposure will drive the lipid into the lens matrix. Once

another blink occurs, the hydrophilic polymer moieties will again flip to the anterior surface. This occurrence is especially true for lenses which have higher contact angles and for a contact lens wearer with dry eyes, more unstable tear films, and thus shorter tear break up times. This wetting/de-wetting cycle can occur after every blink and therefore thousands of times a day, thus allowing lipid to continuously accumulate on and in the lens material.

In the 1990's, Bontempo and Rapp's *in vitro* studies found that FDA Group II lens materials were the lens materials that were prone to the highest masses of lipid deposition.[1] They therefore published a theory for lipid deposition on conventional hydrogel contact lens materials called the "push/pull" theory.[1] The theory outlined that the forces involved in lipid deposition onto conventional hydrogel lenses were the material, which pulled lipid in to the lens, and the water component, that pushed the lipid into the matrix of the lens.[1] This theory can be modified for silicone hydrogel lens materials as the incorporation of silicone will provide a strong pull of lipid into the lens material and then the lipid is forced to "hide" within the matrix due to air exposure during the inter-blink period. This creates a more powerful push/pull dynamic for lipid deposition on silicone hydrogel lens materials. The mechanism of lipid deposition introduced here, is much different than experienced with in-vial incubations. During in-vial incubations, the lens remains in an aqueous environment and the deposition is mainly driven by hydrophobic/hydrophilic interactions with the lens and the ATS.

To-date, the effect of intermittent air exposure on *in vitro* lipid deposition on contact lens materials has not been examined. Peters and Millar analyzed the stabilizing effects of phospholipids on tear break up time (TBUT) using a Tearscope and a very complex upright model blink eye system.[25] Their blinking model eye held a contact lens and was able to spread ATS over the surface of the contact lens using a solenoid and an artificial eyelid. However, this study only examined TBUT and lipid deposition was not examined. In 2004, Copley and Radke presented a poster at the Association of Research in Vision Science and Ophthalmology (ARVO) conference which outlined an experiment that used a model blink to analyze the wettability of lens materials.[26] Their model included a blink cell that held a single contact lens and the ATS used was pumped in and out of the cell, thus raising and lowering the liquid levels. In this experiment a layered ATS was used by spreading a single lipid layer on the anterior surface of the liquid.[26] Once again, lipid deposition was not quantified or compared with in-vial incubations.[26]

In vitro models for lipid deposition onto various contact lens materials have proven to be valuable for the examination of extraction efficiency (Chapter 4), the effect the tear film composition (Chapter 5),[18] the effects of ATS concentration,[20] the effect of ATS replenishment (in house data), the effect of incubation time[19] and the efficiency of hydrogen peroxide solutions to remove lipid from contact lens materials (Chapter 6).

The model blink cell unit introduced in this paper has effectively modeled the exposure to air experienced by contact lenses during the inter-blink period. Further

work is needed to determine if the changes in deposition are a phenomenon specific to early stages of deposition or if the effects are cumulative over a longer period of time for all lens materials and lipids. It also may be interesting to see if the lenses incubated with the model blink cell contain a higher mass of oxidized lipids than conventional in-vial incubations and to compare that to *ex vivo* lens data. Additionally, since the model blink cell elicits a different mode of deposition than in-vial incubations, it may be prudent to examine the depth of lipid penetration into the lens matrix using both incubation models.

The ATS used in this experiment was a homogenous mix of all incorporated components and did not incorporate the human biophysical tear film structure. It is well known, that the human tear film is composed of three main phases: the anterior lipid layer, the larger aqueous phase and the mucin glycocalyx phase that covers the epithelium.[27-29] The model blink cell unit could support a layered ATS, with mechanical modifications, to make the device similar to a Langmuir trough. It would be of great interest to see if the deposition pattern and masses deposited differed with a change in ATS structure. The experiment presented here, was a pilot study for this device and therefore further experimentation is needed.

It is true that an *in vitro* model will never be able to fully mimic human contact lens wear, as there are just too many variables and individual differences in home/work environment, diet, disease and tear film composition. However, the incorporation of a complex ATS and now air exposure has increased the validity of this lipid deposition model.

7.6 CONCLUSION

In conclusion, this *in vitro* blink cell model has demonstrated that lipid deposition kinetics can be impacted by air exposure and that lipid deposition profiles are contact lens and lipid dependent. *In vitro* models must begin to use more physiologically relevant incubation solutions and conditions that mimic contact lens wear within the natural tear film if *in vitro* data is to be extrapolated to the *in vivo* situation. These methodologies will provide hitherto unavailable information on the way in which lipid interacts with silicone hydrogel materials and will be of interest to the contact lens industry, clinicians and other areas of biomaterial research.

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8 OVERALL DISCUSSION AND CONCLUSIONS

Since the release of silicone hydrogel contact lens materials on the market in 1999, practitioners and researchers around the world have found that these materials are more prone to lipid deposits in the form of calculi and oily films, when compared to the traditional conventional hydrogel materials.[1-4] It is well known that silicone hydrogels, with their incorporation of siloxane groups are more hydrophobic and less wettable and therefore more prone to lipid deposition. [5-7] Many companies have therefore incorporated a wetting agent or surface coating to reduce the hydrophobic effect of their lenses.

Historically, lipid deposition has not been the focus of extensive research as conventional hydrogel materials are hydrophilic and more susceptible to protein deposition, especially for group IV materials.[3, 8-17] For this reason, there has been a relative dearth of information on lipid deposition, the factors that influence it, and its impact on lens wear. Now that silicone hydrogels have been on the market for over ten years and are becoming a popular choice for lens wearers around the world, researchers are scrambling to assess all of the factors that influence lipid deposition and how to manage it.

It is clear that a vast amount of research needs to be completed to even begin to comprehend the complex interactions that occur during human contact lens wear. Therefore, many researchers have begun to conduct both *in vitro* and *ex vivo* experiments to assess the many unanswered questions. There are many advantages

and disadvantages of conducting both *in vitro* and *ex vivo* studies. Some of the main advantages of *in vitro* research are that all of the variables in the system can be controlled, unique experimental techniques can be utilized that may not be suitable for clinical studies, and that *in vitro* models can be as complex or simplistic as required. The main purpose of the research conducted in this thesis was to start from the beginning and to slowly, yet systematically build up a model for lipid deposition which incorporates and analyzes the many different interactions and variables.

The optimization and assessment of a new artificial tear solution was described in Chapter 3. In this chapter, the formulation for an updated incubation solution was tested for its chemical stability, physical stability, and for the lens parameter stability following incubation. Lipid deposition onto two different lens materials following incubation in two different artificial tear solution iterations was also examined. From the plethora of small individual experiments conducted it was found that the optimized complex artificial tear solution remains stable in its pH, osmolality, homogeneity, and surface tension throughout a four week incubation. When various contact lenses are incubated within this solution, their base curve, centre thickness, and diameter remain stable with no clinically significant changes. When lipid deposition was examined using a radiochemical experiment, it was found that the addition of lactoferrin and immunoglobulin G to the ATS significantly increased deposition. Overall, the cholesterol and phosphatidylcholine deposition was lower than other *in vitro* and *ex vivo* lipid deposition publications; however

differences in protocol were noted. Despite this, the hierarchy of deposition on the various lens materials was consistent with other studies.

The efficiency of (2:1) chloforom:methanol as a lipid extraction method was assessed on both silicone hydrogel and conventional hydrogel lenses for both model lipids in Chapter 4. Through the use of radiolabeled lipids, both of the model lipids could be assessed for their extraction from several contact lens materials by calculating the lipid remaining in the used incubation solutions. Overall, it was determined that three extractions of 3 hrs at 37°C in 2 mL of (2:1) chloroform: methanol delivered an extraction efficiency of >90% for all lens materials examined. This (2:1) chloroform: methanol protocol was found to be efficacious for phosphatidylcholine extraction for four out of the six lens materials, however lotrafilcon B and balafilcon A had extraction efficiencies ≤85%. Therefore, three other extraction methods were examined to see if the lipid recovery and thus the extraction efficiency could be improved. Ultimately, it was found that extractions of 3 hrs at 37°C in 2 mL of (60:50:1:4) chloroform: methanol: acetic acid: water were able to increase phosphatidylcholine extraction efficiency to approximately 100%. This experiment revealed the need that all lens materials being utilized have to be tested with the corresponding extraction protocol, as different lens materials may bind lipids with different strengths.

The effect of artificial tear solution composition on cholesterol and phosphatidylcholine deposition after 3 and 14 days of incubation was assessed in chapter 5. Balafilcon A, senofilcon A and etafilcon A contact lens materials were

incubated in four different compositions and complexities of artificial incubation solutions using radiochemical experiments. The four artificial tear solutions tested were: a single lipid tear solution (SLTS), a lipid tear solution (LTS) containing five common lipids, a protein tear solution (PrTS) and an artificial tear solution (ATS) containing the LTS and the PrTS, at physiological concentrations. It was found that the incubation solution composition had a profound effect on the deposition profile of each radioactive lipid and the pattern of deposition was directly correlated to the contact lens properties, lipid properties, and interactions between the solution components. The experiment supported the theory that single component and even moderately complex artificial tear solutions have a very different effect on lipid deposition when compared with a fuller tear solution containing a range of lipids, proteins, and mucin.

The sensitivity and reliability of the radiochemical protocol and the ability for hydrogen peroxide solutions to remove lipid from contact lenses was examined in Chapter 6. Lenses were incubated in the artificial tear solution daily for eight hours and then either stored or cleaned with one of two commercially available hydrogen peroxide cleaning solutions for the remaining sixteen hours. This cycle was continued for one week and then the lenses were extracted and cholesterol and phosphatidylcholine was quantified. Both ClearCare and AOSept hydrogen peroxide solutions both showed decreased deposition over the control, however the improvement was minimal and possibly not clinically relevant.

Chapter 7 probed the effect of intermittent air exposure on lipid deposition using a custom-built model blink cell. Due to the hydrophobicity and ease of polymer chain rotation for silicone hydrogel lenses, it was found that most lenses exposed to air experienced an increase in cholesterol and phosphatidylcholine deposition after a 10 hour cycling period when compared with lenses that were simply submerged for the incubation period. This model blink cell device was designed to mimic the effect of tear film breaking in between blinks during human contact lens wear and this study found that lipid deposition increases as tear break up time decreases.

Overall, this thesis has contributed greatly to our understanding of lipid deposition and the *in vitro* model built here can be utilized in various ways in the future to assess other aspects and variables of lipid and protein deposition. For instance, the model developed here has already been used to assess the effect of lipid concentration in the incubation solution on lipid deposition, the kinetic uptake of lipids on a range of materials, and the effect of replenishing the incubation solution on deposition.[18, 19]

Future research using the model blink cell alone has a vast range of abilities in the ophthalmic industry including: assessing the penetration profile of lipids during submersion and intermittent air exposure with fluorescently tagged lipids, tear film stability and tear break up time and the factors that effect it, the release of pharmaceuticals from contact lenses, the effect of air exposure and light on lipid

deposition, the ability of contact lens cleaning solutions to remove lipid from various lens materials, and the substantivity of surfactants on contact lens materials.

In general, future research in lipid deposition should focus on how the deposited lipid changes in structure and how the contact lens surface and matrix change because of deposition. Other questions to be examined include: Does UV light, exposure to aerosols and chemicals in disinfecting and multipurpose solutions create oxidized lipids and by-products which can signal an inflammatory response? Do oxidized lipids and their by-products affect comfort of a contact lens? How much lipid should remain on a contact lens following cleaning and which lipids should be preferentially removed? What effect do various pharmaceuticals have on meibum composition and contact lens wear? Does lipid preferentially deposit in the periphery or central portion of the lens? What effect does MGD treatment have on lipid profiles and contact lens wear?

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APPENDIX A: COPYRIGHT PERMISSIONS

UNIVERSITY OF WATERLOO

From: Roland Hall
To: Holly Lorentz
Subject: RE: Question about writing my PhD thesis
Date: July 7, 2011 12:04:45 PM
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Hi Holly,
This is an interesting question and situation. I think it is appropriate to provide an updated revised version of the Introduction from your Master's thesis. You need to be upfront about this, identifying that your PhD is a continuation from your MSc and that the Introduction is an updated and revised version beyond what was presented in your MSc thesis. You can cite your MSc thesis. I think your situation is much like issuing a revised version of a book chapter when a new edition of the book is issued. Some part s are lightly modified and other parts are new or have been modified considerably.

Hope this helps.
Cheers, Roland

_____
Roland Hall
Professor & Associate Dean of Science for Graduate Studies
Department of Biology, University of Waterloo
200 University Avenue West, Waterloo, ON, N2L 3G1, Canada
Tel. (+1) 519-888-4567 x32450
Fax: (+1) 519-746-0614
Email: rihall@uwaterloo.ca

From: Holly Lorentz [mailto:hmelchin@sciborg.uwaterloo.ca]
Sent: July 6, 2011 1:47 PM
To: Roland Hall
Cc: Holly Lorentz Lorentz
Subject: FW: Question about writing my PhD thesis

Hello Dr. Hall
I have a question for you about writing my PhD thesis. I have already contacted academic integrity and they have suggested that I contact you. My correspondence with them is below.
Here is my story: I completed my MSc thesis here at UW working on the same line of research as I am for my PhD thesis. In my MSc thesis I had a long in-depth Introduction outlining basics of my research and current literature from my area of

research. Now when I am starting to write my PhD thesis Introduction I need to cover a lot of the same material. Of course some of it will be different and some will have to be updated but is it possible to use sentences, paragraphs and/or sections from my MSc thesis in my PhD thesis? How would I reference my own MSc thesis? Do I have to re-write my entire MSc introduction and paraphrase my own work? I really would like to know what I am allowed to do in this area .

I would appreciate any help you can give me – I understand the serious issues of plagiarism and I am trying to be extraordinarily cautious so I do not make any fatal errors.

I have spoken with my supervisor, Dr. Lyndon Jones, and he hopes that I will be able to update my MSc introduction with recent references and information.
Thank you for your help and guidance in this manner

Holly Lorentz

Holly Lorentz BSc, MSc
PhD Candidate in Vision Science
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hmelchin@scimail.uwaterloo.ca

From: Faye Schultz [mailto:fschultz@uwaterloo.ca]
Sent: June 3, 2011 1:23 PM
To: Holly Lorentz
Subject: RE: Question about writing my PhD thesis
-->
Hello Holly,
Thank you for contacting the Office of Academic Integrity with your questions. My suggestion is that you should first discuss these questions with your supervisor, and then the Associate Dean, Graduate Studies from Science if further clarification is required. There are some basic principles to be aware of such as acknowledging previous work (including your own) and there are different disciplinary conventions, so your advisor and the Associate Dean, Roland Hall (rihall@uwaterloo.ca) are in the best position to advise you. You can certainly draw upon your thesis, given there will be some of the very same literature involved, but you need to be clear to indicate where and how you do this within your dissertation.

Regarding your question on copyright according to the Graduate Studies Calendar, "The author is the immediate owner of the copyright in the original work, except in certain cases where he or she is under an employment contract. However, students including works by other authors in their document, must obtain prior permission from the copyright holder."

Please refer to the link in the calendar which refers to copyright and University ownership of the physical/electronic document (in your case the thesis) as part of the Library Collection: http://gradcalendar.uwaterloo.ca/page/GSO-Students-Work

I hope these suggestions are helpful to you.
Sincerely,
Faye Schultz

From: Holly Lorentz [mailto:hmelchin@sciborg.uwaterloo.ca]
Sent: June 3, 2011 11:29 AM
To: Faye Schultz
Subject: Question about writing my PhD thesis
Hello
I am hoping you can help me or direct me to someone who can help me.
I am currently starting to write up my PhD thesis and I have come across a conundrum.

I completed my MSc thesis here at UW working on the same line of research as I am for my PhD thesis. In my MSc thesis I had a long in-depth Introduction outlining basics of my research and current literature from my area of research. Now when I am starting to write my PhD thesis Introduction I need to cover some of the same material. Of course some of it will be different and some will have to be updated but is it possible to use sentences and/or paragraphs from my MSc thesis in my PhD thesis???? Do I have to ask for permission since I believe UW now holds copyright on my MSc thesis? Do I reference my own MSc thesis or is this not necessary? Do I have to re-write my entire MSc introduction and paraphrase my own work? I really would like to know what I am allowed to do in this area.

I would appreciate any help you can give me – I understand the serious issues of plagiarism and I am trying to be extraordinarily cautious so I do not make any fatal errors

Thanks
Holly

Holly Lorentz BSc, MSc
PhD Candidate in Vision Science
Centre for Contact Lens Research

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hmelchin@scimail.uwaterloo.ca

SPRINGER SCIENCE AND BUSINESS MEDIA

From: Essenpreis, Alice, Springer DE
To: hmelchin@sciborg.uwaterloo.ca
Subject: WG: Use of a couple of figures
Date: October 31, 2011 10:33:48 AM
-->
Hello Holly,
You are allowed to use the figures requested in the way you described below, provided that the original Springer source will be credited to the figures.
Thank you.
Kind regards,
Alice

_
Alice Essenpreis
Springer
Rights and Permissions

_
Tiergartenstrasse 17 | 69121 Heidelberg GERMANY
FAX: +49 6221 487 8223
permissions.Heidelberg@springer.com
www.springer.com/rights

_
Von: Holly Lorentz [mailto:hmelchin@sciborg.uwaterloo.ca]
Gesendet: Freitag, 21. Oktober 2011 05:29
An: Essenpreis, Alice, Springer DE
Betreff: RE: Use of a couple of figures
Hello
I have read over the conditions reported for the use of the figures I have requested (below) and I have recently found out that all theses at the University of Waterloo are available publicly and therefore can be searched and downloaded, without passwords etc. Does this change my permissions for use of these figures?

I have contacted Dr. Knop (the first author of both articles) via email to gain his permission for use of these figures, but I have not yet heard back.
Thank you for all of your help

Holly

Holly Lorentz BSc, MSc
PhD Candidate in Vision Science
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extension # 37009 (lab) or #36210 (office)
hmelchin@scimail.uwaterloo.ca

From: Essenpreis, Alice, Springer DE [mailto:Alice.Essenpreis@springer.com]
Sent: October-17-11 10:16 AM
To: Holly Lorentz
Subject: AW: Use of a couple of figures
-->
Hi,
Yes, we would appreciate it very much if the author is informed as well.
Kind regards,
Alice

Von: Holly Lorentz [mailto:hmelchin@sciborg.uwaterloo.ca]
Gesendet: Montag, 17. Oktober 2011 16:09
An: Essenpreis, Alice, Springer DE
Betreff: RE: Use of a couple of figures
Hi
Thank you so much for this permission.
From the details of your email do I understand it correctly that I am also required to contact the original author of the articles to obtain their permission for use of the figures?
Thank you again
Holly

From: Essenpreis, Alice, Springer DE [mailto:Alice.Essenpreis@springer.com]
Sent: October 17, 2011 9:51 AM
To: hmelchin@sciborg.uwaterloo.ca
Subject: WG: Use of a couple of figures
-->
Dear Ms. Lorentz,
Thank you for your e-mails.
We were attending the Frankfurt Book Fair last week, therefore, the delayed reply.
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charge, for the use indicated in your enquiry. Licenses are for one-time use only with a maximum distribution equal to the number that you identified in the licensing process.

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Kind regards,

Alice Essenpreis
Springer
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FAX: +49 6221 487 8223
permissions.Heidelberg@springer.com
www.springer.com/rights

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Von: Holly Lorentz [mailto:hmelchin@sciborg.uwaterloo.ca]
Gesendet: Dienstag, 11. Oktober 2011 09:31
An: Permissions Heidelberg, Springer DE
Betreff: Use of a couple of figures
Hello
I am currently writing up my PhD thesis and would like to use a couple figures from a couple different journal articles in my thesis introduction.

I tried going on the Copyright Clearance RightsLink – but it instructed me to contact Springer directly.

I would like to use:
1) Figure 5 (morphology of a single meibomian gland) from Knop N, Knop E. [Meibomian glands. Part I: anatomy, embryology and histology of the Meibomian glands] Meibom-Dru¨sen Teil I: Anatomie, Embryologie und Histologie der Meibom-Dru¨sen. *Ophthalmologe*.2009;106:872–883.
2) Figure 4 (pathophysiology of obstructive MGD) from Knop E, Knop N. [Meibomian glands, Part IV: functional interactions in the pathogenesis of meibomian gland dysfunction (MGD).] Meibom-Dru¨sen, Teil IV: Funktionelle Interaktionen in der Pathogenese der Dysfunktion (MGD). *Ophthalmologe*. 2009;106:980–987

For both figures I wish to use the English versions as published in Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. Invest Ophthalmol Vis Sci. 2011 Mar;52(4):1938-78.

Please let me know if I have permission to use these two figures, how you want me to cite the permission and if I am required to pay for this permission
Thank you
Holly Lorentz

Holly Lorentz BSc, MSc
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hmelchin@scimail.uwaterloo.ca

From: Debbie Chin
To: Holly Lorentz
Subject: RE: Use of a couple figures in my PhD thesis
Date: October 4, 2011 11:43:43 AM
-->
Dear Dr. Lorentz,

The two figures from the Knop article (#1 and #3 in your email) were reprinted from a different journal. You will need to obtain permission from them to use the figures. You can see the citation of the original articles in the figure legends: http://www.iovs.org/content/52/4/1938.full?sid=cbf8d9c2-43f5-4360-9faf-2d24f73e0627#F3 and http://www.iovs.org/content/52/4/1938.full?sid=cbf8d9c2-43f5-4360-9faf-2d24f73e0627#F25.

Permission is granted to reprint the following figure in your PhD thesis:
Figure 1 from Green-Church KB, Butovich I, Willcox M, Borchman D, Paulsen F, Barabino S, Glasgow BJ. The International Workshop on Meibomian Gland Dysfunction: Report of the Subcommittee on Tear Film Lipids and Lipid-Protein Interactions in Health and Disease. *Invest Ophthalmol Vis Sci*. 2011;52:1979-1993.

A reprint of the material must include a full article citation and acknowledge ARVO as the copyright
holder.

Best regards,
Debbie Chin
Association for Research in Vision and Ophthalmology
1801 Rockville Pike, Suite 400
Rockville MD 20852 USA
Direct: +1.240.221.2926 | Main: +1.240.221.2900 | Fax: +1.240.221.2370
www.arvo.org
2012 ARVO Annual Meeting
May 6 – 10 | Fort Lauderdale, Florida
www.arvo.org/am

From: Holly Lorentz [mailto:hmelchin@sciborg.uwaterloo.ca]
Sent: Monday, October 03, 2011 2:58 PM
To: Debbie Chin
Subject: RE: Use of a couple figures in my PhD thesis
Hello Debbie
Please let me thank you for allowing me to utilize a few figures from IOVS in my PhD

thesis.

The figures I wish to use as part of my PhD thesis are the following:
Figure 3 (morphology of a single meibomian gland) from: Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. Invest Ophthalmol Vis Sci. 2011 Mar;52(4):1938-78.

Figure 1 (A proposed model of the precorneal tear film…) from: Green-Church KB, Butovich I, Willcox M, Borchman D, Paulsen F, Barabino S, et al. The international workshop on meibomian gland dysfunction: report of the subcommittee on tear film lipids and lipid-protein interactions in health and disease. Invest Ophthalmol Vis Sci. 2011 Mar;52(4):1979-93.

Figure 25 (pathways and proposed sequence of events …) Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. Invest Ophthalmol Vis Sci. 2011 Mar;52(4):1938-78.

Please let me know how you wish me to cite and acknowledge IOVS and ARVO for the use of these figures.

Cheers
Holly Lorentz

OPTOMETRY AND VISION SCIENCE

From: Zadnik, Kurt
To: Holly Lorentz
Subject: RE: Permission for use of my own article
Date: October 3, 2011 2:52:28 PM
-->
Hi Holly,
Sounds good. Go ahead.
Cheers,
Kurt
****
Optometry and Vision Science
Kurt A. Zadnik, Managing Editor

The Ohio State University, College of Optometry
338 West 10th Avenue
Columbus, OH 43210
Tel: (614) 292-4942; Fax: (614) 292-4949;
e-mail: ovs@osu.edu
http://ovs.edmgr.com
****

From: Holly Lorentz [mailto:hmelchin@sciborg.uwaterloo.ca]
Sent: Monday, October 03, 2011 2:48 PM
To: Zadnik, Kurt
Subject: Permission for use of my own article

Hi Kurt

Thank you very much for this permission. I do want to make one amendment to my request: I would like to be able to use the entire section subtitled "lipid deposition on contact lens materials", including Figures 2,3 and 4 from my paper: Lipid deposition on hydrogel contact lenses: how history can help us today. Optom Vis Sci 2007;84:286-95.

Of course I will use the citation you listed:

"Reproduced with permission from: Lorentz H, Jones L. Lipid deposition on hydrogel contact lenses: how history can help us today. Optom Vis Sci 2007;84:286-95. ©The American Academy of Optometry 2007."

Please confirm that this is still approved – This description will better cover the portions I wish to use.

Thank you

Holly Lorentz

From: Zadnik, Kurt [mailto:ovs@osu.edu]
Sent: September-27-11 12:53 PM
To: Holly Lorentz
Subject: RE: Permission for use of my own article

-->

Hi Holly,

Permission is granted to use the excerpt and figures, as described, from your original paper. Please use the following, so that the proper credit is given to OVS and the AAO:

"Reproduced with permission from: Lorentz H, Jones L. Lipid deposition on hydrogel contact lenses: how history can help us today. Optom Vis Sci 2007;84:286-95. ©The American Academy of Optometry 2007."

Best of luck with your thesis,

Kurt

****

Optometry and Vision Science
Kurt A. Zadnik, Managing Editor
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http://ovs.edmgr.com
****

From: Holly Lorentz [mailto:hmelchin@sciborg.uwaterloo.ca]
Sent: Monday, September 26, 2011 1:00 PM
To: Zadnik, Kurt
Subject: Permission for use of my own article

Hello

My name is Holly Lorentz and I have a article published in OVS (Lorentz H, Jones L. Lipid deposition on hydrogel contact lenses: how history can help us today. Optom Vis Sci. 2007 Apr;84(4):286-95.) and I would like to use a significant portion of this article in my PhD thesis. I am unable to use the entire article (due to new data and research completed since the original review paper's publication), but I would like to use the portion titled "lipid deposition on contact lens materials" specifically pertaining to past research completed on lipid deposition on conventional hydrogel lens materials, including Figures 2,3 and 4.

Please let me know if this is possible and how I can obtain official permission for this request. I do not know if this request fits under normal rights and permission requests listed on your website.

Thank you

Holly

Holly Lorentz BSc, MSc
PhD Candidate in Vision Science
Centre for Contact Lens Research
School of Optometry
University of Waterloo
200 University Avenue West
Waterloo, Ontario, Canada N2L 3G1
1-519-888-4567
extension # 37009 (lab) or #36210 (office)
hmelchin@scimail.uwaterloo.ca