Development of an *in vitro* eye model to better represent *in vivo* physiological conditions

by

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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

The purpose of this thesis was to optimize a novel *in vitro* blink model, the OcuBlink, to best mimic the physiological characteristics of the human eye. By improving this *in vitro* model, the results from *in vitro* studies using this model can be more representative of *in vivo* studies.

Methods

The first experimental chapter of this thesis, Chapter 3, explores the results of active lysozyme deposition on contact lenses using the blink model. An *ex vivo* active lysozyme deposition study was referenced to determine the ideal flow rate for the blink model. These parameters were used to determine the active lysozyme deposition data for several other lens materials. The blink model was directly compared to a simple vial at 8 hours of incubation to show the difference between the two *in vitro* models.

The experiments in Chapter 3 led to several developmental improvements to the blink model design. Chapter 4 explores the changes to the blink model from its initial design through different iterations to incorporate a heated system to simulate ocular temperatures.

Chapter 5 uses the new blink model improvements to study contact lens dehydration. The effect of incubation temperature, incubation solution, and *in vitro* model design were explored. The comparison between the vial system and the blink model showed a difference in water content patterns over time.

Results

With *ex vivo* data as a reference, the blink model was able to replicate the active lysozyme deposition data on etafilcon A lenses. The parameters of the blink model were used to determine active lysozyme deposition on other contact lenses. This study provided the expected range for *ex vivo* active lysozyme deposition for these lens materials as this data was not available in the literature at the time of the study. The comparison of the *in vitro* models showed that contact lens material plays a large role in active lysozyme deposition patterns over time.

The different iterations of the blink model show how different materials and designs can improve and progress *in vitro* testing of ocular studies. The most significant addition to the blink model was the incorporation of a heated element to allow studies to be conducted at ocular temperatures.

The improved blink model showed a decrease in water content for all lenses for an increase in incubation temperature. The incubation solution did not have an effect on water content for most tested lenses, however, lens material played a major role. All lenses decreased in water content after the first hour of incubation. With the blink model, lenses showed a recovery in water content over 16 hours, however the lenses showed a plateau effect in a simple vial model. The recovery in water content on the blink model has not been seen on other *in vitro* or *in vivo* studies to date and will need further testing to better understand the phenomenon.

Conclusion

Although the data has yet to be validated with *in vivo* data, this thesis shows that the blink model has promise as a predictive tool for *in vivo* studies. The advanced *in vitro* blink model can be adjusted per study as required to fulfil experimental requirements. The ultimate goal of the blink model is to produce results that are more representative of *in vivo* data compared to more simplistic

in vitro models while minimizing the cost and time of animal models and clinical trials where appropriate.

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List of Abbreviations

ANOVA	analysis of variance
ATS	artificial tear solution
BSA	bovine serum albumin
CAD	computer-aided design
CH	conventional hydrogel
CL	contact lens
CLD	contact lens discomfort
CNC	computer numerical control
DD	daily disposable
ELISA	enzyme-linked immunosorbent assay
EWC	equilibrium water content
FDA	the Food and Drug Administration
MPS	multipurpose solution
OT	ocular temperature
PBS	phosphate buffered saline
PDMS	polydimethylsiloxane
pHEMA	2-hydroxyethyl methacrylate
PLA	polylactic acid
PMMA	Polymethylmethacrylate
PVA	polyvinyl alcohol
PVP	polyvinyl pyrrolidone
RT	room temperature
RU	reusable
SD	standard deviation
SH	silicone hydrogel
SLA	stereolithography
UV	ultraviolet
TFOS	Tear Film & Ocular Surface Society

1 Thesis Introduction

1.1 Contact Lenses

1.1.1 Contact lens history

The first concept of a contact lens (CL) was introduced in the 1500s by Leonardo da Vinci.[1, 2] In the following years, several theorists, such as René Descartes and Thomas Young, explored refraction and accommodation of the eye.[1, 2] In the mid-1800s, John Herschel had used animal jelly to correct for corneal irregularities using a spherical glass on the eye.[1, 2] By the late 1800s, glass scleral and corneal lenses were being produced and tested, but discomfort and lack of oxygen to the eye became immediate problems.[1, 2] In the late 1930s, Theodore Obrig made the first all-plastic scleral CL with polymethylmethacrylate (PMMA).[2] Just over a decade later, Kevin Tuohy manufactured and obtained a patent for a corneal CL.[2] In 1952, Otto Wichterle produced 2-hydroxyethyl methacrylate (pHEMA) for human prosthetics and gained a patent for soft CLs the next year.[2]

In the 1960s to 1970s, the development of corneal lenses improved and increased in popularity.[1, 2] However, discomfort and corneal hypoxia were still common.[1, 2] In the 1970s, silicone elastomers were used to create highly oxygen permeable lenses with the introduction of surface treatments for increased wettability.[1] When silicone hydrogel materials were used for CL synthesis in the late 1990s, they were proposed to solve corneal hypoxia.[3] While silicone hydrogel materials did increase oxygen permeability to the eye, the increased hydrophobicity led to decreased wettability and an increase in lipid deposition.[3] Since then, CL design and manufacturing have improved significantly, and CLs are now used worldwide by over 140 million people.[4] The further development of siloxane-based materials for CLs led to various “generations” of silicone hydrogel materials.[5] As new CL materials improve, the problems of comfort and

deposition still exist. There has been a focus on changing CL modality, leading to the rise of daily disposable CLs, which provide the additional benefit of a decreased rate of CL complications.[3]

1.1.2 Contact lens materials

Contact lens materials are separated into categories of rigid and soft CLs. Soft CL are further categorized into conventional hydrogel (CH) materials and silicone hydrogel (SH) materials. CH lens materials are categorized by the Food and Drug Administration (FDA) into four numerical groups based on water content and ionicity. SH lens materials can be further broken down into three different generations.

CLs were originally made of glass,[1, 3] which gradually evolved to materials such as HEMA, for better durability and ease of manufacturing.[3] Early CH materials had low oxygen transmissibility and corneal edema occurred frequently.[1, 3] Further, some CH lens materials were modified with hydrophilic monomers in either the packing solution or in the polymer matrix for added comfort.[3]

The incorporation of silicone into hydrogel materials caused a major change in CL manufacturing.[3] SH lens materials had increased oxygen permeability compared to CH lens materials.[1, 3] However, incorporating silicone into CL materials made the lenses more hydrophobic and resulted in an increase in lipid deposition.[3] To address these issues, SH CLs were initially designed with a surface coating to overcome these hydrophobic effects.[3, 5] For example, first generation SH lens materials lotrafilcon A and lotrafilcon B have a hydrophilic plasma coating and balafilcon A has a plasma oxidation surface treatment to increase surface wettability.[5] As new lens material and manufacturing methodology was explored, there was an increase in understanding of corneal hypoxia and the effect of CL design and material properties on corneal health.[3]

The second generation of SH CL materials no longer required surface treatments, but instead incorporated internal wetting agents for increased comfort.[3, 5] Polyvinyl pyrrolidone (PVP) was the internal wetting agent for both senofilcon A and galyfilcon A lens materials.[5] As the development of CL materials improved, different modifications of SH CL materials emerged, with modifications to the lens polymer matrix that removed the need for any surface treatment or internal wetting agents.[5] These lens materials, such as comfilcon A, were categorized as the third generation of SH CL materials.[5]

1.1.3 Contact lens modality

Although lens material is an important factor in deciding which CL is optimal for individual use, CL wearing modality also plays a major role.[1, 3] CLs would initially only be worn for a few hours at a time due to discomfort and corneal hypoxia.[1, 3] As the lens materials improved to increase oxygen permeability, lenses were able to be worn for longer periods of time and overnight.[1, 3] Cases of ocular infections and microbial keratitis became frequent, most often due to poor hygiene and lens care.[1] When disposable CLs were introduced in the 1990s, lens care became less of a problem.[1] The emergence of SH materials to increase oxygen permeability, continual research on ocular health and proper lens care, and an increase in daily disposable CLs resulted in an improvement in CL comfort and reduced incidence of infections.[1, 3] However, not all individuals improved by changing from CH lens materials to SH materials or from extended lens wear to daily disposable lenses.[6] There are many underlying factors that affect the comfort and complications of CL wear, which is why both CH and extended wear lenses are still prescribed to patients today, albeit in reduced numbers.

1.2 Contact lens discomfort

Even with the success of millions of CL wearers worldwide, up to half experience some degree of contact lens discomfort (CLD).[7] Contact lens discomfort was defined as "a condition characterized by episodic or persistent adverse ocular sensations related to lens wear, either with or without visual disturbance, resulting from reduced compatibility between the contact lens and the ocular environment, which can lead to decreased wearing time and discontinuation of contact lens wear" by the Tear Film & Ocular Surface Society (TFOS) International Workshop on Contact Lens Discomfort.[7] Approximately 12 to 51% of contact lens wearers drop out of contact lens wear, primarily due to CLD.[7]

The stability of the tear film plays an important role in CLD. The state of the tear film, and subsequently CLD, is influenced by several factors, including the tear film components, the lens material, length of lens wear, type of CL, lens care, the ocular environment, the external environment, and the lifestyle of the individual.[7, 8] The tear film components are able to interact with the CL, which can cause irritation and dryness.[7-11] The lens fit on the eye,[7] as well as the wettability,[8, 10, 12] and lens material[8, 11] are properties of the CL that play a role in the comfort of the lens on the ocular surface. Individuals in low humidity environments may experience more CLD compared to those in high humidity environments.[9] The individual's lifestyle and ocular condition also varies the level of CLD.[8, 11] The CL cleaning solution, regime, and wear time is also important to CLD.[7, 8, 13] With so many factors affecting CLD, it is important to understand the individual components of the tear film and how they interact with the CL.

1.2.1 Tear Film biochemistry

The tear film is about 3 to 6 µm thick[9, 14, 15] and consists of several layers.[16] The most anterior layer of the tear film is the lipid layer, which ranges between 50-100 nm in thickness,[9]

overlying an aqueous-mucin phase.[14] Figure 1.1 depicts a CL sitting in the aqueous and mucin layer of the tear film.

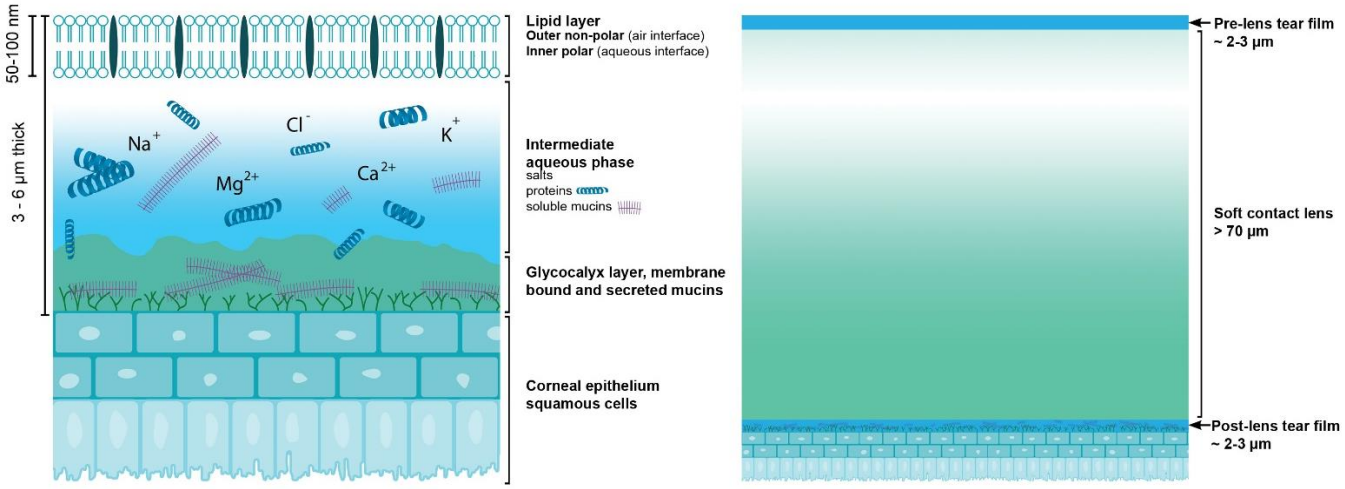

Figure 1.1. Layers of the tear film (left) and with a contact lens (right). Used with permission of CORE (Centre for Ocular Research and Education), University of Waterloo.

1.2.1.1 Lipid layer

The lipids in the tear film are produced mainly by the Meibomian glands in the eyelids, with additional lipids produced by the glands of Moll and Zeiss.[9] These lipids are spread across the surface of the eye by the blinking motion of the eyelid.[9] The lipid layer functions to lower tear evaporation,[9, 14, 16] prevent overflow of tears onto the skin,[9, 14] and protect against small foreign particles.[9, 16] The lipid layer is further separated into two phases, the outer phase being non-polar and the inner phase, a thin polar layer.[8, 9, 14] The outer non-polar layer acts as protection and prevents evaporation of the tear film.[8, 9] The inner polar layer aids in tear film stability and acts as a surfactant.[8, 9]

1.2.1.2 Aqueous and mucin phase

The aqueous portion of the aqueous and mucin phase is produced mainly by the lacrimal gland and accessory glands of Krause and Wolfring.[9, 14] This portion of the tear film contains salts, electrolytes, proteins, cytokines, and other nutrients to make up the bulk of the tear film.[9, 14, 16, 17] The aqueous layer functions to protect, remove waste and act as a source of nutrients for the cornea.[9, 14, 16] Electrolytes, such as sodium, potassium, and calcium, aid in regulating osmolarity and pH.[9, 14] The concentration of proteins in the tear film is dependent on the type of tears.[9] Tear proteins such as lysozyme, lactoferrin, and lipocalin-1, tend to stay consistent for basal, reflex, and closed-eye tears.[9, 14] Secretory immunoglobulin-A is more prominent in closed-eye tears and is less prominent in reflex tears.[9] While some of these proteins are secreted by lacrimal glands and accessory glands, other proteins, such as albumin and IgG are from plasma leakage.[11, 14] The most abundant protein in tears is lysozyme, making up over 30% of the total proteins, followed by lactoferrin.[11]

The mucin portion is produced mainly by conjunctival and corneal epithelial cells[9, 14, 16] as well as conjunctival goblet cells.[9, 14] The mucin layer functions as protection against potential pathogens[9, 14, 16] and from shear stress due to the blinking motion.[9, 14] On the surface of the corneal epithelium, a layer of glycocalyx sits between the mucin layer and the cornea as shown in Figure 1. This acts as an additional protective layer against potential pathogens, helps to anchor the aqueous layer, and aids in the wettability of the tear film.[8, 11, 14]

1.2.2 Tear film interaction with contact lenses

When a CL is worn, it sits in the aqueous and mucin phase of the tear film.[7, 9] The addition of a contact lens changes the normal tear film structure.[7, 9, 16] The disruption to the lipid layer results in a less stable tear film.[7, 9, 16] Tear film evaporation rates are subsequently increased,

leading to a thinner tear film layer and increased tear break up time.[7, 9, 11] These changes to the tear film on the surface of the CL have been associated with CLD.[7, 11]

1.2.2.1 Tear film deposition

When the tear film is destabilised, due to the addition of a CL for example, it causes an increase in tear evaporation and consequently, the aqueous-mucin phase decreases in volume.[7-9, 16] This can cause the lipid layer to come into contact with the CL surface, resulting in lipid deposition onto the CL,[9] as shown in Figures 1.2 and 1.3. The amount and rate of deposition onto CL varies for both protein and lipid deposition, in which both are dependent on individual tear film composition, lens replacement frequency, and cleaning solution and regimens.[7, 8, 11, 12]

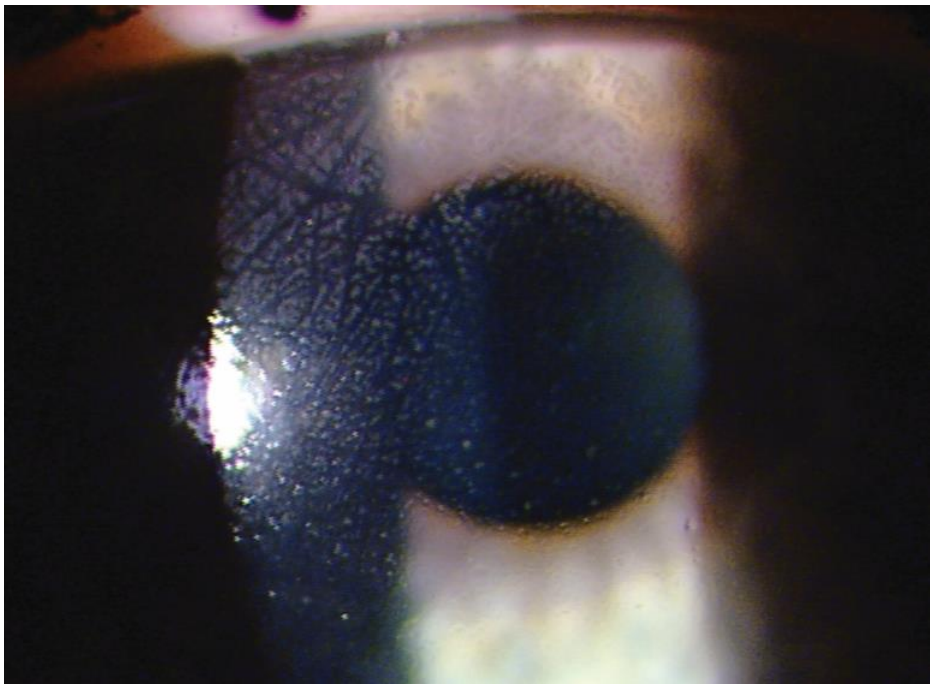

Figure 1.2. A hydrogel contact lens with a layer of deposition shown through a slit lamp. Image used with permission; Luensmann D, Jones L. Albumin Adsorption to Contact Lens Materials: A Review. Cont Lens Anterior Eye 2008;31:179-87.

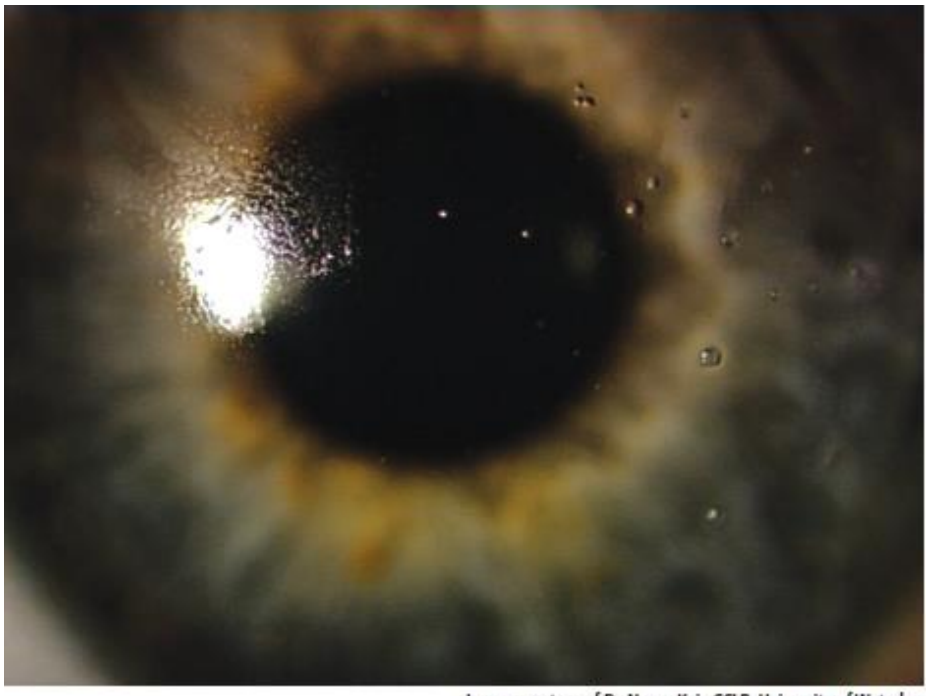

Figure 1.3. A silicone hydrogel contact lens with visible deposits as a thin layer and as small bumps. Image courtesy of Dr. Nancy Keir; https://www.clspectrum.com/supplements/2010/october-2010/the-current-realities-of-silicone-hydrogel-lenses/the-genesis-of-silicone-hydrogels.

1.2.2.1.1 Lipid deposition

Lipid deposition is associated with lens hydrophobicity, environmental factors, and lifestyle.[8, 12] For example, diabetics who wore CLs while taking certain medications were found with lower concentrations of potassium in their tear film, which was correlated with an increase in lipid deposition.[18] Analysis of tear film lipid deposits of individuals with extended lens wear and considered to have heavy and frequent deposition, were found to be similar to Meibomian gland secretions.[19] These lipids are not soluble in aqueous solutions and are difficult to remove with CL cleaning solutions.[19] Lipid deposition varies depending on the individual's cleaning regime and their own tear film composition.[8] Although the lipid composition varies per individual, in many cases, cholesterol was found to be the main component of the lipid deposits.[20]

When the tear film is disrupted, small patches of dry areas form on the lens surface. These dry patches reveal hydrophobic regions which promote lipid deposition in the area.[8] The lipids are then able to deposit further onto the lens material, creating a larger area for further lipid deposition,[8, 12] and become even more difficult to remove with CL cleaning solutions. The silicone moieties of SH material lenses allow for increased oxygen permeability, but the increased hydrophobicity may cause a higher rate of lipid deposition.[10-12] Additionally, lens materials with N-vinyl pyrrolidone had an increase in lipid deposition and lenses with polyvinyl alcohol showed relatively less lipid deposition.[21] Lipid deposition is also shown to be cumulative over time and does not reach a plateau.[8, 12]

1.2.2.1.2 Protein deposition

For protein deposition, the lens polymer material, lens ionic charge, and water content of the lens are factors that strongly affect deposition.[8, 10, 11] The interaction between the ionic charge of the protein and the lens material affects how strong the protein binds to the lens.[10] For example, etafilcon A, a negatively charged lens material, often shows a high amount of lysozyme deposition.[10] This is due to the strong ionic charge of lysozyme, a positively charged protein that is abundant in the tear film.[10, 22] However, this does not mean negatively charged proteins will not bind to lens materials. Albumin is a protein that is negatively charged, but has been found to deposit onto lens materials, regardless of the ionic charge.[23]

Proteins deposit on the surface of the lens material and can embed themselves further into the polymer matrix,[8] with less than 50% removed with cleaning solutions.[10] Surface modifications to CLs were used to decrease protein deposition into the bulk of the CLs.[10] There is also competitive surface binding between protein and lipid deposition, where proteins have been shown to displace some lipids.[8] SH lenses tend to deposit more lipids and less protein than CH lenses,

however, a higher percentage of protein denaturation was observed in SH lenses.[10] As more tear film components deposit on the lens material, there is a decrease in lens wettability and a decrease in the quality of vision.[8, 9] These factors lead to further discomfort and can potentially lead to discontinuation of CL wear.[7-9, 11]

1.2.2.2 Protein denaturation

There is an increased amount of denatured protein on SH materials compared to CH materials.[10, 11] Proteins retain their structure through intermolecular interactions (i.e., ionic bonds, hydrophobic bonds, hydrogen bonds, disulfide bonds, and van der Waals forces),[24] which could be disrupted when coming into contact with a hydrophobic surface, leading to conformational changes and denaturation.[10, 11] As a result, a higher amount of protein denaturation is seen in SH material CLs due to their hydrophobic lens properties.[8, 10, 11] When proteins denature, their biological functions often change or are nullified. In some cases, these denatured proteins can cause immunological reactions.[10]

1.2.2.3 Lipid oxidation

Lipids in the tear film are susceptible to oxidative degradation, causing lipids to break down to various intermediate and end products of lipid oxidation, such as malondialdehyde and 4-hydroxynonenal.[11, 12] The degradation of these lipids have been suggested to play a role in lens wettability and CLD.[11, 12] There are two major pathways of degradation, enzymatic degradation and autoxidation.[11, 12]

Enzymatic breakdown of lipids, particularly phospholipids, can result in various by-products, such as malondialdehyde.[12] As phospholipids are suggested to be involved in tear film stability, degradation of these lipids can destabilize the tear film and increase lipid and protein

deposition.[12] Some of the end products of enzymatic degradation have also been found to be precursors to ocular surface inflammation.[7]

Lipid autoxidation occurs in situations where lipids are exposed to a high concentration of oxygen and ultraviolet (UV) radiation, such as lipids on the tear film exposed to sunlight.[11, 12] Other factors that influence the rate of autoxidation include the level of antioxidants and lipid structure. Antioxidants prevent oxidative damage to the cornea via UV radiation[12] and unsaturated lipids are more susceptible to oxidative degradation.[9, 11, 12] One such end product of oxidative degradation is malondialdehyde, which has been known to cause tissue damage.[11] The concentration of malondialdehyde has been shown to be higher in intolerant CL wearers compared to tolerant CL wearers, suggesting more lipid degradation and oxidative stress in intolerant CL wearers.[7]

The addition of a CL to the ocular surface causes lipids to bind to the surface and bulk of the lens almost immediately[11] which exposes them to autoxidation.[11, 12] Although the effect of the autoxidation end products have not been confirmed, it has been suggested to contribute to discomfort in CL wearers.[11, 12]

1.2.2.4 Inflammatory effects

With the addition of a CL, the tear film is disrupted, leading to an increased rate of tear evaporation and an unstable tear film, both of which have been associated with ocular surface inflammation.[9] The cleaning regime and lens modality both affect the amount of deposition on the CL as well as the resulting effect on the eye. Even though the tear film includes antimicrobial proteins and mucins which trap and remove pathogens from the eye,[11, 16, 25] any residual proteins left on the lens after cleaning can contribute to an immune response. There are multiple causes of

ocular surface inflammation, and in most cases, inflammation is caused by several overlapping factors.

1.2.2.4.1 Deposition and lens care

Denatured proteins that have been deposited onto the lens surface or into the bulk of the lens can cause an immune response.[10, 11] The denatured proteins can create scaffolds on the lens surface, leading to an increase in microbial attachment.[10, 25] The immune response triggered by the protein deposition and denaturation can cause more proteins to deposit on the lens, creating an on-going cycle of protein deposition, microbial attachment, and ocular inflammation.[10, 11]

It has been shown that SH CL wearers using their lenses on a reusable basis tend to have more corneal inflammatory events than CH CL wearers.[10, 25, 26] Although the lens material may play a role in the increase of inflammatory events, the cleaning solutions and regime are also important. Several studies have shown that using a hydrogen peroxide cleaning solution may be more beneficial than a multipurpose solution (MPS).[13, 25, 27] Most MPS contain preservatives, which have been associated with corneal epithelium toxicity.[25, 27] There has also been a reported increase in bacterial adherence and corneal epithelial damage when using MPS compared to hydrogen peroxide.[13, 25, 27] The preservatives from MPS may be absorbed into a CL and slowly released onto the eye, causing further toxicity and corneal damage.[13] The amount of discomfort due to toxicity varies per individual, but higher levels of toxicity are likely to cause CL dropout.[27]

1.2.2.4.2 Immune response

The immune response due to protein deposition and use of preservatives causes an increase in inflammatory cytokines to the ocular surface.[13] Extended inflammatory events can cause ocular surface damage.[13] Neutrophils, recruited to sites of infection during an inflammatory response, help to remove bacteria.[25] However, extended inflammation can cause necrotic neutrophil debris

to accumulate, resulting in an increase of bacterial adherence to the lens.[25] This may, once again, add to the on-going cycle of deposition and ocular inflammation.

Corneal infiltrative events are inflammatory reactions from bacterial toxins and enzymes, and can occur in healthy non-CL wearers.[28] However, the use of CLs increase risk and severity of corneal infiltrative events, especially for extended and overnight wear.[28, 29] The bacterial bioburden on worn CLs and eyelid margins was found to be associated with the development of corneal infiltrative events.[28, 29] As SH materials have a higher risk of bacterial adhesion compared to CH materials, there is a relatively higher risk of a greater bacterial bioburden on SH lenses.[29]

1.2.2.4.3 Extended lens wear

CLs that are worn for six or more consecutive nights without removal are considered to be worn on an "extended wear" schedule, whereas CLs that are removed at the end of each day are considered to be worn on a "daily wear" schedule.[30] It has been shown that extended CL wear increases the risk for microbial keratitis compared to daily CL wear.[25, 26] However, several studies have shown a "survivor effect," where CL users who are less susceptible to immediate inflammatory events are more successful with long term CL wear.[25, 26]

Protein deposition, denaturation, low tear stability, lens material, cleaning solution and regime, bacterial adhesion, autoimmune response, and wear time are all factors that can cause an inflammatory event. Although there are many factors that play a role in the inflammation of the ocular surface, it is clear that these factors contribute to each other and cause a cycle of inflammatory events. It is important to understand and study the effect of CL wear and the ocular environment in order to decrease the rate of CL discomfort and dropout.

1.3 Modeling contact lens wear with *in vitro* methods

In order to explore the effect of CLs on the eye, researchers use *in vitro* models, animal models, or human clinical trials.

1.3.1 *In vitro* vial testing

In vivo testing, using animal models or human trials, are expensive, time consuming, and often raise ethical concerns.[31-33] As a result, many researchers use *in vitro* models as a preliminary stage of their research.[34] For experiments, such as a comparison of dehydration rates between lens types, a simple *in vitro* model would suffice,[34] as shown in Figure 1.4. In these types of experiments, a static vial would often be used as an inexpensive, easily attainable, and quick method of testing.[34, 35] Although the results may not provide the information of the compounding effects of the complexity of the human eye, it provides researchers with information to further improve future studies.[17, 32, 34-37]

Figure 1.4. A simple vial *in vitro* model, often used for preliminary experiments. Courtesy of Dr. Chau-Minh Phan.

Using *in vitro* models allows researchers to quickly screen products, such as CL materials, to determine which materials would be most likely to be suitable for in-eye wear, without having to harm an animal in the process.[36] The biggest benefit of *in vitro* models, despite their simplicity, is that they provide information to improve future *in vitro* and *in vivo* studies with minimal cost, time, and material loss, and the additional benefit of no animal harm.[32, 33, 35, 36]

In vitro models can be used to study tear film deposition onto CL materials.[38] *In vitro* models are the more cost and time efficient option, and have often shown similar results to *in vivo* studies.[17, 31, 35, 38] The *in vitro* models also allow for manipulation of the variables involved in a controlled environment.[17, 32, 37] In these tear deposition studies, single proteins can be added or removed from complex tear solutions to determine the effect on the CL material.[32, 38] Such experiments are not easily achieved *in vivo* due to the many variables of the human ocular system and potential immune responses.[17] Although these experiments do not show the biological effect of the human body, they are useful in determining and understanding the role of specific components of the tear film.[17, 32, 35, 37, 38]

1.3.1.1 Advancement of *in vitro* testing

In the past, to study toxicity effects or irritation of products to the eye, the Draize test was considered the standard methodology.[31, 39] In brief, a small amount of a product, usually for topical administration, would be placed on the skin or eye of a small mammal. After a pre-determined time frame has passed, the product is removed and the animal is observed for several days for any adverse effects, such as blindness or swelling. As the United States government was establishing agencies to promote general welfare during the 1940s, the FDA adopted the methods proposed by John H. Draize and his colleagues.[39] Their work mainly used rabbits to assess the effect of topical exposure to the eye and skin.[39] The FDA then used these techniques to determine the toxicity effect

of several cosmetic products, insecticides, and other substances.[39] As the testing involves causing discomfort and pain to animals, many ethical concerns were raised over the methodology of the Draize test.[31, 39] Additionally, the test involves a subjective scoring system, further raising doubts on the methodology and reproducibility.[31] Since rabbits and humans are anatomically and physiologically different, there were many concerns over the relevancy of some of the test results.[31]

Different methodologies in research may include using cell culture models instead of animal models to study the effect of toxicity on cells.[33] These *in vitro* models allow for high throughput testing of several compounds with varying conditions.[17] Positive and negative controls, commonly used in the past, are still readily used in the controlled environment of *in vitro* experiments and variables can be manipulated to test for different conditions.[17, 32] In addition to being cost and time efficient, these models are easy to monitor and can be easily reproduced.[32]

Although animal model testing is still important and provides information that *in vitro* models cannot, the advancement of these *in vitro* studies, allows for a lower frequency for animal model testing. The conditions and properties of certain products can be thoroughly screened and tested using *in vitro* models before animal or human clinical trials, reducing the amount of resources required. Although *in vitro* studies may not show the complex interactions of the human body, they provide valuable information that could guide the development of *in vivo* studies.[17, 32, 35-38]

1.3.2 *In vitro* eye models

The nature of static vials to test the effect of CL materials on the eye was too simplistic to account for the many variables associated with complex human eye conditions.[40] *In vitro* testing often used a simple vial that covered a CL in a solution containing the components of interest. These conditions do not represent the small tear volume in human eyes and result in an inaccurate

portrayal of tear film components accumulated on the lens.[41, 42] When testing for cholesterol uptake onto CL materials from an artificial tear solution, the accumulation of cholesterol in a simple vial was considerably higher than on an advanced *in vitro* eye model.[43] Specifically for this eye model, CLs were placed vertically between moving "eye" and "eyelid" structures with tear fluid flowing at a physiological flow rate.[43] The structures were programmed to move and rotate to simulate a blink mechanism.[43] The cholesterol accumulation determined was suggested as being more comparable to physiological conditions than the simplistic vial model.[43]

A separate drug release study compared a simple vial to a more advanced *in vitro* eye model using an antifungal drug, fluconazole, demonstrating that a simple vial had a burst release and plateau profile, whereas the *in vitro* eye model had a more sustained release.[44] In this eye model, the CL is also placed vertically between two structures representing the eye and eyelid, with a physiological flow rate of tear fluid.[44] In this eye model, the eye structures were stationary to represent a closed eye model, but still suggested to more accurately portray physiological results.[44] Both these studies showed varying results, but the *in vitro* eye model results are predicted to follow ocular condition patterns more closely than the simple vial model.[43, 44]

1.3.2.1 Current *in vitro* eye models

The difference between simple vials and the human eye led to the development of more advanced *in vitro* models. Many researchers developed their own eye model instead of using a simple vial method.[41, 43-47] In order to recreate a more physiologically accurate model, microfluidic models were used in drug release studies.[45, 47] These microfluidic models would use pumps to administer the correct tear flow to mimic the tear flow rate of human eyes.[47] In many cases, more advanced tear solutions were used to further optimize the models to mimic ocular physiological conditions.[17, 40-42, 46, 48] The microfluidic models used a microfluidic chip designed with

physiological ocular flow in order to mimic the flow rate of tears.[45, 47] These microfluidic chips were then placed on a base structure, made of polydimethylsiloxane (PDMS) and silicone mix, and attached to a syringe pump for tear flow administration.[45, 47] These *in vitro* eye models created a more sustained drug release profile in comparison to a simple vial model.[44, 47] The simple vial models had a drug release profile that show a burst release effect before reaching a plateau.[44] Although these *in vitro* models allowed for more control over the experimental conditions, researchers acknowledged that their models still required further improvements.[41, 44, 45, 47]

Other types of *in vitro* models used a variety of materials to build an artificial eye platform. PDMS was often chosen as the material used to create eye models. PDMS was used to create microfluidic models[47] as well as eyeball and eyelid structures.[43, 44, 46, 48] With more advanced tear solutions, administered using a controlled pump,[47] the *in vitro* models were used to analyze the effect of several factors that affect CL wear, such as the effect of certain tear film components and the deposition on different lens materials.[41, 42, 48] Drug release and tear film deposition onto CL materials were able to be analyzed under different variables with these more advanced models.[46] In addition to using PDMS, researchers acknowledged the need for more biocompatible materials to use for eye model components, taking into consideration the hydrophobicity and corneal surface structure for more accurate *in vivo* conditions.[43, 44, 49] CL deposition and drug release kinetics are now approaching the likes of *ex vivo* and *in vivo* results due to the progression of *in vitro* models.

To improve the potential relevance of toxicity studies, *in vitro* models included corneal epithelium cells to determine how cells would react under certain conditions without having to use *in vivo* trials.[50, 51] This cost effective and high throughput method of analysis allowed for more control of experimental variables and could be used to study microbial growth on corneal epithelial cells.[50, 51]

Several researchers had recognized the missing features of their *in vitro* models, including the effects of eye and CL orientation,[43, 44] temperature,[45, 49] humidity,[49] air exposure,[40, 49] and tear composition interactions.[17, 40] *In vitro* models were developed to focus on these features by developing vertical eye platforms to incorporate the effect of gravity and better mimic *in vivo* conditions.[43, 44, 48] These conditions promoted fluid exchange and replenishment to the ocular surface in low tear volumes and physiologically relevant tear flow rates.[44, 48, 52] Temperature and humidity could be controlled by placing *in vitro* models into enclosed environmental-controlled chambers.[40] Air exposure could be replicated by physical movement of eyeball and eyelid pieces of certain *in vitro* platforms or by changing the tear volume that covered the surface of the *in vitro* eye model.[48] By adjusting these variables, changes in the interaction of tear film components, such as deposition of proteins or lipids, to the CL materials could be demonstrated.

1.3.2.2 Future development of *in vitro* eye models

As more advanced *in vitro* models are developed, several factors are considered to be needed in all models. *In vitro* models should be inexpensive, feasible, reproducible, allow for high throughput testing, decrease experimental time, and could accurately model *in vivo* testing.[41, 44, 49, 50]

Some areas of advancement for future *in vitro* models as suggested by previous model developers are to create the proper tear film conditions in the presence of a CL,[44, 47] consideration of the tear components[44] and tear film thickness,[45, 47] and the replenishment of tears.[50] The ocular temperature,[44, 45] corneal surface structure,[49] orientation[44] and material of both the CL and eyelid and eyeball materials[43, 44, 48, 49] have also shown to have various impacts on *in vitro* studies. For example, having CLs placed vertically incorporates the effect of gravity, and low tear volume and tear flow of an *in vitro* model does not result in the burst release pattern seen in a simple vial drug

release study.[44] Instead, it demonstrates a slower, sustained release that can be modified by the many controllable factors of the *in vitro* model. As stated in the study, further testing is needed to determine which drugs will be viable for clinical use, however, by changing the variables of the *in vitro* model, the effect of temperature, lens material, tear volume and many more can be observed and analyzed prior to clinical testing, providing valuable information that would otherwise be difficult to monitor in animal studies.[44] Many studies have also stated that air exposure,[44, 45, 47, 49] blink movement,[44, 46, 49, 52] and shear force[46] have an effect on tear film deposition onto CL materials and should be factors to consider when trying to simulate ocular conditions using *in vitro* models.

1.3.3 OcuBlink

1.3.3.1 Features

The OcuBlink is a novel *in vitro* eye model designed to overcome many of the missing features of currently available *in vitro* models.[53] The development of the OcuBlink model stems from the previous *in vitro* model, the OcuFlow (Figure 1.5). The OcuFlow model was designed to incorporate low tear flow and volume using artificial tear solutions,[43, 48, 54] however, the materials of the OcuFlow model was not representative of physiologic conditions.[43, 49] Building on the success of the OcuFlow model, the OcuBlink uses a polyvinyl alcohol (PVA) material that is biocompatible and wettable instead of the hydrophobic PDMS for the eyeball and eyelid,[53] depicted in Figure 1.6. PVA is able to mimic the hydrophilic conditions of the ocular surface and the *in vitro* model uses a blink mechanism that matches the physiological eyelid blinking motion;[53] whereas the previous OcuFlow model had used a rotational movement of the eyelid pieces.[48] The blink rate of the OcuBlink model was arbitrarily set to 6 blinks per minute to encompass both functionality of the blink model and reference human blink rates.[55] The blink rate can be adjusted

as required per experimental conditions. The low tear flow and volume of the OcuBlink model can be adjusted during experiments. Air exposure and the shear stress of the eyelid during the blinking motion can be tested with this model.[53] The addition of the blinking mechanism allows for a tear film to be spread on the surface of the eye, which allows for testing tear stability (i.e., tear break up time) using this model.[53] The materials used for the OcuBlink model, such as PVA for eyeballs and eyelids and 3D-printer ink, are inexpensive and readily available.[53] A larger model of the OcuBlink is also used for high throughput testing. Different iterations of the OcuBlink models in the future are focused to tackle the issues of ocular temperature conditions and using corneal epithelial cells for more representative data.

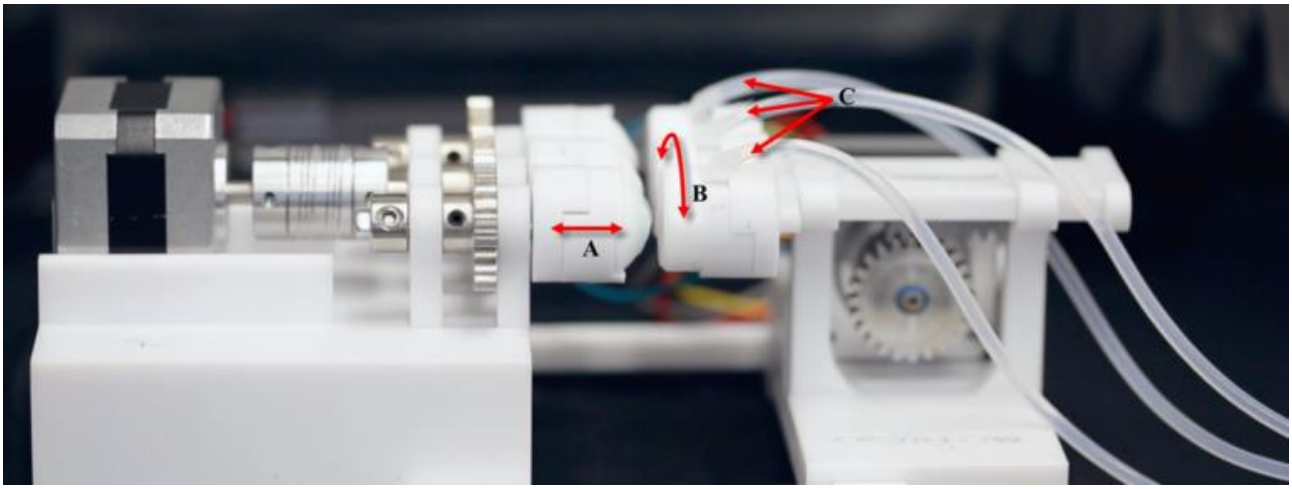

Figure 1.5. The OcuFlow model. Red arrows depict the movement of the two eyelid components (A and B) and for the flow of tears (C). Courtesy of https://core.uwaterloo.ca/ocuflow/.

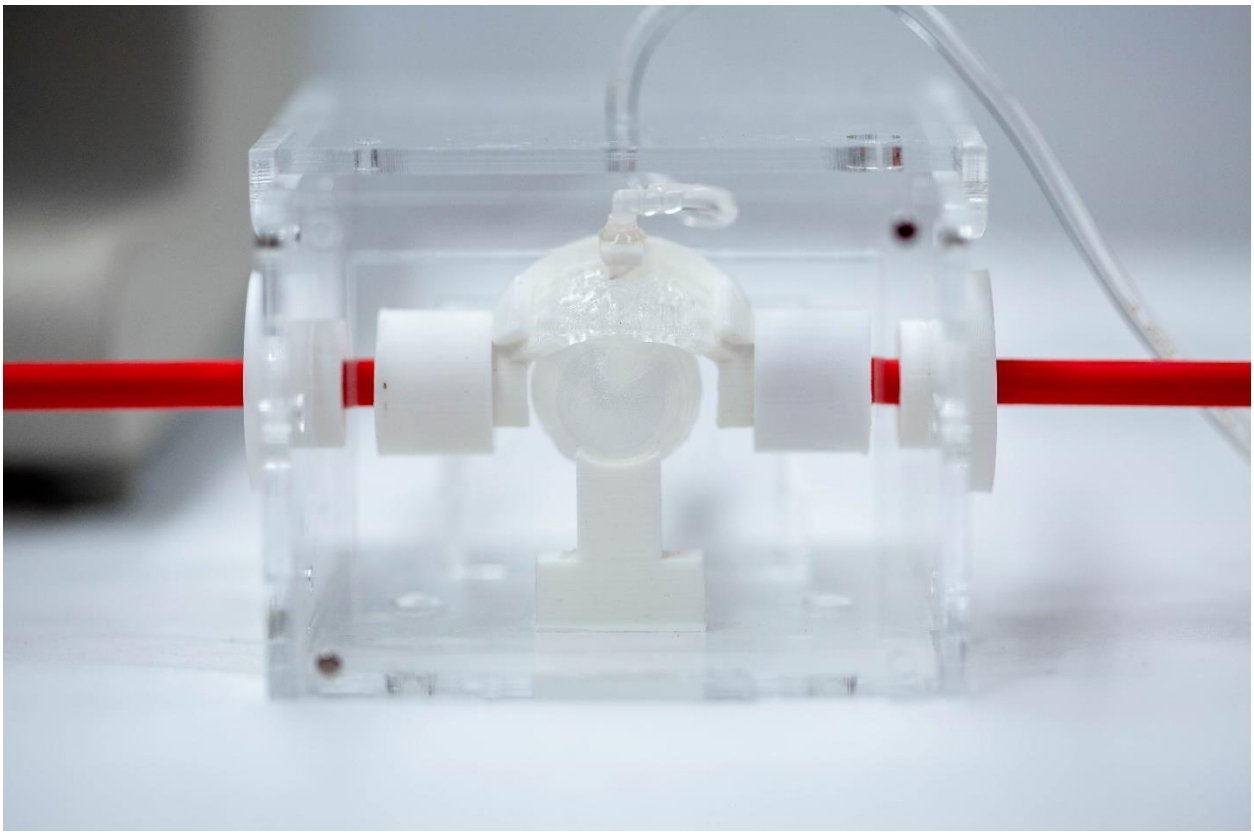

Figure 1.6. The OcuBlink model: a wettable material used for the eyelid and eyeball components. An artificial tear solution is administered from the top of the eyelid and spread across the surface of the eye during the blinking mechanism.

1.3.3.2 Clinical/Industry/research applications

The OcuBlink is designed to mimic ocular physiological conditions that exceed the testing limitations of a simple vial to provide information in a controlled testing environment. Having a more advanced *in vitro* model allows for researchers in academia and industry to quickly screen through products and experimental designs. The more closely an *in vitro* model is able to resemble ocular physiological conditions, the more valuable the information it can provide. By screening early stage developmental projects using an *in vitro* model saves time and money for researchers that would otherwise be spent on animal testing or clinical trials. The additional benefit of *in vitro* models allows for certain parameters to be changed and adjusted for further study, contributing to

the understanding of certain mechanisms.[17, 53] These controlled experiments provide valuable information that can be used to further advance the design of *in vitro* models and for adjusting *in vivo* trials. The ability to modify and change these parameters would not be possible with *in vivo* studies due to the amount of variables involved.[41] Therefore, the advancement of *in vitro* models plays an important role in industry, academia, and clinical testing. The OcuBlink is designed as an improved ocular model used for advanced *in vitro* testing.[53]

2 Thesis rationale

The human ocular surface is a complex environment and is comprised of a wide variety of proteins and lipids within the tear film.[8, 9] When foreign matter encounter the ocular surface, an innate physiological response causes a cascade of different chemical and biological changes.[9] When a CL is placed on the eye it disrupts the tear film,[7, 9] and various proteins and lipids deposit onto the lens.[8, 11] This in turn may cause dryness or irritation to the eye, which is impacted by the material that the lens is manufactured from.[7, 9, 11]

In order to develop CLs to reduce the severity of CL dryness or dropout, CLs go through several rounds of testing. Typically, initial *in vitro* tests precede *in vivo* animal testing, prior to human clinical trials. To improve the time to commercialisation and prevent a loss of valuable resources and reduce costs, it is beneficial to improve the lens materials in the earliest stages of testing.

In vitro testing is often undertaken using very simplistic systems, typically consisting of nothing more than a glass vial with a CL immersed in solution.[34] However, this is clearly not representative of the complicated physiological conditions of the human eye. *In vitro* testing allows for high throughput, controlled testing conditions to manipulate certain variables for a better understanding of certain characteristics.[34, 35] These characteristics can include the interaction of the CL material with certain components of the tear film,[56] physical stability, uptake and release of preservatives contained in contact lens solutions[57, 58] and how CL materials react after certain amounts of time in solution.[38] The physiological conditions of the human eye are complex and there are many interactions that arise due to the CL placed onto the eye that a simple vial cannot replicate. As a result, more advanced *in vitro* eye models have been developed in order to bridge the benefits of *in vitro* testing to the results of *in vivo* testing.[41, 43-47]

The overarching goal of this thesis is to show the development of a novel *in vitro* blink model, OcuBlink. This *in vitro* blink model was developed to include a blink mechanism, adjustable tear flow administration, and a heated element while using wettable materials to best mimic the physiological conditions of the human eye. The first experimental chapter (Chapter 3) explores how the blink model is used to replicate historical data from an *in vivo* study that quantified active lysozyme deposition on CLs.[59] By adjusting the parameters, the blink model was able to replicate the *in vivo* data and these conditions were used on other CLs to add to the literature pool. As there were no other *in vivo* results available at the time of the study on other daily disposable lenses on active lysozyme deposition, the blink model provides an estimate for where *in vivo* data may fall.

Chapter 4 of this thesis describes the challenges encountered while using the blink model and what changes were made to adapt and improve the model for precise results and ease of operation. In the following chapter (Chapter 5), these improvements were put to use in a different version of the blink model to include a heating element. This chapter explores the effect of incubation solutions and temperature prior to a comparison of a simple vial system to the sophisticated blink model. The changes in water content determined from this study shows the relative dehydration of CL materials using the blink model over time to provide results representative of lens wear.

The final chapter summarizes the discussion and reviews some of the possible drawbacks of the blink model and where the model may be improved to develop an *in vitro* model that truly mimics human ocular conditions.

3 Lysozyme deposition on contact lenses in an *in vitro* blink-simulation eye model versus a static vial deposition model

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ABSTRACT

Purpose: To evaluate active lysozyme deposition on daily disposable (DD) CL using a novel *in vitro* blink model.

Methods: Three CH DD CL materials (etafilcon A, omafilcon A, nelfilcon A) and three SH DD CL materials (delefilcon A, senofilcon A, somofilcon A) were tested. The device blink rate was set to 6 blinks/min with a tear flow rate of 1 µL/min using an artificial tear solution (ATS) containing lysozyme and other typical tear film components. After incubation at 2, 4, or 8 hours, lenses were removed, and lysozyme activity measured. A separate experiment was conducted with lenses incubated in a static vial containing 480 µL of ATS.

Results: Etafilcon A deposited significantly higher amounts of active lysozyme (402 ± 102 µg/lens) than other lens materials after 8 hours ($p < 0.0001$). Etafilcon A had a higher amount of active lysozyme using the blink model compared to the static vial ($p = 0.0435$), whereas somofilcon A ($p = 0.0076$) and senofilcon A ($p = 0.0019$) had a higher amount of lysozyme activity in the vial compared to the blink model.

Conclusion: The blink model can be tuned to provide quantitative data that closely mimics *ex vivo* studies and can be used to model deposition of lysozyme on CL materials.

Keywords:

In vitro, eye model, blink, lysozyme activity, contact lens, polyvinyl alcohol

3.1 Introduction

Despite their worldwide success,[4] CLs continue to result in non-optimal comfort[7] and adverse immune responses,[60-62] that lead to discontinued use in approximately 25% of wearers. The deposition and denaturation of tear film components has been suggested as a contributor to discomfort and inflammatory events.[7, 59-62] As a result, understanding the deposition of various tear film components on CLs would be helpful in designing improved materials that may ultimately result in enhanced comfort and longer wearing times.

While a significant number of *in vitro* studies have investigated tear film deposition on CL,[42, 56, 63-67] the impact of deposition on CL success remains unclear and is highly debated. One of the underlying problems is that current *in vitro* eye models used to study tear deposition on CL are still rudimentary. Most *in vitro* studies are performed in a highly simplistic, static glass vial containing the lens exposed to a deposition solution, which limits the ability to mimic the complexity of the ocular environment.[17, 40, 42, 56, 60, 63, 65-67] Factors such as a low tear volume, low tear flow and replenishment, intermittent air exposure, and the blink mechanism, could potentially impact deposition of tear components and are absent from vial-based models.[40, 43, 48] Therefore, the results obtained from *in vitro* studies may not be predictive of *in vivo* outcomes. Increasing the quality of *in vitro* testing prevents unnecessary loss of money, time, and resources during the development of new materials.

To more appropriately mimic physiological conditions, several groups have developed their own unique *in vitro* eye models.[40, 43, 44, 48, 52, 54] To this end, our group has previously developed an *in vitro* model designed to physiologically mimic representative tear flow, tear volume, air exposure, and the mechanical rubbing produced during blinking.[44, 48, 52, 54] Not surprisingly, the results obtained for protein and lipid deposition on CL using this model were

significantly different from those obtained from the simple vial model.[48, 54] To improve on previous designs, we have developed an *in vitro* model capable of simulating the physiological blink mechanism, which may better model tear film deposition on CLs, and provide further insights on the underlying mechanisms involved in tear film deposition.

One major protein deposited on CLs is lysozyme, a bacteriolytic enzyme found at high concentrations in the human tear film.[48, 64-66, 68-70] For this reason, it is often used to model protein deposition on a wide variety of CL materials.[48, 64-66, 68-70] As lysozyme deposits on CL materials, it may undergo conformational changes that can alter or reduce its activity,[61, 62, 71] which could trigger adverse immune responses.[61, 62, 71] The purpose of this study was to evaluate lysozyme activity on CLs using a novel blink-incorporated eye model.

3.2 Methods

3.2.1 Contact lenses

Three CH DD CLs (etafilcon A, omafilcon A, nelfilcon A) and three SH DD CL materials (delefilcon A, senofilcon A, somofilcon A) were tested in this study. The properties of the CLs are listed in Table 3.1.

Table 3.1 Contact lenses materials evaluated in the study.

Commercial Name	1 Day Acuvue Moist	Proclear 1 Day	Dailies Aqua Comfort Plus	Clariti 1 day	Acuvue Oasys	Dailies Total 1
United States Adopted Name	etafilcon A	omafilcon A	nelfilcon A	somofilcon A	senofilcon A	delefilcon A
Manufacturer	Johnson & Johnson	CooperVision	Alcon	CooperVision	Johnson & Johnson	Alcon
FDA classification	IV	II	II	V	V	V
Water content (%)	58	60	69	56	38	33
Dk/t	25.5	28.0	26.0	86.0	121.0	156.0
Monomer composition	HEMA, MA	HEMA, PC	PVA, FMA, PEG	Not disclosed	mPDMS, DMA, HEMA, siloxane macromere, PVP, TEGDMA	Not disclosed

HEMA, poly(2-hydroxyethyl methacrylate); MA, methacrylic acid; PC, phosphorylcholine; PVA, polyvinyl alcohol; FMA, N-formylmethyl acrylamide; PEG, polyethylene glycol; mPDMS, monofunctional polydimethylsiloxane; DMA, *N*,*N*-dimethylacrylamide; PVP, polyvinyl pyrrolidone; TEGDMA, tetraethyleneglycol dimethacrylate

3.2.2 Reagents

All materials were purchased from Sigma Aldrich (St. Louis, MO) unless otherwise specified.

3.2.3 Fabrication of moulds and eyelids

The fabrication of the eyeballs and eyelids for the blink model was conducted as previously described.[53] In brief, the eye model was designed using computer-aided design (CAD) software. The eyeball dimensions were 22.5 mm (height) × 22.5 mm (width) × 24.0 mm (axial length). The moulds for the eyeball were machined from an aluminum block using a computer numerical

control (CNC) lathe. The eyelids and all other supporting structures were fabricated using a 3D-printer (Prusa3D, Prague, Czech Republic).

3.2.4 Synthesis of eyeball and eyelid

The procedures for the synthesis of the eyeballs and eyelids were adopted from methods described by Hyon et al.[72] In brief, PVA was added to a mixture of dimethyl sulfoxide and Milli-Q water (8:2) to achieve a concentration of either 15% or 23% weight/volume (w/v). The mixture was stirred and then heated in an oven at 120°C for 1.5 hours. After the heating step, the 15% (w/v) PVA was used to fabricate the eyelid, while the 23% (w/v) PVA was poured into the eyeball mould. Subsequently, the eyelid and eyeball moulds were held at -80°C for 30 minutes for gelation to occur. The gelled eyeballs and eyelids were then removed from the moulds and placed in 500 mL of Milli-Q water, renewed daily for 3 days to remove the dimethyl sulfoxide. Before starting the experiments, the eyeballs and eyelids were incubated in 300 mL of phosphate buffered saline (PBS) for 12 hours.

3.2.5 Artificial tear solution

The ATS used in this study was previously described by our group.[17] The ATS contains various salts, proteins, and lipids, listed in Table 3.2.[17]

Table 3.2 Artificial tear solution components.

Salts (mg/mL)	Lipids (mg/mL)	Proteins (mg/mL)
NaCl (5.26)	Oleic acid (0.0018)	Mucin (0.15)
KCl (1.19)	Oleic acid methyl ester (0.012)	Albumin (0.20)
$Na_3C_6H_5O_7$ (0.44)	Triolein (0.016)	Lysozyme (1.90)
Glucose (0.036)	Cholesterol (0.0018)	Lactoferrin (1.80)
Urea (0.072)	Cholesteryl oleate (0.024)	
$CaCl_2$ (0.07)	Phosphatidylcholine (0.0005)	
Na_2CO_3 (1.27)		
$KHCO_3$ (0.30)		
Na_2HPO_4 (3.41)		
HCl (0.94)		
ProClin 300 (200 μL/L of solution)		

3.2.6 Set-up of blink model

The set-up for the blink model is shown in Figure 3.1. The blink motion and blink rate were controlled by an Arduino board (WEMOS D1 R2 Wifi ESP8266) and attached motor (Adafruit Industries, New York, NY). The blink rate was set to 6 blinks/min. Tubing was attached to the top of an eyelid support structure, which was then connected to a commercial microfluidic pump (PHD Ultra, Harvard Apparatus, Holliston, MA) to deliver ATS to the system. The ambient temperature and humidity were kept at 23°C ± 4°C and 50% ± 5%, respectively.

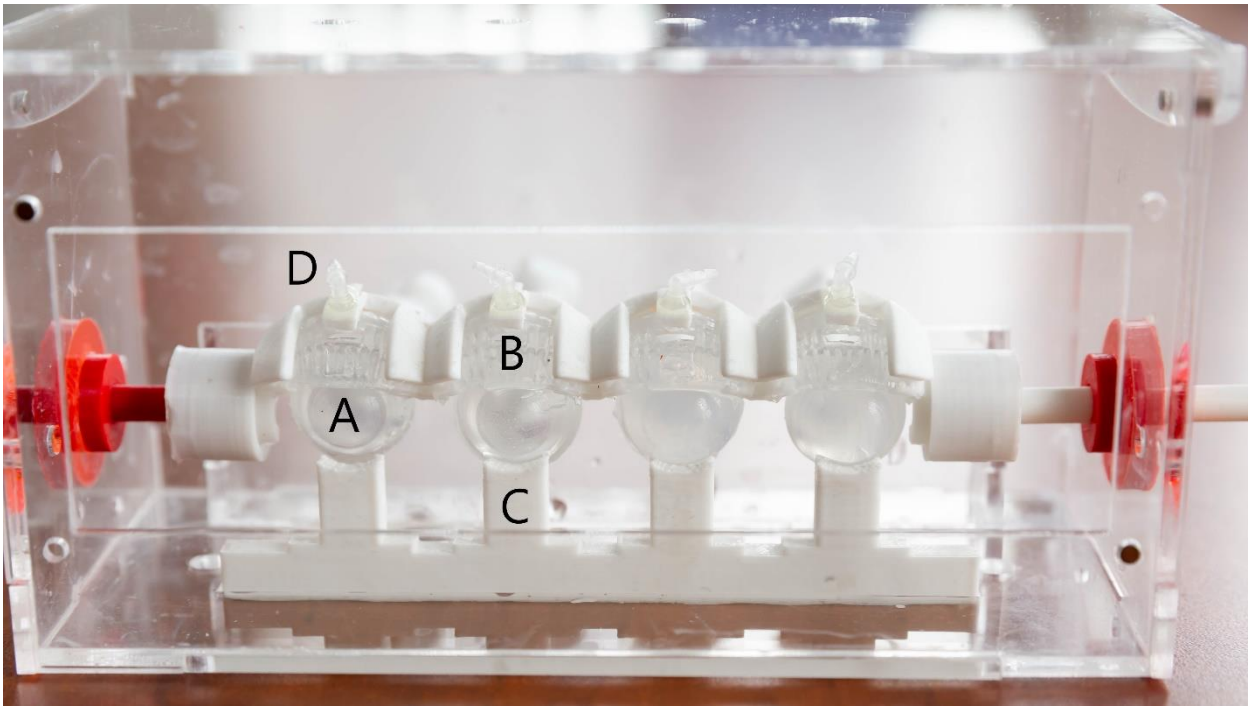

Figure 3.1 *In vitro* eye blink model used in this study. (A) PVA eyeball (B) PVA eyelid with 3D-printed support structure (C) 3D-printed lower eyelid (D) tubing connector for fluid input.

3.2.7 Preliminary flow rate determination

For the preliminary flow rate determination, etafilcon A was selected as the test CL because there are published *ex vivo* results (asymptomatic participants) on lysozyme deposition for this lens type, see Table 3.3.[59] The ATS flow was tested at three different rates: 0.3 µL/min, 1.0 µL/min, and 2.0 µL/min, to determine one which would closely yield lysozyme activity matching previous *ex vivo* results.[59]

Table 3.3 Preliminary flow rate testing under different flow conditions of active lysozyme.

Time (hours)	*Ex vivo*[59] (µg/lens)	0.3 µL/min (µg/lens)	1.0 µL/min (µg/lens)	2.0 µL/min (µg/lens)
2	122 ± 1	23 ± 8	137 ± 14	317 ± 35
4	225 ± 5	253 ± 37	201 ± 155	467 ± 70
8	342 ± 14	186 ± 29	401 ± 102	929 ± 118

3.2.8 Experimental design

The blink model was equilibrated for 30 minutes before the addition of CLs to ensure adequate tear flow on the eyelid and eyeball. The lenses were removed from the blister pack and placed directly on the blink model. After t = 2, 4, and 8 hours, the lenses were removed from the blink model. Another set of the same lenses was incubated in vials, immersed in 480 µL of ATS for 8 hours on an orbital shaker at 60 rotations per minute. 480 µL of ATS represents the amount of ATS equivalent to 8 hours on the blink model at 1.0 µL/min. All lenses were tested in triplicate for both the blink model and vial system. After completing their incubation period, all lenses were stored at -80°C until further analysis. Control CLs were removed directly from the blister pack solution and stored at -80°C until further analysis.

3.2.9 Extraction of contact lenses

The extraction of lysozyme from CLs was conducted as previously described.[73] In brief, CLs were removed from the -80°C freezer and placed in an extraction buffer containing 1:1 acetonitrile and 0.2% trifluoroacetic acid for 24 hours in the dark. All lenses, with the exception of etafilcon A and senofilcon A, were incubated in 1 mL of extraction buffer. For etafilcon A, 6 mL of extraction buffer was used. For senofilcon A, the lenses were extracted in 1 mL of 1:1 acetonitrile and 0.02% trifluoroacetic acid.[73-76] After the extraction period, the lens extracts were aliquoted, and the solvents were dried down using a commercial speed vacuum concentrator (Savant Speed Vac, Halbrook, NY).

3.2.10 Lysozyme activity assay

Lysozyme activity using a micrococcal activity assay was conducted as previously published.[69, 73] In brief, the extracts were re-suspended in PBS containing bovine serum albumin (BSA) and then added to a 48-well plate of *Micrococcus lysodeikticus* diluted to an optical density of 0.9-1.0. The absorbance of the samples was measured at 450 nm by light spectrophotometry (SpectraMax M5e plate reader, Molecular Devices, Sunnydale, CA). The change in absorbance was compared to a reference standard curve to determine the amount of active lysozyme per CL. Each lens was read in triplicate and corrected by subtraction of control sample value readings.

3.2.11 Statistical Analysis

Statistical analysis and graphs were plotted using GraphPad Prism 8 software (GraphPad, La Jolla, CA). All data are expressed as mean ± SD. The difference in amount of total active lysozyme deposited at t = 8 hours was tested using a one-way analysis of variance (ANOVA) with a post-hoc Tukey's multiple comparisons test. Etafilcon A was analyzed separately because it deposited significantly more lysozyme than any other lens type. A one-way ANOVA with a post-hoc Tukey's multiple comparisons test was used to test the difference in active lysozyme deposition across the different time points. A two-way ANOVA with a post-hoc Tukey's multiple comparisons test was used to test the difference of active lysozyme deposition across lens types and time.

Another two-way ANOVA with a post-hoc Sidak multiple comparisons test was used to test the differences in active lysozyme deposition between the model systems. Since etafilcon A was separated from prior analysis, a t-test was used to test the difference in active lysozyme deposition between the two model systems. Statistical significance was achieved at the level of $p < 0.05$.

3.3 Results

3.3.1 Determination of Flow Rate

The difference in active lysozyme was tested for flow rates 0.3 µL/min, 1.0 µL/min, and 2.0 µL/min. 1.0 µL/min was selected as the flow rate of choice due to its similarity to *ex vivo* data previously published[59] in comparison to the other tested flow rates, see Table 3.3.

3.3.2 Deposition study

Etafilcon A was associated with the greatest amount of active lysozyme deposition per lens compared to all other lens materials at 8 hours ($p < 0.0001$). Deposition of active lysozyme increased significantly over the 8 hours for etafilcon A ($p = 0.0049$). Deposition at $t = 8$ hours was significantly greater than that of $t = 0$ hours ($p = 0.0033$), and $t = 2$ hours ($p = 0.0339$). (Figure 3.2A)

For the deposition of active lysozyme for other materials, both time ($p < 0.0001$) and lens type ($p < 0.0001$) were significant factors. For all lens types, the deposition of active lysozyme at $t = 8$ hours were significantly greater than $t = 0$ hours ($p < 0.0001$ for omafilcon, $p < 0.0001$ for nelfilcon, $p = 0.0390$ for somofilcon, $p = 0.0100$ for senofilcon, and $p < 0.0001$ for delefilcon). (Figure 3.2B)

Table 3.4 summarizes the amount of active lysozyme on CLs for each time point using the *in vitro* blink model.

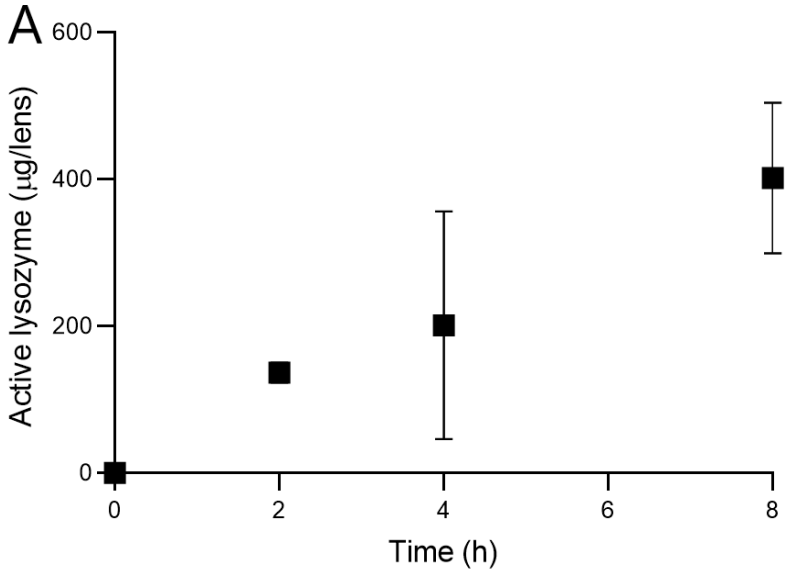

Figure 3.2A Active lysozyme per lens for etafilcon A (■) over 8 hours.

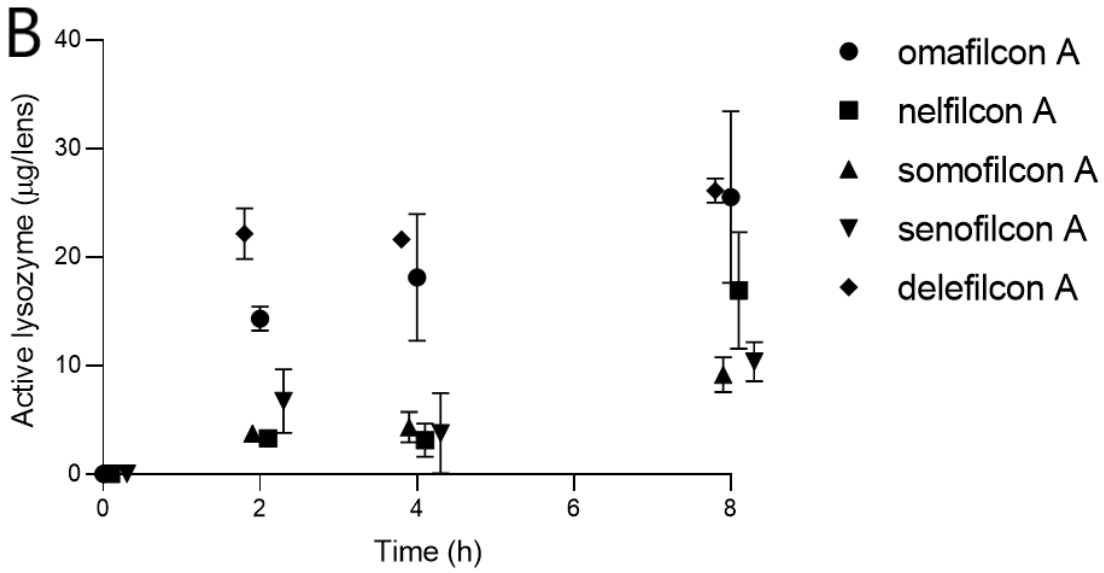

Figure 3.2B Active lysozyme per lens over 8 hours for omafilcon A (●), nelfilcon A (■), somofilcon A (▲), senofilcon A(▼), and delefilcon A (◆).

Table 3.4 Amount of active lysozyme deposited per lens with the *in vitro* blink model.

Incubation time (hours)	*In vitro* testing (µg/lens) (n=3 each)					
	Conventional hydrogel materials			Silicone hydrogel materials		
	Etafilcon A	Omafilcon A	Nelfilcon A	Somofilcon A	Senofilcon A	Delefilcon A
2	137 ± 14	14 ± 1	3 ± 0.5	4 ± 0.6	7 ± 3	22 ± 2
4	201 ± 155	18 ± 6	3 ± 2	4 ± 1	4 ± 4	22 ± 0.2
8	402 ± 102	26 ± 8	17 ± 5	9 ± 2	10 ± 2	26 ± 1

3.3.3 Blink model vs vial

Table 3.5 compares the amount of lysozyme activity in the blink model to a vial at 8 hours. Etafilcon A deposited more active lysozyme per lens in the blink model system than the vial system ($p = 0.0435$). In contrast, somofilcon A ($p = 0.0076$) and senofilcon A ($p = 0.0019$) both deposited more active lysozyme per lens in the vial system than the blink model. There was no significant difference in active lysozyme deposition between the blink model and the vial system for the other three lens types ($p > 0.6389$).

Table 3.5 Comparison between blink model to vial system over 8 hours of active lysozyme.

8 hour incubation	Etafilcon A (µg/lens)	Omafilcon A (µg/lens)	Nelfilcon A (µg/lens)	Somofilcon A (µg/lens)	Senofilcon A (µg/lens)	Delefilcon A (µg/lens)
Blink model	402 ± 102	26 ± 8	17 ± 5	9 ± 2	10 ± 2	26 ± 1
Vial model	228 ± 13	30 ± 3	13 ± 4	24 ± 9	28 ± 2	32 ± 7

3.4 Discussion

This study has successfully evaluated the deposition of active lysozyme on various CL materials using a novel blink-incorporated *in vitro* eye model. Several parameters on the blink model can be adjusted, including blink and flow rates, to match physiological conditions. A previous study reported the amount of *ex vivo* active lysozyme on etafilcon A after 2, 4, and 8 hours to be 122 ± 1 µg/lens, 225 ± 5 µg/lens, and 342 ± 14 µg/lens respectively.[59] These data were used to determine the most appropriate flow rate (1.0 µL/min) for the blink model in the preliminary phase of this study.

Overall, there was an increase of active lysozyme deposited on all lens types over the 8 hours, which are similar to published *ex vivo* studies.[59, 70, 77] Consistent with previously published results, etafilcon A deposited the highest amount of active lysozyme.[38, 60, 65, 66, 69, 70, 73, 76, 78, 79] Within the first 24 hours, the percentage of active lysozyme compared to total lysozyme is >95%.[73] These results suggest that the amount of active lysozyme deposited onto CL materials for the short duration of this study is suggestive of total lysozyme deposition. However, quantitative amounts of total lysozyme were not determined in this study but will be for future work.

3.4.1 Blink model vs vial model

Previous studies have noted differences in the amount of lysozyme deposition on CL from a vial compared to a blink system.[43, 48] In the current study, there were also notable differences in the amount of active lysozyme per lens for some lens types between the blink model and the traditional vial incubation, whereas other lens materials showed no difference between the models. While active lysozyme deposition has been generally observed in higher quantities for etafilcon A, a greater amount of deposition observed in the blink model compared to the vial system may be due to the additional contribution of air exposure. The intermittent air exposure causes the tear film on the lens surface to break and evaporate, which may facilitate deposition of lysozyme on the lens surface. This phenomenon of increased deposition due to air exposure has been previously observed with the deposition of lipids on CL.[40] However, in SH materials such as senofilcon A and somofilcon A, there was a relatively lower amount of active lysozyme with the blink model than the vial system. The reason for this may be that air drying of the tear film exposed the relatively hydrophobic lens surface to lysozyme, which resulted in lysozyme denaturation and reduction of activity.[40]

The amount of active lysozyme deposited on the other CH materials (omafilcon A and nelfilcon A) were similar in both deposition systems. Omafilcon A is a non-ionic material that is known to have low protein deposition and spoilation.[76] Nelfilcon A, also a non-ionic material, has previously been shown to have little lysozyme deposition.[73] Thus, it is not surprising to see no significant difference between the two deposition systems for either of these lens materials. Delefilcon A is a lens with a SH-core and a CH-like surface to increase wettability and lubricity.[80] As a result, delefilcon A has a lens surface with lower hydrophobicity than other SH CLs materials. Without the silicone properties of a SH lens on the surface, there is less lysozyme denaturation compared to other SH lenses, as shown in this study.

Lens material and tear film composition have a large effect on lysozyme deposition, as shown in many studies.[38, 40, 56, 65, 70, 77] Air exposure affects the hydrophobicity of the material interface, causing an increase in lipid deposition, which in turn competes with lysozyme deposition.[40] The blink mechanism in this *in vitro* model provides a more accurate lysozyme deposition profile, however, lens material contributes more to the variability in deposition. Similarly, the total immersion of a CL in a vial does not provide the air exposure that occurs during a blink.

Another point of relevance that differentiates the blink and vial-based models relates to the availability of a low tear volume that is constantly replenished. The adjustable tear flow rate of this *in vitro* model not only provides a low tear flow but also the act of replenishing the tear film over the ocular surface. A potential confounder in static vial experiments is the rapid saturation of a lens when kept in the same solution, leading to potentially lower deposition results.[65] As the lens material has a significant impact on lysozyme deposition, having a high throughput *in vitro* model

allows for several lens materials to be tested simultaneously prior to animal models or clinical trials.

3.4.2 Blink Model Design

The blink model in this study utilized PVA to simulate the eyeball and eyelid. The highly wettable and flexible material has previously been used as an artificial cornea and eyeball [81, 82] due to its high water content and resistance to degradation.[72, 83] The material is also relatively inexpensive. Additionally, studies have shown PVA is able to absorb lysozyme, but at low concentrations.[81] The amount of lysozyme adsorbed onto PVA is less than that adsorbed onto a conventional soft CL.[72] Further studies need to be conducted to evaluate whether these absorption rates are similar to that of the cornea and conjunctiva.

3.4.3 Experimental Design

The CLs chosen for this experiment included etafilcon A as the representative CL for a positive control due to its ability to deposit a high amount of lysozyme.[60, 66] Three SH and three CH lenses were chosen with a range of varying ionic charge and water content. Specifically, delefilcon A was chosen due to its SH-core and a CH-like surface.[80]

3.4.4 Clinical Importance

While there are numerous tear film proteins that deposit on CLs, lysozyme was selected as a model because it is the most abundant protein in the tear film.[84] The deposition of lysozyme on CLs can change the conformation of the protein, which could lead to an inflammatory response.[78, 79] Thus, high levels of denatured proteins have been associated with ocular inflammatory responses such as CL-associated papillary conjunctivitis, giant papillary conjunctivitis and reduced CL comfort. [38, 60, 75, 76]

The data from currently published *ex vivo* and *in vitro* studies on lysozyme deposition were collected on biweekly or monthly replacement CLs,[38, 59, 74, 76, 79] and this study contributes to the literature pool, with an advanced *in vitro* blink model. By using the blink model and etafilcon A as a reference lens, lysozyme deposition patterns can potentially be used to predict that determined on other lens types. However, further experiments will be required to validate the predicted data. Using an improved model for *in vitro* testing could lead to more efficient use of research funds by, in situations where appropriate, replacing animal models or clinical trials. This *in vitro* blink model acts as a high throughput device that is relatively inexpensive and produces minimal waste. The model is designed to be used for many testing platforms ranging from CL physical property assessments, ocular drug testing, to research and education purposes. This versatile model can be adjusted as needed for many variables, such as flow rate, blink rate, time of wear for CL, and addition of ocular drugs.

This study provides new *in vitro* data for DD CLs with a novel blink model that can be easily adjusted as needed for a variety of experiments. It also demonstrates a notable difference between a vial system to a more sophisticated *in vitro* model. However, as many *ex vivo* studies test biweekly and monthly CLs, limited data is available to compare results. Conducting *ex vivo* studies in parallel to the blink model to evaluate *ex vivo* data fitting, and to guide model parameter settings, would be a valuable next-step for this work.

3.5 Conclusion

In conclusion, a comparison of active lysozyme deposition on DD CL materials from the eye blink model to *ex vivo* data shows the ability of this model to mimic active lysozyme deposition observed on *ex vivo* CLs by tuning the parameters of the *in vitro* model. The results in this study using both the blink model and a vial showed that etafilcon A deposits the most active lysozyme

per lens. With additional work and tuning in the future, the *in vitro* blink model in this study could be used to predict lysozyme activity on a wide variety of CL materials.

4 OcuBlink Improvements

ABSTRACT

Purpose: To explore the process of the OcuBlink model design and improvements.

Methods: This chapter shows the development process for the OcuBlink designs through several iterations.

Results: The OcuBlink model started as an *in vitro* blink model that can be easily adjusted and tuned for different experiments. The blink model structure was transitioned to a tight fit 3D-printed design for reliable and repeatable results. As the blink model design was developed, the ease of use and set up improved. Less waste was produced as the full wettable eye material, PVA, was changed to a reusable 3D-printed structure for a partial eye that can be either PVA or silicone. With the silicone eye, experiments could be held at ocular temperatures to produce more representative data to the human eye.

Conclusion: The change in the OcuBlink design has improved ease of use, ease of set up, decreased waste produced, improved repeatability, and produces data that is more representative to *in vivo* ocular conditions than previous iterations.

Keywords:

In vitro, eye model, blink model, polyvinyl alcohol

4.1 Introduction

Most products undergo a multistep process before it is consumer-ready. With products such as contact lenses or multipurpose solutions, thorough *in vitro* and *in vivo* testing is required before commercialization. Product testing typically involves stages of experimental design, mathematical models, *in vitro* testing, and animal models before reaching human clinical trials. As animal and clinical testing are likely the most expensive and extensive of the stages, improving the testing stages prior to these phases would decrease expenses significantly and likely help with screening early iterations, providing a "fast-fail" process. The development and improvement of a sophisticated *in vitro* model to test early versions of contact lens materials or contact lens coatings would permit high throughput testing at a lower cost before reaching animal models.

Simple *in vitro* testing of contact lenses for uptake and release of solution components or interaction with various components of the tear film often involves nothing more complicated than a static vial filled with a solution, into which the contact lens under test is immersed.[17, 42, 56, 63, 65-67, 85] Using simple vials for *in vitro* testing allows for an easy and relatively controlled environment for experiments,[17, 85] avoiding the need for ethics approvals and remuneration associated with animal or human testing.[17, 85] The benefits of lower time and costs with more control make simple vials a very convenient *in vitro* testing platform.[17, 65, 85] However, a static vial model does not represent the complexity of the human eye, lacking factors such as the low tear volume, tear turnover, intermittent exposure to the atmosphere between blinks and the blink motion itself, all of which likely play a role in tear film deposition on contact lens materials.[40, 43, 44, 46, 48, 52, 54]

In many *in vitro* experiments, the limitations of published studies tend to be an inaccurate portrayal of *in vivo* contact lens wear.[42, 56, 65-67, 85] Some studies note that lenses are kept in the same solution for the entire duration of the experiment,[42, 65-67] leading to a misrepresentation of

protein or lipid deposition on the lens surface. Others note a lack of air exposure to the lens, which affects the deposition pattern for tear film components.[17, 42, 65-67] More specific studies use a single component solution instead of a complex tear solution,[38, 76, 86-88] which results in data that does not fully represent the competition between tear film components that deposit on contact lens materials.[56, 65, 66, 89, 90]

Several previously published *in vitro* models have incorporated air exposure into their design.[41, 43, 44, 46, 48, 52, 54] Our group's initial *in vitro* model (termed 'OcuFlow'), was initially a closed eye model.[52] In subsequent studies, the OcuFlow was designed with air exposure and mechanical shear stress in mind.[43, 44, 46, 48, 54] In all drug release studies, the OcuFlow model showed a constant, sustained release pattern when compared to the rapid burst release observed in a static vial model.[44, 46, 52, 54] The OcuFlow model was also used to characterize lysozyme[48] and cholesterol[43] deposition patterns on several daily disposable contact lens materials. The deposition patterns observed using the OcuFlow model was more complex than the static vial model.[43] In order to build on these improvements on *in vitro* eye models, a blink mechanism was designed in addition to adjustable tear flow rates on a more advanced *in vitro* model, the OcuBlink.[53, 91, 92] The addition of the blink mechanism creates an artificial tear film on the surface of the eye that is susceptible to a measurable tear break-up time.[53]

To further improve on the current iteration of the eye model, the purpose of this thesis chapter is to review the previous iterations of the OcuBlink model and how it has developed into an eye model that can be tuned to ocular temperatures.

4.2 Methods and Materials

4.2.1 OcuBlink Iterations

OcuBlink is a new *in vitro* eye model that uses a wettable material and includes a blink mechanism. The blinking mechanism is controlled by an Arduino board (WEMOS D1 R2 Wifi ESP8266) and an attached motor (Adafruit Industries, New York, NY). The blink rate is arbitrarily set to 6 blinks per minute, based on human blink rates[55] and to maintain a functional rate for experimental practicality. The blink rate can be changed per study based on the experimental parameters required. For example, an increased blink rate could be used for experiments focused on dry eye studies, where blink rates tend to be higher.[93] To administer the tears to the system, tubing is placed above the eyelids, held in place at the top of the outer chamber. The tubing is connected to a commercial microfluidic pump (PHD Ultra, Harvard Apparatus, Holliston, MA). The OcuBlink system, depicted in Figure 4.1, is encased in an acrylic chamber, with metal bearings to hold the rods used for the blink mechanism in place. A separate acrylic rod at the back of the chamber holds the eyeball in place and can be adjusted.

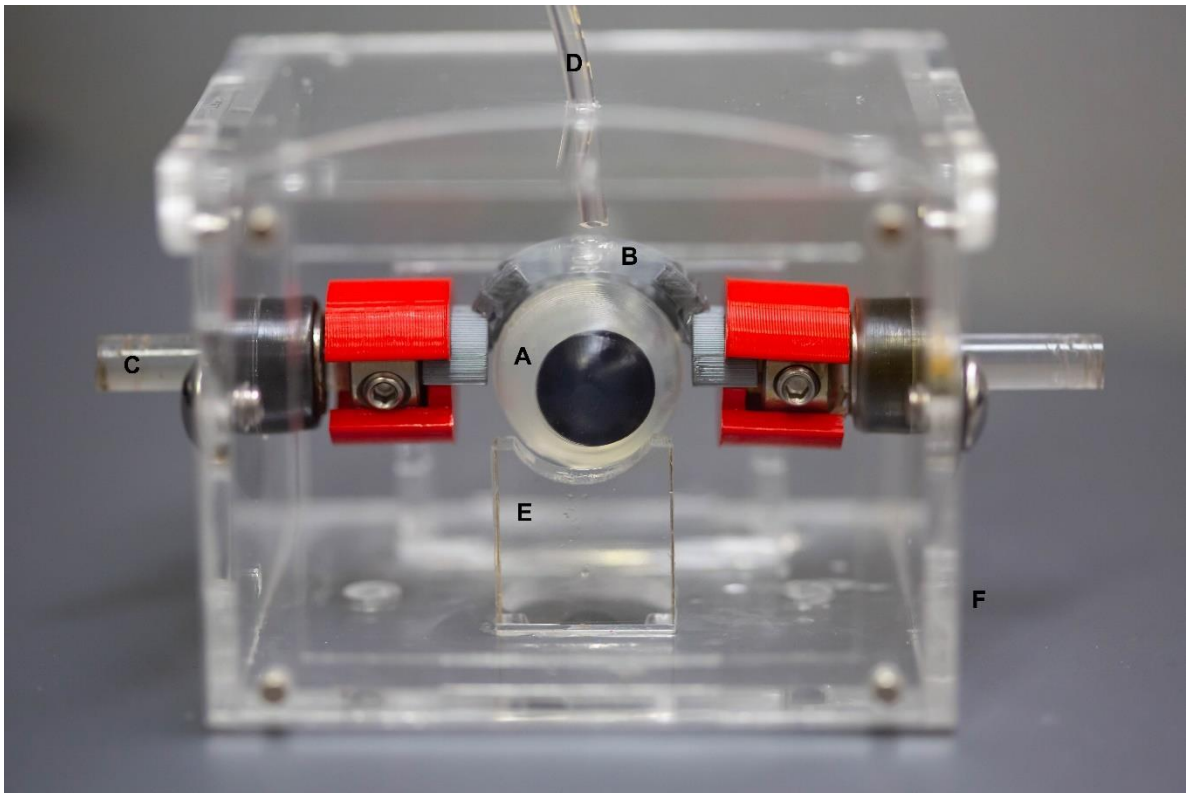

Figure 4.1. OcuBlink 1.0 (A) eyeball (B) eyelid (C) acrylic rod to attach to motor (D) tubing to administer tear fluid (E) lower eyelid (F) acrylic chamber.

The eyeball and eyelid are made of a wettable material based on the methods proposed by Hyon et al.[72] Briefly, PVA is added to a mixture of dimethyl sulfoxide and Milli-Q water (8:2) and stirred. The mixture is heated for 1.5 hours at 120°C and poured into moulds. Filled moulds are then held at -80°C for 30 minutes to set. Once removed, eyes and eyelids are placed in 500 mL of Milli-Q water, renewed daily for 3 days to remove the dimethyl sulfoxide.

4.2.2 OcuBlink 2.0 setup protocol

To set up OcuBlink 2.0 for a simple experiment with contact lenses, eyeballs are soaked in PBS for 12 hours prior to starting the experiment. After soaking, eyes can be placed onto the back support rods of the OcuBlink system. Eyes can be aligned by turning the rods to change the distance from the chamber. Eyelids are then attached to their support structure and side connectors.

Once the eyelid is placed on top of the eyeball, rods are inserted into the chamber to the eyelid connectors. One rod will be connected to the Arduino motor for the blink mechanism (Figure 4.2). Plastic tubing will be threaded through the top of the chamber and connected to the eyelid support structure. The tubing is connected to a syringe filled with ATS, previously published by our group.[17] The syringes are attached to the microfluidic pump to administer the tears at the desired flow rate. Once the OcuBlink system is set up, the tears and blink mechanism runs for 30 minutes to equilibrate the system. After 30 minutes, contact lenses can be placed on the eyes for experimental testing.

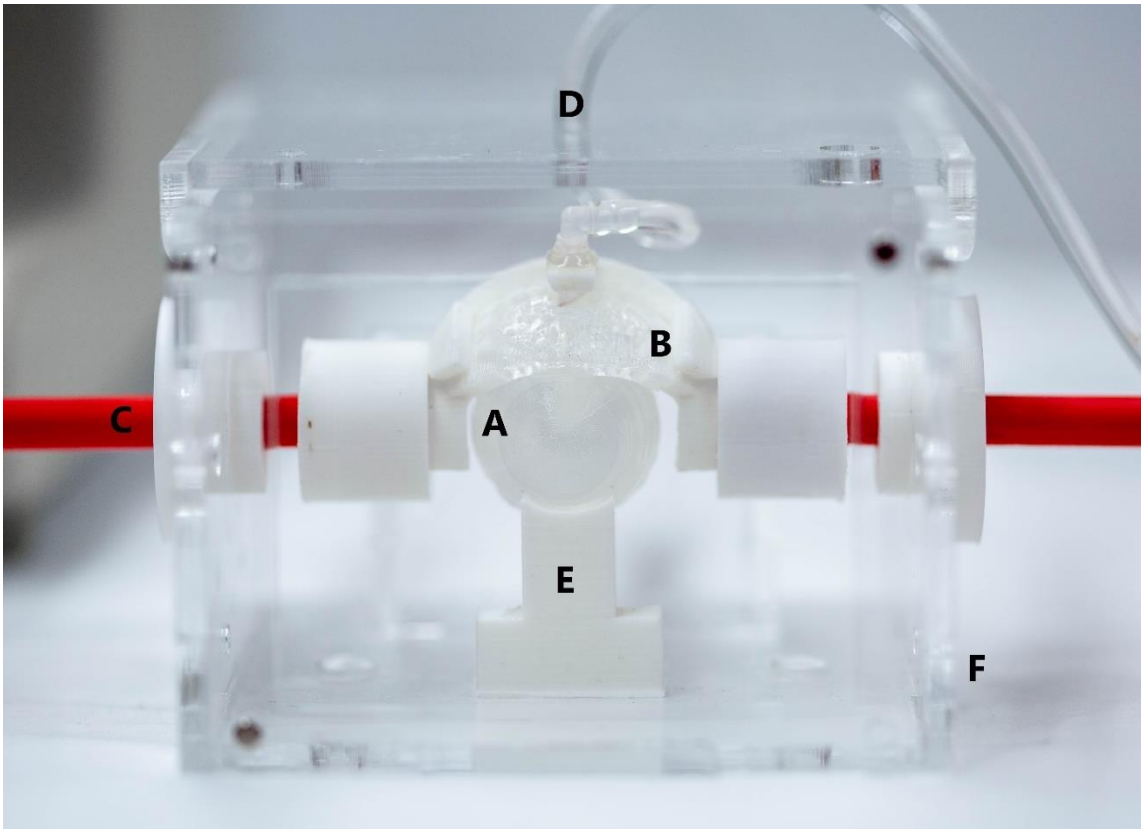

Figure 4.2. OcuBlink 2.0 (A) eyeball (B) eyelid (C) 3D-printed rod to attach to motor (D) tubing to administer tear fluid (E) lower eyelid (F) acrylic chamber.

4.2.3 OcuBlink 3.0 setup protocol

To set up OcuBlink 3.0, the eyeball support structure fits into the OcuBlink chamber. Plastic tubing connected to the heated water bath and pump is threaded through the back of the chamber and connected to the inlet and outlet of the eyeball structure. The pump is turned on to allow all air bubbles to be pushed out of the system. Eyelids are attached to their support structure and side connectors. Once the eyelid is placed on top of the eyeball, rods are inserted into the chamber to the eyelid connectors. One rod is connected to the Arduino motor for the blink mechanism. Plastic tubing is threaded through the back of the chamber and connected to the eyelid support structure. The tubing is connected to a syringe filled with ATS. The syringes are attached to the microfluidic pump to administer the tears at the desired flow rate. Once the OcuBlink 3.0 system is set up, the tears, blink mechanism, and heated system runs for 30 minutes to equilibrate the system. After 30 minutes, contact lenses are placed onto the eyes for experimental testing.

Figure 4.3. OcuBlink 3.0 XL (A) eyeball (B) eyelid in white, with eyelid support structure in black (C) 3D-printed rod to attach to motor (D) tubing to administer tear fluid (E) black 3D-printed lower eyelid with resin support structure (F) 3D-printed chamber.

4.3 Discussion

4.3.1 OcuFlow

Our *in vitro* blink model, OcuBlink, is the successor to a previous *in vitro* model by our group, called the OcuFlow (Figure 4.4). The OcuFlow design included tear flow, low tear volume, air exposure, and mechanical wear of blinking. The model was able to successfully observe the release of multiple drugs from CL materials over time [44, 46, 52, 54] and characterize lysozyme deposition patterns on CLs.[48] The drug release studies involving the OcuFlow platform had shown a sustained release pattern that better mimicked *in vivo* results when compared to the burst release of a static vial model.[44, 46, 52, 54] The air exposure and low tear volume allows the OcuFlow to act as a better *in vitro* model compared to a static vial, providing more accurate results. However, the OcuFlow design used a hydrophobic material for the eyeball and eyelid structures, unlike the smooth hydrophilic ocular surface.[44, 48]

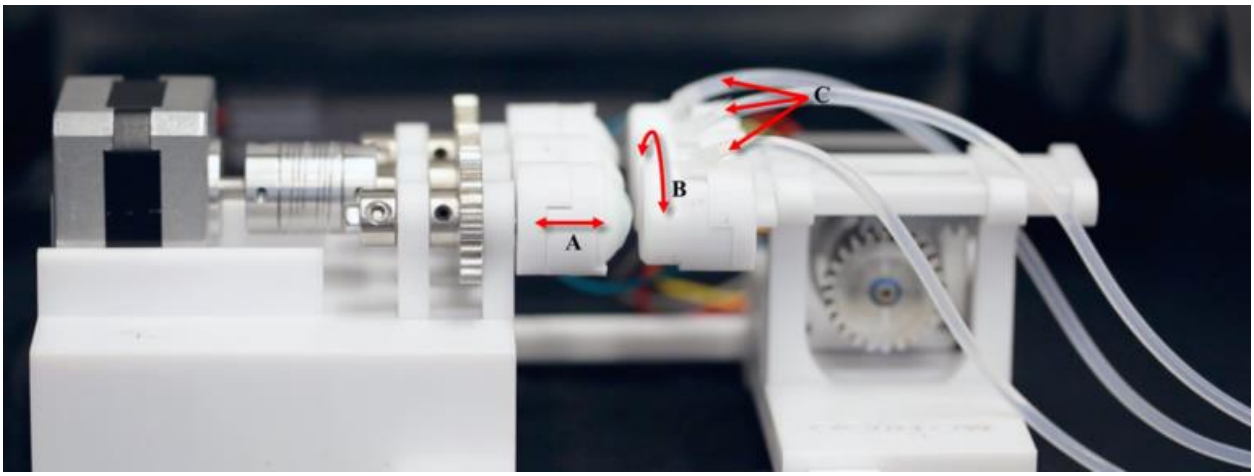

Figure 4.4. OcuFlow (A) horizontal motion of eyelid (B) rotational motion of eyelid (C) tubing for tear fluid.

4.3.2 OcuBlink 1.0

The new OcuBlink model incorporates a blinking mechanism and retains the low tear flow design of the OcuFlow. The tear flow and blink rate of the OcuBlink model can be tuned if

necessary. The PVA-based material used for the eyeball and eyelid has a very high water content and has previously been used as an artificial cornea and eyeball.[72, 81-83, 94-96] The new wettable material is an improvement to the previously hydrophobic material as it better mimics ocular conditions.

4.3.3 OcuBlink 2.0

However, the metal bearings used for the system required tinkering with Allen keys. Once the metal bearings began to rust, alternative materials were explored, resulting in OcuBlink 2.0 (Figures 4.5 and 4.6).

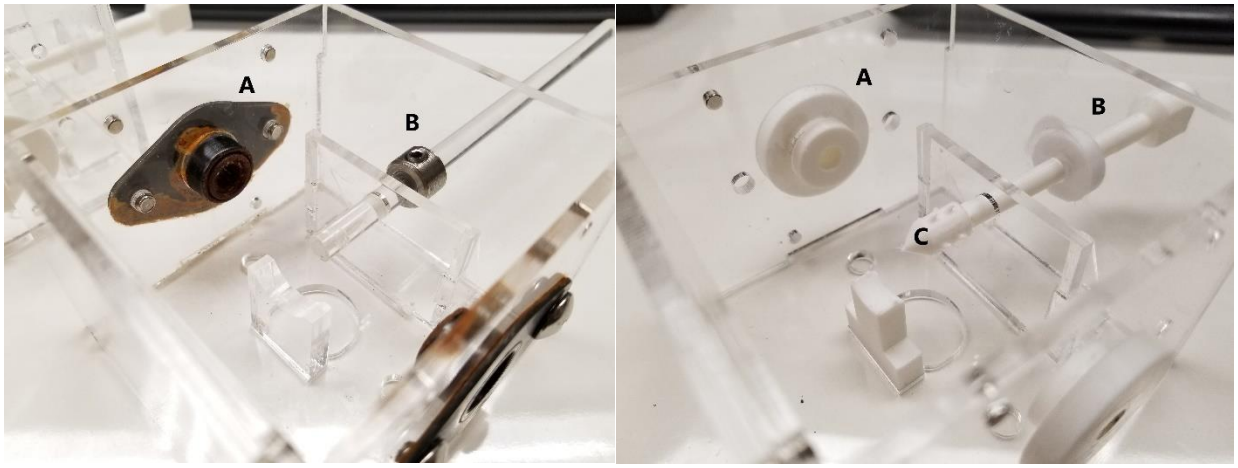

Figure 4.5. OcuBlink 1.0 (left) (A) rusted metal bearings (B) adjustable acrylic rod using Allen keys.
Figure 4.6. OcuBlink 2.0 (right) (A) 3D-printed bearings (B) adjustable 3D-printed rod (C) increased grip for eyeball.

OcuBlink 2.0 uses 3D-printed (Prusa3D, Prague, Czech Republic) bearings instead of metal, so no Allen keys are required, instead, it is a simple plug and play system with fine adjustments by turning rods as needed. A new 3D-printed structure support for the eyelid allows for better tear flow administration (Figure 4.7). In addition, the eyelid design itself was changed from a smooth surface to one with channels to help with the tear distribution across the front

surface of the eye (Figure 4.8). OcuBlink 2.0 uses eyeballs made with 23% PVA for better consistency in experimental results. Prior to running experiments, eyeballs were always soaked in PBS to better mimic ocular conditions.

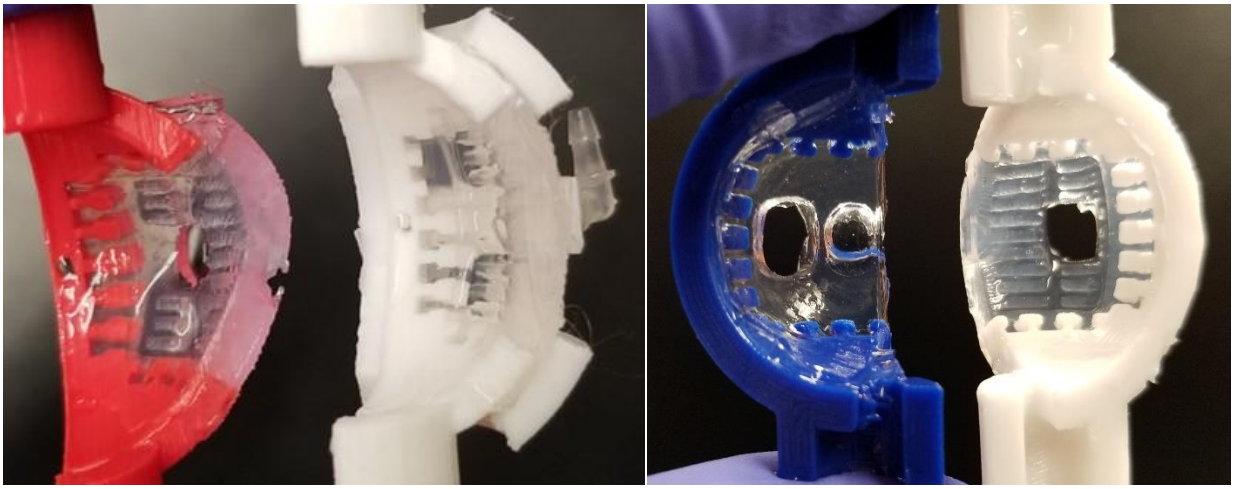

Figure 4.7. (left) Improved eyelid support structure. Support structure prevents eyelid displacement as shown in red. Eyelid support structure improves tear fluid administration.
Figure 4.8. (right) Improved eyelid design. Channels added to eyelid design to improve tear distribution over front surface of eye.

With the new upgrades to the system, tear flow rates were tested to determine what would match *ex vivo* data.[59] With the new standardized design, OcuBlink 2.0 was able to test lysozyme activity on contact lenses that matched *ex vivo* data as shown in Chapter 3.

4.3.4 Development for OcuBlink 3.0

Testing of the OcuBlink 2.0 system at room temperature resulted in minimal issues. However, ocular temperature is around 34°C.[97-99] When testing the OcuBlink system in a 34°C incubator, the eyeball, eyelid, and contact lenses would dehydrate. The conditions were similar to someone wearing lenses in a 34°C sauna instead of ocular temperature. Even if water reservoirs were added to both the OcuBlink chamber and the incubator, humidity was never above 50%. Many of the support structures are 3D-printed parts that are not meant to withstand high heat and

mechanical stress, which resulted in multiple damaged parts that needed to be frequently replaced. With the dehydration of the eyeball and eyelid, scratches were formed on the surface of the contact lenses after a few hours under the blinking system (Figure 4.9). Most tested lenses at this temperature resulted in deformation of the lens while on the eye as the dehydration rate was too high (Figure 4.10). To improve the design in order to withstand ocular temperatures, OcuBlink 3.0 was designed.

Figure 4.9. Scratched contact lens on OcuBlink 2.0. A contact lens with a scratched front surface due to dehydration of lens and eyelid during blinking at 34°C for 16 hours.

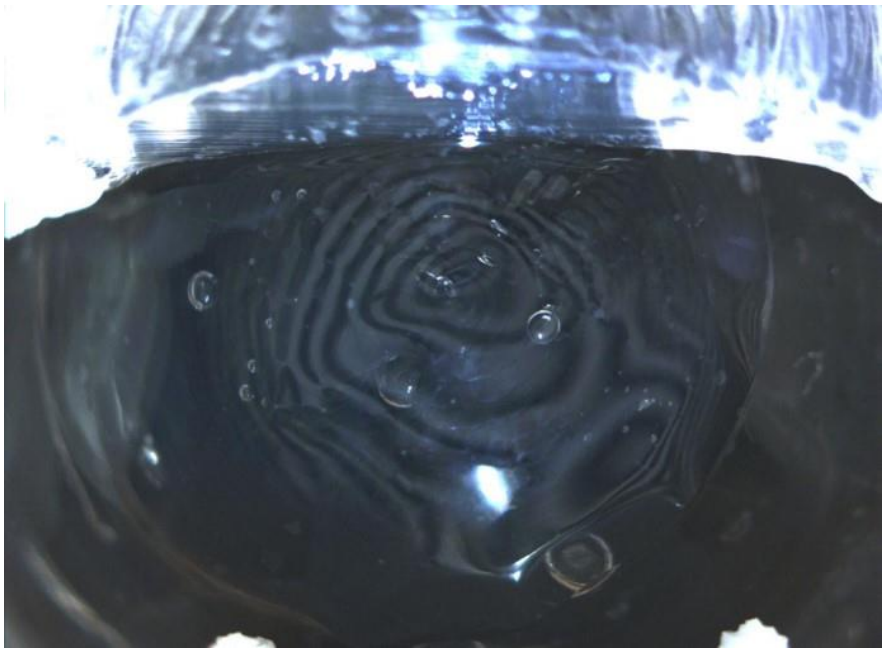

Figure 4.10. Distorted contact lens on OcuBlink 2.0. A contact lens distorted after 8 hours of blinking at 34°C.

4.3.5 OcuBlink 3.0

Instead of placing the whole system in a 34°C chamber, only the eyes would be heated to prevent a sauna-like environment. Heated water would run through the back of the eyes through a heated water bath (Aquasonic Model 50D, VWR, PA, USA) and a pump system (Auto Dosing pump, Jebao, Guangdong, China). In order to do this, a new eyeball design was created. The eyeball structure is a stereolithography (SLA) resin-based 3D-print (Photon S, Anycubic, Shenzhen, China) and therefore re-usable. This new hollow structure allows the heated water through with small inlets and outlets on either end. The SLA resin-based print material allows for structures to be filled with water without leakage, unlike the previous polylactic acid (PLA) 3D-printed structures.

Since the PVA-based material has very high water content, the eyes would dehydrate and deform, ultimately falling off the new 3D-printed structure. A silicone material was tested and proved to stay on the eyeball structure even after 18 hours of heated use. The new eyeball structure

only requires a small amount of silicone on the surface of the eyeball structure, decreasing waste production (Figure 4.11).

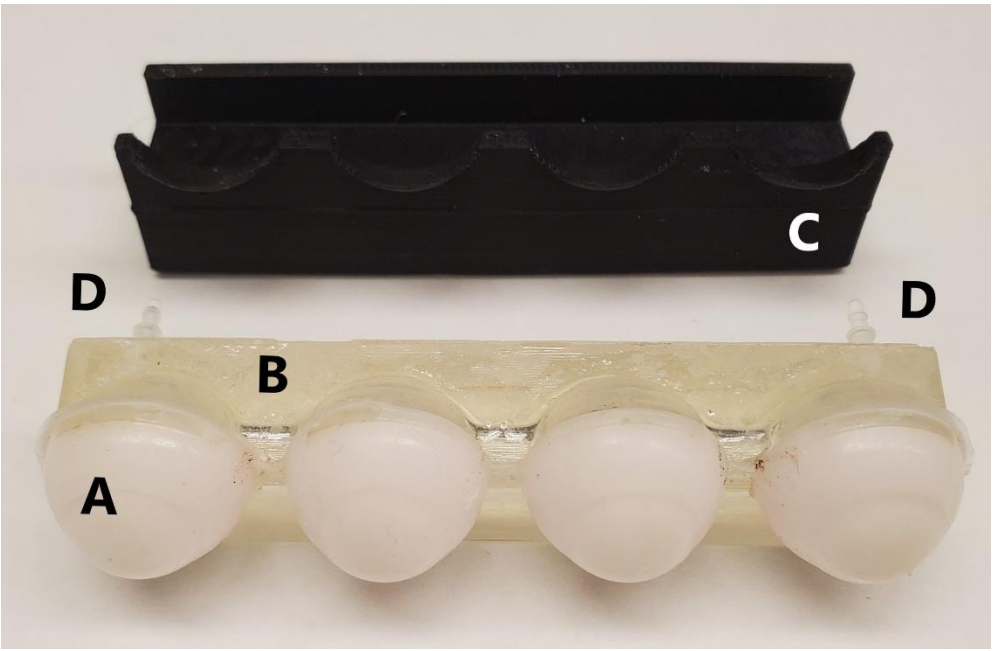

Figure 4.11. OcuBlink 3.0 XL eye structure. (A) silicone eye (B) resin eye structure (C) 3D-printed eye structure base (D) inlet and outlet for heated water.

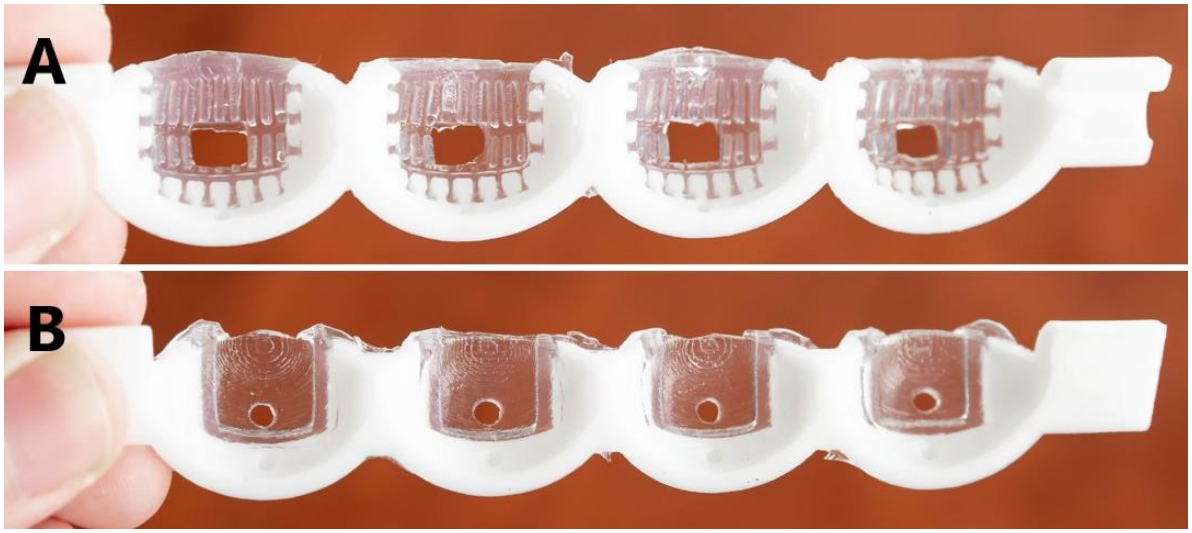

Figure 4.12. OcuBlink eyelid comparison. (A) OcuBlink 2.0 eyelids with channels. (B) OcuBlink 3.0 eyelids, very thin to assist in tear film distribution.

The OcuBlink 3.0 eyelid became much thinner in design (Figure 4.12). This thin eyelid does not require the addition of channels as the tears would easily spread across such a thin layer to cover the front surface of the eye. The moulding process of creating the eyelids became much quicker with the use of silicone moulds instead of 3D-printed moulds (Figure 4.13). All of the new OcuBlink 3.0 designs had decreased the amount of material used, consequently saving in material cost, time of manufacturing parts, and waste production. As many of the structural parts are re-useable and sturdier, less waste was produced.

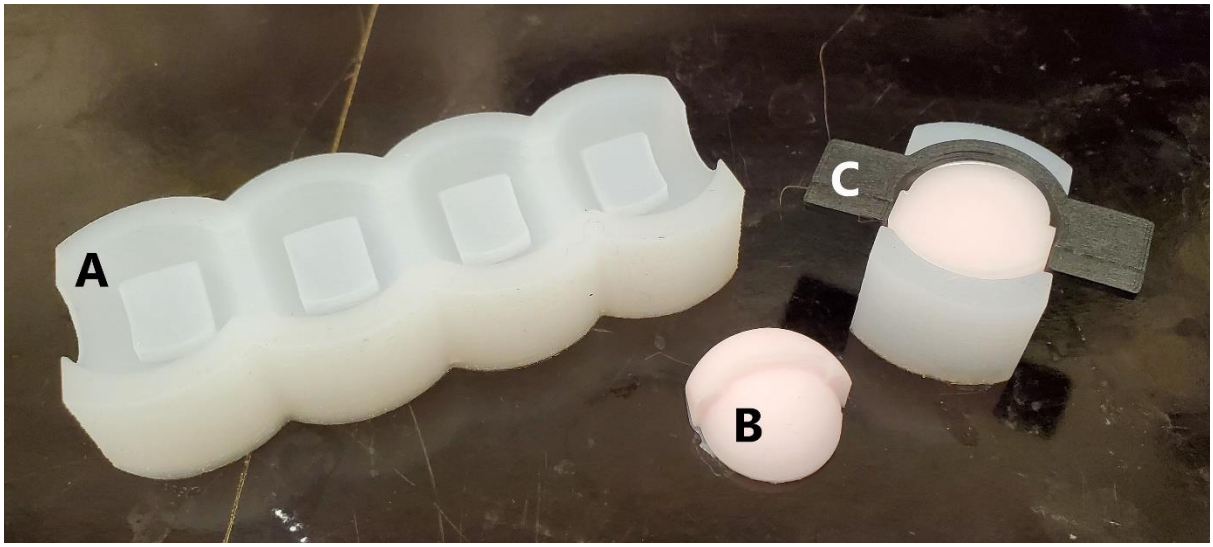

Figure 4.13. Silicone moulds for eyelids. (A) XL eyelid mould. (B) Counter mould for single eyelid. (C) 3D-printed eyelid structure.

With the change in eyeball structure, a new chamber was designed so that there would be no adjustments needed. All structures would be properly aligned and a tight fit (Figure 4.14). This significantly improved the setup time for the OcuBlink system. Within the new enclosed OcuBlink chamber (Figure 4.15), heated water was pumped through the system, keeping humidity high (>95%) and preventing dehydration of the eyelids and CLs. As the eyeballs are made of silicone, dehydration of the eyes was not an issue. In order to maintain regularity in room temperature and humidity, all OcuBlink 3.0 experiments were run in a larger, monitored humidity chamber to

maintain the ambient temperature and humidity. Table 4.1 summarizes and compares the changes in the OcuBlink iterations to the OcuFlow model and a simple vial model in reference to key features of *in vivo* ocular conditions.

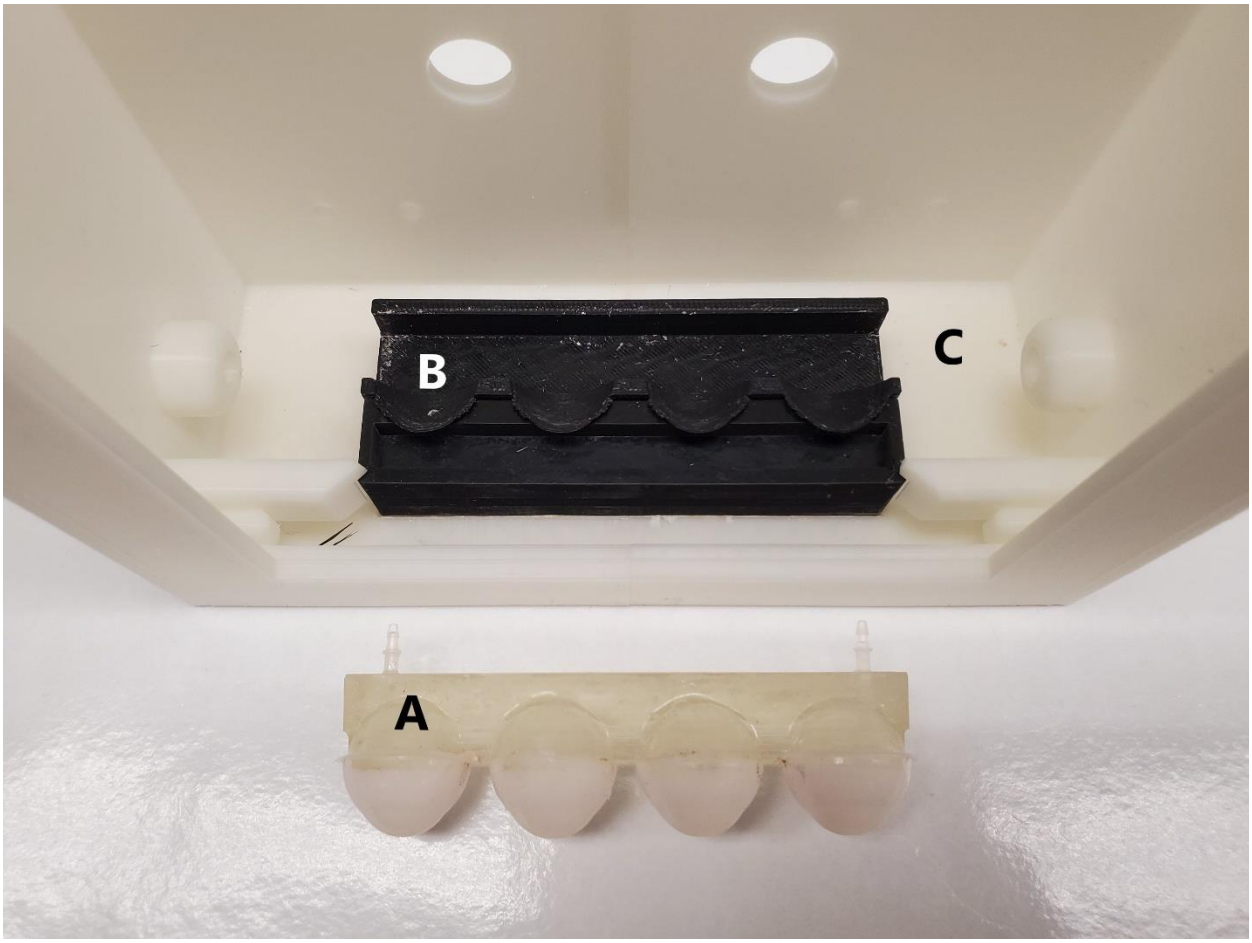

Figure 4.14. OcuBlink 3.0 XL set up. (A) eye structure. (B) eye structure base. (C) 3D-printed chamber. All pieces are a tight fit, no adjustments required during set up.

Figure 4.15. OcuBlink 3.0 XL enclosed. Acrylic sheets used to enclose the OcuBlink chamber to maintain humidity and temperature.

Table 4.1 Comparison of *in vitro* eye models to *in vivo* ocular features.

In vivo	Vial	OcuFlow	OcuBlink 1.0	OcuBlink 2.0	OcuBlink 3.0
Eye	×	PDMS	PVA	PVA	Silicone
Eyelid	×	PDMS	PVA	PVA with channels	Thin PVA
Ocular temperature	✓	×	×	×	✓
Air exposure	×	✓	✓	✓	✓
Blink	×	✓	✓	✓	✓
Shear stress	×	✓	✓	✓	✓
Biological interactions	×	×	×	×	×

PDMS, polydimethylsiloxane; PVA, polyvinyl alcohol; ×, this feature is not represented in this model; ✓, this feature is represented in this model.

4.4 Conclusion

With the development of the newest iteration of the OcuBlink eye model, *in vitro* testing can occur under heated ocular conditions to provide more accurate results. While retaining the features of previous OcuBlink iterations, the heated system is an adjustable addition that provides more information and control to ocular experiments.

5 Effects of temperature and blinking on contact lens dehydration of contemporary soft lens materials using an *in vitro* blink model

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ABSTRACT

Purpose: To evaluate the effects of temperature and blinking on CL dehydration using an *in vitro* blink model.

Methods: Three silicone hydrogel (delefilcon A, senofilcon A, comfilcon A) and two conventional hydrogel (etafilcon A, omafilcon A) CL materials were evaluated at 1 and 16 hours. The water content of the CLs was measured using a gravimetric method. Lenses were incubated on a blink model, internally heated to achieve a clinically relevant surface temperature of 35°C. ATS was delivered to the blink model at 4.5 µL/min with a blink rate of 6 blinks/min. A comparison set of lenses were incubated in a vial containing either 2 mL of ATS or PBS at 35ºC.

Results: Increasing temperature to 35°C resulted in a decrease in water content for all tested CLs over time ($p \leq 0.0052$). For most CLs, there was no significant difference in water content over time between ATS or PBS in the vial ($p > 0.05$). With the vial system, water content decreased and plateaued over time. However, on the blink model, for most CLs, the water content significantly decreased after 1 hour but returned towards initial water content levels after 16 hours ($p > 0.05$).

Conclusion: The reduction in water content of CLs on the eye is likely due to both an increase in temperature and dehydration from air exposure and blinking.

Translational relevance: This study showed that the novel, heated, *in vitro* blink model could be used to provide clinical insights into CL dehydration on the eye.

Keywords:

Contact lens, dehydration, in vitro, eye model, blinking.

5.1 Introduction

CLs have seen great adoption globally, with over 140 million wearers worldwide.[4] Prior to commercialization, CLs must go through thorough testing of the device quality as well as ensuring the CLs are safe to use and will not change their parameters when worn. Some parameters that are tested include changes in lens dehydration,[100, 101] lens diameter,[102] and lens thickness.[103] These parameters can vary due to the nature of the CL material.[100]

Soft CLs are made of water-containing (hydrogel) materials and can be categorized as conventional hydrogels or silicone hydrogels, depending on the composition of the material.[3] Hydrogels are composed mainly of water held together by a crosslinking network of polymer side chains.[104] The crosslinking network creates a scaffolding structure, which gives the material the ability to keep its shape but remain malleable to enable them to conform to the shape of the anterior ocular surface.[104, 105] The crosslinking structure allows for some permeation, such as oxygen transfer, making them an attractive material for manufacturing.[100] As CLs are exposed to varying environmental conditions, the properties of the lens may undergo physical changes due to dehydration during in-eye wear.[100, 104]

When CLs are worn, they are exposed to the environment and begin to dehydrate due to both evaporation[106] and changes in temperature as they go from the blister pack or lens case (at room temperature) to ocular temperature once placed onto the eye.[106, 107] As hydrogels dehydrate, the crosslinking structure begins to deform as empty spaces, previously occupied by water molecules, begin to form.[104] Although lens dehydration may not have a direct effect on CL discomfort,[108] the lens diameter,[102] fit[37] and oxygen transmissibility[109] may change due to dehydration, and as a result, lens comfort may decrease.[108] As variability between patients and tear

film composition differs greatly, CL dehydration may not have the same effect on all patients.[37, 106]

Several studies have reported varying results on water content and dehydration under different conditions. Most studies agree that lens material composition impacts the dehydration of CLs, primarily due to their initial water content.[37, 101] However, the effects of environmental conditions, such as temperature and humidity, have varying results, with some studies reporting no significant difference[110] and others reporting significant impacts on dehydration.[37]

Previous dehydration studies compared the effect of temperature on CL properties but submerged the lenses in saline, which does not explore the effects of tear film components and air exposure.[102, 107] Other studies directly compared *in vitro* and *in vivo* results, however, both studies had submerged their *in vitro* lenses in blister pack solutions at room temperature, whereas the *in vivo* lenses were exposed to tears, ocular temperature and air from blinking.[34, 100] The current study used a blink model that incorporates an artificial tear solution and a blinking mechanism at ocular temperatures, in an attempt to provide more physiologically representative data.

The aim of this study was to evaluate the effects of temperature and blinking on the dehydration of CLs using an advanced *in vitro* blink model. This high throughput model allowed for results to be collected in a more controlled, physiologically relevant environment and showed potential to be used as a predictive tool for *in vivo* lens dehydration data, in addition to providing insights into CL dehydration on the eye.

5.2 Methods

5.2.1 Contact lenses

Three commercially available SH (delefilcon A, comfilcon A, senofilcon A) and two CH (etafilcon A, omafilcon A) lens materials were tested in this study (n = 5 for the vial system and n = 4 for the blink model). Of those, senofilcon A and etafilcon A were assessed in both DD and reusable (RU) modalities. Thus, the total quantity of tested CLs equated to four SH and three CH materials. The properties of the CLs are listed in Table 5.1.

Table 5.1 Contact lenses materials evaluated in the study.

USAN	**Delefilcon A**	**Senofilcon A**	**Etafilcon A**	**Omafilcon A**	**Comfilcon A**	**Senofilcon A**	**Etafilcon A**
Commercial Name	DAILIES TOTAL1	ACUVUE OASYS 1-DAY	1-DAY ACUVUE MOIST	Proclear 1 Day	Biofinity	ACUVUE OASYS	ACUVUE 2
Manufacturer	Alcon	Johnson & Johnson	Johnson & Johnson	Cooper Vision	Cooper Vision	Johnson & Johnson	Johnson & Johnson
FDA classification	V	V	IV	II	V	V	IV
Water content (%)	33	38	58	60	48	38	58
Dk/t	156.0	121.0	25.5	28.0	160.0	147.0	25.5
Monomer composition	Not disclosed	mPDMS, DMA, HEMA, siloxane macromere, PVP, TEGDMA	HEMA, MA	HEMA, PC	M3U, FMM, TAIC, IBM, HOB, NMNVA, NVP	mPDMS, DMA, HEMA, siloxane macromere, PVP, TEGDMA	HEMA, MA
Wear modality	DD	DD	DD	DD	RU	RU	RU
Lens material	SH	SH	CH	CH	SH	SH	CH

USAN, United States Adopted Name; mPDMS, monofunctional polydimethylsiloxane; DMA, *N*,*N*-dimethylacrylamide; HEMA, poly(2-hydroxyethyl methacrylate); PVP, polyvinyl pyrrolidone; TEGDMA, tetraethyleneglycol dimethacrylate; M3U, αω-bis(methacryloyloxyethyl iminocarboxy ethyloxypropyl)-poly(dimethylsiloxane)-poly(trifluoropropylmethylsiloxane)-poly(methoxy-poly(ethyleneglycol)propylmethylsiloxane; FMM, α-methacryloyloxyethyl iminocarboxyethyloxypropyl-poly(dimethylsiloxy)-butyldimethylsilane; TAIC, 1,3,5-triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione; IBM, isobornyl methacrylate; HOB, 2-hydroxybutyl methacrylate; NMNVA, N-methyl-N-vinyl acetamide; NVP, N-vinyl pyrrolidone; MA, methacrylic acid; PC, phosphorylcholine; DD, daily disposable; RU, reusable lenses; SH, silicone hydrogel; CH, conventional hydrogel

The CLs selected for this experiment were chosen to represent different lens materials (CH and SH), varying modalities (DD and RU) and to encompass materials with varying water content values across the range typically used by clinicians. Etafilcon A and senofilcon A were chosen specifically to compare any differences within lens modality within the same lens material, as both are commercially available as DD and RU lenses. Delefilcon A was chosen due to its unique water gradient design, which has a SH-core and a CH-like surface.[80]

5.2.2 Reagents

All materials were purchased from Sigma Aldrich (St. Louis, MO) unless otherwise specified.

5.2.3 Artificial Tear Solution

The ATS used in this study was previously described by our group.[17, 43, 66] The ATS contains various salts, proteins, and lipids (see Table 5.2).

Table 5.2 Artificial tear solution components.

Salts (mg/mL)	Lipids (mg/mL)	Proteins (mg/mL)
NaCl (5.26)	Oleic acid (0.0018)	Mucin (0.15)
KCl (1.19)	Oleic acid methyl ester (0.012)	Albumin (0.20)
$Na_3C_6H_5O_7$ (0.44)	Triolein (0.016)	Lysozyme (1.90)
Glucose (0.036)	Cholesterol (0.0018)	Lactoferrin (1.80)
Urea (0.072)	Cholesteryl oleate (0.024)	
$CaCl_2$ (0.07)	Phosphatidylcholine (0.0005)	
Na_2CO_3 (1.27)		
$KHCO_3$ (0.30)		
Na_2HPO_4 (3.41)		
HCl (0.94)		
ProClin 300 (200 µL/L of solution)		

5.2.4 Experimental design for vial incubation

Lenses were incubated in vials with 2 mL of incubation solution, either PBS or ATS. At room temperature (22°C ± 2°C), lenses were placed on an orbital shaker at 60 rotations per minute for 1 or 16 hours. To mimic ocular temperature,[97-99] lenses were placed in a shaking incubator (New Brunswick Innova, Marshall Scientific, Hampton, NH) at 35°C for 1 or 16 hours. After the incubation period, lenses were removed from the vials and water content was measured.

5.2.5 Synthesis of eyeball and eyelid for blink model

The procedures for the synthesis of the eyelids of the blink model were adopted from methods described by Hyon et al.[72] In brief, PVA was added to a mixture of dimethyl sulfoxide and Milli-Q water (8:2) to achieve a concentration of 15% weight/volume (w/v). The mixture was then stirred and heated at 120°C for 1 hour before being poured into a mould. The moulds were then stored at -20°C for 24 hours for gelation. After the eyelids gelled, they were removed from the moulds and placed in 500 mL of Milli-Q water, renewed daily for 3 days to remove any residual dimethyl sulfoxide.

The eyeball structure was designed using CAD software and 3D-printed (Photon S, Anycubic, Shenzhen, China) with SLA resin as a 4-piece hollow structure. This design allowed heated water to be pumped through the model to achieve and simulate an ocular surface temperature of 35°C ± 0.8°C. The surface of the eyeball was coated with silicone material to limit the absorption of tear film components on the eye model, as shown in Figures 5.1A and 5.1B. Furthermore, the silicone polymer does not absorb water from the contact lenses, which could potentially exacerbate any measured dehydration.

5.2.6 Set-up of blink model

The set-up for the blink model is shown in Figure 5.1A and Figure 5.1B. The blink motion and blink rate were controlled by an Arduino board (WEMOS D1 R2 Wifi ESP8266) and attached motor (Adafruit Industries, New York, NY). The blink rate was set to 6 blinks/min. Tubing was attached to the top of an eyelid support structure, which was then connected to a commercial microfluidic pump (PHD Ultra, Harvard Apparatus, Holliston, MA) to deliver ATS to the system. In preliminary trials, flow rates close to reported tear flow values of 1µL/min[111] were insufficient to maintain a reliable flow rate of fluid on the eye. We hypothesize that the increase in temperature caused an increase in evaporation rates, which caused the CLs to shrink on the blink model. Through trial and error, an appropriate tear flow rate of 4.5µL/min was chosen for this experiment. A secondary pump (Auto Dosing pump, Jebao, Guangdong, China) administered 40°C water from a water bath (Aquasonic Model 50D, VWR, PA, USA) through the back of the eyeballs to achieve a surface temperature of 35°C. A video demonstrating surface heating of the eyeball is available upon request. The blink model was placed in a humidity chamber to maintain an average temperature and humidity of 23.6°C ± 1.6°C and 91.8% ± 3.2%, respectively.

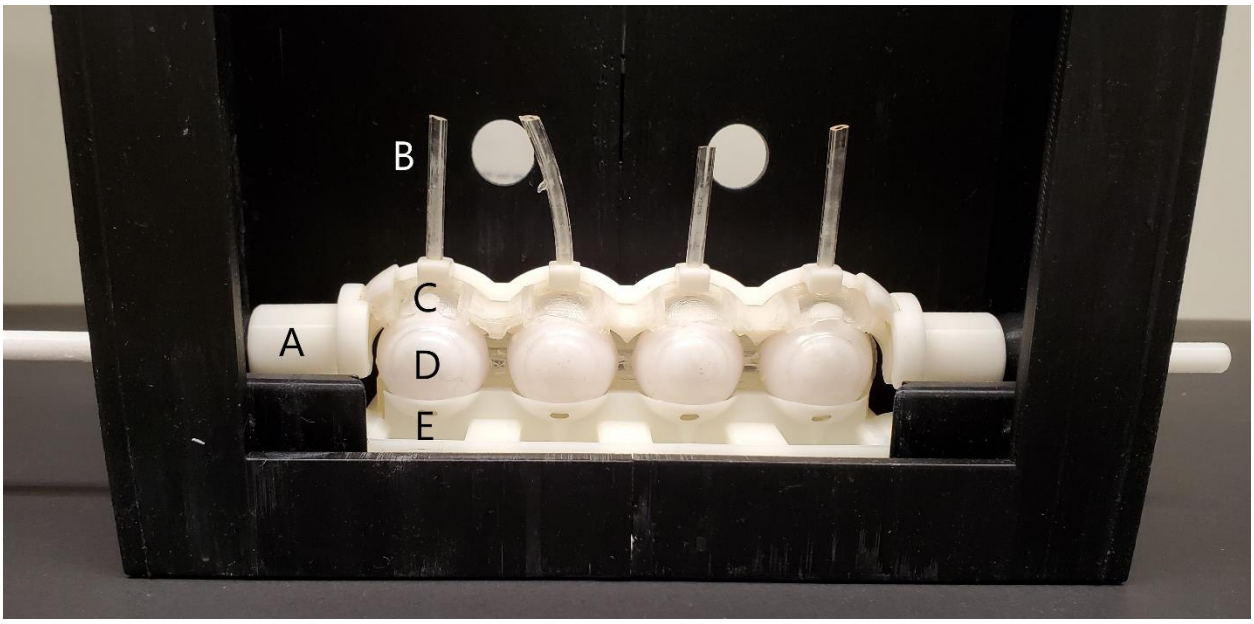

Figure 5.1A *In vitro* eye blink model used in this study. (A) Connector from eyelid to blink motor. (B) Tubing for artificial tear fluid. (C) Eyelid. (D) Silicone eyeball. (E) Lower eyelid with trough to hold excess tear fluid.

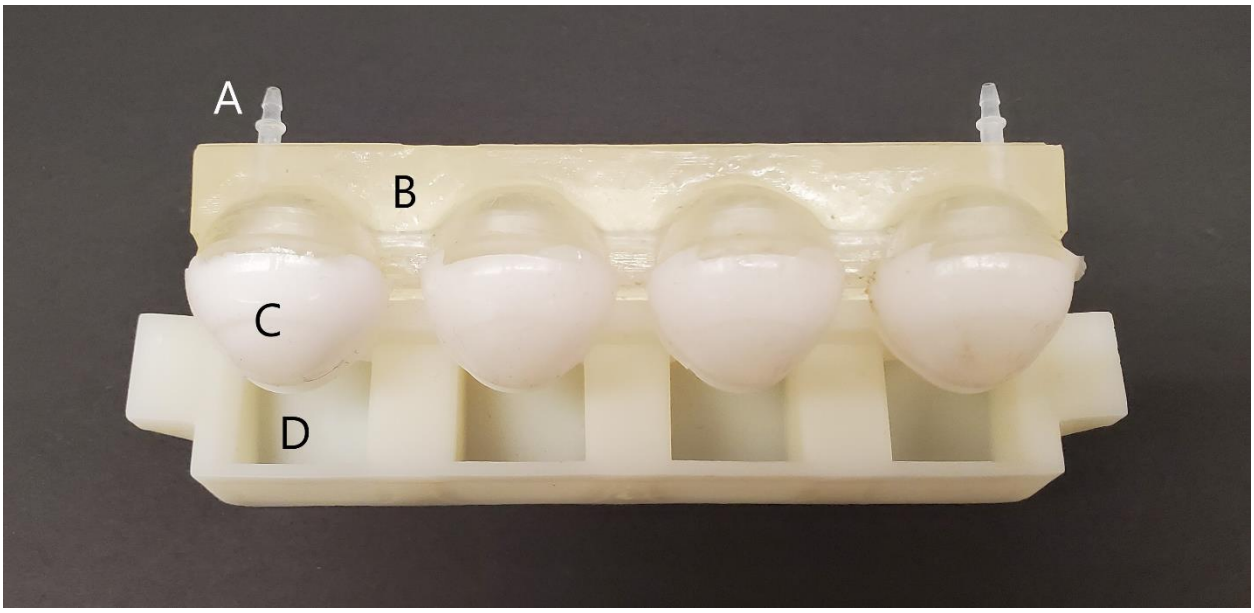

Figure 5.1B Top view of eyeball structure. (A) Inlet and outlet for heated water. (B) Hollow eyeball structure to allow heated water through. (C) Silicone eyeball. (D) Lower eyelid with trough to hold excess tear fluid.

5.2.7 Experimental design for blink model

The blink model was equilibrated for 30 minutes prior to the addition of CLs to ensure adequate tear flow on the eyelid and eyeball. The lenses were removed from the blister pack and placed directly on the blink model. After 1 or 16 hours, the lenses were removed, and water content measured. Time points of 1 and 16 hours of lens incubation were chosen to explore the immediate and extended effect of lens dehydration for a daily disposable CL worn across a typical day.

5.2.8 Water content determination

The procedure to measure content from the CLs was adopted from Jones et al.[37] In brief, lenses were removed from their blister pack or vial incubation and gently blotted on lens paper to remove any excess solution on the lens surface. Once blotted, the lens was placed on a digital balance (Sartorius M100, Göttingen, Germany), and the wet weight recorded. Lenses were then heated at 105°C for 1 hour and then cooled in a desiccator for 30 minutes before weighed again to determine the dry weight. The two measurements were then used to determine the water content of the lens from the blister pack and from each individual time point using equation 1.

$$\%\, Water\ content = \left(\frac{wet\ weight - dry\ weight}{wet\ weight}\right) \times 100 \qquad (1)$$

5.2.9 Relative dehydration

Determination of relative dehydration was adopted from Jones et al.[37] In brief, using the water content values calculated from equation 1, the relative dehydration was determined using equation 2. Initial equilibrium water content (EWC) was determined from the CL taken out of the blister pack, and the final EWC was determined after incubation on the blink model or in the vial.

$$Relative\ \%\ dehydration = \left(\frac{initial\ EWC - final\ EWC}{initial\ EWC}\right) \times 100 \qquad (2)$$

5.2.10 Statistical Analysis

Statistical analysis and graphs were plotted using GraphPad Prism 8 software (GraphPad, La Jolla, CA). All data are expressed as a percentage in mean ± SD. With the vial system, a two-way ANOVA with a post-hoc Sidak multiple comparison test was used to test the differences in water content between time and incubation temperature for both PBS and ATS conditions. A second two-way ANOVA with a post-hoc Sidak multiple comparison test was used to test the differences in water content between time and incubation solution for both room temperature and ocular temperature conditions.

Unpaired t-tests between lens materials were used to test the difference in water content for both the vial and blink model systems. A two-way ANOVA with a post-hoc Sidak multiple comparisons test was used to test the differences in water content between lens material and time for both the vial and blink model. A two-way ANOVA with a post-hoc Sidak multiple comparisons test was used to test the differences in water content between lens material and model systems, for both 1 and 16 hours. Statistical significance was achieved at the level of $p < 0.05$.

5.3 Results

5.3.1 Water content from vial incubation

The water content of all tested CLs in PBS and ATS over time at both room temperature (RT, 22°C) and ocular temperature (OT, 35°C) in the vial system is summarized in Table 5.3.

Table 5.3 Equilibrium water content of various lens materials measured after vial incubation in two test solutions and at two temperatures.

Contact lens material NWC (n=5 each)	PBS				ATS			
	EWC (%) at RT (Mean ± SD)		EWC (%) at OT (Mean ± SD)		EWC (%) at RT (Mean ± SD)		EWC (%) at OT (Mean ± SD)	
	1hr	16hr	1hr	16hr	1hr	16hr	1hr	16hr
Delefilcon A 33% SH DD	34.48 ± 0.00	34.48 ± 0.00	29.09 ± 1.21	29.09 ± 1.21	29.55 ± 2.61	34.01 ± 1.05	28.55 ± 1.48	29.05 ± 2.20
Senofilcon A 38% SH DD	36.32 ± 1.93	35.17 ± 1.09	32.63 ± 2.31	32.26 ± 0.00	35.94 ± 1.62	34.38 ± 0.00	32.23 ± 1.55	30.84 ± 2.57
Etafilcon A 58% CH DD	53.01 ± 0.72	52.69 ± 0.88	50.00 ± 0.00	46.95 ± 1.09	54.54 ± 0.67	55.12 ± 0.63	53.33 ± 0.00	50.69 ± 0.94
Omafilcon A 60% CH DD	59.62 ± 0.55	59.38 ± 0.00	58.33 ± 0.59	58.06 ± 0.00	58.06 ± 0.00	58.59 ± 0.72	57.78 ± 0.63	57.23 ± 0.77
Comfilcon A 48% SH RU	48.06 ± 2.45	48.89 ± 1.01	47.32 ± 1.86	46.52 ± 1.73	49.23 ± 1.72	49.63 ± 0.83	46.12 ± 1.47	47.69 ± 2.11
Senofilcon A 38% SH RU	35.71 ± 0.00	35.71 ± 0.00	30.77 ± 0.00	30.77 ± 0.00	35.71 ± 0.00	34.76 ± 1.30	30.77 ± 0.00	30.77 ± 0.00
Etafilcon A 58% CH RU	55.61 ± 0.60	55.06 ± 1.16	52.52 ± 0.83	52.82 ± 0.68	53.69 ± 0.78	54.81 ± 0.60	51.61 ± 0.00	47.54 ± 1.01

NWC, nominal water content; PBS, phosphate buffered saline; ATS, artificial tear solution; EWC, equilibrium water content; SH, silicone hydrogel; CH, conventional hydrogel; DD, daily disposables; RU, reusable. RT, room temperature, 22°C ± 2°C; OT, ocular temperature 35°C ± 0.8°C.

5.3.2 Lens water content as a function of temperature (vial) and time

Overall, lower water content was observed for OT compared to RT. For all lenses, this difference in water content was statistically significant ($p \leq 0.0052$), except omafilcon A and delefilcon A at 1 hour in ATS ($p > 0.05$). Water content for comfilcon A was only significantly lower after 1 hour in ATS ($p = 0.0029$).

5.3.3 Lens water content as a function of incubation solution (vial) and time

For most lenses tested, there were no significant differences in water content due to incubation solution. There was no significant difference for senofilcon A (DD) or comfilcon A for either incubation solution or time ($p > 0.05$). For delefilcon A, there was no significant difference except for 1 hour incubation at RT ($p < 0.0001$), where PBS incubation had a higher water content

than ATS incubation. For senofilcon A (RU), there was no significant difference except for 16 hour incubation at RT ($p = 0.0277$), where PBS incubation had a higher water content than ATS incubation. Etafilcon A (RU) showed significant differences RT incubation at 1 hour ($p = 0.0004$) and OT incubation for 16 hours ($p < 0.0001$), both with a higher water content value in PBS over ATS incubation. Omafilcon A showed significant differences for all conditions except 1 hour incubation at OT ($p \leq 0.0277$), all with a higher water content value in PBS over ATS incubation. Etafilcon A (DD) showed a significant difference for all conditions ($p \leq 0.0014$), with a higher water content value for ATS over PBS incubation.

5.3.4 Lens water content as a function of lens material

An unpaired t-test between SH and CH lens materials showed a significant difference in water content for both vial ($p = 0.0296$) and blink model ($p = 0.0135$) incubation systems. Both systems showed a higher mean water content value for CH lens materials (51.82% vial and 51.09% blink system) compared to SH lens materials (34.59% vial and 34.89% blink system) when incubated in ATS over 16 hours at OT.

5.3.5 Lens water content on blink model

The water content of all tested CLs on the blink model over time at ocular temperature (35°C) is shown in Figure 5.2 and summarized in Table 5.4.

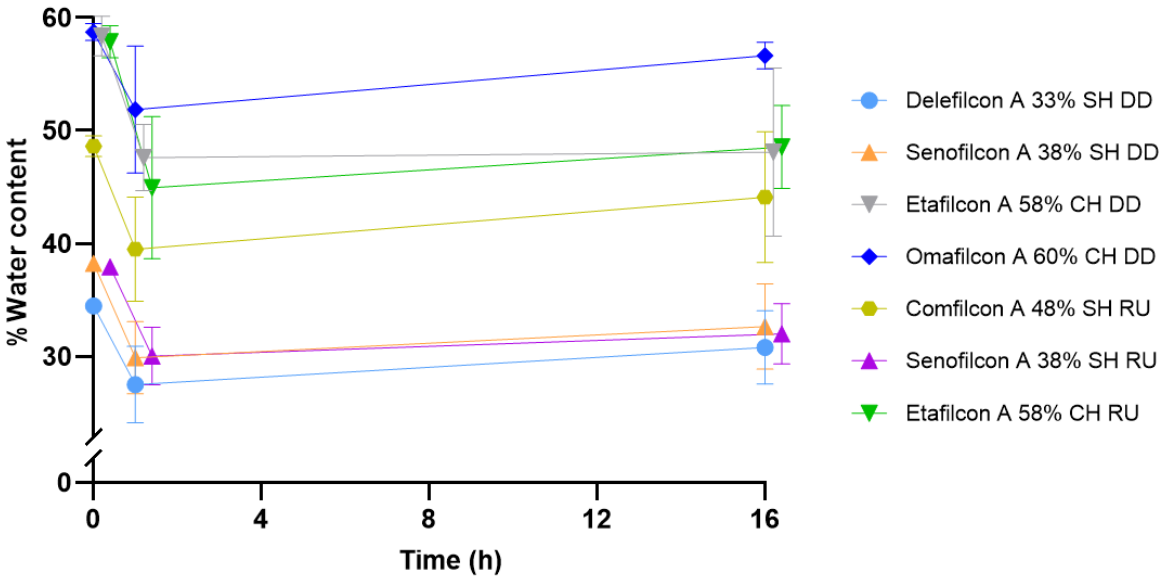

Figure 5.2 Percent water content of contact lenses over time on blink model system with artificial tear solution at ocular temperature (35°C). SH, silicone hydrogel; CH, conventional hydrogel; DD, daily disposables; RU, reusable; error bars represent standard deviation.

Table 5.4 Equilibrium water content of various lens materials measured after blink model incubation at OT with ATS over time.

Blink model (n=4)	**Delefilcon A (DD)**	**Senofilcon A (DD)**	**Etafilcon A (DD)**	**Omafilcon A (DD)**	**Comfilcon A (RU)**	**Senofilcon A (RU)**	**Etafilcon A (RU)**
EWC (%) after 1 hour (Mean ± SD)	27.50 ± 3.39	29.89 ± 3.20	47.61 ± 2.94	51.85 ± 5.63	39.50 ± 4.62	30.03 ± 2.56	44.95 ± 6.31
EWC (%) after 16 hours (Mean ± SD)	30.79 ± 3.25	32.65 ± 3.77	48.08 ± 7.46	56.64 ± 1.18	44.10 ± 5.79	32.00 ± 2.67	48.55 ± 3.69

OT, ocular temperature 35°C ± 0.8°C; ATS, artificial tear solution; EWC, equilibrium water content; DD, daily disposables; RU, reusable.

5.3.6 Lens water content as a function of lens material and time

All lenses showed a significant decrease in water content after 1 hour of incubation in the vial system compared to the blister pack ($p \leq 0.0254$). After 16 hours of incubation in the vial system, only comfilcon A did not show a significant decrease in water content ($p > 0.05$).

All lenses showed a significant decrease in water content after 1 hour of incubation on the blink model compared to the blister pack ($p \leq 0.0266$). Both etafilcon A lens modalities showed

an additional significant decrease in water content after 16 hours of incubation on the blink model ($p = 0.0004$ for DD and $p = 0.0016$ for RU). After 16 hours of incubation with ATS on the blink model, etafilcon A had the greatest decrease in water content (10.30% ± 7.46), and omafilcon A had the least decrease of water content (2.08% ± 1.18).

5.3.7 Change in water content between vial incubation and blink model

After 1 hour of incubation at OT in ATS, there was a significant difference in water content between the two incubation systems for all lenses ($p \leq 0.0364$) except delefilcon A, and senofilcon A (DD and RU). All lenses had a higher water content in the vial system compared to the blink model. After 16 hours of incubation at OT in ATS, there were no significant differences in water content between the two incubation systems for any of the CLs tested.

A comparison of water content between the two incubation systems allows for the determination of how much change in water content is due to certain factors. The difference in water content between RT and OT incubation in ATS in the vial system determines the change in water content due to heating alone. The difference in water content between the blink model and vial system at OT determines the change in water content due to other factors that are not due to heating. The results are summarized in Table 5.5.

Table 5.5 Water content after 16 hour incubation in ATS.

Vial model (n=5)	**Delefilcon A (DD)**	**Senofilcon A (DD)**	**Etafilcon A (DD)**	**Omafilcon A (DD)**	**Comfilcon A (RU)**	**Senofilcon A (RU)**	**Etafilcon A (RU)**
EWC (%) at RT (Mean ± SD)	34.01 ± 1.05	34.38 ± 0.00	55.12 ± 0.63	58.59 ± 0.72	49.63 ± 0.83	34.76 ± 1.30	54.81 ± 0.60
EWC (%) at OT (Mean ± SD)	29.05 ± 2.20	30.84 ± 2.57	50.69 ± 0.94	57.23 ± 0.77	47.69 ± 2.11	30.77 ± 0.00	47.54 ± 1.01
Blink model (n=4)	**Delefilcon A (DD)**	**Senofilcon A (DD)**	**Etafilcon A (DD)**	**Omafilcon A (DD)**	**Comfilcon A (RU)**	**Senofilcon A (RU)**	**Etafilcon A (RU)**
EWC (%) at OT (Mean ± SD)	30.79 ± 3.25	32.65 ± 3.77	48.08 ± 7.46	56.64 ± 1.18	44.10 ± 5.79	32.00 ± 2.67	48.55 ± 3.69
Change in water content							
Due to heating in a vial (ΔEWC between RT and OT)	-4.97 ± 2.20	-3.53 ± 2.57	-4.43 ± 0.94	-1.36 ± 0.77	-1.94 ± 2.11	-3.99 ± 0.00	-7.28 ± 1.01
Due to heating and air exposure on blink model (ΔEWC between blink model at OT and vial at RT)	-3.22 ± 3.25	-1.73 ± 3.77	-7.04 ± 7.46	-1.95 ± 1.18	-5.53 ± 5.79	-2.76 ± 2.67	-6.26 ± 3.69

ATS, artificial tear solution; EWC, equilibrium water content; RT, room temperature; OT, ocular temperature; DD, daily disposables; RU, reusable.

5.3.8 Relative percent dehydration

Figure 5.3 shows the relative percent dehydration of the tested CLs on the blink model at OT. At 1 hour, delefilcon A had the lowest amount of relative dehydration and etafilcon A (RU) had the highest amount of relative dehydration. At 16 hours, omafilcon A showed the lowest amount of relative dehydration, and etafilcon A (DD) showed the highest amount of relative dehydration. However, no statistical significance was determined.

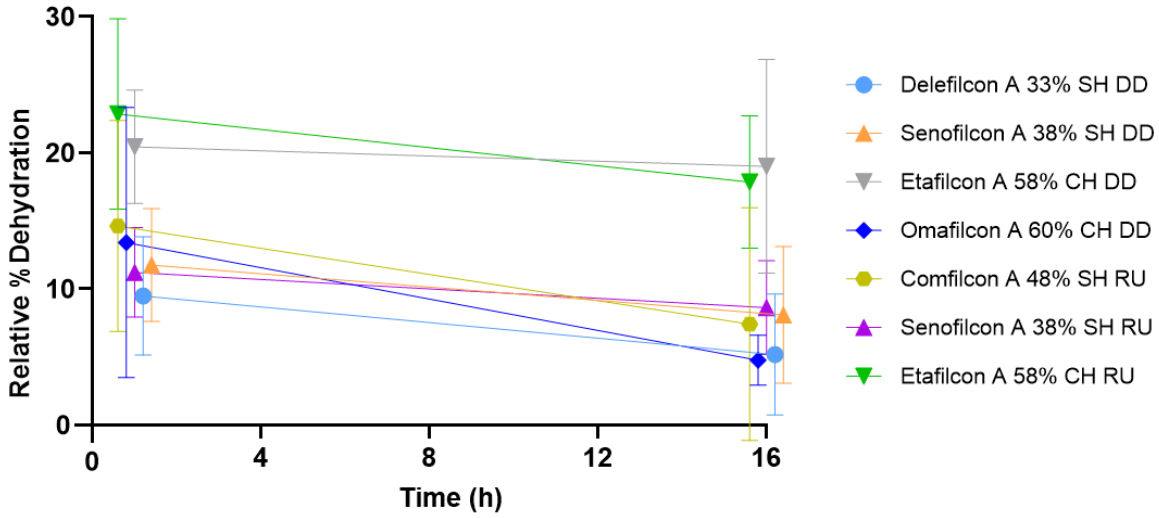

Figure 5.3 Relative percent dehydration of contact lenses over time on blink model with artificial tear solution at ocular temperature (35°C). SH, silicone hydrogel; CH, conventional hydrogel; DD, daily disposables; RU, reusable; error bars represent standard deviation.

5.4 Discussion

This study examined the effects of incubation solution and temperature on the water content of several contemporary CL materials. In addition, the study also examined the dehydration of CLs using an advanced *in vitro* blink model. To summarize, an increase in temperature was accompanied by a decrease in water content of all tested CLs. For most CLs, vial incubation solution did not have an effect on water content. Over time, the water content of CLs incubated in the vial model plateaued, whereas with the blink model, there was recovery in water content after 16 hours of incubation.

5.4.1 Water content from vial incubation

The results from this study support previously published studies[37, 100, 110] on the lens material being a significant contributing factor to differences in CL water content. Initial water content plays an important role in the loss of water in CL materials.[100] The water in polymer material can be categorized as free water, loosely bound water, or tightly bound water.[100, 112] Free

water in the polymer material does not interact with the polymer, and dehydration of free water occurs quickly.[100] Tightly bound water directly interacts with the polar side chains of the polymer material through hydrogen bonding, in which dehydration of tightly bound water is unlikely to occur at room temperature.[100] Loosely bound water in the polymer material is in a state between the free and tightly bound water forms, where they interact with the polar side chains of the polymer material but are more strongly associated with water molecules via hydrogen bonding.[112] CLs with higher nominal water content have a greater amount of loosely bound water.[37, 112] As a result, the rate of dehydration for high water content CLs is often greater and occurs more rapidly.[37, 101, 109, 113, 114] This was demonstrated in this study, with both etafilcon A lens modalities with the second highest nominal water content, 58%, having the highest relative percent dehydration (Figure 5.3). However, omafilcon A, the lens material with the highest nominal water content in this study at 60%, did not show a high dehydration rate due to the presence of phosphorylcholine, demonstrating that the lens material does play a role in dehydration of CLs, as reported in literature.[100, 114, 115] In addition, the hydrophobicity of different lens materials plays a role in the strength of hydrogen bonding to water molecules in the hydrogel.[112] As a result, each lens material will have different water-binding capabilities and water content, and consequently will have different rates of dehydration.

However, the hydrophobicity of the lens material is not the only factor affecting lens water content and dehydration rates. The solution in which the lens resides, temperature, and air exposure, may also play a role in the dehydration rates of CL materials.[112]

5.4.2 Change in water content due to temperature

Based on the results of the vial study, a change in temperature from RT (22°C) to OT (35°C), causes a reduction in water content for all the CLs tested in this study. This phenomenon

has been previously reported[106, 107] and may be due to different lens polymer configurations, which may cause water to be expelled at different rates.[100] The change in temperature can cause the hydrogel matrix to contract and shrink, causing the expulsion of water molecules within the hydrogel matrix.[104] This study demonstrated that the components of the CL materials likely have an effect on the rate of dehydration. For example, omafilcon A, had the highest water content, but did not show a rapid dehydration rate in comparison to other high water content materials. Omafilcon A contains phosphorylcholine, which has a high affinity for water, as reported in literature,[100, 114, 115] which consequently leads to a slow dehydration profile.

5.4.3 Change in water content due to incubation solution and lens material

The lens materials may behave differently when incubated in distilled water, PBS,[112] or ATS.[116] Salts and proteins in the surrounding solution can interact with the polymer side chains[116] as well as cause the lens material to swell.[100] Comparing the lens materials after the 16 hour incubation, in both solutions, showed no statistically significant difference between the incubation solutions ($p > 0.05$) for most lenses. However, the CH lenses in this study, etafilcon A (DD) and omafilcon A, showed a significant difference between both incubation solutions when incubated at OT for 16 hours ($p < 0.0001$ and $p = 0.0277$, respectively), whereas etafilcon A (RU) showed a significant difference between incubation solutions when incubated for 16 hours at OT only ($p < 0.0001$). As the amount of bound water is expected to be similar in both high and low water content lenses, the amount of free water is greater for high water content CLs.[112] Etafilcon A is a lens material that has been previously shown to have high dehydration rates driven by osmolarity due to its ionic lens material and high water content.[37, 101, 113-115] In this study, etafilcon A (DD) has lower water content in PBS whereas etafilcon (RU) has lower water content in ATS after 16 hours

of incubation at OT. It is interesting, but unclear, why the two different modalities of the same lens material had different dehydration patterns when incubated in the two solutions.

Omafilcon A has previously been shown to have a lower dehydration rate relative to other high water content CLs.[110, 114, 115] It has been proposed that this is due to the presence of phosphorylcholine, which has a high affinity to water.[100, 114, 115] As the ATS is a more complex solution with several components, it is not surprising to see omafilcon A with higher water content in PBS compared to ATS after 16 hours of incubation at OT.

5.4.4 Water content determination using the blink model

The blink model was used to evaluate the change in water content of CLs as a function of both time and lens material. The blink model incorporates the use of ATS, OT, and a blink mechanism to best mimic physiological conditions. After 1 hour of incubation on the blink model, all lenses showed a statistically significant decrease in water content ($p \leq 0.0266$). However, after 16 hours of incubation, most CLs recovered their water content to blister pack values, with no statistically significant difference ($p > 0.05$). It is hypothesized that the combination of high humidity levels (>90%) and the blink mechanism causes additional movement of the polymers in the CL material when compared to the vial system.[100] This may allow for greater water binding over time. The only CLs that continued to show a significant decrease in water content after 16 hours, were both etafilcon A lens materials (DD: $p = 0.0004$; and RU: $p = 0.0016$).

Additionally, after 16 hours, etafilcon A showed the largest overall decrease in water content with the blink model (10.30% ± 7.46%). These results are in agreement with previously published studies on ionic, high water content CLs having a greater and faster dehydration rate compared to low water content CLs.[37, 101, 113-115] In contrast, omafilcon A had the lowest overall decrease in water content on the blink model (2.08% ± 1.18%). This agrees with published studies

based on the presence of phosphorylcholine of omafilcon A. The high affinity for water of this lens material shows a different dehydration pattern to other high water content CLs.[100, 114, 115] Both *in vitro*[37, 100] and *in vivo*[100, 114, 115] studies have previously shown these results and demonstrate that this *in vitro* blink model is an excellent model to use when predicting dehydration patterns.

5.4.5 Change in water content over time between vial incubation and blink model

On both incubation systems, the CLs showed a statistically significant decrease in water content from the blister pack after 1 hour of incubation in ATS at OT ($p \leq 0.0266$). With vial incubation, all the CLs, except comfilcon A ($p > 0.05$), showed a statistically significant decrease in water content after 16 hours of incubation compared to the blister pack ($p \leq 0.0004$). However, with the blink model system, only the etafilcon A lens modalities (DD: $p = 0.0004$; and RU: $p = 0.0016$) showed a significant decrease in water content at 16 hours of incubation, whereas all other changes in water content of the other CL materials were not statistically different ($p > 0.05$). When the lens materials were incubated in the vial system, CLs demonstrate a plateau of dehydration (Figure 5.4), whereas with the blink model, CLs show signs of recovering water content after 16 hours (Figure 5.2). We suspect that the blinking mechanism allows replenishment of the tear film, similar to the physical reflex of increased blinking during dry eye symptoms, which prolongs the time to CL dehydration, allowing a slight recovery for the CLs on the blink model. It is also hypothesized that the increased humidity levels working together with the blinking mechanism may increase the movement of the polymers of the CL materials, allowing for greater water binding over time. The vial CLs may have reached a saturation point as the lenses are fully immersed in solution, as shown in previously published studies.[43] Due to the high humidity (>90%) on the blink model, we suspect that the blink model lenses also approach their saturation level, but delayed due to the increased humidity. A high humidity value was chosen for this study to test the effects of

other variables without the addition of low humidity, which has previously been shown to change water content values.[37, 106] Compared to *in vivo* studies, we would expect much lower water content values due to lower humidity levels. Further investigation with varying humidity is required to explore these results.

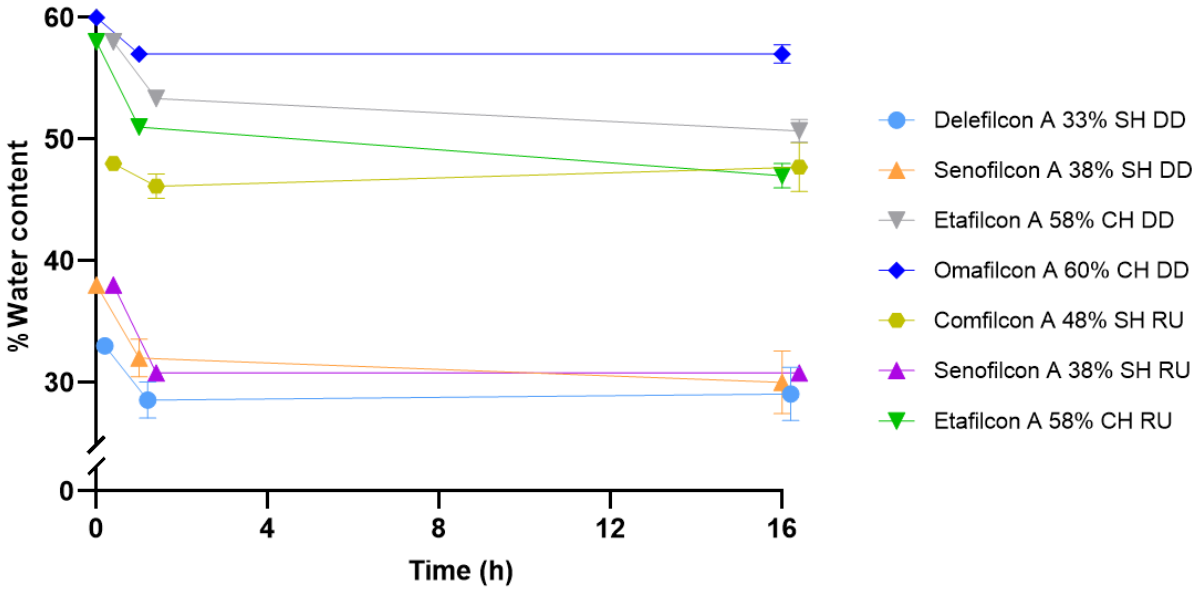

Figure 5.4 Percent water content of contact lenses over time in vial system with artificial tear solution at ocular temperature (35°C). SH, silicone hydrogel; CH, conventional hydrogel; DD, daily disposables; RU, reusable; error bars represent standard deviation.

Between incubation systems, at 1 hour, there were only significant differences between the vial system and blink model for both etafilcon A lens modalities, omafilcon A, and comfilcon A. After 16 hours of incubation, no significant differences were seen between the two incubation systems.

By comparing the change in water content between the two incubation systems, the percent change due to heating was determined and summarized in Table 5.5. The change in water content from the vial incubation was only due to the heating of the incubation solution. The differences in

water content between the vial incubation and the blink model may be due to air exposure from the blink mechanism of the blink model that could further cause varying degrees of deposition, shear stress, and other additional interactions that have not been investigated in this study. For the majority of the tested CLs, the results of this study demonstrate that the amount of water loss can largely be attributed to the increase in temperature of the incubation solutions alone. For etafilcon A (DD) and comfilcon A, the amount of water loss is mainly due to a combination of heating, dehydration, and air exposure. As from previously published studies, [37, 101, 109] high water content lens materials tend to have higher rates of dehydration compared to CLs with low water content. Further work on the blink model will be required to determine which additional factors cause a change in water content of lens materials.

5.4.6 Clinical Significance

Water content and dehydration of CLs have varying results regarding its correlation to comfort. Some studies report that CL dehydration plays a major role in the production of reduced comfort,[37] whereas others report no correlation.[108, 110] However, several studies agree that further testing is required to better understand the effect of CL dehydration on lens comfort.[37, 100, 110] This study attempted to determine the loss in water content due to temperature as well as dehydration over time. For some of the CLs tested in this study, half or more of the CL dehydration was due to heating the material to ocular temperature. For other CLs, temperature had a lower effect on dehydration. The factors that would have caused lens dehydration besides heating could be lens polymer composition, air exposure, shear stress from the blinking mechanism, or other factors that remain to be determined. Dehydration observed in the first hour of incubation in both *in vitro* models do not reflect the discomfort scores in *in vivo* studies,[117, 118] suggesting that dehydration may not have a direct association with end of day discomfort. This is further supported as the water

content after 16 hours of incubation on the blink model recovers to initial blister pack water content values.

A better understanding of how lens materials react due to changes in temperature can help clinicians determine the lens materials which are best suited for certain consumers. This study shows that higher temperatures will lead to a decrease in water content in all lens materials. Certain CL materials that have better retention of their water content, therefore, may be better suited for use in hotter climates. It has been previously published that the dehydration of some lens materials can affect lens fit[37] and oxygen transmissibility,[109] resulting in discomfort and potentially other clinical problems. As a result, having a better understanding of CL dehydration allows for more informed choices in lens materials and subsequently, lens comfort.

Overall, the average dehydration of all test lenses combined was 5.91% ± 4.79% after 16 hours of incubation when using the blink model. Comparatively, two clinical studies[119, 120] showed that the decrease in water content of DD CLs was less than 5% after 12 hours of wear. We suspect that our results should differ from *in vivo* data as this study was conducted at a humidity level of > 90%, and varying humidity levels have previously been shown to affect dehydration rates.[37, 106] At lower humidity levels, a higher rate of dehydration is expected. However, it is unclear whether or not a 5% change in water content is enough to cause dryness or discomfort for CL wearers, therefore, further testing is required. Based on the results from this study, there is variability seen between all the CLs tested, warranting further testing in addition to comparison to *in vivo* data. By using more advanced *in vitro* models and changing the parameters, more information on CL dehydration can be explored.

5.5 Conclusion

In conclusion, this study compared the effects of incubation temperature, incubation time, and incubation solution on CL dehydration of various lens materials in both a vial system and an advanced *in vitro* blink model. Increased temperature resulted in a decrease in water content for all CLs. Furthermore, with the novel features of the *in* vitro blink model, when lenses were exposed to air exposure during blinking, CL dehydration was observed. However, the majority of the reduction in water content occurred within the first hour and did not reduce further over time. For most tested lenses, the type of incubation solution did not have an effect on water content. CL materials, however, played a major role in differences in water content. A novel finding with the blink model was a recovery in water content demonstrated after 16 hours of incubation. Further work is required to investigate this phenomenon. Overall, by examining the water content values, the amount of dehydration due to change in temperature could be determined with the two incubation systems. Generally, employing more advanced *in vitro* models that better mimic the ocular environment *in vitro* will provide more representative data to translate laboratory results to real world CL wear scenarios.

6 General discussion and Future Work

The OcuBlink *in vitro* blink model was developed to mimic the physiological conditions of the human eye. The blink model aimed to maintain the major benefits of *in vitro* testing, including low cost, ease of access, controlled, high throughput, and rapid experimental data collection.[34, 35] The blink model aimed to act as a quick screening tool in situations where a simple *in vitro* vial experiment would suffice, but provide results that would fall closer to *in vivo* experimental studies. This would provide savings on time, cost, and avoid ethical concerns of animal or human trials where applicable. [31-33] Although the blink model is not able to precisely mimic the human body, it is useful for improving our understanding of the interaction of the tear film with contact lens materials.

Rather than a static vial filled with solution, the blink model incorporated a blink mechanism. A static vial results in oversaturation of a CL in solution and does not incorporate intermittent air exposure as seen with blinking. Often this leads to an inaccurate representation of accumulation of tear film components on the lens than what would be seen *in vivo*.[43, 65] The blink motion is crucial to the formation of a tear film on the surface of the eye and be representative of intermittent air exposure to CLs,[40] hence a distinguishing factor to our *in vitro* model. To mimic the low tear flow rate and volume of the human eye, the blink model used a pump to administer ATS. A wettable, hydrophilic material, PVA, was used for the eyeball and eye lid to simulate the properties of the ocular tissues of the anterior eye.[53, 81, 82]

6.1 Experimental summary and discussion

To test the ability of the blink model to replicate *in vivo* results, an active lysozyme deposition study was chosen as the reference point.[59] This study by Subbaraman et al. collects total and active lysozyme data from patient-worn etafilcon A lenses.[59] The blink model was able to

replicate the active lysozyme data to fall within the range of the *in vivo* results after adjustments to the model. Several other lenses were tested under the same conditions to produce *in vitro* results that were not available in the literature at the time of the study. With etafilcon A, high quantities of active lysozyme deposition were seen on the blink model, in agreement with other published studies.[38, 60, 65, 66, 69, 70, 73, 76, 78, 79] We hypothesize that the intermittent air exposure of the blink model was the cause of the significantly greater amount of active lysozyme deposition on etafilcon A when compared to the vial system. Intermittent air exposure causing a break in the tear film may facilitate protein deposition on CLs, as previously seen with lipid deposition.[40] With senofilcon A and somofilcon A, two SH lens materials, a significantly lower amount of active lysozyme deposition was seen with the blink model compared to the vial system. We hypothesize the air exposure caused an increase in lysozyme denaturation on the hydrophobic lens materials, leading to a reduction in lysozyme activity.[40] With the remaining three lens materials tested, omafilcon A, nelfilcon A, and delefilcon A, no significant difference was seen between the two incubation systems. In agreement to previously published studies, these lens materials exhibit low levels of lysozyme deposition, and may not show a significant difference in the amount of active lysozyme deposition.[73, 76] In general, the blink model provided a more accurate reflection of the degree of active lysozyme deposition, however, lens material played a bigger role in the variability to deposition. The blink model was designed to predict *in vivo* results, as well as potentially replace animal models or clinical trials where appropriate. Although a validation study is required, the *in vitro* model is a promising tool for future *in vitro* studies.

Although it is reported in the literature that active lysozyme makes up over 95% of total lysozyme,[73] we attempted to collect total lysozyme data to complete the study. An enzyme-linked immunosorbent assay (ELISA) kit was used for total lysozyme data collection. However, after

several attempts, the ELISA kit results did not produce results to fall within range of the expected values. The lysozyme values for the blink model were below the range of the kit and the control values were much less than expected. Other ELISA kits were incompatible with the hen egg lysozyme used in the ATS and as a result, the total lysozyme data was not included in the study.

The blink model went through several iterations to improve general design, ease of use, and structural stability. What started as an adjustable blink model became a tight fit model after standardizing and improving the manufacturing of several parts. All structural parts of the blink model migrated to a 3D-printed design to cut down on cost and improve durability. The biggest change in the blink model came after attempting to use the blink model in an incubator to replicate ocular temperatures. The wettable, hydrophilic PVA used for the eyeballs and eyelids would dehydrate and scratch the surface of CLs placed on the eye section. Even with increased humidity, the PVA (which is mainly composed of water) would deform when dehydrated.

These experiments led to the development of a heated blink model. The eyeball design was changed to allow heated water to be pumped through the back of the eyes instead of placing the whole model into an incubator. The 3D-printed eye structure was changed to a resin material to prevent water leakage. As the PVA eyes would still dehydrate and fall off in this design, a silicone eye was used instead. The new silicone eye model allowed CLs to stay on the eyes at ocular temperatures for hours with no degradation. Slight changes to the eyelid and overall structural design allowed for decreasing material cost, manufacturing time, waste production, and set up time for more sturdy and reusable parts.

Using the new heated blink model, a CL dehydration study was performed to determine the dehydration rate of several CLs. CLs dehydrate due to evaporation[106] and changes in temperature during wear when moving from room temperature (RT, 22°C) in the blister pack or lens case to ocular temperatures (OT, 35°C) when placed on the eye.[106, 107] As the hydrogel material of the CLs dehydrate, their crosslinking structure deforms as water molecules leave behind empty pockets.[104] This change due to dehydration may cause a change in lens diameter,[102] lens fit,[37] and oxygen transmissibility,[109] which may in turn decrease lens comfort.[108] As each lens material behaves in a different way, the heated blink model is a good tool to test CL dehydration, hoping to produce *in vivo* results without human clinical studies.

This study showed that increasing incubation temperature decreased CL water content and that incubation solution did not have an effect on the water content of most CLs tested. However, with the CH lens materials, (omafilcon A and both etafilcon A lens modalities), there was a significant difference between the incubation solutions after 16 hours of incubation at OT. CH lens materials generally have higher nominal water content values, meaning the amount of free water is more prominent in CH lens materials. As free water evaporates rapidly, the loss of water content is expected with these lenses.[112] It is interesting to note that the etafilcon A modalities behaved differently with incubation solution; the DD lens had a higher water content value in ATS, and RU lens in PBS. With omafilcon A, there was a higher water content value in PBS, which was expected due to the presence of phosphorylcholine in the lens material, which as a high affinity for water,[100, 114, 115] as ATS is a more complex solution with several components.

The blink model and vial model both showed a decrease in water content, however the CLs on the blink model showed a recovery pattern, whereas the vial model CLs plateaued. We hypothesize that the combination of high humidity and the blinking mechanism causes additional

movement for the CL material polymers, which may allow for greater water binding over time[100] when compared to the vial system. The blinking mechanism acts to replenish the tear film, similar to the increased blinking reflex during dry eye symptoms, which may prolong CL dehydration and allow for slight recovery on the blink model. However, future work will be required to better understand this phenomenon. The increase in water content on the blink model also supports that CL dehydration does not correlate to end of day discomfort from lens wear. Several studies have concluded that ocular fatigue and cumulative lens wear plays a larger role on end of day discomfort instead of CL dehydration.[117, 118, 121, 122] For most CLs, the amount of water loss can be attributed mainly to heating alone. The blink model can be used as a predictive model for CL dehydration rates *in vivo*, however, validation studies would be required.

Overall, the blink model could be adjusted to match *in vivo* active lysozyme deposition results and may act as a predictive tool for both deposition and dehydration studies. Several components of the blink model are hypothesized to contribute to more representative data, such as the blinking mechanism, intermittent air exposure, incubation at OT, and low tear flow volume when compared to a simplistic *in vitro* glass vial model. As the development of the blink model can always be improved, we aim to continually bridge the gap from *in vitro* models to *in vivo* data.

6.2 Limitations and future work

The blink model has potential to be used as a predictive model for *in vivo* studies based on its ability to replicate current *in vivo* results. However, validation studies still need to be performed along side *in vivo* experiments for a direct comparison of data. Once validated, the *in vitro* blink model may replace animal model or clinical trials for certain studies to decrease cost and time, while still providing accurate data.

The active lysozyme deposition study was chosen as a representative study to test the ability of the blink model to replicate *ex vivo* data from the literature. Lysozyme was chosen as the representative protein because it makes up 30% of the proteins in the tear film,[11] allowing it to be more easily detected in assays and there is an abundance of studies available in the literature for references. It must be noted that other proteins in the tear film, such as albumin and lactoferrin, are also proteins of interest to be studied with the blink model and may be explored in future studies.

The tear film components may deposit onto the materials used on the blink model, such as the eye, eyelid, and the tubing for tear input. As a result, future studies would explore deposition on the blink model materials and determine if there is a significant influence on the study results. Future iterations of the blink model will also explore the different materials to use for the model's eye. In this thesis, wettable and biocompatible PVA eyes and heat resistant silicone eyes were explored. Future work will explore eye materials that can encompass both features to best improve the blink model.

The feature that will likely attract the most attention is the incorporation of epithelial cells on the blink model. Future work will explore adding human corneal epithelial cells to the surface of the eye on the blink model. Epithelial cells will either be grown on the eye itself or will be grown on a membrane and added to the eye prior to experimental testing. The success of this feature will open the door to many studies involving biological interactions and bridge the gap between *in vitro* and *in vivo* experiments.

Additional future studies for the blink model include exploring changes in CL parameters, effects of solutions on CLs, and drug delivery studies. Changes to the blink model design will also include a more physiological relevant eyelid and separating the lipid and aqueous phase of the

tears. By continually improving the blink model, we hope to add to the literature with results that can be attained *in vitro* and decrease the need for animal models or clinical trials as well as improve efficiency in costs, time, and resources.

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