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Chan, Amanda

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EVALUATION OF *IN VITRO* CONTACT LENS FRICTION:
EFFECTS OF RECOMBINANT HUMAN PROTEOGLYCAN 4 AND TEST COUNTERFACE

by

Amanda Chung Yan Chan

A THESIS

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ABSTRACT

In vitro contact lenses friction significantly correlates with contact lens discomfort. In this thesis, *in vitro* friction testing was conducted to evaluate the effects of recombinant human PRG4 (rhPRG4) as an ocular surface boundary lubricant and to evaluate both biological and synthetic test counterfaces. The objectives were to (1) assess the potential effect of different lens types and incubation times on the friction of rhPRG4-incubated contact lenses and (2) evaluate mucin-soaked PDMS as a synthetic test counterface for *in vitro* friction testing of contact lenses. Results of this thesis work demonstrated that a) rhPRG4 may be useful as a friction reducing lubricant on some, but not all, silicone hydrogel contact lenses; and b) as a synthetic counterface, PDMS_{mucin} can exhibit similar friction coefficients compared to biological counterfaces on certain silicone hydrogel lenses. Overall, results led to an improved understanding of PRG4 and *in vitro* contact lens friction.

PREFACE

This thesis is presented in a manuscript-based type format; therefore, there is some repetition in the Introductions and Methods between chapters. While none of the chapters have been submitted for publication at the time of thesis submission, it is the author's intent to submit Chapter 2 for publication in the future.

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LIST OF SYMBOLS, ABBREVIATIONS AND NOMENCLATURE

Symbol	Definition
μ	Coefficient of friction
$\langle\mu_{\text{kinetic}}\rangle$	Kinetic coefficient of friction
ADDE	Aqueous tear-deficient dry eye
AFM	Atomic force microscopy
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
ATS	Artificial tear solution (proteins and lipids)
ATLS	Artificial tear solution (lipids alone)
CHO	Chinese hamster ovary
CV	Coefficient of variation
DE	Dry eye disease
DEWS	Dry Eye Workshop
ECM	Extracellular matrix
EDE	Evaporative dry eye
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
HA	Hyaluronan
ISCLR	International Society for Contact Lens Research
LSD	Least Significant Difference post hoc test
MUC5AC	Purified pig gastric mucins
PBS	Phosphate buffered saline

PDMS	Polydimethylsiloxane
pHEMA	Poly (2-hydroxyethyl methacrylate)
PMMA	Poly (methyl 2-methylpropenoate)
PRG4	Proteoglycan 4
PVP	Polyvinylpyrrolidone
RGP	Rigid gas permeable lenses
rhPRG4	Recombinant human PRG4
SD	Standard deviation
SEM	Standard error of the mean
TFOS	Tear Film & Ocular Surface Society
UV	Ultraviolet
VAS	Visual analog scale

Chapter 1 BACKGROUND

1.1 OCULAR ANATOMY

1.1.1 THE OCULAR SURFACE AND TEAR FILM

The ocular surface consists of the cornea and the conjunctiva, a thin mucous membrane covering the outer surface of the eye and the inner surfaces of the eyelids. The tear film exists on the corneal surface and historically, it is thought to be composed of three layers which together enables it to perform numerous functions. These three layers include the outermost lipid layer, the middle aqueous layer and the innermost mucus layer.

The outermost lipid layer, also known as the Meibomian layer, prevents tear film contamination from the external environment, reduces tear evaporation, and provides a smooth optical surface for light refraction [1]. This outermost layer is around 0.2 μm thick and is produced by Meibomian glands [2]. The middle aqueous layer, functions to lubricate and prevent infection of the ocular surface. This layer is 3-4 μm thick, making up much of the tear volume. Most of the aqueous fluid is secreted from the lacrimal glands and the conjunctival epithelium and contains nutrients such as inorganic salts, glucose, oxygen and proteins, as well as antibacterial factors to the cornea. This layer also functions to spread tears, flushing away debris, toxins and foreign bodies from the cornea [3]. Lastly, the 1 μm thick inner mucus layer is composed of mucin that functions to lubricate and protect the cornea. It also allows for the aqueous layer to anchor to the corneal epithelium, providing protection against shear forces. The inner mucus layer is hydrophilic, promoting the spread of the tear film over the ocular surface which aids in preventing damage to the epithelium during blinking [4]. Overall, all three layers function to maintain the health of the ocular surface and prevent infection [2].

1.2 DRY EYE DISEASE

The Tear Film & Ocular Surface Society (TFOS) Dry Eye Workshop (DEWS) II report in 2017 established the working definition of dry eye disease: Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles [5]. Dry eye disease is also known as keratoconjunctivitis sicca and is one of the most common diagnoses in ophthalmology. Studies have shown that up to 20% of adults aged 45 years or more experience dry eye symptoms [6]. Dry eye disease is believed to affect over 30-40 million people in the United States [7] and 100-344 million people worldwide [8] [9].

As described in the TFOS DEWS report, there are two main classes of dry eye: aqueous tear-deficient dry eye (ADDE) as well as evaporative dry eye (EDE), though many hybrid forms of dry eye exist [10]. ADDE is caused when there is a failure of tear secretion from the lacrimal gland and can be further defined as Sjogren syndrome dry eye and non- Sjogren syndrome dry eye. On the other hand, EDE is the result of water loss from the exposed ocular surface despite normal secretory function. Specifically, the EDE can be classified as caused by intrinsic or extrinsic factors. Extrinsic factors include deficiency in Vitamin A or contact lens wear. The use of contact lenses may induce dry eye disease, leading to reduced or even discontinued contact lens wear.

1.2.1 CONTACT LENS INDUCED DRY EYE

Symptoms of dry eye are much more prevalent in patients who wear contact lenses compared to the population who do not wear contact lenses [11]. In a study by Nichols et al., over 50% of

contact lens wearers self-reported that they had dry eye disease, leading to symptoms that have caused as many as 20% of lens wearers to reduce their lens wearing time [12]. It has been reported that contact lens wearers are 12 times more likely to report symptoms of dry eye compared to those with perfect vision who do not wear contact lens [12]. This is largely because contact lens wear interferes with normal tear film structure and function, as the lens itself separates the tear film into two layers. While the tear film layer is only a few micrometers thick, the introduction of a contact lens (~100 μm) can lead to major changes in the tear film structure and function. Increased tear film evaporation of both these two layers (pre- and post-lens) may cause the films to become thinner than usual which may lead to ocular discomfort [13]. Although symptoms of dry eye are similar between contact lens wearers and non-lens wearers, contact lens wearers are also more likely than non-lens wearers to experience increased intensity of their dry eye symptoms toward the end of the day [13].

1.3 CONTACT LENS: A BACKGROUND

Contact lenses have been recognized by the Food and Drug Administration (FDA) as Class II or Class III medical devices since 1976. Regular-wear contact lenses are classified as Class II devices (medium risk devices and require greater regulatory control to provide a reasonable assurance of the device's safety and effectiveness) whereas extended-wear or overnight lenses are classified as Class III (highest level of risk to patients and are therefore subject to the highest level of regulatory control). Similarly, contact lenses are classified as a Class II device by Health Canada. Contact lenses are thin lenses placed on the cornea of the eye, and float on a thin layer of tear fluid. Contact lenses help correct a variety of vision disorders, including near-sightedness, far-sightedness, and astigmatism. Leonardo Da Vinci first came up with the basic concept of contact lenses in 1508,

when he described how submerging the eye in a glass bowl filled with water could alter the corneal power [14]. The first written description of a device analogous to a contact lens is believed to be written by Sir John Herschel in 1823. It was in the late 1880's when the first contact lenses made of glass were invented independently by three separate men: Eugene Kalt, a French ophthalmologist, Adolph Eugen Fick, a Swiss ophthalmologist, and August Müller [15].

Optometrist William Feinbloom invented the first plastic lens in 1936 [14], while Kevin Tuohy filed a patent for the first corneal contact lens in 1948. These lenses were made entirely of poly (methyl 2-methylpropenoate) (PMMA). As PMMA did not allow for oxygen to permeate through the lens and caused many issues such as corneal hypoxia, inventors strived to find a material that was better suited for contact lenses. In the mid-1950's, Newton Wesley developed the first commercially successful rigid contact lens. From the 1970s to the 1990s, many variations of oxygen-permeable rigid materials were developed, including rigid gas permeable lenses (RGP), made from durable plastic that allows for oxygen to permeate through the material.

In 1954, Otto Wichterle and Drahoslav Lím invented the first hydrogel material, poly 2-hydroxyethyl methacrylate (pHEMA), which allowed for oxygen permeability in the lens [16]. Bausch & Lomb eventually acquired the patent for pHEMA and began the commercialization of the lenses in 1971 after receiving FDA approval. Before 1971, almost all contact lenses were made from PMMA, but the introduction of pHEMA initiated the transition towards soft contact lenses due to the benefits of increased comfort, reduced adaptation time and easier fitting procedures. By 1994, FDA had listed 34 unique soft contact lens materials [17].

Hydrogels consist of a network of water-swollen, polymeric structures and can be divided into two groups: conventional hydrogel materials and silicone hydrogels. The main component in hydrogel material consists of pHEMA. Additional monomers are added to alter the ionicity and water content of the material, which will improve the flexibility and wettability of the material [18]. However, in conventional hydrogel material, there is low oxygen permeability due to low solubility of oxygen in water. Water content in hydrogels can only be improved by increasing the thickness of the material. However, thicker lens designs are often less comfortable than thinner ones and result in higher reports of contact lens discomfort [19].

1.3.1 SILICONE HYDROGEL LENSES

The most recent breakthrough in contact lenses has been the launch of silicone hydrogel lenses in 1998 by Alcon (Fort Worth, TX, USA). The incorporation of silicone in these new lenses has greatly improved the oxygen permeability of the lens, due to the excellent solubility of oxygen in silicone. Contact lenses may lead to diminished corneal oxygen flow, which can result in edema, epithelial microcysts, limbal hyperemia, and neovascularization [18]. However, the addition of silicone, an extremely oxygen-permeable material, to the hydrogel lens matrix has reduced the incidence of serious hypoxia-related complications to almost zero [20]. Furthermore, silicone hydrogel lens materials have a greater elastic moduli and is therefore stiffer and easier to handle compared to conventional hydrogels [21].

Due to all the benefits of using silicone hydrogels compared to conventional hydrogels, they are becoming much more popular in the market. In 2013, silicone hydrogel lenses represented 67% of the soft contact lens market, while hydrogels represented the other 33% [22]. In 2015, 72% of soft

lens prescriptions in Canada and 81% of soft lens prescriptions in the US were silicone hydrogels [23]. Like conventional hydrogels, silicone hydrogels are formed from the cross-linking of water-swollen, hydrophilic chains of monomeric units into a matrix-like polymer. The material properties and unique attributes of a polymer depends on the interactions of chemical groups and the cross-linking of the polymeric structures [21]. For silicone hydrogel lenses in particular, siloxy groups are present in the material, which has silicon directly bonded to the carbon and oxygen atoms [24]. The presence of silicon-oxygen bonds has led to reduced protein deposition in addition to the enhanced oxygen permeability. Many laboratory studies have found that significantly less protein deposition was found on silicone hydrogel lenses when compared to conventional hydrogel lenses, which may lead to increased wettability and decreased friction [11]. Currently, there are 15 different silicone hydrogel materials available to practitioners, with many developments in the science of the material as well as their production methods over the last decade [24]. In order to be suitable for contact lenses, the silicone hydrogel must be optically transparent with a refractive index similar to a cornea (1.37), and must be biocompatible in the human eye [21].

Although silicone is highly oxygen permeable, it is also highly hydrophobic. Even when the silicone is embedded in a hydrogel matrix, the silicone can migrate to the lens–air interface. This leads to the formation of hydrophobic areas, reducing surface lubrication and potentially creating discomfort during blinking [25]. To address this challenge, many different techniques have been developed to make the silicone hydrogel lenses more wettable. Material scientists have tried surface treatments to encapsulate the silicone, as well as adding internal wetting agents to the lens matrix to improve surface moisture. Recently, many silicone hydrogel lenses on the market have been advertising different wettability technologies to improve the comfort of the lens.

Subtle differences in the chemical composition of materials can impact a wide variety of common lens properties, including oxygen transport, dehydration, parameter stability and deposition. All these factors can affect lens performance. As described by Jones, the properties of an ideal contact lens material should meet or exceed the cornea's oxygen requirements, be physiologically inert, provide excellent in-eye wetting, resist deposition, provide dimensional stability and reasonable durability, be optically transparent, require minimal patient care and be easy and cost-effective to manufacture [26]. A summary of various silicone hydrogel lenses and their material properties are listed in Table 1.

Table 1 Properties of Various Silicone Hydrogel Lenses

Lens	Material Name	Wear Duration	Manufacturer	Oxygen Transmissibility (Dk/t)	Water Content (%)	Bulk Modulus (MPa)
Dailies Total1*	delefilcon A	Daily	Alcon	156	33-80	0.7
Acuvue TruEye	narafilcon A	Daily	Johnson & Johnson	118	46	0.66
Clariti 1Day	somofilcon A	Daily	CooperVision	86	56	0.5
MyDay	stenfilcon A	Daily	CooperVision	100	54	0.4
Acuvue Oasys	senofilcon A	Daily/ Biweekly	Johnson & Johnson	147	38	0.73
PureVision 2	balafilcon A	Monthly	Bausch & Lomb	130	36	n/a
Biofinity	comfilcon A	Monthly	CooperVision	160	48	0.75
Air Optix Aqua	lotrafilcon B	Monthly	Alcon	138	33	1
Ultra	samfilcon A	Monthly	Bausch & Lomb	163	46	n/a
Acuvue Vita	senofilcon C	Monthly	Johnson & Johnson	147	41	n/a
Air Optix Hydraglyde	lotrafilcon B	Monthly	Alcon	138	33	1
Biofinity Energys	comfilcon A	Monthly	CooperVision	160	48	n/a

*Dailies Total1 contains both hydrogel and silicone hydrogel material

1.3.1.1 ACUVUE OASYS

Acuvue Oasys, known as senofilcon A (Johnson & Johnson Vision Care Inc., Jacksonville, FL, USA) was the first non-surface-treated silicone hydrogel to become commercially available. The lens material features HYDRACLEAR technology, which consists of a long chain high molecular weight internal wetting agent based on polyvinylpyrrolidone (PVP). PVP is a polyvinyl that is designed to reduce hydrophobicity at the lens surface by shielding the silicone at the material interface. It acts as a hydrophilic humectant, attracting and retaining moisture so that the lens is hydrated throughout the wearing day [27]. This creates better wettability and lens surface smoothness. Acuvue Oasys exhibits superior ultraviolet (UV) blocking capabilities, with reported Class 1 UV protection, blocking > 90% of UV-A and > 99% of UV-B rays [28].

Acuvue Oasys contains 38% water, has an oxygen transmissibility of 147 Dk/t, and is one of the most popular lenses on the market. It has been reported to deliver an exceptionally high level of patient and practitioner satisfaction, and is particularly successful in patients with symptoms of contact lens induced dryness [29]. The Oasys is marketed as a biweekly lens, but Johnson & Johnson has recently introduced a 1-day option into the market, known as ACUVUE OASYS 1-Day with HydraLuxe Technology. Furthermore, Johnson & Johnson has also released a monthly lens known as Acuvue Vita. Acuvue Vita uses HydraMax Technology to help maximize and maintain lens hydration and reduced evaporation through maintaining uniform lipid density and distribution through the lens.

1.3.1.2 BIOFINITY

A unique silicone hydrogel lens on the marketplace that contains no surface treatments or wetting agents is the Biofinity lens, also known as comfilcon A (CooperVision, Scottsdale, NY, USA). As silicone is hydrophobic, a surface treatment or the incorporation of an internal wetting agent within the bulk material is typically required. However, Biofinity lenses have no additives, coatings, wetting agents, or surface treatments, and yet claims to be made from a unique, naturally water-loving material that helps them stay moist and comfortable all day long. This is believed to be due to CooperVision's patented Aquaform technology, which optimizes the relationship between oxygen and water, creating a softer, more flexible lens material. According to CooperVision, natural wettability is provided by binding water to the lens; therefore, there is no need for additional surface treatments or wetting agents. The silicone macromers in the Aquaform Technology lenses lock water into the lens keeping them moist even after periods of extended wear [30]. A study by CooperVision reported that patients believed the Biofinity lens had a higher quality of vision and felt less dry compared to Acuvue Oasys [31].

The Biofinity lens contains 48% water and oxygen transmissibility of 160 Dk/t [32]. In other silicone hydrogel lenses, there appears to be a trend of lower oxygen transmissibility with increasing water content, but Biofinity is unique as it has high water content and high oxygen transmissibility.

1.3.1.3 AIR OPTIX PLUS HYDRAGLYDE

Alcon's Air Optix plus Hydraglyde (lotrafilcon B) was recently introduced to the market and received Premarket Approval in 2016 [33]. This lens consists of the proprietary Smartshield

technology found in all Air Optix lenses, which claims to help prevent silicone from reach the surface of the lens, helping the lens retain moisture. It is claims to be able to resist irritating deposits of lipids [34], [35] with the incorporation of Hydraglyde Moisture Matrix in its manufacturing process. This block copolymer adsorbs onto the lens while the other side of the polymer attracts moisture, creating longer lasting lens surface wettability. Like the Air Optix Aqua, there is a plasma surface treatment on the Air Optix Hydraglyde lens, which is created through a fusion process. Lenses are permanently modified in a gas plasma reactive chamber using a mixture of trimethylsilane oxygen and methane. This creates a thin (25nm) plasma layer, which exhibits a high refractive index and a hydrophilic surface [32]. In the gas plasma reactive chamber, the silicone components on the surface of the lenses are transformed into hydrophilic silicate compounds. Glassy silicate areas are created, and the hydrophilicity of these areas bridges over the underlying lens material. This plasma surface coating prevents silicone in the lens material from being exposed to air, which promotes moisture retention and minimal deposit build-up [36]. Air Optix Aqua claims to have less bulk cholesterol deposits on the lens compared to other silicone hydrogel lenses such as Acuvue Oasys and Biofinity after a month of wear. This is of importance, as these hydrophobic spots on the surface of the contact lens can attract lipids and protein, resist rewetting, and could cause discomfort [37].

However, one disadvantage of the Air Optix Aqua and Air Optix plus Hydraglyde lenses are that they have the lowest water content (33%) compared to all the other silicone hydrogel lenses, which may affect lens wettability [38].

Although lenses with surface treatments advertise high hydrophilicity at the lens surface, analysis of the surface of Air Optix Aqua has shown that these surface treatments have only been partially

effective at masking the silicone [32]. The silicone exposed on the lens surface can result in hydrophobic behaviour, which may lead to decreased lens comfort.

1.3.2 CONTACT LENS WEAR

Contact lenses are used by over 140 million people in the world, with 90% of wearers wearing soft lenses [22]. The U.S. Center for Disease Control and Prevention estimated that there are 40.9 million contact lens wearers in the United States age 18 years and older in 2015, which is 16.7% of the U.S. adult population [39]. This means that an estimated one in six adults in the United States wears contact lenses. Baird also estimates that the 2016 worldwide contact lens market was approximately \$7.2 billion [40]. However, it is important to note that contact lens wearers exhibited a dropout rate of 16% to 35% in 2008-2010 [41], [42], with contact lens discomfort being the primary reason for the discontinuation [41], [43].

1.3.3 CONTACT LENS DISCOMFORT AND FRICTION

There have been many studies conducted on lens material characteristics with the goal of improving comfort for contact lens wearers. As previously seen in Table 1, each lens has different material characteristics which may affect lens performance. Confounding factors such as lens design, lens modulus, surface characteristics, and the modality of wear (daily vs extended wear) all may play a role in the comfort response [38]. The primary cause of intolerance and discontinuation of contact lenses use has been due to discomfort and dryness symptoms [43]–[45].

1.3.3.1 WETTABILITY

Many surface characteristics of polymers have been taken into consideration, such as lens wettability, which relates to cohesive and adhesive forces within and between components respectively. With stronger cohesive forces, surface tension increases which decreases wettability. In the contact lens industry, wettability is used to describe the ability of the tear film to spread and remain on the surface of a contact lens. Higher wettability is important in preventing lipid deposition on lenses [46]. One way to measure *in vitro* wettability is via contact angles, in which the angle made by the fluid on the lens surface is measured. Lower contact angles, where fluid is able to spread over the surface more generally indicates higher wettability while higher contact angles indicate low wettability. Theoretically, lower contact angles should provide enhanced comfort, but clinical evaluations have shown that large differences in contact angles measured resulted in minimal difference in reported in-eye comfort between different silicone hydrogel products [32]. Furthermore, studies have shown that angles obtained from the same lenses can vary due to the differences in methodology or experimental conditions [47]. To date, no physical measurement currently exists (*in vitro*, *ex vivo*, or *in vivo*) that can completely quantify wettability of lens material [48], and it remains to be determined whether wettability measurements are clinically relevant to contact lens comfort [49].

1.3.3.2 OXYGEN TRANSMISSIBILITY

The bulk material properties of lenses may also play a role in contact lens discomfort. Another well-investigated lens property is oxygen transmissibility, measured in Dk/t, where D is the diffusivity (cm²/sec), k is the solubility (ml O₂/ml of material x mm Hg) and t represents the thickness of the lens. Despite the assumption that higher oxygen transmissibility in silicone

hydrogel lenses would result in greater comfort for lens wearers, there has not been conclusive evidence from studies that have been conducted in this area. Although some studies have shown that lenses with higher oxygen transmissibility are more comfortable compared to lenses with lower oxygen transmissibility, these studies also have experimental design issues where oxygen transmissibility could not be directly attributed to lens comfort [48]. In separate studies conducted by Morgan and Efron [50], Santodomingo et al. [51] as well as Brennan et al. [52], each found that the lenses in their studies with the highest Dk/t did not result in superior comfort for the lens wearer. Therefore, the TFOS workshop on contact lens discomfort concluded that there have been no Level I evidence studies (evidence from a systematic review or meta-analysis of all relevant randomized control trials) that can provide an answer to the question of whether oxygen levels influence comfort [38].

1.3.3.3 ELASTIC MODULUS

The modulus or the “stiffness” of a contact lens is an important mechanical parameter that has also been hypothesized to play a role in the comfort of a lens. Stresses on the lens materials caused by repeated application and removal of the lens or blinking can cause deformation or fractures on the lens that is irreversible. It is possible that this may lead to a loss of optical performance or user discomfort. Silicone hydrogels tend to have a higher modulus compared to conventional hydrogel lenses due to the incorporation of the silicone. Lens geometry, specifically the lens thickness profile, also plays a role in the modulus of a lens. Lenses with higher moduli are better for users as they are easier to handle, however they tend to not conform easily to the shape of the eye [32]. Studies with minimized bias have reported that no differences in comfort were found between hydrogels and silicone hydrogels that could be attributed to modulus [53], [54].

1.3.3.4 WATER CONTENT

Lastly, the water content and ionicity of a lens has also been investigated as a possible factor of contact lens comfort. Though water content has been shown to affect clinical performance of a lens, the effect of water content of a lens on lens comfort is unclear [38]. Silicone hydrogels tend to have a low equilibrium water content compared to hydrogels. Many studies have been conducted with differing opinions on how water content affects lens comfort. Some studies have shown that low equilibrium water content lenses have led to improved comfort, however the potential influence of material properties other than water content or ionicity has prevented the research community from drawing conclusions. To date, no studies have been able to definitively demonstrate a direct impact of water content and ionicity on contact lens discomfort for silicone hydrogel lenses [48]. On the other hand, a number of studies have demonstrated that dryness and discomfort of lenses were independent of the amount of dehydration or water content of the lenses used [11]. As for ionicity, lenses that are negatively charged on the surface will attract more positively charged tear film proteins. Silicone hydrogels tend to bind to lipids more than proteins as they tend to be more hydrophobic, which may contribute to contact lens-induced inflammation. However, there is no evidence of significant correlation between protein or lipid deposition to comfort [55].

1.3.3.5 CONTACT LENS FRICTION

Although factors such as oxygen transmissibility, lens modulus and water content may have been hypothesized to play a factor in contact lens comfort, recent studies have not found any relationship between comfort and these lens properties [38]. Instead, it has become apparent that contact lens friction has a strong correlation to contact lens comfort and has become an expanding area of

scientific interest. Recent studies have shown that contact lens friction is the strongest indicator of contact lens comfort [56], [57], suggesting that the frictional properties of lenses are an important design consideration when manufacturing soft lenses. To measure *in vitro* friction, horizontal and normal friction forces between two sliding surfaces are measured.

On the ocular surface, friction can occur between the cornea and the eyelid during blinking, or between the eyelid and contact lens if a contact lens is inserted on the eye. By dividing the horizontal friction force by a normal force, friction can be calculated. Kern et al. examined the relationship between subjective comfort and contact lens coefficient of friction among various soft contact lens materials, reporting that a strong relationship between lens coefficient of friction and subjective comfort [56], suggesting that lens friction should be taken into account when optimizing lens wear. Similarly, Brennan and Coles et al. conducted a multiple regression analysis to correlate contact lens material properties with end of day lens comfort, finding that contact lens friction is the sole contact lens property that is an indicator of comfort [57], [58]. In their studies, end-of-day comfort values obtained from over 700 separate 1-month wearing trials were used to correlate with coefficient of friction data from Roba et al. [59] and Ross et al. [60], finding that significant correlations ($r^2 > 0.83$; $p < 0.01$) existed between end-of-day comfort and coefficient of friction [57]. On the other hand, separate regression analyses for oxygen transmissibility, modulus and water content showed that only coefficient of friction remained in the equation as a predictor of comfort. Many independent studies from many research groups around the world have also attempted to measure friction using various methods [61]–[64], all collectively suggesting that there is a need to study and understand the tribology at the ocular surface.

1.4 BIOTRIBOLOGY

1.4.1 TRIBOLOGY

Tribology is the study of surfaces moving relative to one another, and the word tribology originates from the Greek word “tribos,” meaning “to rub”. The area of tribology includes three key topics: friction, wear and lubrication. Friction is defined as the resistance to relative motion, while wear is the loss of material due to that motion, and lastly, lubrication is the use of a fluid (or a gas or solid in some cases) to minimize both friction and wear. When describing tribology within the area of biology, in which the tribological phenomena is occurring in either the human body or in animals, the term “biotribology” is used. Biotribology is a relatively new term and was introduced in the early 1970s and is a subject covering many areas such as biological, physical and material science, as well as engineering and medicine. Contact lens and ocular surface friction falls in the field of biotribology.

1.4.2 MODES OF LUBRICATION

Lubrication can be defined as introducing another material to modify the interaction and control friction and wear of interacting surfaces. Generally speaking, there are two types of lubrication, known as boundary lubrication and hydrodynamic lubrication. Boundary lubrication is the phenomenon in which there is solid to solid contact of the interacting surfaces. The boundary lubricant is usually only a few molecules thick and functions by reducing the inter-molecular forces between the surfaces. In the second type of lubrication, known as hydrodynamic lubrication, there is either a thin layer of fluid that is present in the intervening space, separating the two moving surfaces, or the motion produces a layer of fluid on which the moving surface planes over the counterface.

Classically, the transition between different lubrication regimes while speed is increased for liquid-lubricated sliding surfaces is outlined through the Stribeck curve [65]. This empirical relationship was observed on hard, non-porous materials. This curve describes three sliding regimes, including the hydrodynamic (fluid-film), mixed, and boundary lubrication regimes. On the x-axis of the Stribeck curve is the Hersey number, a dimensionless number calculated by multiplying the dynamic viscosity with the sliding speed and dividing by the load. On the y axis is the friction coefficient, μ . The Stribeck curve was named after Richard Stribeck, a German scientist and engineer who investigated the film-forming properties of lubricants in journal bearings. Stribeck found a distinct correlation between frictional properties and films of lubricant formed between two surfaces.

At low speeds, Stribeck observed that it is mainly the two surfaces that interact and determine the friction. This is known as the boundary lubrication regime, in which the chemical and physical natures of the surfaces and lubricants are very important, and the lubricant is normally capable of being adsorbed on the surface [66]. The friction is represented by the coefficient of friction, μ , which is the ratio between the frictional force and normal force. As speed increases, the lubricant is transported into the space between the surfaces and the upward forces of the lubricant will push the surfaces apart. This is known as the mixed friction regime, where friction decreases as surfaces are pushed further and further apart. Lastly, minimum friction is reached when the surfaces are no longer touching. This is the elastohydrodynamic or hydrodynamic friction regime where the load is supported mainly by the lubricant. In this regime, there is either a thin layer of fluid that is present in the intervening space (hydrostatic lubrication), or the motion produces a layer of fluid on which the moving surface planes over the counter face (hydrodynamic lubrication).

Recent theoretical advances have led to the discovery of polymer brush lubrication, another lubrication mechanism frequently used as a benchmark to gain insight into biological lubrication systems. A polymer brush is formed when polymers are attached by one end to an interface at relatively high coverage as a surface coating. The polymer will stretch away from the interface to avoid overlapping, forming a polymer "brush". Brushes can be used to stabilize colloids, reduce friction between surfaces, and to provide lubrication in artificial joints [67]. Since many biological lubrication systems utilize polymer brushes to enhance lubrication in the boundary regime, polymer brushes are also becoming more popular for friction reduction in artificial polymer applications. Pult et al. investigated the mechanical forces during spontaneous blinks from a tribological perspective, at both low and high sliding velocities in a healthy subject. They concluded that the coefficient of friction of the ocular surface appears to be strongly comparable to that of hydrophilic polymer brushes at low sliding velocities. Because of the fluid film between the two sliding interfaces, there is no wear. However, in cases of dry eye, the full fluid film lubrication regime is not maintained at high blinking speeds, which could lead to increased shear rates and wear on the interacting interfaces [68].

1.5 FRICTION ON THE OCULAR SURFACE

Cells at the ocular and eyelid surfaces are subject to significant friction forces generated by the sliding motion during a blink which are normally mediated by lubricants in the tear film. In the case of a compromised tear film where tear film constituents and properties can be altered, increased tissue wear can occur due to higher friction during blinking [69]. The frictional forces that are exhibited during blinking can be exacerbated by contact lens wear. With the presence of a contact lens sitting on the corneal surface of the eye, the tear film is altered and there exists some

motion of the lens during the blink. Understanding the mechanisms of ocular surface – contact lens lubrication is important for ocular surface homeostasis and future contact lens development.

1.5.1 CURRENT *IN VITRO* OCULAR FRICTION TESTING METHODS

Various methods of friction measurement on the ocular surface have been proposed and tested. In each method, the principle for determining the coefficient of friction is the same, where the measurement is a ratio of the frictional force to the normal load applied on the sample during frictional motion. However, there are many differences in the testing methodology and set up of the studies, including differences in the counterface material and geometry, normal force pressure, lubricating fluid, method of lens sample preparation and type of movement. Overall, the use of a microtribometer has been the most common method used to measure the coefficient of friction of soft contact lenses.

Dunn et al. and Rennie et al. have tested the interface between an elastic substrate with a hard sphere, as the use of a hard sphere allowing the quantification of sample deformational behavior under applied load [70], [71]. In both studies, a glass sphere was articulated against a contact lens using a microtribometer, and Dunn et al. showed that a low modulus and high water content soft surface hydrogel layer provided consistent low friction sliding under boundary lubrication in an aqueous environment. Roba et al. also used a microtribometer, in which a contact lens was articulated against a functionalized glass disk in which the glass was mucin-coated and silanized. The study found that PVP-containing lenses exhibited the lowest friction compared to non-PVP lenses, and the best measurement protocol was found to consist of a sliding speed of 0.1 mm/s, using mucin-coated glass as a counter surface and using a lubricant based on packing solution

containing lysozyme and serum. Zhou et al. studied the friction of senofilcon A contact lenses by articulating the lens against a stainless-steel ball in a saline solution, finding that solid-solid contact dominated the friction when sliding velocity ranged from 0.01 cm/s to 0.5 cm/s. They also observed that the coefficient of the friction increased when velocity increased [72]. Hofmann et al. also utilized a microtribometer, articulating contact lenses against human corneal epithelial constructs and accessing corresponding cell damage [73].

Besides the microtribometer, other methods of testing have been employed, such as a qualitative finger rubbing method used to determine lens lubricity. Tucker et al. employed this method, finding that the method was highly repeatable but only by an experienced investigator [61]. However, this method does not provide the means to differentiate all lens types. Tucker et al. also investigated a quantitative inclined plane method, in which a glass plate is adjusted to a desired angle in a PBS bath. The contact lens is then placed at the top of the glass plate and attached to a stainless-steel weight to initiate movement. A minimum critical angle is then determined, which maintains the movement of the lens over a distance of approximately 100 mm. Finally, by calculating the tangent of the critical angle, the kinetic coefficient of friction could be measured. It was found that experimental lenses with different surface chemistries had significantly lower friction compared to a control lotrafilcon B lens [61].

Atomic force microscopy (AFM) is another method utilized by researchers to determine the surface mechanical and tribological properties of lenses. The use of an atomic force microscope allows for the high-resolution examination of the contact lens surface as it provides an analysis of the surface topography and roughness of a lens surface. In atomic force microscopy, a sharp

microfabricated tip is attached to a cantilever which is scanned across a sample. The deflection of this cantilever, caused by the forces developed between the tip and the sample, is monitored using a laser and photodiode and is used to generate an image of the surface. Not only does AFM provide high quality, three dimensional images, but topographic information can be obtained from aqueous, non-aqueous or dry lenses which eliminates the need for lens sample preparation. Kim et al. used this technique to determine the surface properties of pHEMA-based soft contact lenses. When comparing hydrated and non-hydrated lens surfaces, they found that lenses in saline solution had significantly reduced surface friction and adhesive force compared to those measured for the surface-dehydrated contact lens [74]. Rudy et al. also used AFM to investigate the elastic modulus, frictional, and adhesive properties of commercial contact lenses, finding that the frictional properties of plasma surface treated lenses such as balafilcon A exhibited coefficients of friction five times those of a non-plasma treated lens, such as delefilcon A [63]. Huo et al. found that AFM measurements in saline revealed large disparities between the coefficients of friction of the three lenses, with Pure Vision and Air Optix Aqua lenses exhibiting coefficients of friction approximately five times greater than that of Acuvue Oasys lenses [75].

In terms of testing lubricating fluids, there have been many research groups investigating different molecules and how they affect ocular friction. Sterner et al. investigated the coefficient of friction of two macromolecules commonly found in ocular biomaterials, polyvinylpyrrolidone (PVP) and hyaluronan (HA) along with two known model glycoproteins, bovine submaxillary mucin and α_1 -acid glycoprotein. PVP and HA was shown to be efficient boundary lubricants in phosphate buffered saline and tearlike fluid when surface-anchored, while the glycoproteins were only found to be lubricating when adsorbed on hydrophobic surfaces [64].

Overall, it remains clear that determining the coefficient of friction of soft contact lenses is a challenge. Currently there is no industry standard for the *in vitro* evaluation of friction. With the numerous methodologies and techniques available to measure ocular friction, noticeable differences can be observed among the literature values for the coefficient of friction for different lenses. Until a standardized method of friction testing is established, analysis of coefficient of friction between different studies and research groups will remain difficult. There is a desire in the research community and among contact lens companies for a standardized method to measure the coefficient of friction precisely and accurately, now that the relevance of contact lens friction and comfort is becoming more well known. Although each *in vitro* test setup has its own advantages and disadvantages, it remains unclear whether these test setups would be representative of the *in vivo* friction in the eye.

1.6 PRG4 AS AN OCULAR SURFACE BOUNDARY LUBRICANT

1.6.1 PROPERTIES OF PRG4

Proteoglycan 4 (PRG4), also known as lubricin, is a mucin-like lubricating glycoprotein originally discovered in synovial fluid. PRG4 has a molecular weight of 2.3×10^5 g/mol and 1404 amino acids within the sequence. PRG4 is amphiphilic in nature, allowing it to bind to both hydrophilic and hydrophobic surfaces due to its hydrophobic, positively charged ends and a hydrophilic negatively charged O-linked glycosylated amino acid backbone [76]. The amino acid backbone is heavily glycosylated with short polar (-GalNAc-Gal) and negatively charged (-GalNAc-Gal-NeuAc⁻) sugar groups that are O-linked to threonine residues [77]. PRG4 does not have glycosylated end domains. Instead, the N-end contains somatomedin-B-like and heparin-like domains while the C-end contains homeopexin-like domains [77].

1.6.2 ARTICULAR JOINT LUBRICATION

In articulating joints, PRG4 is synthesized and secreted by chondrocytes (cartilage cells) and synoviocytes (synovial tissue cells) and functions to protect the articular cartilage. PRG4 is an established, critical cartilage boundary lubricant in synovial fluid [78]. Joints deficient in PRG4 may have cartilage degradation, loss of joint lubrication and increased shear stress, as well as significant pain [76]. PRG4's large central mucin-like domain is believed to contribute to the protein's boundary lubrication of the cartilage surface possibly through repulsive hydration forces or charge repulsion [79]. It is believed that PRG4 also provides similar lubrication properties on the ocular surface.

1.6.3 PRG4 ON THE OCULAR SURFACE

Recently, PRG4 has been discovered at the ocular surface [76], where it plays a key role in ocular surface health. It was hypothesized that PRG4 is transcribed and translated in ocular surface epithelial cells and is secreted and adsorbed to both the cornea and conjunctiva. This enables PRG4 to reduce friction and prevent shear stress between these tissues, similar to its role in the lubrication of articular joints. Schmidt et al. were able to show that PRG4-deficient knockout mice showed significant evidence of corneal damage through corneal fluorescein staining, while in the other hand, the ocular surface of the wild type mice showed relatively little damage [76].

Indeed, PRG4 has been shown to act as an ocular surface boundary lubricant, at a human eyelid-cornea interface [76], at cornea – model contact lens biomaterial interfaces [80], and even a human eyelid – commercial contact lens biointerface [81] using a novel *in vitro* ocular surface friction

test. Given PRG4's presence at the ocular surface and established lubricating function, it is likely that it plays a role in the natural lubrication of ocular biointerfaces and potentially commercial contact lenses. Indeed, Samsom et al. showed that bovine PRG4 functioned as an effective boundary lubricant between silicone hydrogel contact lens against human eyelid and cornea tissues [81]. A statistically significant decrease in kinetic friction was observed when PRG4 was added to senofilcon A lenses.

1.6.4 RHPRG4

Recently, technological advances in protein expression systems have resulted in full-length recombinant human PRG4 (rhPRG4) being available for study from Lμbris BioPharma (Boston MA, USA). This form of PRG4 is purified from media condition by mammalian cells transfected with the PRG4 gene after culture in bioreactors and was characterized and was shown to demonstrate appropriate higher order structure, O-linked glycosylations, and ocular surface boundary lubricating ability similar to that of native protein [82]. Thus, rhPRG4 has the potential to be used clinically in humans.

1.6.4.1 RHPRG4 EYE DROPS CLINICAL TRIAL

A two week, randomized, double-masked study was carried out to evaluate the safety and efficacy of rhPRG4 as an eyedrop in patients with moderate dry eye disease [83]. This study compared rhPRG4 eye drops with commercially available 0.18% sodium hyaluronate (HA) eye drops in patients with moderate dry eye. The study was a parallel group study with a 1-week follow up and subjects over 18 years of age with moderate dry eye for at least 3 months were eligible to participate in the study. Results of the study showed that rhPRG4 was safe and well tolerated with

no sign of significant adverse effects. Overall, the study found that rhPRG4 produced significant improvement in signs and symptoms of dry eye disease compared to the HA drops. Specifically, primary outcomes such as the visual analog scale (VAS) for foreign body sensation, burning and stinging, itching, pain, sticky feeling, blurred vision and photophobia (discomfort or pain to the eyes due to light exposure) were investigated. Specifically, the VAS is a psychometric response scale measurement instrument for subjective characteristics or attitudes that cannot be directly measured. rhPRG4 supplementation led to reductions foreign body sensation, burning/stinging, pain, sticky feeling, blurred vision, and photophobia in at least one eye compared to baseline measurements. It is believed that the rhPRG4 preferentially stabilized the tear film, significantly improved damaged to the ocular surface epithelium, and reduced inflammation to the eyelid and conjunctiva. This leads to rhPRG4 providing immediate relief from dry eye symptoms and almost an immediate restoration of a competent tear film [83].

Overall, the results of this study indicate that rhPRG4 eyedrops shows promise towards improving the signs and symptoms for dry eye disease. Since contact lenses usage may induce dry eye disease, there is potential towards incorporating the usage rhPRG4 eyedrops with contact lenses as well.

1.7 INTRODUCTION TO THESIS

There are two main overall goals of this thesis. First, to understand which commercial lenses are compatible with rhPRG4 for friction reduction as a first step for potential clinical use. Second, to contribute towards the development of an artificial counterface similar to eyelid tissue that can be utilized in standardized ocular friction testing. Aspects of these contributions are outlined below.

1.7.1 HYPOTHESIS AND AIMS

There are two hypotheses to this thesis:

1. The incubation of silicone hydrogel lenses in rhPRG4 will result in a reduction of friction, even when tested in lubricant baths devoid of rhPRG4. It is of interest to understand how the coefficient of friction on the ocular surface varies with various lens types, lubricants, and solution incubation time.

2. PDMS can be employed as a test counterface for the *in vitro* testing of contact lenses, resulting in similar friction coefficient values to that of human eyelid tissue

The hypotheses will be tested by the following specific aims:

1. Assess the effect of rhPRG4 incubation time and lubricant bath on *in vitro* coefficient of friction of commercial lens at an eyelid-lens biointerface
2. Evaluate mucin-soaked PDMS as a synthetic test counterface for the *in vitro* friction testing of contact lenses

An outline of this thesis is provided below:

Chapter 1 provides a background on ocular anatomy, dry eye disease, contact lenses, biotribology and PRG4, emphasizing the importance of the coefficient of friction and its correlation to ocular comfort.

Chapter 2 investigates the effect of proteoglycan 4 on the kinetic coefficient of friction of commercial contact lenses.

Chapter 3 evaluates mucin-soaked PDMS as a synthetic test counterface for the *in vitro* friction testing of contact lenses.

Chapter 4 provides an overall summary of the thesis, discussion of the major findings, and suggestions for future work.

Chapter 2 INVESTIGATING THE EFFECT OF PROTEOGLYCAN 4 ON THE KINETIC COEFFICIENT OF FRICTION OF COMMERCIAL CONTACT LENSES

2.1 ABSTRACT

Objective: *In vitro* contact lenses friction significantly correlates with end of day contact lens discomfort [56], [57]. Proteoglycan 4 (PRG4) is a mucin-like lubricating glycoprotein that naturally exists on the ocular surface [76] and functions as an effective boundary lubricant to reduce friction between human eyelid tissue and commercial contact lenses [81]. Recombinant human PRG4 (rhPRG4) has been shown to adsorb to commercial contact lenses [84], and may also function as an effective friction reducing lubricant either in the form of a rewetting drop or as an overnight solution. The objective of this study was to assess the effect of instantaneous and overnight incubation in rhPRG4 on the *in vitro* coefficient of friction of commercial silicone hydrogel contact lenses.

Methods: A custom biomechanical friction test was developed to articulate a human eyelid edge over commercial contact lenses (Acuvue Oasys, Biofinity, Air Optix Hydraglyde) at an effective sliding velocity of 0.3 mm/s and under physiological loads to determine the kinetic coefficient of friction (Mach-1, Biomomentum Inc.). To simulate using rhPRG4 (Lubris BioPharma, LLC) as a rewetting drop, lenses were tested in the following order against the same lid edge: 1) incubated overnight and tested in a bath of phosphate buffered saline (PBS), and 2) incubated overnight in PBS and instantly incubated with 200 uGu/ml rhPRG4 30 seconds prior to testing in PBS. To simulate using rhPRG4 as an overnight rhPRG4 solution, lenses were tested in the following order

1) incubated overnight and tested in PBS, and 2) incubated overnight in rhPRG4 and tested in PBS. Effect of treatment was assessed by a repeated measures one way ANOVA.

Results: Acuvue Oasys lenses incubated in rhPRG4, both instantaneously and overnight, followed by friction testing in PBS had lower friction compared to lenses incubated and tested in PBS. For Biofinity, lower friction was only observed during the overnight incubation test. No differences in friction were observed for Air Optix Hydraglyde following overnight rhPRG4 incubation.

Discussion: This study demonstrates that incubation in rhPRG4, either instantaneously or overnight, can reduce *in vitro* friction of a commercially available silicone hydrogel lens. These results are consistent with previous studies demonstrating that rhPRG4 is able to adsorb to lenses and reduce *in vitro* friction. Collectively these results, when combined with the recent clinical trial data demonstrating rhPRG4 is able to reduce signs and symptoms of dry eye, suggest that rhPRG4 could potentially be an effective friction reducing rewetting drop or overnight soak solution, and thus possibly improve *in vivo* contact lens comfort.

2.2 BACKGROUND

There are over 140 million contact lens wearers in the world [22], but many of these wearers suffer from signs and symptoms of dry eye disease, including discomfort, dryness, and red eyes. Dry eye disease affects over 30-40 million people in the United States [7] and 100-344 million people worldwide [8] [9]. One major non-disease related cause of dry eye is contact lens wear, which can interfere with proper distribution of the tear film on the ocular surface [85], leading to reduced or even discontinued contact lens wear. With every blink, cells in the surfaces of the eye are subject to significant friction forces, which may be exacerbated with contact lens use. Recent data suggests that *in vitro* contact lens friction is inversely correlated with comfort [57]. Therefore, the lower the friction in the eye, the more comfortable the contact lens may potentially be.

Proteoglycan 4 (PRG4), also known as lubricin, is a mucin-like lubricating glycoprotein naturally occurring at the ocular surface and has been shown to adsorb onto commercial contact lenses [84]. PRG4 has been found to function as an ocular surface boundary lubricant, reducing *in vitro* friction between human eyelids and commercial contact lens [81]. Recently, full length recombinant human PRG4 has been expressed from Chinese hamster ovary cell lines and is available for study. Given PRG4's presence at the ocular surface and established lubricating function, rhPRG4 has the potential to be used clinically in humans.

Recently, a clinical trial on rhPRG4 drops was completed in Europe, comparing rhPRG4 eyedrops with HA drops in patients with dry eye [83]. rhPRG4 drops were found to be generally superior to HA drops, improving both signs and symptoms in moderate dry eye subjects. This shows that there is potential in the commercialization of rhPRG4 as an eyedrop, and it would be interesting to

determine whether rhPRG4 eyedrops will show a reduction in friction when used with contact lenses. Development of a rhPRG4 eyedrop may contribute towards more comfortable contact lens wear.

rhPRG4 can be incorporated with contact lens use in multiple ways. For example, rhPRG4 can be coated on a lens surface, developed as an eye drop solution or developed as an overnight soak solution. In terms of a rhPRG4 lens coating, one issue that may arise would be lens sterilization. During the last step in the lens manufacturing process, lenses are typically sterilized by autoclave. However, this sterilization uses high heat which may denature rhPRG4 and affect its lubrication properties. Incorporating rhPRG4 into overnight soak solutions for contact lenses may be a feasible option, but it may also be an expensive option for users as larger amounts of rhPRG4 solution would be required. Considering the outcome of the recent eye drop clinical trial where rhPRG4 improved signs and symptoms of dry eye [83], there is interest in determining whether rhPRG4 can be incorporated into an eye drop solution.

Therefore, it is of interest to investigate how friction is affected with various commercial lenses, lubricants and incubation times of rhPRG4. It is hypothesized that the incubation of silicone hydrogel lenses treated with rhPRG4 will result in a reduction of friction, even when tested in lubricant baths devoid of rhPRG4. Therefore, the objective of this investigation was to assess the potential effect of different types of lenses and incubation times on the coefficient of friction of rhPRG4-soaked contact lenses tested in various lubricants.

2.3 MATERIALS

Human eyelids were excised from fresh cadavers from the University of Calgary Body Donation Program. The average age of donors was 85.75 years ($n = 8$), with two male and six female donors. Approval for use and appropriation of these tissues was obtained from the University of Calgary Conjoint Health Research Ethics Board. Each eyelid was excised from the cadaver using a scalpel and washed in PBS before being stored in -80°C . Eyelid edges were separated from the tarsal plate and eyelashes and other debris was removed prior to storage.

Commercially available lenses were purchased and utilized for testing. Daily disposable silicone hydrogel contact lenses Acuvue TruEye (narafilecon A, Johnson & Johnson Vision Care Inc., Jacksonville, FL, USA) were used to precondition the human eyelid, while bi-weekly silicone hydrogel contact lenses Acuvue Oasys (senofilcon A, Johnson & Johnson Vision Care Inc., Jacksonville, FL, USA) and monthly silicone hydrogel contact lenses Biofinity (comfilcon A, CooperVision, Scottsdale, NY, USA) and Air Optix plus Hydraglyde (lotrafilecon B, Alcon, Fort Worth, TX, USA) lenses were purchased.

Phosphate buffered saline (PBS) solution (GIBCO Dulbecco's Phosphate-Buffered Saline, Thermo Fisher Scientific, Waltham, MA, USA) was the saline solution used in this study. rhPRG4 was obtained from Lubris BioPharma, LLC (Boston, MA, USA) and was re-suspended in PBS at a concentration of $200\ \mu\text{g}/\text{mL}$, as assessed by spectrophotometry (Protein A280, Waltham, MA, USA). To adhere the eyelid tissue onto the testing apparatus, ethyl cyanoacrylate (Krazy Glue, Elmer's Products, Atlanta, GA, USA) was utilized.

2.4 METHODS: *IN VITRO* FRICTION MEASUREMENT TEST

In vitro ocular friction testing was carried out using the Mach-1 micromechanical testing system by Biomomentum Inc. (Laval, QC, Canada). The Mach-1 is a multiple-axis tester that can be used in various configurations to evaluate the mechanical properties of tissues and soft materials. It consists of a multiaxial load cell and can operate with 4 degrees of freedom. A custom, *in vitro* ocular surface – contact lens friction test was adapted from previously described methods [81]. Using a custom program on the Mach-1, the machine imitates a trajectory similar to the blinking of an eye, with the resulting force data analyzed in order to determine the coefficient of friction between the biointerfaces. In particular, the kinetic friction coefficients, $\langle \mu_{\text{kinetic}} \rangle$, was calculated by obtaining raw force data, transposing the data to normal and tangential forces at five displacements, and calculating the slope of the regression line formed by these data points.

In previous studies with cadaver eyelid tissue, tests were completed using a BOSE ELF3200 with axial and rotation actuators and a combination of a torsional and an axial load cell [80]–[82]. In this test set up, human eyelid tissue was articulated in a rotational configuration against contact lenses. With the rotational configuration, plowing friction losses are minimized because the opposed surfaces remain in contact, and fluid pressure effects are minimal at relatively slow velocities after the initial pressure dissipates. Also, with the use of an annular geometry, the variation in sliding velocity is reduced. However, this test setup does not mimic the physiological conditions on the ocular surface, in which the lid edge is articulated over a curved contact lens surface during a natural blink. Furthermore, in the rotational test method, the annulus was excised from the tarsal plate of the eyelid instead of the lid wiper, in order to maintain a uniform sample. As the lid wiper is less than 2 mm thick [86], it was impossible to cut an annulus from this area.

As the lid edge is hypothesized to be subject to most of the shear forces during blinking, a custom eyelid holder was created with the new Mach-1 test in order to hold the eyelid edge and better replicate the sliding interface that occurs during natural blinking.

2.4.1 SAMPLE PREPARATION

Before testing, a 5 mm section of the lid edge was cut using a scalpel and thawed, with the remaining section of the lid edge refrozen. Typically, 4-5 samples can be harvested from each eyelid. This 5 mm piece was then mounted onto a custom metal annulus and glued to the metal eyelid holder using the ethyl cyanoacrylate adhesive as shown in Fig. 1.

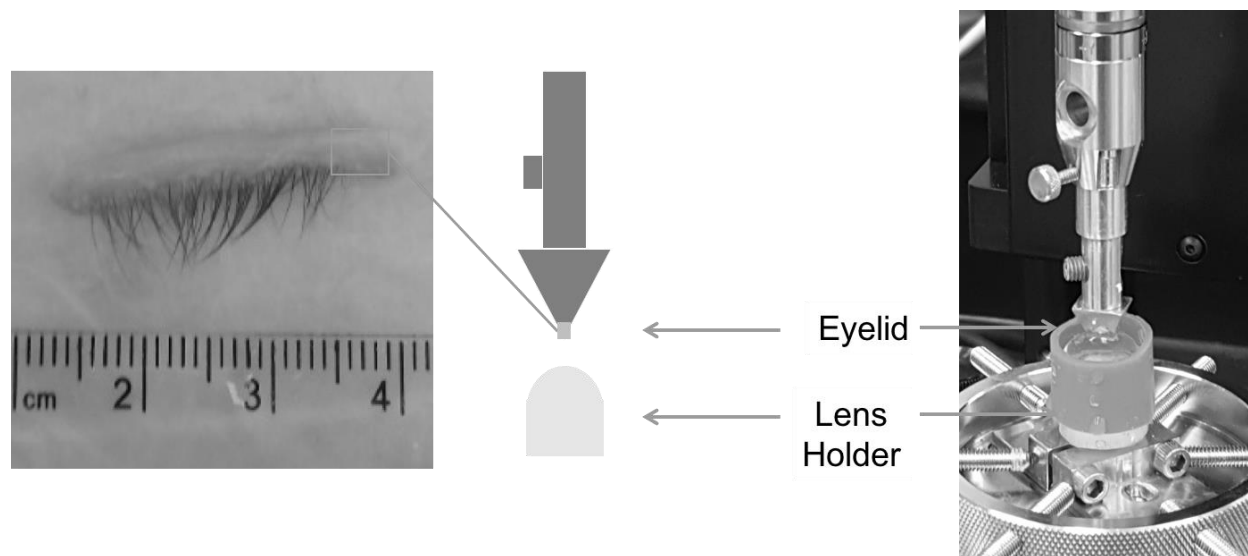


Figure 1 Eyelid sample preparation and test setup

Fresh contact lenses were taken out of their original blister packaging, then washed in PBS to remove any remaining solution from the blister package. Contact lenses requiring an overnight soak in solution were taken out of their original blister packaging, washed in PBS, then placed in solution (PBS, or rhPRG4) in an Eppendorf tube. Prior to testing, all lenses were washed in PBS once more and then mounted on a polydimethylsiloxane (PDMS) mold using cyanoacrylate. Three

to four small drops of cyanoacrylate were applied at the sides of the contact lens, anchoring the lens. Special care was taken to ensure that the lens center that would be in contact with the eyelid did not have any contact with the ethyl cyanoacrylate. A silicone rubber sleeve was fitted around the lens and mold apparatus in order to create a bath to hold 0.8 mL of the lubricant fluid.

2.4.2 FRICTION TEST SETUP

Before commencing the friction testing, the load cell was first calibrated using a 17N calibration weight. Next, the lens holder was mounted onto the Mach-1 holder (Fig. 9). Once the test samples were ready for testing, contact on the lens was found by probing the lens in nine different locations using a spherical stainless steel probe ($r = 0.5 \text{ mm}$) to determine the center point of the lens. Coordinates from the center point were then entered into the program so that the articulation of the annulus would always occur from the center point. The stainless-steel probe was then removed and replaced with the eyelid holder.

A precondition sequence was employed at the commencement of every new test with a new eyelid with the purpose of clearing the lid-lens interface from contaminants. It was also used to compress the eyelid as it is a viscoelastic material that is prone to stress relaxation. This preconditioning test also gave the tester an understanding of what amount of initial strain was suitable and the increasing strain required to attain the 5 target loads required for the friction test, as described below.

After preconditioning, the desired test sequence was then carried out. The precondition lens was removed and replaced with the lens of interest, while the eyelid was washed three times in a PBS

bath. The eyelid was then programmed to find contact with the lens. After the annulus was in contact with the lens at the programmed strain level, a wait period of 40 seconds commenced, permitting the eyelid to stress relax. Next, the eyelid was articulated in the x axis to -5 degrees, 10 degrees, -10 degrees and finally 5 degrees along the radius of curvature of the lens back to the center position (Fig. 2). This was repeated five times at a sliding speed of 0.3 mm/s and at different axial loads between 1-20 kPa to obtain data at different pressures. After testing, the eyelid was washed in PBS and a new lens or lubricant was replaced into the testing system. Typically, 0.8 mL of fresh lubricant was added for each test. In addition to the precondition, a maximum of 4-5 test sequences can be tested on one single lid edge sample.

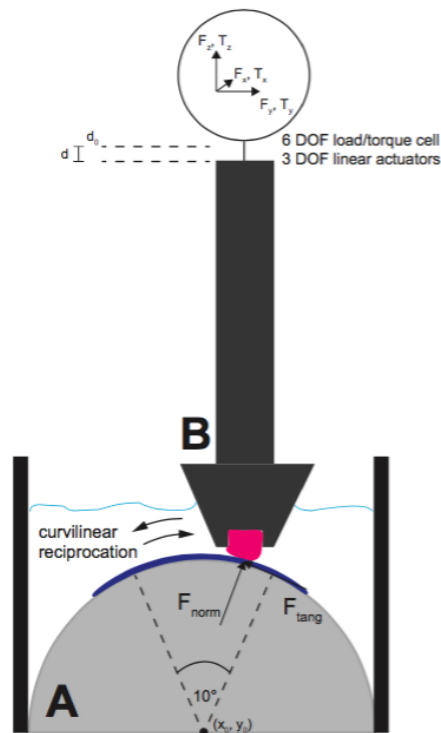


Figure 2 Articulation of the eyelid edge over the contact lens on the Mach-1 [87]

2.4.3 CALCULATION AND ANALYSIS OF FRICTION

The raw x and y force data obtained from the Mach-1 during the last 5° of translation in both the positive and negative directions was transposed to give tangential and normal forces. Typically, force data was taken from 5-7 points corresponding to pressures of 1-20 kPa. Finally, a line of best fit was fitted for all the different pressure points, with the slope of this line being the coefficient of friction value for the test. This regression line was fit to these points of data in order to determine the kinetic coefficient of friction of the shear or normal force (Fig. 3). Analysis of data was completed with PyFrictionTools, a custom python package designed for analyzing data from the Biomomentum Mach-1. PyFrictionTools is an open source project under the MIT software license and is available at <https://github.com/mlsamsom/PyFrictionTools>.

Equation 1 Calculation of coefficient of friction

$$\mu = \frac{dF_{shear}}{dF_{normal}}$$

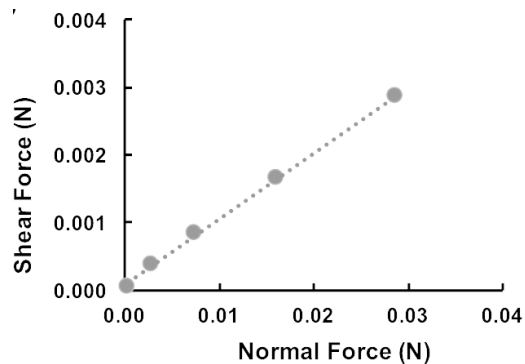


Figure 3 Regression line used to determine kinetic coefficient of friction

2.4.4 STATISTICAL ANALYSIS OF EXPERIMENTAL DATA

Statistical analysis was conducted using SPSS Statistics 24 (IBM SPSS Statistics, Armonk, NY, USA). Q-Q normality plots were generated for each set of data to verify the assumption that the experimental values were normally distributed and that parametric tests could be utilized. As each set of tests were completed with multiple measurements against the same human eyelid tissue, the tests were analyzed using a one way repeated measures analysis of variance (ANOVA) statistical test. For tests comparing only two different conditions, a Student's paired t test was conducted instead of an ANOVA. Mauchly's test of sphericity was used to determine if sphericity was violated prior to determining the appropriate significance value to use. The least significant difference (LSD) post hoc was conducted when significance was found with the ANOVA. Partial eta squared values (η_p^2) was also reported to indicate effect size. Values of 0.0099, 0.0588, and 0.1379 represented benchmarks suggested by Cohen [88] to define small, medium, and large effects respectively. All data is represented as the mean \pm standard error of the mean (mean \pm SEM).

2.5 PRELIMINARY TESTS: RHPRG4-COATED CONTACT LENSES

Overall, the goal of this study was to understand which commercial lenses are compatible with rhPRG4 for friction reduction as a first step for potential clinical use. As it was unknown whether the lubrication properties of rhPRG4 remain after high temperature sterilization, preliminary tests were conducted to see how rhPRG4 behaved after being autoclaved. It is hypothesized that rhPRG4 would denature under high temperature sterilization and not be suitable as a contact lens coating. Therefore, the objective was to determine if there was an observable difference in the lubricating properties of rhPRG4 on a silicone hydrogel lens changes in autoclaved compared with un-autoclaved lenses. Acuvue TruEye and Acuvue Oasys were the lenses chosen for testing.

The test sequence (Fig. 4) was as follows against the same eyelid: 1) the eyelid was first preconditioned against a TruEye lens in PBS, followed by 2) overnight incubation of the lens of interest in PBS and tested in PBS as a negative control. Next, lenses that were soaked overnight in rhPRG4 were 3) autoclaved then tested in PBS, and 4) not autoclaved and directly tested in PBS. Lastly, lenses were 5) soaked and tested in rhPRG4 as a positive control.

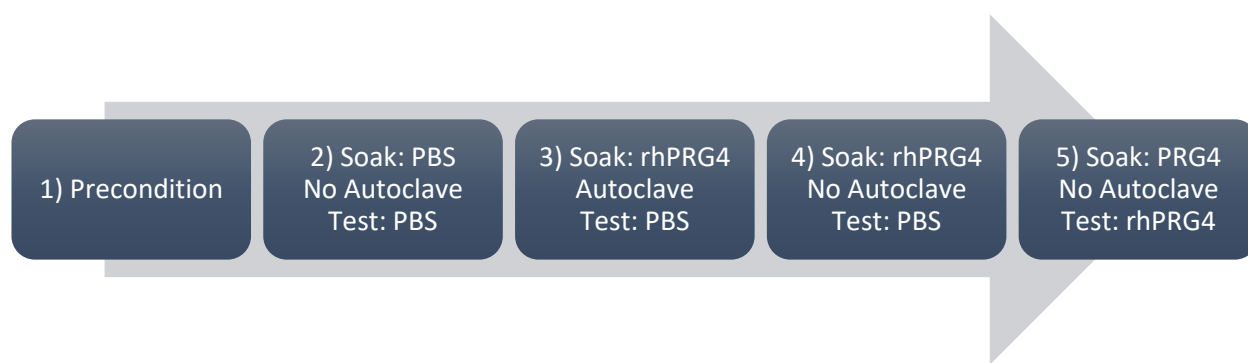


Figure 4 Test sequence for autoclave experiments

Fig. 5 shows the results of friction testing ($n = 3$) for the Acuvue TruEye (narafilecon A). The kinetic coefficient of friction is shown on the vertical axis and different soaking and testing conditions are on the horizontal axis. Statistical analysis was completed using a repeated measures ANOVA and LSD post hoc, which showed that friction values differed statistically significantly between test conditions ($F(3, 6) = 38.152, p < 0.05, \eta_p^2 = 0.950$). LSD post hoc tests found a significant difference ($p < 0.05$) between the lenses that were autoclaved in rhPRG4 (0.234 ± 0.026) compared with the lenses soaked and tested in PBS (0.114 ± 0.006), non-autoclaved lenses tested in PBS (0.123 ± 0.013), and the non-autoclaved lenses tested in rhPRG4 (0.100 ± 0.012). These test results provided evidence that the autoclaving lenses may lead to the denaturing of rhPRG4 and the loss of its lubrication properties. As TruEye was a daily disposable silicone hydrogel, the next

set of tests focused on an extended wear, multiple use lens. Acuvue Oasys (senofilcon A) was chosen as the test lens.

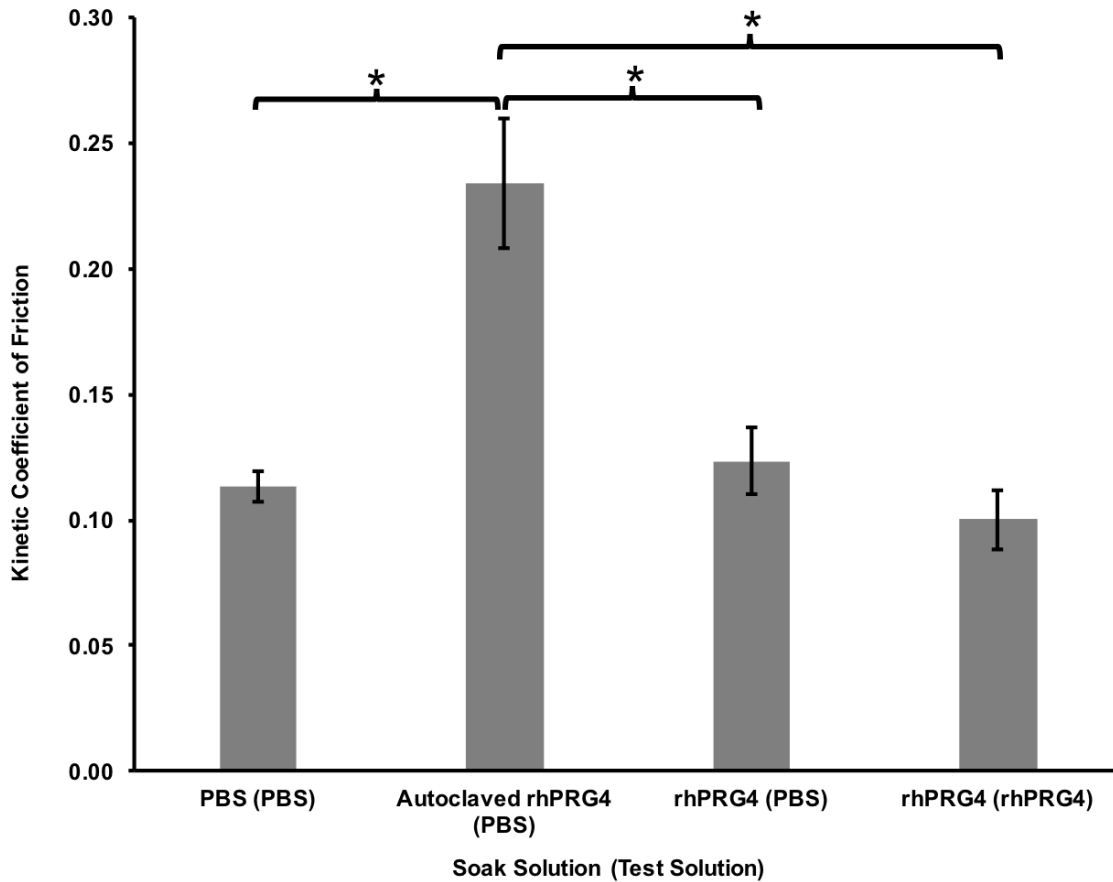


Figure 5 The effect of autoclaving on the coefficient of friction of TruEye lenses (n = 3). * denotes p < 0.05.

Fig. 6 shows the results of autoclave sterilization on the coefficient of friction for the Acuvue Oasys lens (n = 6). A repeated measures ANOVA determined that friction values differed statistically significantly between test conditions ($F(3, 18) = 9.021, p < 0.05, \eta_p^2 = 0.601$). Post hoc tests using showed that lenses soaked and tested in PBS (0.119 ± 0.010) were significantly higher in friction ($p < 0.05$) compared to lenses autoclaved in rhPRG4 and tested in PBS (0.101 ± 0.010), non-autoclaved lenses soaked in rhPRG4 and tested in PBS (0.089 ± 0.007), and

non-autoclaved lenses soaked and autoclaved in rhPRG4 (0.097 ± 0.007). Although there was no significant difference between autoclaved and non-autoclaved lenses that were incubated in rhPRG4 and tested in PBS, lenses that were autoclaved showed a trend towards having higher friction compared to non-autoclaved lenses. This data, along with the TruEye autoclave experimental data suggests that sterilization may denature rhPRG4, so it was concluded that perhaps rhPRG4-coated lenses may not be the best way to incorporate rhPRG4 with lenses. Thus, this method was not pursued further and other methods of incorporating rhPRG4 into contact lens use were investigated.

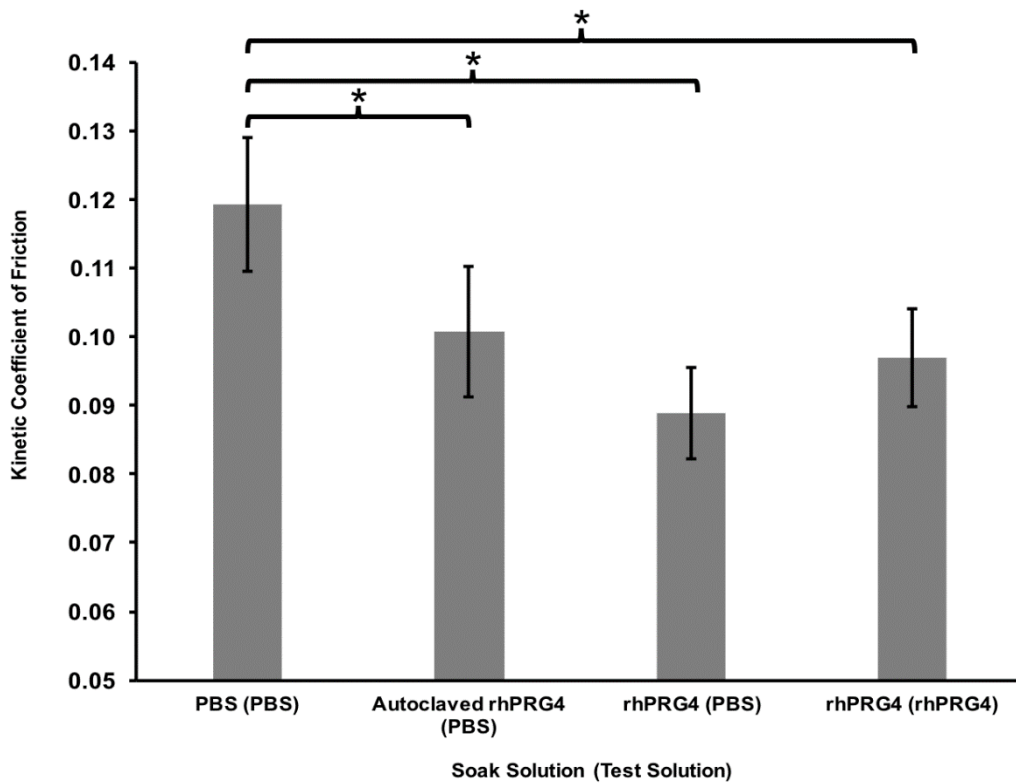


Figure 6 The effect of autoclaving on the coefficient of friction of Acuvue Oasys (n = 6). * denotes $p < 0.05$.

2.6 INCUBATION OF LENSES IN RHPRG4

As it was shown that rhPRG4 may not be feasible as a lens coating, efforts were made to investigate using rhPRG4 as an incubation solution. Both instant and overnight incubation of rhPRG4 was investigated to mimic possible commercial uses of rhPRG4. The instant incubation of lenses would mimic rewetting eye drops, where users would place a few drops in their eye when their eyes felt dry. On the other hand, the overnight incubation of rhPRG4 would mimic commercial overnight soaking solutions where users would place their lenses in to soak overnight before use in the morning.

Two main testing sequences were carried out during friction testing to test both the overnight and instant incubation of lenses in rhPRG4. For each test sequence, lenses were incubated overnight at room temperature for a period of approximately 16 hours in either 0.8 mL of rhPRG4 (200ug/mL) or in PBS before testing.

For the first test sequence with overnight incubation of rhPRG4 (Fig. 7), the eyelid was first 1) preconditioned against a TruEye lens in PBS, followed by 2) a negative control in which the lens of interest was incubated in PBS overnight and tested in a PBS lubricant bath. Next, the lens was 3) incubated in rhPRG4 overnight and tested in a PBS lubricant bath. Lastly, the lens of interest was 4) both incubated in rhPRG4 overnight and tested in a rhPRG4 lubricant bath.

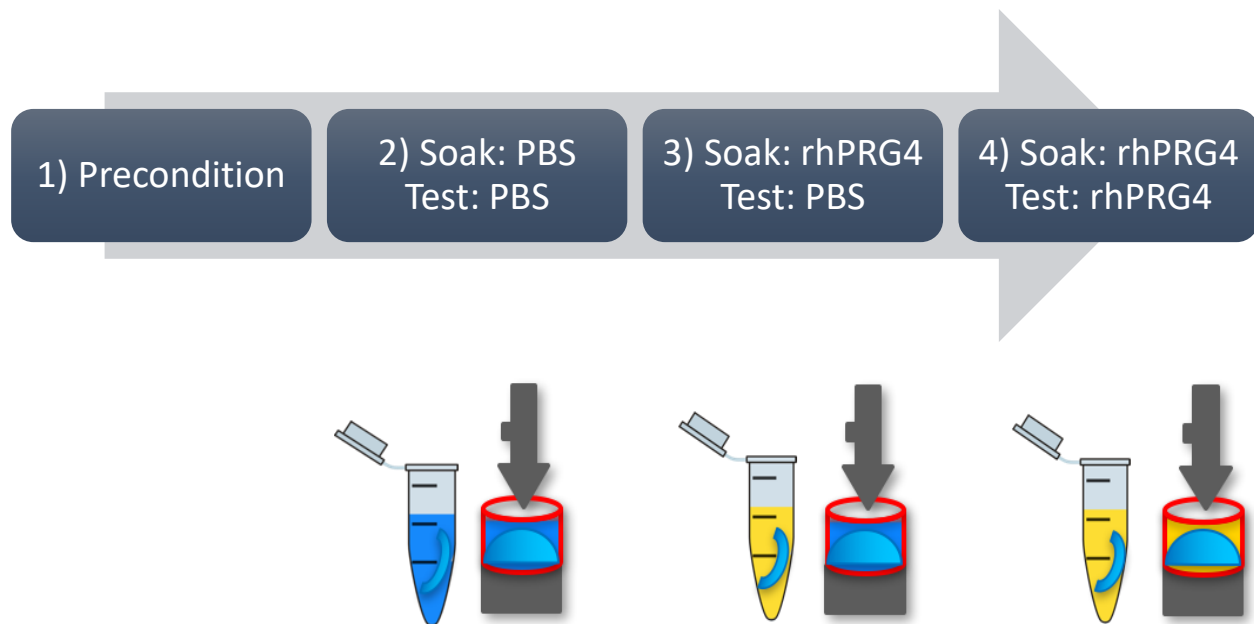


Figure 7 Overnight incubation test sequence, where the blue and yellow liquid represents PBS and rhPRG4 respectively

The purpose behind instant incubation of rhPRG4 in the second test sequence (Fig. 8) was to simulate a contact lens user who would be feeling discomfort and would be wanting to put in eyedrops to provide instant relief. In this second test sequence, the eyelid was once again 1) preconditioned against a TruEye lens in PBS, followed by a negative control where the lens of interest was 2) incubated and tested in PBS. To simulate instant incubation of rhPRG4, 3) the negative control lens was washed in PBS and then 3 drops of rhPRG4 was placed onto the lens using an eye dropper. After 30 seconds, the silicone sleeve was replaced onto the lens and PBS was placed in the bath. Lastly, the lens was 4) incubated in rhPRG4 overnight and tested in a rhPRG4 lubricant bath.

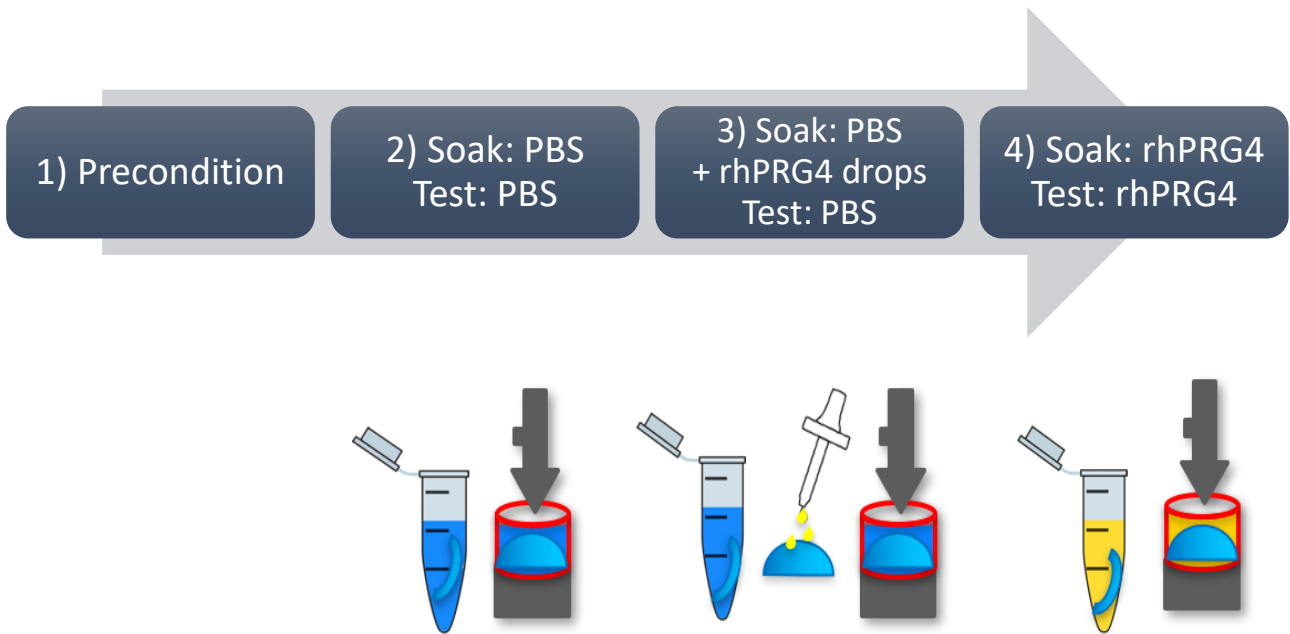


Figure 8 Instant incubation test sequence, where the blue and yellow liquid represents PBS and rhPRG4 respectively

2.7 RESULTS

2.7.1 ACUVUE OASYS FRICTION TESTING RESULTS

2.7.1.1 OVERNIGHT INCUBATION OF ACUVUE OASYS IN RHPRG4

Figure 9 shows the results of the overnight incubation test sequence for Acuvue Oasys ($n = 4$). A repeated measures ANOVA showed that the kinetic coefficient of friction was significantly different ($F(2,6) = 2.815$, $p < 0.05$, $\eta_p^2 = 0.484$) between test conditions, with post hoc tests showing that friction significantly decreased from 0.109 ± 0.001 when soaked and tested in PBS to 0.096 ± 0.004 when soaked in PBS overnight and tested in rhPRG4 ($p < 0.05$). No statistically significant difference was found between lenses soaked and tested in rhPRG4 (0.102 ± 0.006) and the other test conditions.

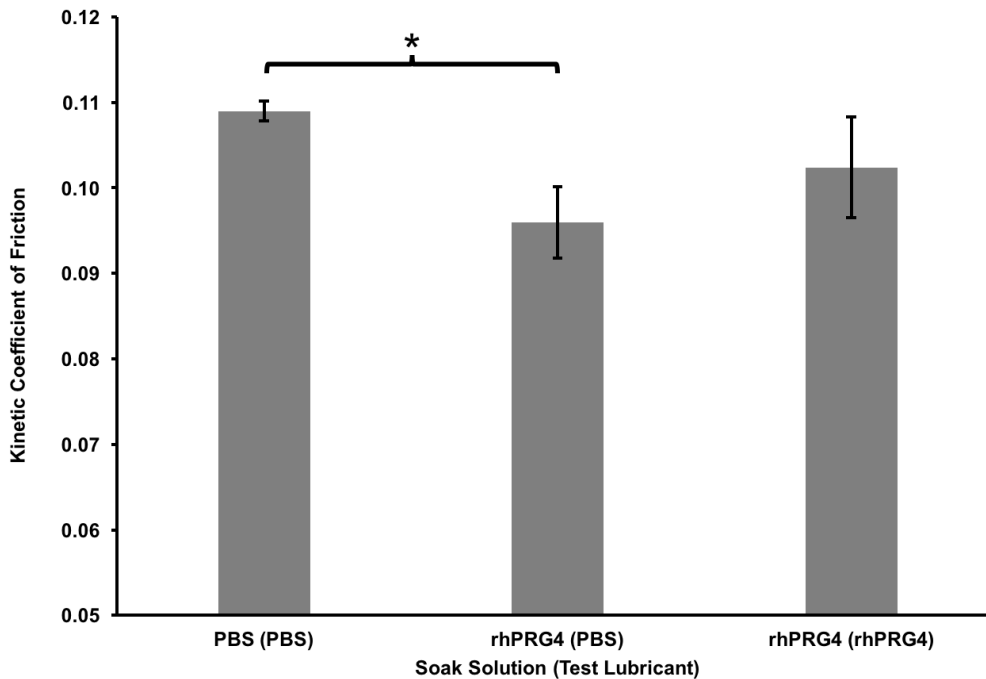


Figure 9 The effect of overnight incubation of rhPRG4 on the coefficient of friction of Acuvue Oasys lenses ($n = 4$).

* denotes $p < 0.05$.

2.7.1.2 INSTANT INCUBATION OF ACUVUE OASYS IN RHPRG4

For the instantaneous incubation test sequence in Fig. 10 ($n = 7$), there was a statistically significant difference between groups as determined by a repeated measures one way ANOVA ($F(2, 12) = 6.048$, $p < 0.05$, $\eta_p^2 = 0.502$). Using a LSD post hoc, friction was found to be statistically lower ($p < 0.05$) when rhPRG4 was instantly added to the lens soaked and tested in PBS (0.092 ± 0.007) compared to the negative control where the lens was soaked and tested in PBS (0.106 ± 0.006). No statistically significant difference was found between lenses soaked and tested in rhPRG4 (0.092 ± 0.008) and the other test conditions.

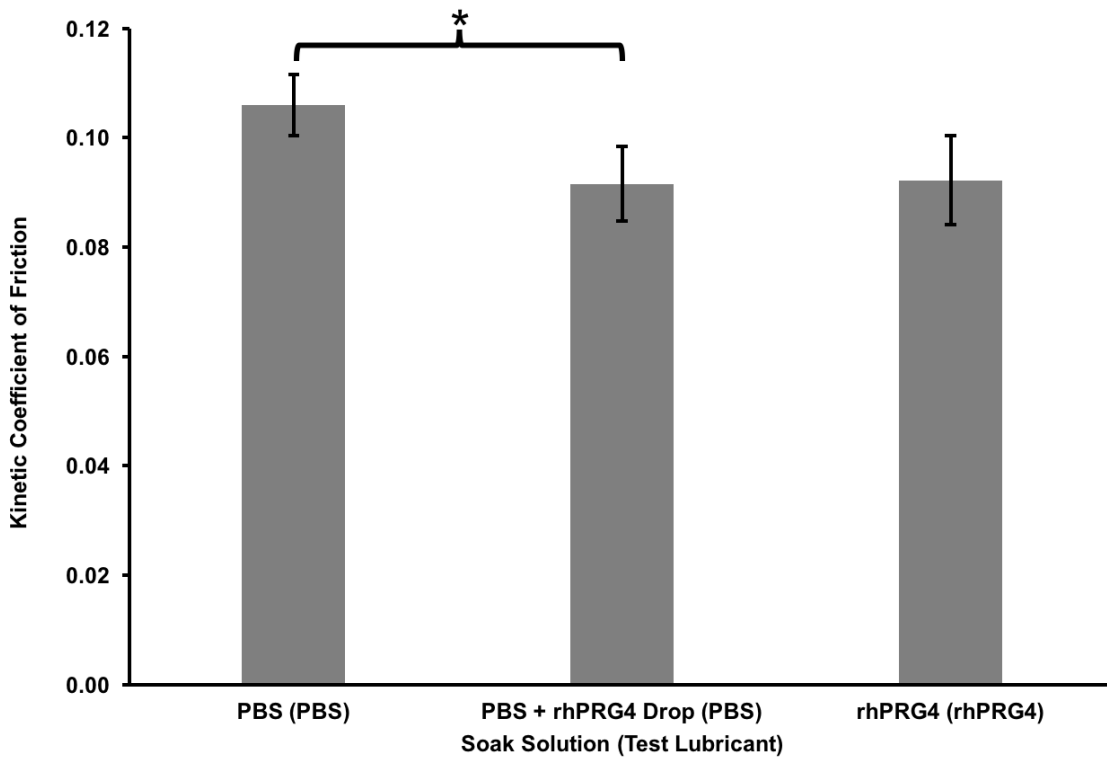


Figure 10 The effect of instantaneous incubation of rhPRG4 on the coefficient of friction of Acuvue Oasys lenses ($n = 7$).

* denotes $p < 0.05$.

2.7.2 BIOFINITY FRICTION TESTING RESULTS

2.7.2.1 OVERNIGHT INCUBATION OF BIOFINITY IN rhPRG4

Fig. 11 displays the results of the overnight incubation of Biofinity lenses in rhPRG4 ($n = 4$). A repeated measures one way ANOVA indicated that there was a statistically significant difference in friction values were between test conditions ($F(2, 6) = 14.307, p < 0.05, \eta_p^2 = 0.827$). LSD post hoc tests found lenses soaked in rhPRG4 and tested in PBS (0.065 ± 0.005) and lenses soaked and tested in rhPRG4 (0.065 ± 0.006) had statistically significantly lower friction ($p < 0.05$) compared to the negative control (0.070 ± 0.006).

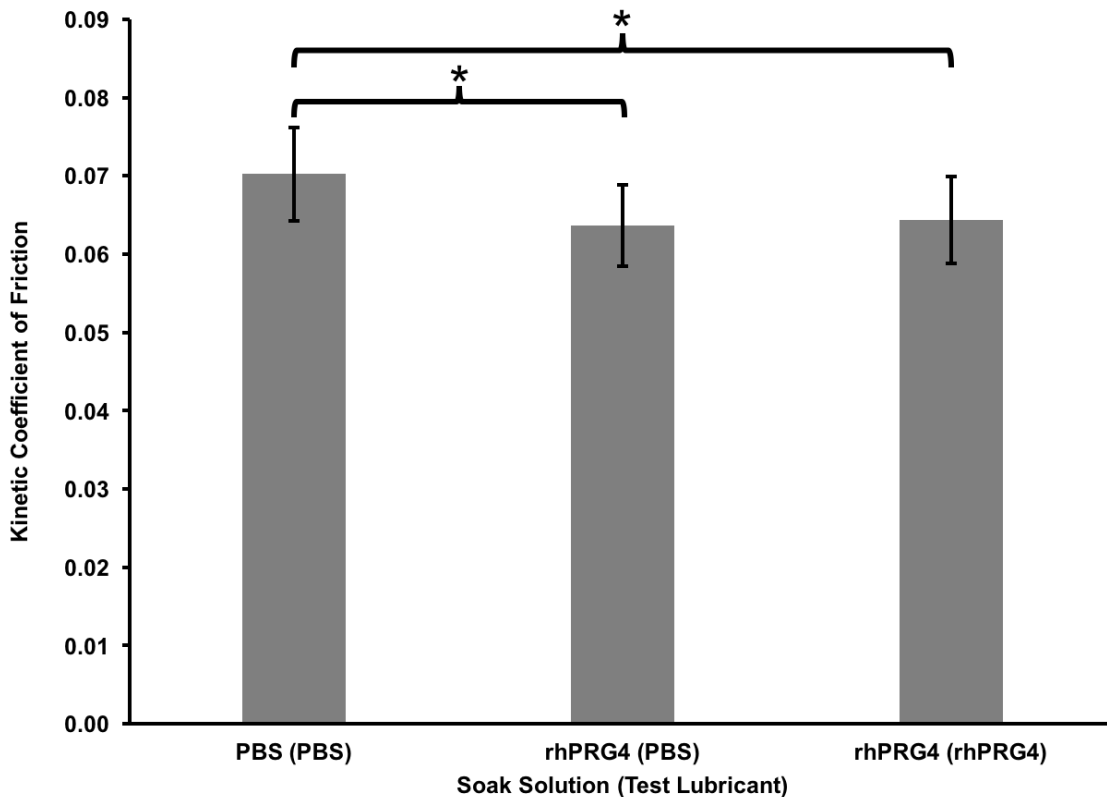


Figure 11 The effect of overnight incubation of rhPRG4 on the coefficient of friction of Biofinity lenses ($n = 4$). * denotes $p < 0.05$

2.7.2.2 INSTANT INCUBATION OF BIOFINITY IN RHPRG4

The results of instant incubation of Biofinity lenses in rhPRG4 is shown in Fig. 12. Due to an eyelid shortage, only 2 repeats of the test ($n = 2$) were completed. Although a repeated measures ANOVA showed no statistical significance was found at this point ($F(2, 2) = 2.621$, $p > 0.05$, $\eta_p^2 = 0.724$), there is a trend towards lower friction with the rhPRG4 drop (0.044 ± 0.001) and the rhPRG4 soak and test (0.042 ± 0.006) compared to the PBS negative control (0.050 ± 0.004). Ideally, more repeats should be completed to determine if a statistical significance can be found.

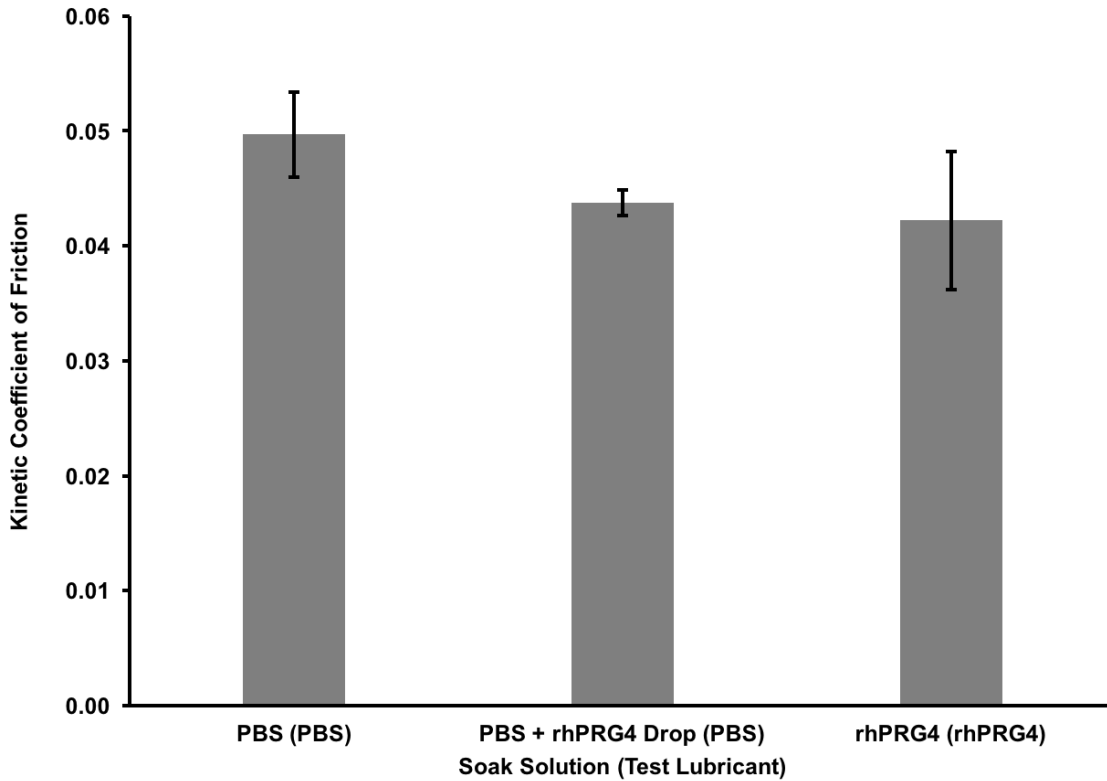


Figure 12 The effect of instantaneous incubation of rhPRG4 on the coefficient of friction of Biofinity lenses ($n = 2$).

2.7.3 AIR OPTIX HYDRAGLYDE FRICTION TESTING RESULTS

2.7.3.1 OVERNIGHT INCUBATION OF AIR OPTIX HYDRAGLYDE IN RHPRG4

Fig. 13 shows the overnight incubation of Air Optix Hydraglyde lenses in rhPRG4 ($n = 3$). When using a repeated measures ANOVA, no significance was found ($F(2, 4) = 1.165$, $p > 0.05$, $\eta_p^2 = 0.368$) between lenses soaked and tested in PBS (0.051 ± 0.014), lenses soaked in rhPRG4 and tested in PBS (0.060 ± 0.021) and lenses soaked and tested in rhPRG4 (0.059 ± 0.021).

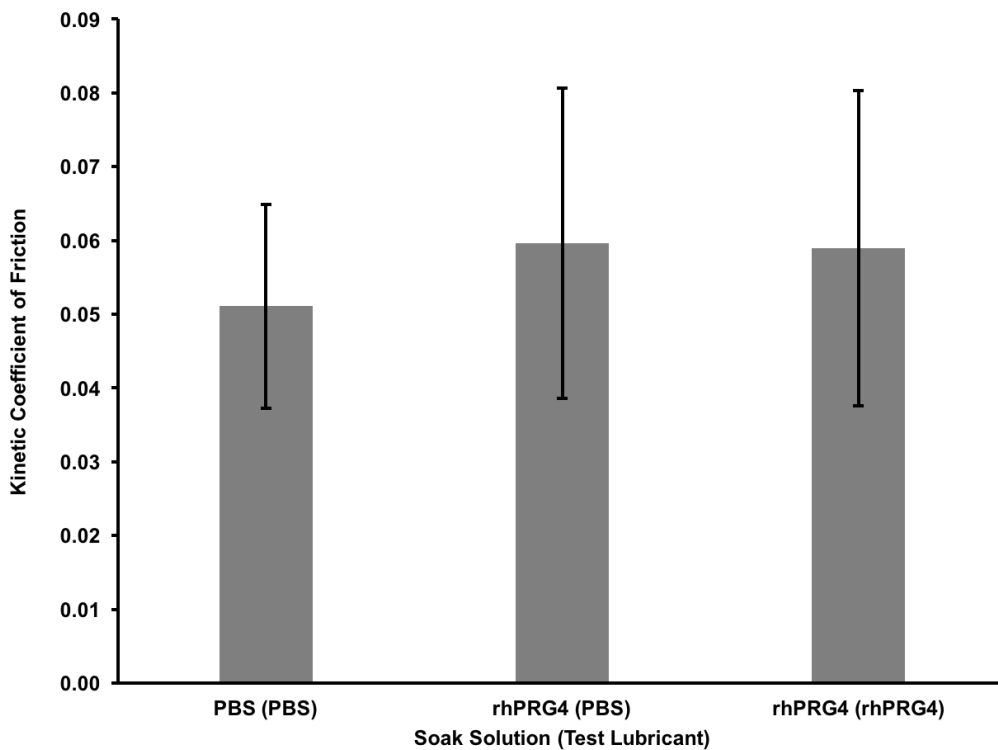


Figure 13 The effect of overnight incubation of rhPRG4 on the coefficient of friction of Air Optix Hydraglyde lenses ($n = 3$)

2.7.3.2 INSTANT INCUBATION OF AIR OPTIX HYDRAGLYDE IN RHPRG4

After considering the results of the overnight incubation of Air Optix Hydraglyde with rhPRG4, and with the dwindling supply of human eyelid tissue, it was decided that it would not be necessary to test the instant incubation. Since results were not statistically significant with the overnight

incubation, it was deemed unlikely that an effect would be seen with the instant incubation. Therefore, this test condition was not tested.

2.7.4 SUMMARY OF MAIN FINDINGS

Table 2 provides a summary of the main findings of the study. rhPRG4 reduced friction in both the soak and eye drop tests for Oasys and the soak test for Biofinity.

Table 2 Summary of the main findings of Chapter 2

Friction	Acuvue Oasys	Biofinity	Air Optix plus Hydraglyde
Soak (overnight incubation)	rhPRG4 reduced friction	rhPRG4 reduced friction	rhPRG4 did not affect friction
Eye drop (instant incubation)	rhPRG4 reduced friction	Results inconclusive	N/A

2.8 DISCUSSION

In this study, the *in vitro* friction of silicone hydrogel lenses incubated in rhPRG4 was evaluated. It was hypothesized that the incubation of silicone hydrogel lenses in rhPRG4 would result in a reduction of friction, even when tested in lubricant baths devoid of rhPRG4. Both the overnight and instant incubation of rhPRG4 was assessed in order to determine the *in vitro* coefficient of friction of commercial lenses at an eyelid-lens biointerface. It was found that rhPRG4 reduced friction in both the overnight and instant rhPRG4 incubation tests for Oasys and the soak test for Biofinity. Incubation of rhPRG4 did not affect Air Optix plus Hydraglyde, which could be due to the surface coating of the lens possibly preventing rhPRG4 from binding.

Similar to Air Optix Aqua, Air Optix Hydraglyde utilizes a unique, permanent plasma technology that results in a smooth surface that resists deposits. Communication with an Alcon representative revealed that the surface coating is a very strong surfactant (amphiphilic molecule with hydrophilic and hydrophobic ends), which can serve to increase wettability and prevent lipid deposition onto the contact lens surface. As it may be difficult for rhPRG4 to bind to Air Optix Hydraglyde due to its hydrophilic surface, it may possibly explain why rhPRG4 incubation did not influence the coefficient of friction for Air Optix Hydraglyde. Previous literature investigating the lubrication behavior of rhPRG4 on artificially controlled hydrophilic and hydrophobic surfaces also found that PRG4 did not lubricate a hydrophilic surface [89], [90].

A major limitation in this study was the amount of human eyelid tissue available for testing. Due to the lack of tissue available, some tests had a low number of repeats, resulting in low statistical power. With the use of eyelid tissue itself, there are also a few limitations. It is assumed that the human cadaver eyelid tissue obtained reflects the physiological properties of the eyelids of a normal group of healthy adults. However, due to the nature of the body donation program, tissue obtained was typically from subjects that are of old age (70+). Typically, more wear can be found on eyelids of older subjects, which may lead to altered mechanical properties of the tissue. This may influence the coefficient of friction measurements obtained. Another major limitation is that there is a relatively large amount of variability between different tissue samples. Because of variability in the tissues used during testing, it is not appropriate to directly compare friction coefficients between different types of lenses as the tests were separate tests conducted using different cadaver eyelids.

It is also important to consider the storage conditions of the tissue. After harvesting, the eyelid tissue was frozen in -20°C until it was defrosted for testing. It is assumed that the tissues were frozen quickly enough to avoid the morphological distortions and damage to the tissue that may result from slow freezing. It is also assumed that the freeze-thaw cycle of the eyelid did not alter any properties of the eyelid tissue, as preliminary testing of fresh vs frozen tissue showed no significant differences in friction. Although there are some limitations with the utilization of cadaver human eyelid tissue, it is believed to be the closest physiologically relevant alternative to testing *in vivo* human eyelid tissue. Multiple studies utilizing cadaver human eyelid tissue as a counter face have led to an improved understanding of *in vitro* contact lens friction testing [81], [90], [82].

This study demonstrates that incubation in rhPRG4, either instantaneously or overnight, can reduce *in vitro* friction of certain commercially available silicone hydrogel lenses. Results of this work indicate that rhPRG4 may be useful as a friction reducing lubricant, either as an eye drop solution or overnight cleaning solution. While results of this work shows that rhPRG4 incubation shows a statistically significant decrease in coefficient of friction for Acuvue Oasys and Biofinity, further studies will need to be carried out to determine whether results can lead to clinical relevance. These results of this study are consistent with previous studies demonstrating that rhPRG4 is able to adsorb to lenses [84] and reduce *in vitro* friction [87]. Collectively these results, when combined with the recent clinical trial data demonstrating that rhPRG4 is able to reduce signs and symptoms of dry eye, suggest that rhPRG4 could potentially be an effective friction reducing rewetting drop or overnight soak solution, and thus possibly improve *in vivo* contact lens comfort. Future directions may involve further *in vitro* tests to further understand and optimize rhPRG4 interaction

with the lens surfaces of silicone hydrogels, or even possibly *in vivo* clinical trials to assess the potential clinical utility of rhPRG4 in improving contact lens comfort.

Chapter 3 EVALUATING MUCIN-SOAKED PDMS AS A SYNTHETIC TEST COUNTERFACE FOR *IN VITRO* FRICTION TESTING

3.1 ABSTRACT

Objective: *In vitro* contact lenses friction significantly correlates with end of day contact lens discomfort. However, currently there is no industry standard for the *in vitro* evaluation of friction. There is a desire in the contact lens research community and among contact lens companies for a standardized method to measure the coefficient of friction precisely and accurately, now that the relevance of contact lens friction and comfort is becoming more well known. It is hypothesized that PDMS can be employed as a test counterface for the *in vitro* testing of contact lenses, resulting in similar friction coefficient values to that of cadaver human eyelid tissue. Thus, the objective of this study was to evaluate mucin-soaked PDMS as a synthetic counterface for *in vitro* friction testing of contact lenses.

Methods: A custom biomechanical friction test was developed to articulate the synthetic biomaterial over commercial contact lenses (Acuvue TruEye, Acuvue Oasys, Biofinity, Air Optix Hydraglyde) at an effective sliding velocity of 0.3 mm/s to determine the kinetic coefficient of friction (Mach-1, Biomomentum Inc). PDMS was chosen as the synthetic counterface and was modified through overnight incubation of MUC5AC (PDMS_{mucin}). The PDMS_{mucin} was articulated against contact lenses in a PBS bath, and effect of treatment was assessed by a repeated measures one way ANOVA.

Results: Pilot tests indicated that a PDMS_{mucin} counterface provided repeatable test results, exhibiting similar friction coefficients as human eyelid edge tissue for non-surface-treated lenses but did not replicate eyelid frictional values for a surface-treated lens. Differences in friction were observed between different types of lenses tested, with a large difference in friction found between surface-treated and non-surface-treated lenses.

Discussion: PDMS_{mucin} replicated frictional values for non-surface treated lenses but did not replicate values for lenses with surface treatments, suggesting that the use of PDMS_{mucin} as an counterface may be useful on some, but not all lenses. Although using PDMS_{mucin} does not completely mimic the mechanical properties of eyelid tissue, it provides a simple start towards finding a more appropriate biomaterial that can fully mimic the physiological and mechanical properties of the human eyelid edge tissue. This biomaterial, along with the Mach-1 testing methodology, can potentially be developed as an standardized testing method for testing *in vitro* contact lens- eyelid friction.

3.2 BACKGROUND

It has been found that the principal contact lens property associated with end-of-day comfort is the measurement of *in vitro* contact lens coefficient of friction. *In vitro* contact lenses friction has been shown to significantly correlate with end of day contact lens discomfort [56], [57]. However, there is currently no standardized method or gold standard for ocular friction testing. Many methods have been investigated, with differences in the testing methodology and set up of the studies, including differences in the counter surface material and geometry, normal force pressure, lubricating fluid, method of lens sample preparation and type of movement [59], [61], [71], [74].

With the numerous methodologies and techniques available to measure ocular friction, noticeable differences can be observed among the literature values for the coefficient of friction for different lenses. Rennie et al. measured the frictional forces between etafilcon A and borosilicate glass, finding frictional values of 0.025 and 0.075 for a range of frictional forces between 0.5 and 2.0 mN [71]. Using senofilcon A and stainless steel, Zhou et al. found that friction was proportional to [sliding speed]^{0.23} and exhibited a value of ~0.1 when observed at sliding speeds of 1 mm/s [72]. Roba et al.'s mucin-coated glass vs contact lenses study reported friction values ranging from 0.011 to 0.562 depending on contact lens type [59], whereas Samsom et al. utilized human cadaver cornea and eyelid tissue to measure the friction, reporting values ranging from about 0.05 to 0.13 depending on lens type and sliding speed [81]. Until a standardized method of friction testing is established, analysis of coefficient of friction between different studies and research groups will remain difficult. It is also uncertain whether *in vitro* test setups would be representative of the *in vivo* friction in the eye.

Although conducting experiments using human cadaveric tissue provides the closest physiologically relevant counterface, there are many limitations associated with using human cadaveric tissues. Human cadaver eyelid tissue obtained is typically from subjects that are of old age (70+) and would have more wear on their eyelid than what is typically found in healthy adults. There is also variability in tissue properties between each subject.

To take a step towards the creation of create a repeatable, standard method of testing that can be repeated between different laboratories, an artificial counterface was created in an attempt to minimize differences in results due to eyelid variability. This synthetic counterface was modified with mucin to mimic the physiological *in vivo* ocular surface. In the eye, the palpebral conjunctiva as well as the lid wiper surfaces are in contact with the lens during blinking. These surfaces are covered by a mucous layer consisting of glycosylated proteins; that is, mucins, which facilitates lubrication [91]. Therefore, PDMS was incubated in mucin to try to recreate the physiological interface in the eye.

It is hypothesized that PDMS can be employed as a test counterface for the *in vitro* testing of contact lenses, resulting in similar friction coefficient values to that of cadaver human eyelid tissue. Thus, the objective of this study was to evaluate mucin-soaked PDMS as a synthetic counterface for the *in vitro* friction testing of contact lenses.

3.3 METHODS AND MATERIALS

All methods and materials are utilized in this study are similar to those used in the cadaveric eyelid study as previously described in Chapter 2, with the artificial surface replacing the eyelid tissue.

Acuvue TruEye and Acuvue Oasys (Johnson & Johnson Vision Care Inc., Jacksonville, FL, USA), Biofinity (CooperVision, Scottsdale, NY, USA) and Air Optix Hydraglyde (Alcon, Fort Worth, TX, USA) were selected for the study and were purchased. The biomaterial chosen for the synthetic eyelid was polydimethylsiloxane or PDMS (Sylgard 184 Silicone Elastomer, Dow Corning Corporation, Midland, MI, USA). This material was chosen based on its mechanical and physiological properties, such as its ability to adsorb mucins and other tear components as well as its viscoelastic properties. To make the PDMS, a silicone elastomer base was combined with the curing agent at a 10:1 mass ratio. The mixture was then centrifuged at 3000 rpm for five minutes to eliminate all air bubbles before it was poured into a petri dish. The PDMS was then left to cure for 30 minutes at 70°C before being left overnight to cure at room temperature. A 3 mm biopsy punch was used to punch PDMS cylinders out of the petri dish. To adhere the PDMS onto the eyelid holder of the testing apparatus, silicone glue (All Purpose Silicone, General Electric, Boston, MA, USA) was employed and was left overnight to cure. Lastly, the PDMS cylinders were incubated in mucins overnight at room temperature before testing the next day. The incubation in mucins prior to testing was completed to better mimic the physiological interface at the ocular surface.

Purified pig gastric mucins were obtained from Dr. Oliver Lieleg, a collaborator from the Department of Mechanical Engineering of Technische Universität München in Germany. As described by Winkeljann et al. [92], the purification process was as follows: Mucus was collected by scraping the surface of pig stomachs, and the extracted mucus was dissolved in PBS (10 mM, 170 mM NaCl adjusted to pH 7.4) and purified through a series of centrifugation and size exclusion chromatography steps. Next, the solution was concentrated and desalinated by cross-flow dialysis

until a conductance of less than 50 μS was reached. Finally, the obtained mucin, MUC5AC, was stored in lyophilized form at $-80\text{ }^{\circ}\text{C}$ until further use. Successful purification of MUC5AC was verified by ELISA [92].

During experimental use, lyophilized MUC5AC was dissolved in PBS at a concentration of 1 mg/mL. Excess MUC5AC in PBS was aliquoted and refrozen at $-80\text{ }^{\circ}\text{C}$ until it was needed. PDMS, already glued onto the eyelid holder, was then incubated in the prepared MUC5AC solution overnight at room temperature before testing the next day.

3.4 EXPERIMENTAL DESIGN

Two testing sequences were employed using the artificial counterface. First, a pilot test was conducted to ensure that the PDMS was a biomaterial which would be a suitable choice as an artificial surface. In the first test sequence, Acuvue TruEye was chosen as the contact lens surface and PBS was utilized as the test lubricant. The PDMS was soaked in mucin overnight (becoming PDMS_{mucin}). During testing, the PDMS_{mucin} was first preconditioned, followed by three consecutive tests with PBS as a lubricant bath (Fig. 14). The PDMS_{mucin} and the contact lens were washed in PBS between each test.

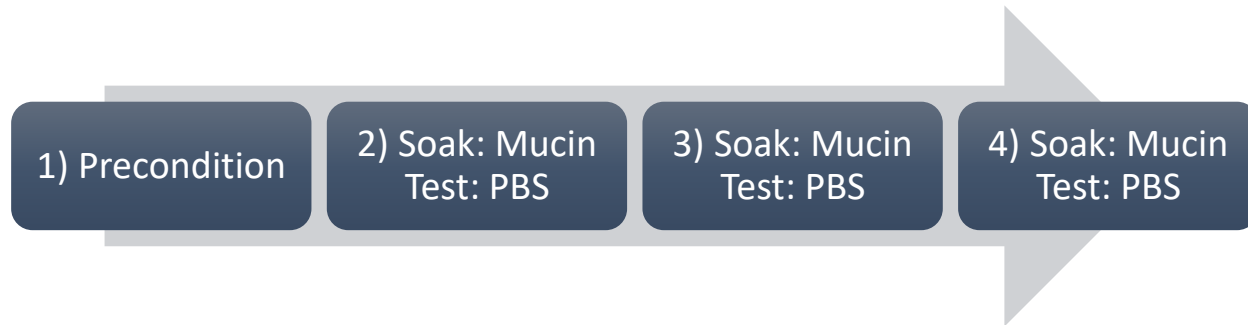


Figure 14 Test sequence for PDMS_{mucin} pilot testing with TruEye lenses

Next, the lenses of interest previously tested with rhPRG4 incubation tests (Acuvue Oasys, Biofinity, Air Optix Hydraglyde) were also tested in the second test sequence. Similar to the first test sequence, the PDMS was soaked in mucin overnight (becoming PDMS_{mucin}) and was tested in PBS, but different lenses were used (Fig. 15). Following the precondition of the PDMS in TruEye, the PDMS_{mucin} was tested against Oasys, Biofinity, and Air Optix Hydraglyde. The order of the lenses was changed with every test so that lens order would not play a role in the outcome of the friction results.

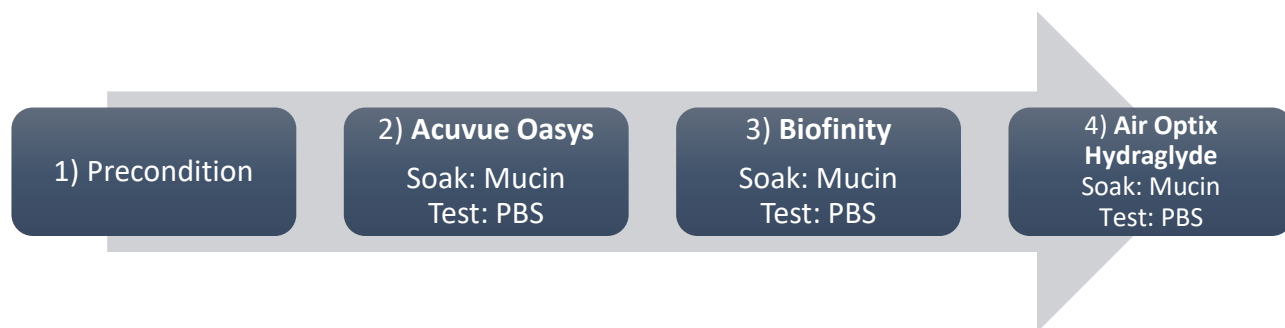


Figure 15 Sample test sequence for PDMS_{mucin} pilot testing with various commercial lenses

3.5 RESULTS

When PDMS_{mucin} was tested against the TruEye (with PBS as the test lubricant) three times in succession ($n = 3$), average friction values were 0.053 ± 0.012 , 0.049 ± 0.008 , and 0.047 ± 0.005 for each PBS test repeat respectively (Fig. 16, 17). A repeated measures ANOVA determined that each repeat of the PDMS_{mucin} in PBS was not statistically significant from each other ($F(2, 4) = 0.509$, $p < 0.05$, $\eta_p^2 = 0.203$). This was expected as there was no difference in test lubricant or test counterfaces. Friction values obtained were within the range of the eyelid tissue tests, providing enough information to verify the hypothesis that PDMS can be employed as a test counter surface for the *in vitro* testing of contact lenses, resulting in similar friction coefficient values to that of a

human eyelid. Thus, it was possible to proceed onto the next set of experiments comparing different types of lenses.

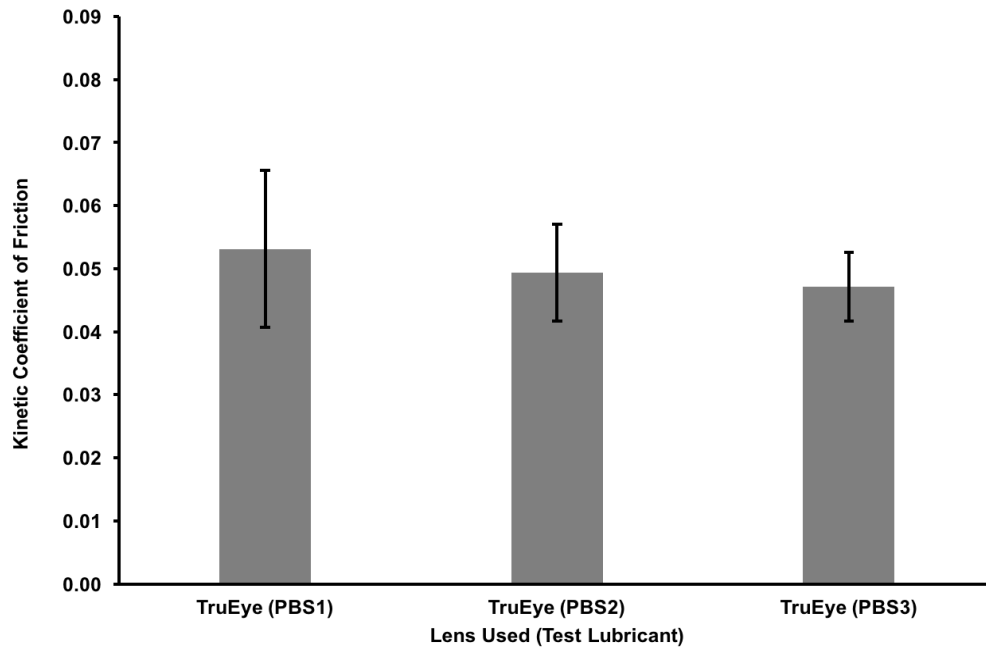


Figure 16 Averaged friction test results for PDMS_{mucin} articulated against Acuvue TruEye tested in PBS (n = 3) demonstrating repeatable results between tests

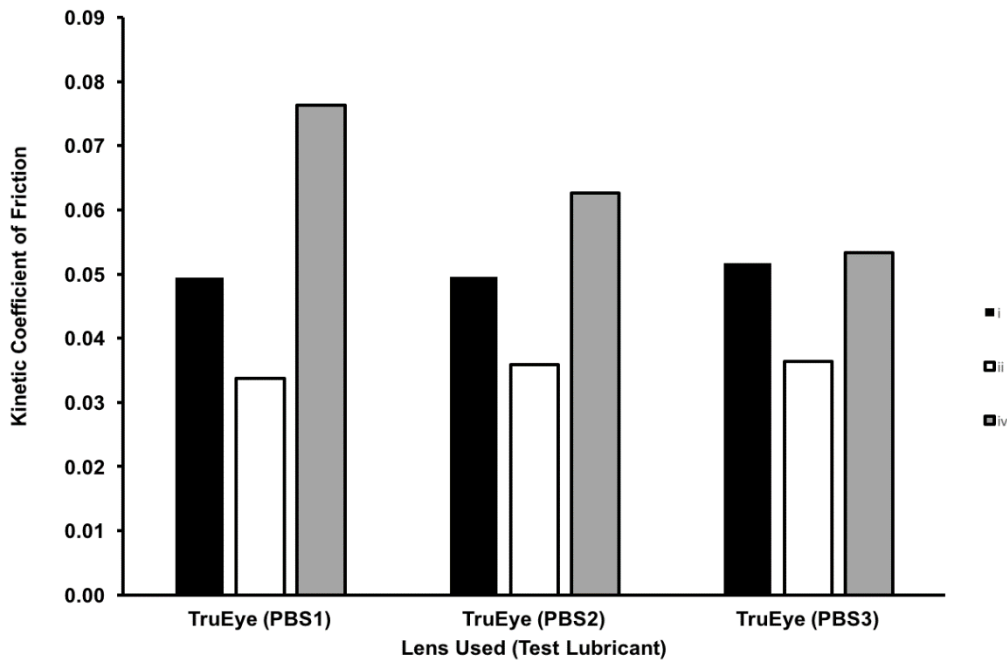


Figure 17 Individual friction test results for PDMS_{mucin} articulated against Acuvue TruEye tested in PBS (n = 3) demonstrating repeatable results between tests

When testing PDMS_{mucin} against the Acuvue Oasys, Biofinity and Air Optix Hydraglyde (n = 3) in PBS, friction values were 0.074 ± 0.011 , 0.055 ± 0.021 , and 0.273 ± 0.017 respectively (Fig. 18, 19). A repeated measures ANOVA determined that friction values differed statistically significantly between lenses ($F(2, 4) = 60.288$, $p < 0.05$, $\eta_p^2 = 0.968$). A LSD post hoc test revealed that the friction of Air Optix Hydraglyde was statistically significantly higher than both Oasys and Biofinity ($p < 0.05$). There was no statistically significant difference in friction between Acuvue Oasys and Biofinity lenses ($p > 0.05$).

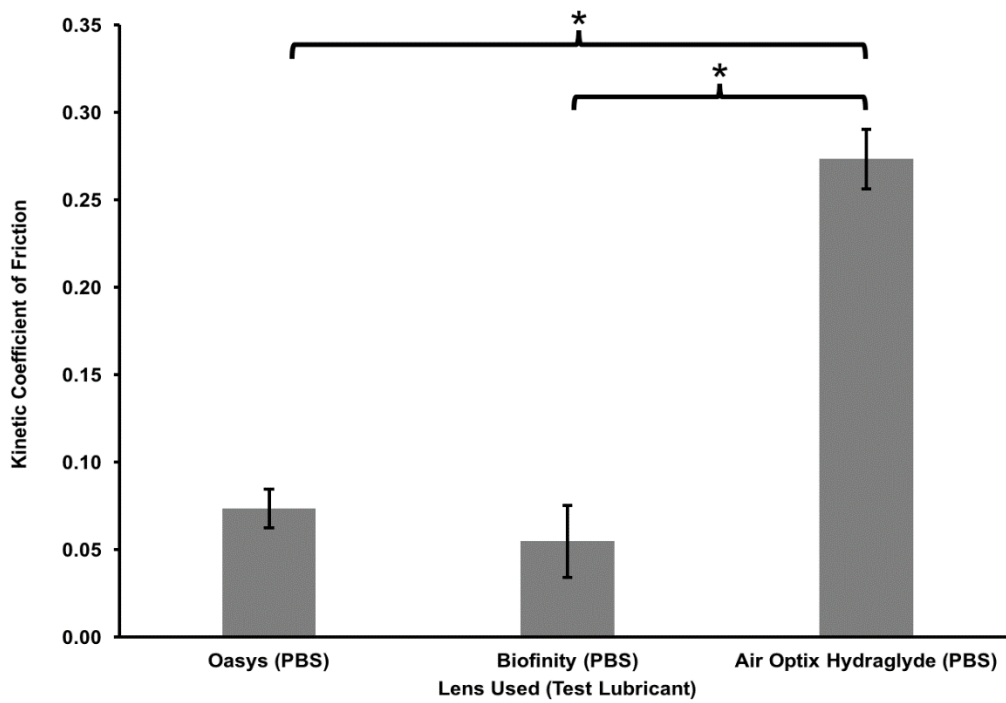


Figure 18 Averaged friction test results for PDMS_{mucin} articulated against multiple commercial lenses tested in PBS (n = 3) demonstrating observable differences between lenses.
 * denotes p < 0.05

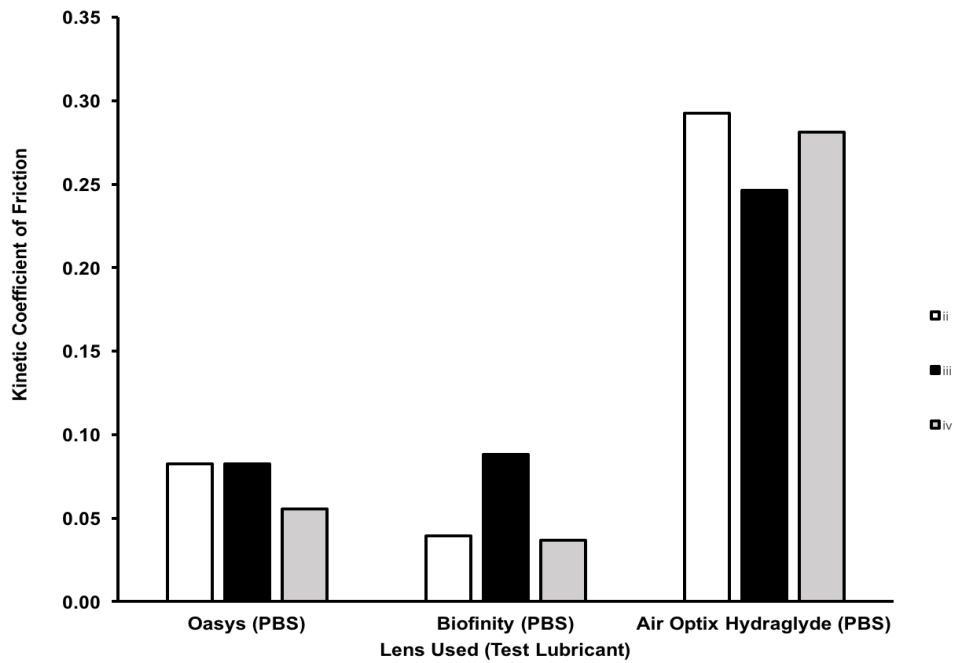


Figure 19 Individual friction test results for PDMS_{mucin} articulated against multiple commercial lenses tested in PBS (n = 3), demonstrating observable differences between lenses.

3.6 DISCUSSION

This study evaluated the feasibility of using mucin-soaked PDMS as a possible test counterface for *in vitro* contact lens friction testing. It was hypothesized that using PDMS_{mucin} may result in comparable friction values to that of cadaver human eyelid tissue. Results from this initial pilot testing with PDMS_{mucin} demonstrated that PDMS_{mucin} can exhibit similar friction coefficients as human eyelid edge tissue for non-surface treated lenses such as Acuvue Oasys and Biofinity, but may not replicate eyelid frictional values for lenses with special surface treatments such as Air Optix Hydraglyde.

Although testing conditions for the PDMS_{mucin} experiments were different compared to the eyelid tissue testing in Chapter 2, a simple comparison was conducted to determine if friction values were within the same range (Table 3). Friction values of the cadaver eyelid negative control (PBS soak, PBS test) in both the instant and overnight incubation tests were compared against the PDMS_{mucin} results. It was found that friction values were comparable between surfaces for the Acuvue Oasys and Biofinity, while friction values for the PDMS_{mucin} were much higher for the Air Optix Hydraglyde. A reason for this large observed difference in friction values between counterfaces may be a result of the SmartShield Technology surface treatment of the Air Optix Hydraglyde lens. This technology is described as a protective shield that helps the lens resist lipid deposits and delivers outstanding wettability and consists of a plasma surface treatment which renders the lens surface hydrophilic [32]. Communication with an Alcon representative revealed that the surface coating is a very strong surfactant, which can serve to increase wettability and prevent lipid deposition onto the contact lens surface. This plasma surface treatment may have prevented the ability of mucin to interact with the lens surface. The hydrophobic domains of mucins are crucial for the adsorption onto and lubrication of hydrophobic surfaces such as PDMS and many human tissue surfaces [93]. The lack of mucin interaction on the lens may have factored into the high friction values observed with the Air Optix Hydraglyde. In comparison, both the Acuvue Oasys and Biofinity do not contain any surface treatments for the lenses and friction values were fairly comparable between the biological and synthetic surfaces.

Table 3 Comparison between friction values from synthetic and biological counterfaces

	Human eyelid tissue		PDMS _{mucin}
Acuvue Oasys	0.109±0.001	0.106±0.006	0.074±0.011
Biofinity	0.070±0.006	0.050±0.004	0.055±0.021
Air Optix Hydraglyde	0.051±0.014		0.273±0.017

Furthermore, in both sets of tests with the PDMS_{mucin}, it was observed that the raw force data differed from the eyelid tissue data in a couple areas. While human eyelid tissues were tested at pressures of 1-20 kPa, the corresponding normal force was typically in the range of 0-0.05N. However, pressures of 1-20 kPa with PDMS_{mucin} resulted in corresponding normal force of only 0-0.01N. A decision was made to test from pressures of 1-35 kPa to obtain normal forces of 0-0.03N so that normal forces were more comparable to those from eyelids. It is possible that the lower resulting forces from the PDMS_{mucin} is due to a smaller contact area of the PDMS_{mucin} compared to the eyelid tissue. The PDMS_{mucin} material was in the shape of a cylinder, which may have had a smaller contact area compared to the flatter shape of the eyelid tissue which also had better viscoelastic properties. This study limitation can be addressed by molding the PDMS into a more rectangular shape and tuning the PDMS to alter its viscoelastic properties for future studies.

Another limitation of this study is that the adsorption of mucin onto the PDMS was not characterized. Mucin adherence levels should be quantified in future studies. Mechanical properties of the PDMS should also be determined and compared to human eyelid tissue. However, this study was simply a pilot study to investigate the viability of PDMS in a very preliminary sense. With the basic data acquired from this study, the properties of PDMS in future studies can be fine-tuned in order to better mimic the mechanical properties of eyelid tissue.

When comparing the friction results of Acuvue Oasys of this study with other studies measuring *in vitro* friction, values span the kinetic friction coefficient values reported. For example, Roba et al. reported values of 0.03 using lenses against mucin-coated glass [59] while Huo et al. reported values of 0.2 when using an atomic force microscope (AFM) to measure contact lens against a

silica colloidal probe [75]. Samsom et al. reported values of 0.09 when also testing Acuvue Oasys against human eyelid tissue, though the friction testing methodology utilized was different [81]. However, it is important to recognize that it is extremely difficult to compare frictional values between different test set ups, different testing methodology and other factors such as counterface material and geometry, normal force pressure, lubricating fluid, sample preparation methods and type of movement. This once again reinforces the need of a standardized testing method and procedure for *in vitro* contact lens friction testing.

Although using PDMS_{mucin} as an artificial counterface does not completely mimic the mechanical properties of eyelid tissue and is one of the limitations of the study, it provides a simple commencement towards finding an ideal biomaterial. This future biomaterial would likely be more complicated in composition and should fully mimic the physiological and mechanical properties of the human eyelid edge tissue. This biomaterial along with the Mach-1 testing methodology can potentially be developed as an American Society for Testing and Materials (ASTM) testing standard for testing *in vitro* contact lens- eyelid friction. This would allow for more consistent *in vitro* tests which can provide reliable friction measurements which can hopefully be translated to *in vivo* testing.

Chapter 4 CONCLUSIONS

4.1 SUMMARY OF FINDINGS

The overall purpose of this thesis work was to contribute to the knowledge of *in vitro* ocular friction testing, specifically by evaluating the effects of rhPRG4 as an ocular surface boundary lubricant and evaluating both biological and synthetic test counterfaces. The effect of rhPRG4 incubation time and lubricant bath on the *in vitro* coefficient of friction of commercial lens at an eyelid-lens biointerface was assessed, and it was found that the incubation of certain lenses in rhPRG4 led to reduced *in vitro* contact lens friction. Furthermore, mucin-soaked PDMS was assessed as a potential synthetic analog of human eyelid edge tissue for *in vitro* contact lens friction testing, and it was determined that PDMS_{mucin} can exhibit comparable friction values to human eyelid for certain lenses.

4.2 DISCUSSION

Results from Chapter 2 demonstrates that incubation in rhPRG4, either instantaneously or overnight, is able to reduce the *in vitro* friction of certain commercially available silicone hydrogel lenses. Overnight rhPRG4 incubation reduced the friction of non-surface-treated Acuvue Oasys and Biofinity lenses, but did not influence surface-treated Air Optix Hydraglyde lenses. It is speculated that the surfactant properties of the plasma surface treatment of the Air Optix Hydraglyde may be a factor on why no effect was observed. It is possible that the plasma polymerization surface treatment of Air Optix Hydraglyde acted as a barrier and prevented rhPRG4 from properly adsorbing onto lenses in the correct orientation. These results are consistent with previous studies demonstrating that rhPRG4 is not able to adsorb into the bulk of lenses with surface treatments [84]. Cheung et al. utilized confocal microscopy to provide a visualization of

rhPRG4 sorption onto lens surfaces, finding that rhPRG4 was exclusively restricted to the lens surfaces with virtually no presence within the bulk of the lens in Air Optix Aqua lenses, which have the same composition as Air Optix Hydraglyde lenses. On the other hand, a uniform distribution throughout the bulk and surfaces of the lens was observed with Acuvue Oasys and Biofinity [84]. These results emphasize that there are fundamental differences in lens material composition between different commercial lenses that needs to be recognized when developing methods to reduce *in vitro* lens friction.

Overall, results from this study, when combined with the recent clinical trial data demonstrating that rhPRG4 is able to reduce signs and symptoms of dry eye [83], suggest that rhPRG4 could potentially be an effective friction reducing rewetting drop or overnight soak solution, and thus possibly improve *in vivo* contact lens comfort. rhPRG4 is currently being developed by Novartis as a drug for the treatment of dry eye disease. With the approval of rhPRG4 as a drug, this thesis work may enable researchers to understand which contact lenses rhPRG4 may work best with for friction reduction on the ocular surface. rhPRG4 has been shown to have potential as an effective boundary lubricant on the ocular surface, but there remains much more work in determining how it interacts with different lens types and test lubricants after various incubation times. Results from this research project aimed to fill those knowledge gaps and provided valuable information towards the development and design of more clinical trials for rhPRG4 and ultimately towards the potential use of rhPRG4 as a rewetting eye drop or in a cleaning solution.

Preliminary testing from Chapter 3 demonstrated that the use of a synthetic biomaterial as an eyelid tissue alternative can provide repeatable results that are within the range of friction of cadaver

human eyelid tissue for certain silicone hydrogel lenses. Numerous previous studies have used glass, mucin-coated silanized glass, and stainless steel as counterfaces. Although using a hard counterface simplifies the quantification of deformational behavior, it does not properly reflect the physiological properties of human eyelid tissue which is much softer. This thesis work capitalized on the soft, porous and tunable properties of PDMS, along with its ability to undergo surface modification with mucin, which allowed for a more physiological relevant representation of the human eyelid during friction testing. This study provides the basis needed to further investigate and develop a biomaterial that can mimic the physiological and mechanical properties of the human eyelid edge tissue. If successful, this biomaterial, along with the Mach-1 testing methodology can potentially be developed as an American Society for Testing and Materials (ASTM) testing standard to test *in vitro* contact lens-eyelid friction.

4.3 LIMITATIONS

With the complexity of coefficient of friction measurements, experimental values of friction coefficients must be treated with caution. Experimental values determined during this study using the Mach-1 system relates to a particular combination of load, concentration of lubricant, orientation of the sample, rate and distance of travel. When using the Mach-1 testing system, great care must be taken to ensure all protocol was properly followed during the friction testing process. Specifically, it was sometimes difficult to cut the cadaver eyelid tissue into the perfect shape and adhere it onto the eyelid holder such that no glue was in contact with the testing surface. Furthermore, if the sample from the eyelid was too thick, the sample was prone to rolling over as it reciprocated over the lens, affecting friction results. It would be of interest, and value, to improve the standard operating protocol of the friction testing method such that user variability is reduced

and the eyelid sample size can be standardized. Furthermore, although these results can provide a useful indication of friction compared to similar materials of similar modulus, careful thought must be undertaken when comparing frictional values across different studies.

While the Mach-1 testing system is quite versatile and powerful, it also has its own limitations when employed in the context of this thesis work. It is assumed that the Mach-1 system is sensitive enough to distinguish the difference in friction measurements between different lens types, test lubricants, and incubation times. The Mach-1 has an actuator resolution of 0.0001 mm under compression. The load cell precision is described as one part in 20 000 of the maximum load while the displacement resolution can be as low as 100 nanometers, depending on the configuration. With the current protocol, it is theoretically possible to resolve friction coefficients down to 0.001 at a lid wiper-contact lens interface.

Another limitation in this study is that during the Mach-1 mechanical friction test, the eyelid is translated by 10 degrees along the curvature of the lens. This test setup does not simulate a full eye blink, but was developed to measure boundary lubrication at a physiologically relevant biointerface. In actuality, an eye blink would have a much higher degree of translation and would move at a faster speed than the 0.3 mm/s used in the program. Furthermore, multiple mode of lubrication may be operative as well in addition to the boundary lubrication regime. Despite all these limitations, the Mach-1 testing system enabled the evaluation of rhPRG4 as an ocular surface lubricant and played a vital role in the comparison between biological and synthetic counterfaces. This thesis work provided the foundation for future studies and potential development and enhancement of the test method.

Lastly, it is important to recognize that the friction testing employed in this study simply provided *in vitro* measurements and are not be fully representative of *in vivo* friction values and the efficacy of *in vivo* measurements. Although statistically significant differences may be found between testing conditions, this may not represent clinical relevance in terms of improved comfort. However, with the abundance of *in vitro* friction work paired with emerging clinical trial data, there is hope that this area of research will play a role in improving contact lens comfort.

4.4 FUTURE WORK

The determination of the frictional properties of soft contact lenses is a complicated process. Despite numerous attempts from multiple research groups to develop a friction testing methodology, the “gold standard” of *in vitro* contact lens friction testing has yet to be agreed upon. However, this need for a standardized method is becoming more apparent now that the role of ocular tribology and its correlation on contact lens discomfort is recognized among the research community. Moving forward, it is of utmost importance to create this industry standard in contact lens friction testing so that scholars can access and utilize standardized data to better understand *in vitro* friction and how it compares the *in vivo* friction within the eye. Further characterization studies and friction testing using the Mach-1 testing method will help elucidate whether it would be a suitable candidate as the standardized industry testing method. The choice of a counterface and testing method will not be simple, but the ultimate goal would be to have a new system that replicates the rank order of lenses in terms of comfort, so that new and even more lubricious (and comfortable) lenses can be developed.

In regard to improving contact lens comfort by reducing ocular friction, results of this thesis work has shown that rhPRG4 incubation shows a statistically significant decrease in the coefficient of friction of some, but not all contact lenses. These studies can be replicated with higher statistical power to verify results, as well as with a higher variety of commercial lenses. It would also be of interest to determine if there is a dose dependent response for rhPRG4, to see if higher or lower concentrations of rhPRG4 would affect friction results. Lastly, given hyaluronan's known boundary lubrication effects, it would be of interest to investigate synergistic lubrication properties of HA and rhPRG4. Indeed, research has already begun in this area, showing that synergistic lubrication was observed for methacryloxy-propyltris (trimethylsiloxy) silane (pHEMA/TRIS) silicone hydrogels [87], [90]. Other areas of research that may be investigated with rhPRG4 includes potential technologies to modify rhPRG4 to enable covalent attachment and binding to lenses [94]–[97] or even the future development of a special lens for the purpose of specific use with rhPRG4. Another area of future work may focus on the mechanobiology of PRG4 on the ocular surface. It would be of interest to understand the effect of contact lens friction on PRG4 expression by corneal epithelial cells, as PRG4 expression by chondrocytes is regulated both chemically as well as mechanically. For example, it would be important to determine whether an increased shear on the ocular surface would cause PRG4 expression to increase or decrease.

Overall, this thesis work made progress towards evaluating the effects of rhPRG4 as an ocular surface boundary lubricant and evaluating both biological and synthetic test counterfaces to improve understanding of *in vitro* contact lens friction. Furthering this thesis work will contribute towards identifying efficacious use of a commercialized rhPRG4 product in terms of improving contact lens wear, as well as contributing towards the development of an *in vitro* test that can

accurately reflect *in vivo* contact lens friction. The ultimate, long term objective of this work is to develop a low friction contact lenses with the goal of improving contact lens comfort and reducing signs and symptoms of dry eye in contact lens wearers.

Though contact lenses are currently very commonly used, they may become even more widely used once contact lenses with electronic components are introduced and receive regulatory approval. Scientists are already working to develop contact lenses with novel capabilities, specifically in three areas: drug delivery, biosensing and visual augmentation. Contact lenses has been thoroughly investigated as a means to deliver pharmaceuticals to the eye in a controlled manner, where optimal concentrations of medication can be slowly released overnight when eyes are closed and not accessible for eye drop application [98], [99]. With intelligent biosensing contact lenses, data can be collected on the dimensions of each individual eye and tear film production upon initial insertion, which could help facilitate determining the most suitable contact lens product for each specific eye [100]. Biosensing contact lenses could also be used to monitor intra-ocular pressure, with electronic sensors and strain gauges transmitting eye pressure measurements via an antenna to a receiver kept on the wearer [101]. Lastly, contact lenses can be utilized in visual augmentation. For example, contact lenses with auto-focusing abilities are being developed [102], as well as lenses that can generate images directly on the contact lens through light emission directed towards the retina [103]. Looking even further into the distant future, it is quite possible that contact lenses become irrelevant with the technological advances and developments into visual prostheses and bionic eyes. For now, until these future technological developments become a reality, what one can accomplish is to continue to develop a low friction, more comfortable contact lens.

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APPENDIX

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Monday, December 4, 2017 at 11:04:30 AM Mountain Standard Time

Subject: Re: Copyright request

Date: Monday, December 4, 2017 at 11:02:04 AM Mountain Standard Time

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