Antifungal Ocular Drug Delivery
via Contact Lenses Using a Novel in Vitro Eye Model

by

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AUTHOR'S DECLARATION

This thesis consists of materials all of which I authored or co-authored: See Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.
Statement of contributions
I would like to acknowledge the names of my co-authors who contributed to this thesis:

- Dr. Lyndon Jones, PhD
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- Hendrik Walther, MSc
- Magdalena Bajgrowicz, MSc
- Huayi Gao
- Jordan Rossy, BSc
Abstract

Purpose:

The purpose of this thesis was to evaluate the potential of contact lenses (CLs) as an antifungal drug delivery device, and to develop an in vitro eye model to test thereof.

Methods:

The first three chapters focused on developing a CL to function as a drug delivery device for natamycin, the only commercially available antifungal:

- In the first experiment (Chapter 3), the in vitro uptake and release characteristics of natamycin from several commercially available CLs were evaluated.

- In the second experiment (Chapter 4), to improve the release characteristics of natamycin from contact lenses, an attempt was made to incorporate novel drug-encapsulated nanoparticles (Dex-b-PLA) within the CLs.

- In the third experiment (Chapter 5), an alternative strategy employing the incorporation of cyclodextrin (CDs) within the CL polymer matrix was evaluated as a potential modification to prolong the release of natamycin.

The second half of the thesis was aimed at developing a sophisticated in vitro ocular model capable of adequately measuring drug release from CLs:

- In Chapter 6, the design of a novel in vitro eye model to simulate the physiological ocular environment was outlined.

- In Chapter 7, this model was used to evaluate the release of the antifungal fluconazole from commercially daily disposable CLs.
In chapter 8, as an extension of the developed \textit{in vitro} eye model, an agar eye model was developed to test the effects of natamycin and fluconazole-releasing CLs on \textit{Candida albicans}.

**Results**

Commercial CLs, after drug incubation with natamycin, will release the drug rapidly within the first half hour, followed by a plateau phase. However, when CL materials were loaded instead with natamycin encapsulated within novel Dex-\textit{b}-PLA nanoparticles, the release duration was extended to 12 hours. Modifying the CL polymer with methacrylated CDs did not significantly improve drug release. On the contrary, high loading of CDs decreased overall drug delivery efficiency, likely resulting from unfavourable arrangements of the CDs within the polymer network.

The developed ocular platform, termed Ocuflow, simulates physiological tear flow, tear volume, air exposure and mechanical wear. When this system was used to analyze the release of fluconazole from commercial CLs, the drug release was sustained for up to 24 hours. This observation significantly contrasts drug release observed in a vial, which typically follows a burst-plateau profile. When CLs releasing natamycin and fluconazole were tested on agar eye models that were inoculated with \textit{Candida albicans}, the growth of the yeast was limited by natamycin-containing CLs. The cell morphology of the yeast also differed noticeably based on drug-lens combinations.
Conclusions

This thesis details potential strategies to develop novel CLs for antifungal ocular drug delivery. The Ocuflow system developed from this thesis is highly versatile; not only can it be used effectively to measure drug release from CLs, but it can also be applied to other in vitro analyses with CLs.
Acknowledgements

I would like to express my sincere gratitude to my supervisors, Lyndon Jones and Lakshman Subbaraman for their continuous support of my PhD study and related research. In addition, I’d like to thank my committee members Maud Gorbet, and David McCanna for sharing their expertise in cell biology and microbiology.
Dedication

Thanks to my mom and dad whom have given up their dreams so that I could achieve mine.
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List of symbols

%                  percentage
°                  degree
°C                 degrees centigrade
g                 gram
h                 hours
L                 liter
M                 molar
mg               milligram
mL               milliliter
mm               millimeter
min              minutes
nm               nanometer
pH                negative logarithm hydronium ion concentration
pKa              logarithmic acid dissociation constant
µg               microgram
µL             microliter
µm               micrometer
**List of abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BAB</td>
<td>blood aqueous barrier</td>
</tr>
<tr>
<td>BRB</td>
<td>blood retinal barrier</td>
</tr>
<tr>
<td>CCLR</td>
<td>Centre for Contact Lens Research</td>
</tr>
<tr>
<td>CFU</td>
<td>colony forming units</td>
</tr>
<tr>
<td>CL</td>
<td>contact lens</td>
</tr>
<tr>
<td>DMAA</td>
<td>N,N-dimethylacrylamide</td>
</tr>
<tr>
<td>DEX</td>
<td>dextran</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>EGDMA</td>
<td>ethylene glycol dimethacrylate</td>
</tr>
<tr>
<td>EVA</td>
<td>ethylene vinyl acetate</td>
</tr>
<tr>
<td>FDA</td>
<td>United States Food and Drug Administration</td>
</tr>
<tr>
<td>FM0411M</td>
<td>(\alpha)-methacryloyloxyethyliminocarboxyethoxypropyl-poly(dimethylsiloxo)-butyldimethylsilane</td>
</tr>
<tr>
<td>HEMA</td>
<td>hydroxyethyl methacrylate</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>HOB</td>
<td>2-hydroxybutyl methacrylate</td>
</tr>
<tr>
<td>IBM</td>
<td>isobornyl methacrylate</td>
</tr>
<tr>
<td>M3U</td>
<td>(\alpha\omega)-bis(methacryloyloxyethyliminocarboxy ethoxypropyl)-poly(dimethylsiloxane)-poly(trifluoropropylmethylsiloxane)-poly((\omega)-methoxy-poly(ethyleneglycol)propyl methylsiloxane)</td>
</tr>
<tr>
<td>MAA</td>
<td>methacrylic acid</td>
</tr>
<tr>
<td>MIC</td>
<td>minimum inhibitory concentration</td>
</tr>
<tr>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td>minimum inhibitory concentration for 90% of microbial isolates</td>
</tr>
<tr>
<td>NVA</td>
<td>N-vinyl amino acid</td>
</tr>
<tr>
<td>NVP</td>
<td>N-vinyl pyrrolidone</td>
</tr>
</tbody>
</table>
MEHQ  4-methoxyphenol
mPDMS  monofunctional polydimethylsiloxane
NaCl  sodium chloride
NP  nanoparticle
NSERC  Natural Science and Engineering Research Council of Canada
PABA  p-amino benzoic acid
PBS  phosphate buffered saline
PBVC  poly[dimethylxiloxy] di [silylbutanol] bis[vinyl carbamate]
PC  phosphorylcholine
PCL  poly(caprolactone)
PDMS  polydimethylsiloxane
PEG  polyethylene glycol
PMMA  poly(methyl methacrylate)
PGA  poly(glycolide)
PLGA  poly(lacto-co-glycolide)
PLA  Poly(D,L-lactide)
pHEMA  poly(hydroxylethyl methacrylate)
PVA  poly vinyl alcohol
PVP  poly vinyl pyrrolidone
TAIC  1,3,5-triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione
TEGDMA  tetraethyleneglycol dimethacrylate
TPVC  tris-(trimethylsiloxy)silyl propylvinyl carbamate
TRIS  methacryloxy propyl tris(trimethylsiloxy)silane
VMA  N-vinyl-N-methylacetamide
Chapter 1 – General Introduction

Since their conception, contact lenses (CLs) have enjoyed considerable success in the vision correction market.¹ With significant progress within the last two decades in material science, nanotechnology, and microelectronics, various novel applications for CLs also have been explored such as drug delivery,²-⁵ intraocular pressure monitoring,⁶-⁹ glucose monitoring,¹⁰-¹⁷ and visual reality augmentation.¹⁸,¹⁹ Not surprisingly, this research has evolved the CL field well beyond its original focus as a simple refractive error correcting device. This evolution has attracted the attention of other disciplines, as well as major technology and pharmaceutical companies such as Google and Novartis.¹⁶ As such, there is enormous potential for unique and rapid growth in this field.

The focus of this thesis is to investigate the potential of CLs as an ocular drug delivery device to treat various ocular diseases, in particular fungal keratitis.²,²⁰ Amongst ocular infections, fungal keratitis is a major concern throughout the world due to its rapid progression to vision loss and potentially blindness.²¹,²² Currently, topical administration using eye drops account for 90% of all ophthalmic formulations.²³-²⁶ However, the ocular structures present numerous barriers to effective drug absorption and penetration.²⁷-²⁹ As a result, multiple dosing is often required for aggressive fungal infections, which in turn leads to problems with patient compliance³⁰,³¹ and potential drug overdose.³² The application of CL as a drug delivery vehicle presents two advantages: (1) drug protection from corneal removal mechanisms and (2) the intrinsic ability of a soft lens material to absorb and release drugs over time. The latter aspect, to increase the amount and extend the release duration of drugs from a CL, will be a primary focus of this thesis.
To better understand the current state in this area, the following introduction chapter will provide an overview of the anatomical features of the eye, ocular drug delivery approaches, development of CLs, and ocular mycoses. The chapter will conclude with current research towards developing a CL that could be useful in the management of fungal keratitis, and potential *in vitro* models to evaluate these devices.
1.1 Anatomy of the eye

Figure 1-1 Anatomy of the eye (Figure courtesy of the National Eye Institute, National Institute of Health,\textsuperscript{33})

The anatomical structures of the eye present several unique ocular barriers for ophthalmic drug delivery. In this regard, the structures of the eye can be divided into the anterior and posterior segments; the division occurs anterior and posterior to the physiological crystalline lens.\textsuperscript{34} This division helps classify the ocular tissues that can be reached using topical treatment such as eye drops, and those tissues which will need invasive or systemic routes.\textsuperscript{34} The main structures of the eye are highlighted in Figure 1-1. Because the focus of this thesis is topical drug delivery, only the structures of the anterior segment will be discussed.
1.2 Anterior segment barriers

The anterior segment includes the cornea, sclera, iris, ciliary body, pupil, trabecular meshwork, Schlemm’s canal, anterior chamber, and the posterior chamber.23, 34 The cornea is the most anterior structure of the eye and consists primarily of an organized, avascular, hydrated collagen network, which is partitioned into three main layers.23, 34 When the eye is closed, the ocular adnexa, consisting of the eyelashes and eyelids, cover the cornea. The conjunctiva, a mucous membrane, covers the front of the eye and lines the inside of the eyelids.

The anterior most layer of the cornea consists of the corneal epithelium, which is several layers thick and held together by tight junctions. The function of the corneal epithelium is to seal off the ocular surface and control the influx and efflux of water and aqueous soluble substances.23, 34 Posterior to the epithelium is the stroma, which accounts for 90% of the corneal thickness. It is composed of transparent networks of collagen fibers. The posterior side of the cornea is covered by a single layer of specialized endothelium, which functions to control corneal hydration and transparency.35, 36

The three distinct sections of the cornea pose a significant barrier for topical drug absorption. The epithelium and endothelium limit the penetration of hydrophilic molecules, while allowing lipophilic molecules to pass through. In contrast, the hydrated stroma behaves in the opposite fashion and prevents lipophilic molecules from passing.34 As such, in order for a drug to successfully penetrate through the cornea, the drug or its delivery vehicle need to possess both hydrophilic and hydrophobic properties.37 Other drug properties, such as molecular size, shape and ionization will also determine a drug’s ability to pass through the cornea.23, 36
The area surrounding the cornea is the limbus, a highly vascularized network comprised of corneal epithelial stem cells and the conjunctiva. Due to the significantly higher surface area and vascular supply, a significantly larger portion of the drugs instilled from topical routes are absorbed into the conjunctiva compared to the cornea. Drug uptake into the conjunctiva is not considered ideal, as these drugs are quickly transferred to the systemic circulation via the surrounding blood vessels and the lymphatic system.

The sclera is a continuation of the cornea, consisting of white opaque collagen fibers organized into very tough tissue. The entire ocular surface is lubricated through the production of tears, which are secreted by the lacrimal and associated glands. The tear fluid functions to lubricate the eye, transport nutrients, remove waste and acts as a first line of defense against pathogens. However, in conjunction with blinking, tear fluid can also act as a drug barrier by...
effectively washing,\textsuperscript{27-29} dispersing,\textsuperscript{40} and draining drugs from the eye following topical administration.\textsuperscript{27, 29}

Posterior to the cornea is the anterior chamber. The iris, which controls the amount of light that enter the eye, separates the anterior chamber from the posterior chamber. The anterior chamber is filled with the aqueous humor, a protein-fluid secreted by the ciliary body.\textsuperscript{41} The posterior chamber contains the aqueous humor and houses the lens, which serves as the physical separation between the anterior and posterior segment of the eye.\textsuperscript{41} The crystalline lens is the most posterior structure in which drugs applied topically can penetrate.\textsuperscript{34} Structures beyond the lens are minimally affected by topical administration.\textsuperscript{38}

\textbf{Table 1-1 Ocular barriers to drug absorption}

<table>
<thead>
<tr>
<th>Ocular barriers</th>
<th>Drug loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea</td>
<td>Hydrophobic and hydrophilic barriers prevent drug penetration.\textsuperscript{34}</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>Drugs absorbed into the conjunctiva are removed by systemic routes.\textsuperscript{23, 37}</td>
</tr>
<tr>
<td>Tear fluid</td>
<td>Dilutes and drains drugs from the ocular surface\textsuperscript{27-29}</td>
</tr>
<tr>
<td>Eyelid</td>
<td>Disperses and removes drugs from the ocular surface\textsuperscript{40}</td>
</tr>
</tbody>
</table>

\textbf{2.1 Ocular drug delivery}

The eye is readily accessible to topical treatment, and thus local therapy is often preferred over systemic therapy to minimize side effects.\textsuperscript{42} Furthermore, the two blood barriers, the blood aqueous barrier (BAB) and the blood retinal barrier (BRB), present a significant hurdle for effective drug absorption via the systemic route.\textsuperscript{36, 42} Topical therapy also bypasses the hepatic first pass metabolism of drugs in the liver.\textsuperscript{37} In the ideal scenario, the administered drug from a topical dosing can treat the disease in a timely fashion with minimal side effects. For this to happen, drug penetration must be effective and exclusive to the targeted corneal tissue. However,
due to the ocular anatomy, in conjunction with mechanisms to remove unwanted substances from the ocular surface, drug penetration is very ineffective. The ocular residence time of a typical eye drop is less than 5 minutes. Consequently, it has been estimated that only between 1-7% of the drugs administered from an eye drop will reach the target tissue, while the remainder is cleared or absorbed into systemic circulation. To compensate for low residence time and poor drug penetration, the viscosity of the drop can be increased or frequent dosing can be employed. However, these strategies come at a cost to convenience, patient compliance and side effects of drug overdosing. In order to effectively address the above problems, the ideal drug delivery vehicle must be able to ensure effective corneal penetration, while concurrently limiting drug loss. Furthermore, treatments for diseases should also preserve visual function in order to ensure acceptance by practitioner and patients.

2.2 Eye drops and ophthalmic formulations

The anterior structures of the eye are readily accessible, and thus eye drops are an obvious choice for the treatment of many ocular disorders. However, for many conditions, frequent dosing is necessary and patient compliance becomes a primary concern. For instance, in the treatment of fungal keratitis, eye drops have to be instilled at hourly intervals over the course of several days to a week. Ophthalmic ointments can significantly improve the ocular residence time of a drug, as the increased viscosity of the ointment makes it more difficult for them to be removed from the ocular surface through tears and blinking. However, ointments are often associated with blurred or “smeary” vision, and their usefulness is typically limited to overnight applications.
2.3 Anterior drug delivery approach

Table 1-2 summarizes currently available drug delivery systems for the anterior eye.

The first commercial drug delivery device for the anterior segment was introduced by Alza in 1974. The ocular insert, branded as Ocusert® pilocarpine, was designed to control intraocular pressure for the treatment of glaucoma by releasing pilocarpine over 7 days. The device consists of a layer of pilocarpine, sandwiched between two semipermeable ethylene vinyl acetate membranes. Drug release was continuous, in contrast to a pulsatile delivery, often observed with eye drops. One of the main disadvantages with the ocular insert was patient discomfort. As such, it was not recommended to wear the device for more than 12 hours. Furthermore, it was difficult to insert and remove, and on occasions, would be expelled from the lid. Over the decades, as the treatment for glaucoma shifted away from pilocarpine, the device became obsolete.

Ocusert® would later inspire the design for other ocular inserts. The Lacrisert® system, from Merck, is a rod-shaped device that is inserted in the conjunctival fornix, designed to release hydroxypropyl cellulose for the treatment of dry eyes for 24 hours. From clinical trials, patients preferred the use of Lacrisert® over frequent dosing with artificial tears. Another insert, from Bausch & Lomb (acquired by Valeant Pharmaceuticals), is the Minidisc Ophthalmic Therapeutic System designed to fit the shape of the superior or inferior sclera. In trials, it was able to release the antibiotics gentamicin and sulfisoxazole for more than 100 hours. It also was reported to be easier to insert and provide greater comfort than the Lacrisert®. Unfortunately, the device was never released commercially.

The next notable ocular surface drug delivery invention were collagen shields. These dissolvable shields, made from porcine sclera, were developed to be used as therapeutic bandages to treat damaged or scratched corneas. Studies were conducted to investigate if these
devices could deliver drugs such as antibiotics, anti-inflammatories, and anti-glaucoma drugs.\textsuperscript{52-55} Unfortunately, while extensive work was conducted, no commercial product of this kind remains available.\textsuperscript{52-55}

Another strategy to improve the residence time of drugs is the development of eye drops with mucoadhesive properties.\textsuperscript{56, 57} One example is Durasite\textsuperscript{®} (Insite vision, California), a polycarbophil vehicle designed to interact with the ocular epithelium and mucin layer. The vehicle is well tolerated in patients.\textsuperscript{38} Azasite\textsuperscript{®} (Inspire Pharmaceuticals, North Carolina) was the first formulation containing Durasite\textsuperscript{®} released onto the market.\textsuperscript{38}

Since a major route of drug loss is through the nasolacrimal duct, the development of a punctal plug may improve residence time. Several studies have examined their effectiveness in the treatment of glaucoma,\textsuperscript{58, 59} allergic conjunctivitis and microbial infections.\textsuperscript{38, 60} While drug residence time is improved with these devices, their overall effectiveness in terms of delivery remains unclear.\textsuperscript{38} Furthermore, they have to be used with caution since they can cause excessive tearing due to them blocking tear drainage from the eye.

The last route of drug delivery to the front of the eye worthy of discussion is through an injectable implant into the anterior chamber. One of these devices, Surodex\textsuperscript{™} (Allergan), is used to manage postoperative inflammation from cataract surgery. The biodegradable device is comprised of poly lacto-co-glycolide (PLGA), which can release anti-inflammatory dexamethasone for 7-10 days. However, from current clinical trials, the device does not seem to produce better clinical outcome than conventional topical anti-inflammatory therapy.\textsuperscript{35}

The use of CLs for ocular drug delivery can overcome several of the drawbacks associated with eye drops and previous ophthalmic drug delivery devices. The advantages of using CLs are that drugs are released directly to the cornea, while protected from corneal
removal mechanisms. Furthermore, CLs can be engineered to deliver drugs over extended time periods, which simplifies dosing regimens.
Table 1-2 Summary of commercially developed anterior segment drug delivery devices. This table has been adopted from Alex Hui’s thesis, with permission.61

<table>
<thead>
<tr>
<th>Device</th>
<th>Manufacturer</th>
<th>Composition</th>
<th>Active agent(s)</th>
<th>Release duration</th>
<th>Treatment</th>
<th>Design</th>
<th>REF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocusert®</td>
<td>Alza Corp</td>
<td>Ethylene vinyl acetate</td>
<td>Pilocarpine</td>
<td>7 days, 12h recommended wear time</td>
<td>Primary open angle glaucoma</td>
<td>Small disc placed in inferior cul-de-sac</td>
<td>49</td>
</tr>
<tr>
<td>Lacrisert®</td>
<td>Merck</td>
<td>Hydroxypropyl cellulose</td>
<td>Hydroxypropyl cellulose</td>
<td>1 day</td>
<td>Dry eye</td>
<td>Rod placed in upper or lower fornix</td>
<td>51</td>
</tr>
<tr>
<td>Minidisc Ocular</td>
<td>Bausch &amp; Lomb (Valeant)</td>
<td>Polyhydroxymethylmethacrylate, hydroxypropyl cellulose, proprietary monomers</td>
<td>Gentamicin and sulfisoxazole</td>
<td>100-300 hours</td>
<td>Prophylaxis against bacterial infections</td>
<td>Miniaturized contact lens to fit sclera</td>
<td>49</td>
</tr>
<tr>
<td>Therapeutic System</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AzaSite®</td>
<td>Inspire Pharmaceuticals</td>
<td>Durasite®, polycarbophil vehicle</td>
<td>Azithromycin</td>
<td>1 day</td>
<td>Bacterial conjunctivitis</td>
<td>Mucoadhesive eyedrop</td>
<td>38</td>
</tr>
<tr>
<td>Surodex™</td>
<td>Allergan</td>
<td>Poly-lacto-co-glycolide (PLGA)</td>
<td>Dexamethasone</td>
<td>7-10 days</td>
<td>Postoperative inflammation</td>
<td>Injection into anterior chamber</td>
<td>35</td>
</tr>
</tbody>
</table>
3.1 Contact lenses

The first documented concept of CLs belongs to Leonardo da Vinci, who approximately 500 years ago, illustrated his ideas for using water to change the refractive power of the eye. While there were many reiterations of his idea over the next centuries, it was not until 1888 that the first functional design of the CL in the form of scleral lenses was proposed. However, because these lenses were originally made from glass, they were uncomfortable and oxygen impermeable, and thus could not be worn for an extended period of time. The first breakthrough in material synthesis came in 1934 with the invention of polymethylmethacrylate (PMMA), an acrylate polymer with desirable optical properties and biocompatibility with the ocular surface. This material would later contribute to the successful development of rigid CLs.

In 1960, two Czech scientists, Otto Wichterle and Drahoslav Lím, revolutionized contact lens fitting with their introduction of the first soft CL material, poly(hydroxyethylmethacrylate) (pHEMA), a biocompatible hydrogel that could absorb and retain a significant amount of water. Compared to rigid lenses made from PMMA, pHEMA lenses had significantly improved comfort. Wichterle described the fabrication of these lenses using a simple and efficient method of spin casting, and his patents would later be acquired by the National Patent Development Corporation in the United States in 1965, and subsequently commercialized by Bausch & Lomb in the early 1970s.

For the next few decades, pHEMA and its polymer derivatives enjoyed considerable success in the CL market. However, adverse symptoms associated with wearing these CLs began to emerge, in particular reduced oxygen permeability to the ocular surface. Extended wear with these CLs would lead to hypoxia-related symptoms, including corneal edema,
vascularization, inflammation, and increased susceptibility to ocular infections. The next era for CLs would aim to address these hypoxia-related problems.

3.2 Silicone hydrogels

The amount of oxygen that can be transported through pHEMA CLs are influenced by both lens thickness and the amount of water content within the lens. This latter factor poses a significant limitation in the amount of oxygen that these lenses can transmit to the ocular surface. One potential solution to the hypoxia dilemma is to use silicone-based lenses, which are well documented to be highly oxygen permeable. However, due to the hydrophobicity of silicone, these lenses have very low wettability and suffer from extensive deposition of hydrophobic tear film components. These problems result in considerable discomfort, which limits their commercial viability.

Thus, the question to solve this oxygen problem was how to incorporate silicone properties into CLs, to provide oxygen transmission, while still preserving the high water content of previous hydrogels for comfort. For many years, the chemistry behind this concept elucidated scientists, as it would be difficult to combine a hydrophobic component, such as silicone, with hydrophilic monomers. Conventional wisdom would predict that the combination of these two components would result in non-uniformity and phase separation, leading to a significant reduction in optical transparency of the final material. In addition, the overall architecture of the silicone hydrogel also had to be co-continuous to allow for proper ion flow and oxygen transmission throughout the lens. It was not until the late 1990s that these design problems would be successfully tackled, and the first generation of silicone hydrogels were finally introduced.
3.3 Generation of silicone hydrogels

Bausch & Lomb and CIBA Vision were first to launch the first generation silicone hydrogels, PureVision (balafilcon A) and Focus Night and Day (lotrafilcon A) respectively. To mask the hydrophobicity of the silicone within their materials, the surfaces of these lenses were modified with plasma processes. PureVision lenses underwent a plasma oxidation process,\(^\text{72}\) whereas Focus Night and Day lenses were coated with an even thin layer of plasma.\(^\text{72}\) These processes successfully improved surface wettability of the materials, but they were also costly and thus new alternative methods were explored. This eventually led to the development of the second generation silicone hydrogels such as Acuvue Oasys (senofilcon A) and Acuvue Advance (galyfilcon) from Johnson & Johnson. In contrast to previous surface treatment approaches, the wettability of these lenses were improved by incorporating an internal wetting agent, polyvinylpyrrolidone.\(^\text{72}\) The third generation silicone hydrogels, Biofinity (comfilcon A) from CooperVision, addressed the wettability issue by incorporating silicone polymers which are inherently wettable.\(^\text{66}\) However, despite considerable efforts over the past decade to improve the wettability of silicone hydrogels, there have been little improvements in CL comfort. Thus, while silicone hydrogels have addressed problems associated with hypoxia, the problem of CL discomfort continues to challenge researchers and manufacturers in this field.
3.4 Contact lens manufacturing

There are three primary techniques for creating CLs: lathe cutting, spin casting, and cast moulding. In lathe cutting, a solid cylindrical or round piece of material is mounted on a spinning shaft, and excess material is cut away from the front and back surface to produce the desired CL shape. This technique was initially used to make glass CLs. However, this practice is time consuming, and highly dependent on the machine operator, which can lead to high variability in lens production. With the introduction of liquid monomers as the starting materials, the technique of spin casting could be used to create CLs. In this method, the liquid mixture is placed into a mould corresponding to the front surface of the lens. As the mould is spun at the desired rotational speed, the centripetal force creates the desired shape for the posterior surface of the lens, and the mixture is subsequently polymerized to produce the lens. The final and most conventional method for creating CLs is cast moulding. In this process, the polymerization mixture is injected between two moulds corresponding to the front and back surface of the lens. The solution is then polymerized in a chamber to produce the CLs.
3.5 Lens chemistry

Soft CLs are produced through a polymerization reaction of a monomer mixture. Many of the important lens properties, such as optical transparency, water content, protein sorption, lipophilicity, oxygen permeability, porosity, and lens modulus are derived from the properties of the monomers in the mixture. For this reason, varying the ratios of the monomers within the mixture will result in different lens materials. For conventional hydrogel CLs, the base monomer is typically hydroxyethyl methacrylate (HEMA), shown in figure 1-2. This monomer, which was first introduced by Witcherle for the synthesis of CLs, produces desirable hydrogels with high water content and low modulus.

**Figure 1-3** chemical structure of A hydroxylethyl methacrylate (HEMA) B poly-hydroxylethyl methacrylate (pHEMA) C ethylene glycol dimethacrylate (EGDMA), a cross-linker. Polymerization reaction occurs at the double bond structure highlighted in red.

**Figure 1-4** chemical structure of other base monomers used in hydrogels A 3-[tris-(trimethylsiloxy)silyl] propyl methacrylate (TRIS) B -vinylpyrrolidone (NVP) C methacrylic acid (MAA) D dimethylacrylamide (DMAA) E poly-vinyl alcohol (PVA)
As the CL industry evolved to produce materials with higher biocompatibility, new monomers were introduced (Figure 1-3). One important monomer, 3-[tris-(trimethylsiloxy)silyl] propyl methacrylate (TRIS), and similar silicone derivatives are responsible for the high oxygen permeability within the lens materials. However, the compatibility of TRIS with HEMA is poor, and thus more compatible hydrophilic monomers such as N-vinylpyrrolidone (NVP), methacrylic acid (MAA), dimethylacrylamide (DMAA) were introduced. The demand for high-throughput, automated, and lower cost manufacturing also have placed pressures to produce new and better monomers. Among them is a daily disposable lens material, nelfilcon A, a poly-vinyl alcohol (PVA) based hydrogel. The demand to improve comfort continues to stimulate the research in developing new CL monomers.

The typical monomer mixture will include the (1) base monomers, which provides the properties of the lens, (2) a cross-linker, which acts to form the polymer meshwork (3) and an initiator. The overall reaction schematic (Figure 1-4) begins with the activation of the initiator, which generates highly energetic free electrons. These free radicals subsequently react with the double bonds within the monomers, which in turn generates highly reactive species that react with other monomers. The result is a rapid polymerization process, as monomers are covalently linked together. This process continues until all the monomers have been exhausted or the free radical generation is stopped.
Figure 1-5 schematic for free radical polymerization of hydroxylethyl methacrylate (HEMA)

Initiator activation can be accomplished by temperature control or light initiation. However, for heat initiated polymerization, the reaction is typically slower and produces variable and unreacted side chains. In contrast, light initiated photo-polymerization occurs rapidly, and the process can be stopped simply by modifying the amount of exposure to the light source.

3.6 Demographics

CL wear has seen several changes over the last decades. Initially, CLs were used primarily to correct spherical refractive error, hyperopia, and myopia, but have extended to include correction for astigmatism and presbyopia. The prevalence for rigid CL wear has decreased. The prevalence for CL extended wear is also decreasing due to the associated complications such as microbial keratitis. Instead, there is an increasing trend towards prescribing daily disposable (DD) lenses. These lenses, which are worn straight from the packaging and replaced daily, overcome the potential complications associated with extended wear. DD lenses also have included silicone hydrogels to provide adequate oxygen permeability, even on a daily wear basis. However, currently the lens cost for DD lenses is a significant factor affecting its widespread use.
4.1 Prevalence of fungal keratitis

Ocular fungal infections (mycoses), in particular that of the cornea (fungal keratitis), while an uncommon occurrence can lead to loss of vision and blindness.\textsuperscript{21, 22} In temperate regions such as the United Kingdom and Northern United States, the prevalence is typically low.\textsuperscript{83-85} However, in tropical regions, particularly South India, Ghana, and China, the prevalence of these infections is high.\textsuperscript{83, 86-90} In these countries, fungal keratitis is a major cause of vision loss.\textsuperscript{91, 92}

4.2 Etiology

Fungal infections are caused primarily by fungal penetration through a compromised corneal epithelium, typically following corneal trauma with vegetable matter.\textsuperscript{93} There also have been numerous case reports of fungal keratitis associated with both therapeutic and daily soft CL wear.\textsuperscript{94-96} Topical steroids also have been implicated with ocular fungal growth.\textsuperscript{93, 97} In North America, the importance of fungal keratitis only became known following the worldwide outbreak of fungal keratitis, associated with a multipurpose contact lens solution (ReNu MoistureLoc), in 2006.\textsuperscript{98-100}

Filamentous fungi are the major etiologic agents causing fungal keratitis with \textit{Fusarium spp} (species) being the primary agents, followed by \textit{Aspergillus spp}.\textsuperscript{91, 93, 95, 97} \textit{Candida spp}, although rare, can also cause fungal keratitis.\textsuperscript{101} These fungal agents, such as \textit{Fusarium spp}, are common plant pathogens in corn crops or onion fields.\textsuperscript{102}

4.3 Clinical features and diagnosis

Fungal ulcers are typically described as dry and painless, with a tough raised surface.\textsuperscript{93, 103, 104} In the early stages, the ulcers appear dendritic and inflammation is minimal compared to
microbial keratitis. Generally, there is an absence of lid edema. Fungal infiltrates are grayish- or yellowish-white, and produce a soft and creamy exudates at the base of the ulcer.\textsuperscript{93, 103, 104}

Laboratory diagnosis of fungal keratitis can be made through a variety of methods. Obtaining a sample of the ulcer from the cornea and then smearing it on a glass slide, followed by direct microscopic evaluation is the most common and rapid method for diagnosis. Stains such as the Giemsa stain, Gram stain, and potassium hydroxide are highly sensitive in detecting fungal elements.\textsuperscript{105, 106} Further diagnosis can be performed using fungal cultures, which are processed within 48 to 72 hours in blood agar or Sabouraud dextrose agar at room temperature (27°C).\textsuperscript{83, 93, 97} The detection accuracy for fungal cultures depends on the severity of the infection and the criteria established for positive culture, but generally is very high.\textsuperscript{83, 93, 97} However, culture techniques are lengthy, requiring anywhere from 2 days to 2 weeks to obtain results. New diagnostic tools to evaluate fungal infections have been explored, notably polymerase chain reaction (PCR) and confocal microscopy. PCR assays only require 4 hours to generate results,\textsuperscript{107} whereas confocal microscopy can be used to noninvasively image the infected cornea.\textsuperscript{108} Unfortunately, these techniques are expensive and not readily accessible in areas where fungal keratitis is highly prevalent.

4.4 Treatment

Current therapies for fungal eye infection are inadequate, as many agents are only fungistatic, requiring a prolonged course of treatment. Fungi that affect the ocular system are rarely encountered among systemic mycoses, and thus therapeutic principles for systemic infections are not applicable to the cornea.\textsuperscript{109}
Drugs to treat mycoses are generally found in two classes of antifungal agents, polyenes and azoles. However, only natamycin, a polyene antifungal, is commercially available and United States Food & Drug Administration (FDA)-approved for ocular treatment. The treatment plan, depending on the severity of the infection, involves applying eye drops at hourly or two-hourly intervals for the first 48 hours. After this period, the frequency may be reduced, but treatment is continued for 6 weeks or until the infection is resolved. The dosing regimen for fungal keratitis is considered very taxing, and patients are often hospitalized to ensure compliance. In cases where topical treatments are ineffective, keratoplasty, or corneal surgery to remove the infected tissue can be employed.

4.5 Fungal agents

4.5.1 Natamycin (Polyene)

In North America, natamycin (pimaricin) is currently the only FDA-approved antifungal for topical ocular administration, formulated as a 5% ophthalmic suspension. It is classified as a polyene antifungal, and similar to other polyenes, has a broad inhibition spectrum against fungi such as Fusarium, Aspergillus, and Candida. The drug binds to ergosterol, a sterol found only in fungal cytoplasmic membranes, and consequently inhibits the functional effects of ergosterol. In many countries, natamycin is the favored drug to treat mycotic keratitis. However, the availability of natamycin is sporadic, and thus the drug is expensive compared to other antifungals. Furthermore, natamycin is insoluble in water (30-50 mg/L) and alcohols, and sensitive to ultraviolet degradation.
4.5.2 Fluconazole, Econazole, Miconazole (Azoles)

The azole class of antifungals are fungistatic at low drug concentrations, and only become fungicidal at higher concentrations. Their mechanism of action occurs via inhibition of 14-α-demethylase, a pivotal enzyme in the ergosterol synthesis pathway. While the majority of azoles are hydrophobic, the development of new azoles in the 1980s have yielded more hydrophilic drugs. Fluconazole is one of the new generation azoles, and it is a stable, low molecular weight, hydrophilic drug. Other attractive properties of fluconazole include high bioavailability and low toxicity, while maintaining a broad inhibition spectrum against fungi. However, it has not been FDA approved, but has been proposed to be an ideal candidate to treat ocular mycoses. Several studies have examined the delivery of fluconazole from CLs. Two other azoles, econazole and miconazole, also have been investigated with CL materials.

4.5.3 Terbinafine, Natifine (Allylamines)

Unlike polyenes and azoles, clinical uses for allylamines are less common. Two of these antifungals, terbinafine and naftifine, have broad range inhibition against yeast and dermatophytes, and are well tolerated. Terbinafine inhibits fungal growth by binding to fungal squale oxidase, which prevents the biosynthesis of ergosterol. Naftifine functions in a similar manner, but also exhibits anti-inflammatory properties. There have been some published studies examining the release of allylamines from hydrogels, but their applications for the eye have not been explored.
**Figure 1-6** Structure of representative antifungal compounds.\textsuperscript{130} Adopted from Phan *et al* with permissions\textsuperscript{3}
5.1 CLs for ocular drug delivery

The idea of using CLs for ocular drug delivery is not new, and has been proposed in the 1960s by the inventor of modern day soft CLs, Otto Wichterle. Unfortunately, there were too many complications associated with early materials, in particular inadequate oxygen transmission to the cornea. The resulting hypoxia-related complications, especially during overnight wear, limited the long term therapeutic potential of CLs. It was not until several decades later that this issue was resolved with the introduction of oxygen permeable silicone hydrogel (SH) CLs in the 1990s. These new lenses, which overcame the significant hypoxic hurdle, re-kindled the interest in developing CLs to deliver drugs.

The use of CLs for ocular drug delivery can overcome several of the ocular barriers that limit effective drug absorption and penetration. When a CL is placed on the cornea, it separates the natural tear film into the pre- and post-lens tear compartment. Several studies suggest that the post-lens compartment has limited tear mixing and exchange. Consequently, drugs released into this compartment from the CL should have prolonged contact with the cornea, resulting in higher bioavailability. Furthermore, CLs can be engineered to deliver drugs over extended periods of time, which significantly simplifies the dosing requirements. For ocular infections where frequent applications are necessitated, this improvement can drastically enhance the treatment regimen.

In developing CLs for ocular drug delivery, the focus has been on extending the release duration of drugs from CLs. The most basic method, simple drug loading via incubating a commercial CL in a pharmaceutical preparation, often results in rapid drug release, which may not be clinically useful. Consequently, various strategies to develop novel CL materials have been explored including molecular imprinting, vitamin E coatings and encapsulation.
using nanoparticles.\textsuperscript{141,142} \textit{In vitro} studies with these materials have shown that they are capable of releasing ophthalmic drugs up to several days.\textsuperscript{124, 140, 142}

5.2 Commercial contact lenses to deliver antifungals

The development of an antifungal contact lens could significantly improve the treatment process for fungal keratitis. There only have been a limited number of antifungal drugs that have been reported for drug delivery with CLs. The following section, adapted from our previous published review paper,\textsuperscript{3} overviews various approaches to modify CLs for antifungal ocular drug delivery. The goal in developing these lenses is to form specific polymer-drug interactions to increase the amount of drugs that can be loaded on the lenses, and to extend the drug release duration. These strategies are summarized in table 1-3.

Several commercial CLs, such as alphafilcon A (Bausch+Lomb), balafilcon A (Bausch+Lomb), etafilcon A (Johnson & Johnson), and lotrafilcon A (Alcon) are FDA approved for use as bandage lenses in corneal wound healing.\textsuperscript{143-148} To reduce infections, antibiotics and anti-inflammatory drugs are often administered with these lenses.\textsuperscript{149} This has sparked the idea of using commercial CLs as a vehicle to deliver drugs to the eye.\textsuperscript{143}

Several studies have examined the ability of commercial CLs to deliver drugs to the eye,\textsuperscript{149-152} but studies specifically examining release of antifungals are limited.\textsuperscript{124} In one study by Peng \textit{et al}, the uptake and release of fluconazole was examined from several commercial lenses.\textsuperscript{124} These lenses were soaked with fluconazole dissolved either in PBS or methanol. However, the method of loading did not affect the release of the drugs from the CLs, which occurred within 1-10h.\textsuperscript{124} According to this study, commercial CLs are not ideal for extended drug delivery.
The effectiveness of drug uptake and release from a CL has been linked to the material properties of the lens. It has been theorized that if the CL cannot form sufficient interactions with the drug, then release occurs rapidly. For hydrophobic drugs, drugs can also bind irreversibly to the lens polymer. These drugs will favor partitioning in the lens polymer rather than the surrounding aqueous media. In contrast, hydrophilic drugs will partition higher in the release media, resulting in rapid drug release from the CLs.

5.3 Commercial contact lenses with Vitamin E coating

A simple method of extending drug release from commercial CLs has been proposed by Peng et al. Their approach was to create diffusion barriers within the lens, accomplished by pre-soaking the CLs in vitamin E. The rationale behind using vitamin E over other similar agents is unclear, besides the fact that ‘it works’ and vitamin E is non-toxic. The amount of vitamin E that can be sorbed is dependent on the CL material. When these Vitamin E-coated lenses were loaded with fluconazole, they could maintain the drug release duration up to one week. Vitamin E coating did reduce ion permeability and oxygen permeability of the lenses, but the authors suggested that these reductions were within acceptable limits. Vitamin E did not leach from the CLs under storage conditions. This method has been applied to other drugs, and also has shown high efficacy in animal studies.


Figure 1-7 Strategies to modify contact lens materials for antifungal drug delivery
Table 1-3 Strategies to deliver antifungal drugs from contact lens materials. Adopted from Phan et al with permissions.

<table>
<thead>
<tr>
<th>Method</th>
<th>Antifungal Drugs</th>
<th>Release Duration</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug soaking with commercial CLs</td>
<td>fluconazole&lt;sup&gt;124&lt;/sup&gt;</td>
<td>1-10 hours (h)&lt;sup&gt;124&lt;/sup&gt;</td>
<td>convenience, easy to use</td>
<td>burst release</td>
</tr>
<tr>
<td>Vitamin E coating</td>
<td>fluconazole&lt;sup&gt;124&lt;/sup&gt;</td>
<td>7 days (d)&lt;sup&gt;124&lt;/sup&gt;</td>
<td>convenience, easy to use, extended release, compatibility with a wide range of drugs</td>
<td>cannot increase total drug release</td>
</tr>
<tr>
<td>Impregnated drug film</td>
<td>econazole&lt;sup&gt;142&lt;/sup&gt;</td>
<td>21 d&lt;sup&gt;142&lt;/sup&gt;</td>
<td>extended release</td>
<td>cannot be used with UV-sensitive drugs</td>
</tr>
<tr>
<td>Cyclodextrin hydrogel</td>
<td>miconazole&lt;sup&gt;126&lt;/sup&gt;, naftifine&lt;sup&gt;129&lt;/sup&gt; and terbinafine&lt;sup&gt;129&lt;/sup&gt;</td>
<td>8h&lt;sup&gt;129&lt;/sup&gt; - 7 d&lt;sup&gt;126&lt;/sup&gt;</td>
<td>increased drug loading, extended release, compatibility with a wide range of drugs</td>
<td>drugs in this studies are not FDA-approved for ocular administration</td>
</tr>
<tr>
<td>Polyelectrolytes hydrogel</td>
<td>terbinafine hydrochloride&lt;sup&gt;128&lt;/sup&gt;</td>
<td>&lt; 12 h&lt;sup&gt;128&lt;/sup&gt;</td>
<td>release can be controlled using pH</td>
<td>can only be used with ionizable drugs, pH sensitive, prone to protein deposition</td>
</tr>
<tr>
<td>Molecular imprinting</td>
<td>fluconazole</td>
<td>1-5 d&lt;sup&gt;125&lt;/sup&gt;</td>
<td>extended release, compatibility with a wide range of drugs</td>
<td>only compatible with one target drug</td>
</tr>
</tbody>
</table>
5.4 Drug-embedded hydrogels

One straightforward approach, provided the drugs are stable to the polymerization methods, is to incorporate the drugs within the polymer mixture before polymerization.\textsuperscript{142, 156, 157} After polymerization by ultraviolet (UV) irradiation, the drugs are entrapped within the matrix of the hydrogel, and the release of the drug is significantly extended.\textsuperscript{142, 156, 157} In a study by Ciolino \textit{et al}, a poly(lactic-co-glycolic) acid (PLGA) film containing econazole was polymerized between two sheets of pHEMA hydrogel.\textsuperscript{142} The efficacy of the lens was tested against \textit{Candida albicans} and was shown to release enough drug to inhibit the growth of the fungi for up to 21 days \textit{in vitro}. Not surprisingly, the potency of the material was dependent on the amount of econazole loaded on the lens.\textsuperscript{142} There are two major drawbacks to this approach. The first is that PLGA-econazole films are opaque.\textsuperscript{142} To achieve optical transparency appropriate for CLs, the center of the material was kept drug free. The second drawback is PLGA is biodegradable when stored in solution.\textsuperscript{142} As a result, the drug film continuously degraded while in storage.

5.5 Cyclodextrin hydrogels

In the pharmaceutical field, cyclodextrins (CD), due to their ability to form complexes with a range of drugs, have been employed in a variety of drug delivery applications.\textsuperscript{158} This versatility is attributed to their unique structure, consisting of a hydrophilic shell and a lipophilic central cavity.\textsuperscript{158} The cavity can host a hydrophobic agent, while the entire CD dissolves readily in solution. They have been shown to increase solubility,\textsuperscript{159} bioavailability,\textsuperscript{159} and stability\textsuperscript{159} of a wide range of drugs, including antifungals.\textsuperscript{160-163} CDs have been polymerized with various hydrogels to produce materials with improved drug uptake and release profiles.\textsuperscript{126, 129, 164-168}
Studies with HEMA-based hydrogels containing α-, β-, and γ-CDs with miconazole show that these hydrogels could release drugs for 1 week. In vitro, these gels inhibited the biofilm formation of *Candida albicans*. In another study, the release of naftifine and terbinafine from β-CD hydrogels were observed for 8 hours.

### 5.6 Polyelectrolyte hydrogels

For the release of charged drugs, poly-electrolytes polymers can be employed to increase material-drug interactions. These polymers contain a large number of ionizable functional groups, which allow them to interact strongly with oppositely charged drugs. Furthermore, the high sensitivity of these hydrogels to changes in pH could be exploited for controlled release. In a study with poly(N-vinyl 2-pyrrolidone/itaconic acid) hydrogels, terbinafine hydrochloride could be absorbed and released from these hydrogels in a controlled manner over 12 hours. One potential drawback of using these types of hydrogels on the eye is that these materials would accumulate significant amounts of tear components.

### 5.7 Molecularly imprinted hydrogels

The idea behind molecular imprinting is to use molecular templates to create specific sites within the polymer matrix that can recognize the target drug. This concept has been filed in a 2008 patent, which outlines the synthesis of a biomimetic contact lens designed to release a wide range of antifungals (azoles, polenes, and allylamines) for large animals. The templates, typically the target drug or a compound structurally similar to the drug, are polymerized with monomers capable of interacting with the target drug. These monomers can include n-vinyl pyrrolidone (NVP), hydroxyethyl methacrylate (HEMA), diethylaminoethyl methacrylate
(DEAM), acrylamide (AM), and polyethylene glycol dimethacrylate (PEGDMA). The one apparent limitation of this approach is that each biomimetic material is only attuned to its target drug.

6.1 In vitro models to evaluate CL drug delivery

In the past, the demands for sophisticated in vitro eye models were low, and extremely simple, static volume, vial-based models were adequate for many research purposes. The current standard for in vitro eye models for evaluating CL drug delivery default to using vial-based assessments. Unfortunately, the volume of fluid in a vial greatly exceeds physiological amounts; thus it should not be surprising that these studies report rapid drug release kinetics. Furthermore, the simple vial model lacks a natural tear flow component and blinking mechanism to expose the material to the atmosphere. These parameters are defining factors of the ocular environment, and will significantly affect the behavior of the CL material on the eye.

The recognition for better in vitro eye models have been acknowledged by research groups across the world. Attempts have been made towards developing models to simulate ocular parameters such as microfluidic tear flow, intermittent air exposure, and in vitro spoilation. Not surprisingly, the results generated from these experiments are considerably different than those obtained with the conventional vial model, and may more closely resemble in vivo data. As the CL drug delivery field advances further in the near future, better in vitro eye models will be necessary to properly assess how these devices will perform on the eye.
7.1 Conclusion

The anatomical structures of the eye present numerous barriers to ocular drug delivery, many of which can be addressed with a CL drug delivery device. The main challenge in developing a CL suitable for ocular drug delivery is to engineer materials capable of sustained drug release over several days. Currently, in vitro models to test CLs are limited, and better models may be necessary to give a better representation of on-eye performance. The successful development of a CL device for ocular mycoses, a potentially blinding infection, will be invaluable not only in improving fungal therapy, but also pushing the ocular drug delivery field forward.
Chapter 2 – Rationale and objectives

Topical eye drops, due to their ease of dosing, accessibility and cost,\textsuperscript{1-4} account for 90\% of all ophthalmic formulations.\textsuperscript{1-4} However, the anatomy of the eye, in fulfilling its role to ‘keep out’ foreign substances, prevents the effective delivery of drugs to the affected ocular tissue. Continuous tear dilution,\textsuperscript{5-7} dispersion and drainage during blinking and tear flow,\textsuperscript{5, 7, 8} non-specific absorption,\textsuperscript{1, 5, 7} and variable drug penetration\textsuperscript{4} limits effective drug bioavailability to only 1-7\% at the target site.\textsuperscript{8} The remaining dose either spills over onto the cheek, drained through nasolacrimal duct or is absorbed into the systemic circulation.\textsuperscript{9} Consequently, to achieve therapeutic drug concentration, multiple dosing over extended periods is often required. This, in turn, exacerbates problems with patient compliance\textsuperscript{10, 11} and the side effects of drug overdose.\textsuperscript{12}

In recent years, new applications for contact lenses (CLs) have been explored, including their use for ocular drug delivery. In the past, CLs have been used as bandage lenses for managing pain, and promoting re-epithelialization in ocular post-surgery or ocular trauma.\textsuperscript{13-19} Typically, to prevent microbial infections, antibiotics and anti-inflammatory drugs are administered concurrently during lens insertion.\textsuperscript{13, 20} This clinical practice resulted in an interest in whether it was feasible to specifically design a CL with drug delivering therapeutic properties. The initial concept of using CLs for ocular drug delivery was suggested as early as 1960,\textsuperscript{21, 22} but at that time (and for several decades later), long term wear with CL without hypoxic compromise was not possible.\textsuperscript{23, 24} It was not until hypoxic problems were solved with the commercialization of silicone hydrogel (SH) materials in the late 1990s, that the safe use of CL for long-term medical applications became a real possibility.\textsuperscript{25, 26} An ophthalmic survey in the United States and Canada suggested that such a concept would be well received by practitioners.\textsuperscript{13}
There are two apparent advantages of using CLs to deliver drugs. The first stems from the physical placement of the CL on the cornea, which effectively separates the natural tear film into pre- and a post-lens (between the lens and cornea) compartments. The physical CL barrier limits tear mixing and exchange in the post-lens tear film, as well as shielding this layer from the blinking reflex. Consequently, it has been proposed that a drug released from a CL into this post-lens area has prolonged contact time with the cornea. Mathematical model simulations for drug delivery with a CL predicts that approximately 50% of the drugs released from a CL can diffuse into the cornea, making it 35 times more efficient than a conventional eye drop. The second advantage of using a CL for drug delivery is the intrinsic ability of any hydrogel material to absorb and release molecules. Thus, in theory, CLs can serve a drug reservoir to release drugs over an extended period of time at a slow and sustained rate. For microbial keratitis, where frequent eye-drop dosing is often necessary, the design of a slow drug releasing CL device would eliminate the need for multiple dosing and simplify the treatment therapy.

Amongst ocular infections, fungal keratitis is a major cause of vision loss and blindness throughout the world. However, since these infections occur predominantly in India and China, their impact has been minimal in North America. While several case reports of fungal keratitis associated with CL wear exist, it was not until a severe outbreak of fungal eye infections associated with a multipurpose solution (Renu MoistureLoc) occurred in 2006 that significant interest in this area arose. Currently, treatment for fungal infections remains inadequate, and only one antifungal (natamycin) is United States Food & Drug Administration (FDA) approved for topical application. The treatment regimen is intensive, requiring dosing at 1-2 hour intervals for two weeks. Thus, there is a definite demand for a better method of treatment for fungal keratitis.
One of the major road blocks to progress in the CL drug delivery field is the limited number of *in vivo* studies to support the effectiveness of this delivery platform. Currently, there only have been three published animal studies to validate this claim. On the *in vitro* side, models to simulate the cornea are also substantially lacking. For instance, the majority of *in vitro* studies to measure drug release from CLs have used a fixed volume vial model, containing a certain amount of fluid, as the accepted standard. However, not only does this model contain too much fluid to properly simulate the tear film, it also lacks both the natural tear flow component and the blinking reflex, both of which are defining factors of the ocular environment. Thus, it is very difficult - and potentially erroneous - to use the data generated from these *in vitro* studies to predict the *in vivo* outcomes of these medical devices.

In recent years, researchers have recognized the limitations of using a fixed volume, static fluid vial model as a method of evaluating drug delivering CLs. Subsequently, to better simulate the ocular environment, several unique *in vitro* eye models have been developed to simulate different on-eye parameters, such as microfluidic tear flow, intermittent air exposure, or deposition of lipid tear film components. Not surprisingly, the results generated from these experiments are significantly different than those obtained with the conventional vial model, and may more closely resemble *in vivo* data. However, the number of parameters emulated on these models are still limited, and while many models have been proposed, no model has been accepted as a “gold standard”. Thus, the development of a physiologically relevant eye model to test the performance of drug delivering CLs will be invaluable in progressing this technology towards a viable commercial product.
In summary, to advance the CL drug delivery technology, it is important to develop new materials as well as a sophisticated in vitro eye model to test these materials. For these reasons, there are two main aims to this thesis:

a) The first objective is to investigate the potential for a CL to function as a drug delivery device for a commercially available antifungal (natamycin). To this end, chapter 3 sets the foundation for subsequent experiments, by evaluating the in vitro uptake and release of natamycin from several commercially available CLs. To improve the release characteristics of natamycin from CLs, chapter 4 details an attempt to incorporate novel drug-encapsulated nanoparticles within the CLs. In chapter 5, an alternative strategy employing the incorporation of cyclodextrin molecules within the CL polymer matrix was evaluated as a potential modification to prolong the release of natamycin.

b) The second objective is to develop a sophisticated in vitro ocular model capable of adequately measuring drug release from CLs. Chapter 6 outlines the design of a novel in vitro eye model to simulate the physiological ocular environment. In chapter 7, the release of the antifungal fluconazole from commercially daily disposable CLs was explored using this model. In chapter 8, as an extension of the developed in vitro eye model, an agar eye piece was developed to test the effects of natamycin and fluconazole releasing CLs on Candida albicans.
Chapter 3– In vitro uptake and release of natamycin from conventional and silicone hydrogel contact lens materials

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3.1 Overview

3.1.1 Objective

To investigate the uptake and release of the antifungal ocular drug, natamycin, from commercially available conventional hydrogel (CH) and silicone hydrogel (SH) contact lens (CL) materials and to evaluate the effectiveness of this delivery method.

3.1.2 Methods

Five commercial SH contact lenses (balafilcon A, comfilcon A, galyfilcon A, senofilcon A, lotrafilcon B) and four CH contact lenses (etafilcon A, omafilcon A, polymacon, vifilcon A) were examined in this study. These lenses were incubated with natamycin solubilized in DMSO, and the release of the drug from these lenses, in Unisol 4 pH 7.4 at 32±1°C, was determined using UV-Visible spectrophotometry at 305 nm over 24 hours.

3.1.3 Results

There was a significant uptake of natamycin between 0 hour and 24 hours (p<0.05) for all CL materials. However, there was no significant difference between any of the lens materials, regardless of their composition (p >0.05). There was a significant difference in release between all the SH materials (p<0.05) and between all CH materials (p<0.05). All CL materials had a significant increase in the release of natamycin until 1 hour (p<0.05), which was followed by a plateau (p>0.05). Overall, the release of natamycin was higher in CH than SH lenses (p<0.001).

3.1.4 Conclusions

All CLs released clinically relevant concentrations of natamycin within 30 minutes, but this release reached a plateau after approximately one hour. Further CL material development will be necessary to produce a slow and sustained drug releasing device for the delivery of natamycin.
3.2 Introduction

Ocular fungal infections, in particular fungal keratitis, while an uncommon occurrence can lead to vision loss and blindness.\(^1,2\) These infections are caused by fungal penetration of a compromised corneal epithelium, typically following corneal trauma with vegetable matter.\(^3\) While the prevalence of fungal keratitis is relatively high in tropical regions such as South India, Ghana, and China,\(^4-9\) it is generally much lower in temperate regions such as the United Kingdom and the Northern United States.\(^4,10,11\) However, there have been numerous case reports of fungal keratitis associated with both therapeutic and daily soft contact lens wear.\(^12-14\) Furthermore, the worldwide outbreak of ocular fungal infections in 2006 associated with the multipurpose contact lens solution ReNu MoistureLoc demonstrated that there is a substantial need for further research in the management of ocular fungal infections.\(^15-17\)

Currently available antifungal treatments often fail to restore vision to its original level following an ocular infection.\(^18,19\) Common topical ophthalmic antifungal formulations suffer from low drug bioavailability as they are drained out through the naso-lacrimal duct.\(^20\) Multiple dosing is therefore required, and patients often are hospitalized to ensure treatment compliance. Thus, the development of a slow-release antifungal drug-delivery device that would provide adequate drug concentration over extended periods of time would be a valuable addition to the options for treating ocular surface infections.

Contact lenses have been suggested for use as ocular drug delivery devices since 1960,\(^21\) but long-term wear complications with low oxygen permeability conventional hydrogel (CH) soft contact lenses have made them undesirable for medical use.\(^23,24\) During overnight or “extended” wear these contact lenses are unable to transmit adequate oxygen to the cornea to maintain normal metabolic activities, for the majority of wearers.\(^23,24\) The introduction of highly oxygen permeable silicone hydrogel (SH) lenses a decade ago has made hypoxic-free overnight
wear possible, and the use of contact lenses as medical devices has become increasingly popular. Several researchers have already developed model contact lens materials for use as drug delivery devices, but no commercial products are yet available.

The only commercially available and FDA-approved ocular antifungal is natamycin (pimaricin). It is a macrolide polyene antifungal with a fairly broad inhibition spectrum against various infectious fungi, including *Fusarium*, *Aspergillus*, and *Candida*. Natamycin elicits its inhibition by binding to ergosterol, a sterol unique to fungal cytoplasmic membranes, which consequently inhibits ergosterol from performing its functional effects. The drug has poor water solubility at physiological pH, and therefore is prescribed as a 5% ophthalmic suspension (Alcon, Fort Worth, TX). The suspension is instilled in the conjunctival sac, and is generally well tolerated.

The kinetics of drug delivery from modern CH and SH contact lens materials is not yet well understood. Several publications have investigated the uptake and release of topical ophthalmic drugs from commercial contact lens materials, but to our knowledge no such work has been undertaken with antifungal drugs. Thus, the purpose of the current study was to investigate the *in vitro* uptake and release of natamycin from five commercially available SH and four CH contact lens materials and to evaluate their potential use as antifungal ocular drug delivery devices.

### 3.3 Materials and methods

#### 3.3.1 Contact lens materials

Five commercially available SH contact lens materials [balafilcon A (PureVision, Bausch & Lomb, Rochester, NY), comfilcon A (Biofinity, CooperVision, Pleasanton, CA), galyfilcon A (Acuvue Advance, Johnson & Johnson, Jacksonville, FL), lotrafilcon B (Air Optix, CIBA]
Vision, Duluth, GA), and senofilcon A (Acuvue OASYS, Johnson & Johnson), and four CH lens materials (etafilcon A [Acuvue 2, Johnson & Johnson], omafilcon A [Proclear, CooperVision], vifilcon A [Focus Monthly, CIBA Vision] and polymacon [SofLens 38, Bausch & Lomb]) were evaluated in the study. All lenses had a dioptric power of -3.00 and base curve of 8.6mm, obtained from the manufacturer in the original packaging. Table 3-1 and Table 3-2 details the properties of the SH and CH contact lenses respectively.
<table>
<thead>
<tr>
<th></th>
<th>PureVision®</th>
<th>Biofinity®</th>
<th>Acuvue® OASYS®</th>
<th>Air Optix®</th>
<th>Acuvue® Advance®</th>
</tr>
</thead>
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<tr>
<td>United States adopted name (USAN)</td>
<td>balafilcon A</td>
<td>comfilcon A</td>
<td>senofilcon A</td>
<td>lotrafilcon B</td>
<td>galyfilcon A</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Bausch &amp; Lomb</td>
<td>CooperVision</td>
<td>Johnson &amp; Johnson</td>
<td>CIBA Vision</td>
<td>Johnson &amp; Johnson</td>
</tr>
<tr>
<td>Centre thickness (mm)</td>
<td>0.09</td>
<td>0.08</td>
<td>0.07</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>36</td>
<td>48</td>
<td>38</td>
<td>33</td>
<td>47</td>
</tr>
<tr>
<td>Oxygen permeability (x 10^{-11})</td>
<td>91</td>
<td>128</td>
<td>103</td>
<td>110</td>
<td>60</td>
</tr>
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<td>Oxygen Transmissibility (x 10^{-9})</td>
<td>101</td>
<td>160</td>
<td>147</td>
<td>138</td>
<td>86</td>
</tr>
<tr>
<td>FDA group</td>
<td>III</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td>Principal monomers</td>
<td>NVP + TPVC + NVA + PBVC</td>
<td>FM0411M + HOB + IBM + M3U + NVP + TAIC + VMA</td>
<td>mPDMS + DMAA + HEMA + siloxane macromer + VMA + TEGDMA + PVP</td>
<td>DMA + TRIS + siloxane macromer</td>
<td>mPDMS + DMAA + EGDMA + HEMA + siloxane macromer + PVP</td>
</tr>
</tbody>
</table>

DMAA, N,N-dimethylacrylamide; EGDMA, ethyleneglycol dimethacrylate; FM0411M, \(\alpha\)-Methacryloyloxyethyl iminocarboxyethyl oxypropylpoly(dimethyldimethylsiloxane)-butyldimethylsilane; HEMA, hydroxyethyl methacrylate; HOB, 2-hydroxybutyl methacrylate; IBM, isobornyl methacrylate; MA, methacrylic acid; mPDMS, monofunctional polydimethylsiloxane; NVP, N-vinyl pyrrolidone; TEGDMA, tetraethyleneglycol dimethacrylate; TPVC, tris-(trimethylsiloxy)silyl) propylvinyl carbamate; TRIS, trimethylsiloxyl silane; M3U, \(\alpha\) \(\omega\)-bis(methacryloyloxyethyliminocarboxy ethyloxypropyl)-poly(dimethylsiloxane)-poly(trifluoropropylmethylsiloxane)-poly(\(\omega\)-methoxy-poly(ethyleneglycol)propyl methyilsiloxane); NVA – N-vinyl amino acid; PBVC, poly(dimethysiloxy) di(silylbutanol) bis(vinyl carbamate); PC, phosphorylcholine; PVP, polyvinyl pyrrolidone; TAIC, 1,3,5-triallyl-1,3,5-triazine-2,4,6(1H,3H,5H)-trione; VMA, N-vinyl-N-methylacetamide.
Table 3-2 Properties of conventional hydrogels used in the study\textsuperscript{57,58}

<table>
<thead>
<tr>
<th>Properties</th>
<th>SofLens\textsuperscript{®} 38 (Formerly Optima FW)</th>
<th>Proclear\textsuperscript{®}</th>
<th>Acuvue\textsuperscript{®} 2</th>
<th>Focus\textsuperscript{®} Monthly</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States adopted name (USAN)</td>
<td>polymacon</td>
<td>omafilcon A</td>
<td>etafilcon A</td>
<td>vifilcon A</td>
</tr>
<tr>
<td>Manufacturer</td>
<td>Bausch &amp; Lomb</td>
<td>CooperVision</td>
<td>Johnson &amp; Johnson</td>
<td>CIBA Vision</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>38</td>
<td>62</td>
<td>58</td>
<td>55</td>
</tr>
<tr>
<td>Oxygen permeability (x 10\textsuperscript{-11})</td>
<td>10</td>
<td>27</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>FDA group</td>
<td>I</td>
<td>II</td>
<td>IV</td>
<td>IV</td>
</tr>
<tr>
<td>Surface treatment</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Principal monomers</td>
<td>pHEMA</td>
<td>pHEMA + PC</td>
<td>pHEMA + MA</td>
<td>pHEMA + PVP + MA</td>
</tr>
</tbody>
</table>

pHEMA, poly(2-hydroxethyl methacrylate); MA, methacrylic acid; NVP, N-vinyl pyrrolidone; PC, phosphorylcholine; PVP, poly(vinyl pyrrolidone).
3.3.2 Preparation of the drug solution

Ophthalmic natamycin eye drops are formulated as a 5% (w/v) suspension in sterile water. The solubility of natamycin in water at physiological pH is 30-50 mg/L, which is low for the purpose of the study. Natamycin is soluble in dimethyl sulfoxide (DMSO) at concentrations up to 3.2 mg/mL. A stock solution of natamycin (EMD Millipore, Billerica, MA, USA) was completely solubilized in a solution of DMSO (Sigma Aldrich, Oakville, ON) at a concentration of 2.6 mg/mL. The experiments and storage of natamycin were performed using amber vials.

3.3.3 Spectrophotometric determination of natamycin

The absorbance spectra of natamycin was determined using a SpectraMax M5 UV-Vis Spectrophotometer (Molecular Devices, Sunnyvale, CA) between 250 to 400nm at 34°C using a 4 mL-disposable methacrylate cuvettes (Fischer Scientific, Ottawa, ON). The spectra had an absorption maxima corresponding to the 305 nm wavelength, which is similar to values reported in the literature. Thus, 305 nm was the wavelength used to detect Natamycin in this study.

3.3.4 Determination of natamycin concentration-standard curve

The stock solution of natamycin was diluted to a range between 1.56 µg/mL to 25.00 µg/mL in an unpreserved saline solution (Unisol 4, Alcon Labs Ltd, Fort Worth, Texas) and in DMSO, to generate a linear calibration curve to correlate absorbance readings to natamycin concentrations.

3.3.5 Uptake studies

Three lenses of each type were removed from their original packaging and placed in 5mL of Unisol 4 in a 12-well clear plate (VWR International, Mississauga, Ontario) and gently shaken.
for 24 hours (h) at room temperature, to remove any packaging solution. The lenses were then pre-treated with 2.5mL of DMSO in a 12-well clear plate for 24 hours at room temperature. The lenses were removed from the DMSO solution and blot dried on lens paper before being transferred into an amber vial (Wheaton, Millville, New Jersey) containing 2 mL of 2.6 mg/mL natamycin in DMSO. The vial was incubated between 32±1°C with constant rotation over 24 h to simulate eye conditions. At specified time intervals (t = 0 min, 1 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h, 24 h), 5 µL of the solution was removed and diluted 400 fold to obtain a reading within the linear range of the standard curve.

### 3.3.6 Release studies

After the 24 hour uptake period, lenses were removed from the natamycin solution and briefly rinsed with DMSO to remove any residual drug solution not sorbed onto the lens. The lenses were then partially dried on lens paper and placed into an amber vial containing fresh 5 mL solution of Unisol 4 (pH 7.4) for SH contact lens materials. CH lens materials were placed in an 8 mL solution of Unisol 4. Preliminary release studies (not shown) determined the adequate volume of Unisol 4 needed to obtain natamycin release concentrations within the linear ranges of the standard curve. The vial was incubated at 32±1°C with constant rotation over 24 h to simulate eye conditions. At specified time intervals (t = 0 min, 1 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h, 24 h), 2 mL of the release solution was removed and measured with spectrophotometry, and then carefully pipetted back into the vial. After the 24 h release period, the lenses were incubated in fresh release solution; the release of natamycin from the lenses was monitored for an additional 24 h.
3.3.7 Metrology studies

Metrology measurements using Chiltern Optimec Soft Contact Lens Analyzer (Malvern, UK) were performed for CH contact lenses, which increased in size following their pre-treatment with DMSO. No such diameter changes were seen with the SH lenses.

3.3.8 Statistical analysis

Statistical analysis was performed using Statistica 8 software (StatSoft, Tulsa, OK). All data are reported as mean ± standard deviation, unless otherwise stated. All calculations took into account volume change associated with sampling. Two repeated measures of analysis of variance were performed to determine the differences across various time points within the same lens type, and the differences between lens types. Post-hoc Tukey’s multiple comparison tests were used when necessary. In all cases, statistical significance was considered significant for p value of < 0.05. Graphs were plotted using GraphPad Prism 5 software (GraphPad, La Jolla, CA).

3.4 Results

The uptake curves over 24 hours for the five SH contact lenses are shown in Figure 3-1, and the uptake curves for the four CH contact lenses are shown in Figure 3-2. The quantity of natamycin uptake (µg/lens) from all contact lens types is summarized in Table 3-3. Figures 3-1 and 3-2 show that there was significant increase in the uptake of natamycin between 0 hour and 24 hours (p<0.05) for both SH and CH lens materials, however, there was no significant difference between any SH and CH lenses (p >0.05). Overall, there were no statistical significances in the uptake of natamycin between SH and CH materials (p>0.05).
Figure 3-1 Natamycin uptake (µg/lens) for balafilcon A (■), lotrafilcon A (●), senofilcon A (▲), comfilcon A (▼), and galyfilcon A (●) over 24 hours as measured by spectrophotometry. The values plotted are the mean ± standard deviation. Galyfilcon A had the highest uptake of Natamycin amongst the silicone hydrogel lenses after incubation (763.5 ± 89.1 µg/lens), but the differences in uptake were not statistically significant (P=0.5576).

Figure 3-2 Natamycin uptake (µg/lens) for omafilcon A (●), etafilcon A (■), vifilcon A (▲), polymacon (▼) over 24 hours as measured by spectrophotometry. The values plotted are the mean ± standard deviation. Polymacon had the highest uptake of natamycin amongst the conventional hydrogel lenses (1264.2 ± 51.5 µg/lens), but the differences in uptake were not statistically significant (P=0.1993).
Table 3-3 Summary of uptake of natamycin into different lens type

<table>
<thead>
<tr>
<th>Lens</th>
<th>60 min</th>
<th>120 min</th>
<th>240 min</th>
<th>480 min</th>
<th>1440 min</th>
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<tr>
<td>Balafilcon A</td>
<td>91.8 ± 43.7</td>
<td>173.6 ± 66.6</td>
<td>401.46 ± 93.9</td>
<td>434.15 ± 52.0</td>
<td>495.0 ± 120.8</td>
</tr>
<tr>
<td>Comfilcon A</td>
<td>93.3 ± 63.2</td>
<td>158.8 ± 36.8</td>
<td>199.8 ± 65.2</td>
<td>247.9 ± 45.4</td>
<td>533.7 ± 24.6</td>
</tr>
<tr>
<td>Galyfilcon A</td>
<td>50.4 ± 42.0</td>
<td>118.1 ± 51.2</td>
<td>151.2 ± 11.1</td>
<td>264.0 ± 40.0</td>
<td>763.5 ± 89.1</td>
</tr>
<tr>
<td>Lotrafilcon B</td>
<td>112 ± 125.6</td>
<td>151.8 ± 135.3</td>
<td>223.3 ± 15.2</td>
<td>224.1 ± 49.2</td>
<td>688.6 ± 102.9</td>
</tr>
<tr>
<td>Senofilcon A</td>
<td>86.9 ± 63.7</td>
<td>162.8 ± 37.6</td>
<td>257.9 ± 84.2</td>
<td>254.4 ± 50.9</td>
<td>632.0 ± 38.1</td>
</tr>
<tr>
<td>Etafilcon A</td>
<td>190.3 ± 102.9</td>
<td>267.3 ± 92.4</td>
<td>453.6 ± 102.7</td>
<td>423.3 ± 70.6</td>
<td>578.7 ± 35.8</td>
</tr>
<tr>
<td>Omafilcon A</td>
<td>124.4 ± 112.2</td>
<td>300.0 ± 111.7</td>
<td>368.0 ± 1.9</td>
<td>338.8 ± 90.8</td>
<td>816.6 ± 53.1</td>
</tr>
<tr>
<td>Vifilcon A</td>
<td>156.8 ± 66.5</td>
<td>355.9 ± 62.8</td>
<td>510.5 ± 88.0</td>
<td>608.2 ± 24.5</td>
<td>970.5 ± 86.6</td>
</tr>
<tr>
<td>Polymacon</td>
<td>56.1 ± 33.3</td>
<td>193.5 ± 67.8</td>
<td>227.5 ± 52.0</td>
<td>476.1 ± 51.2</td>
<td>1264.2 ± 51.5</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± standard deviation of 3 lenses.

The typical release curves over 24 hours for the five SH lens materials are shown in Figure 3-3, and the release curves for the four CH CL materials are shown in Figure 3-4. The quantity of natamycin release (µg/lens) from all contact lens types is summarized in Table 3-4. All the lenses released a statistically significant amount of drug in comparison to the initial time point (p<0.001). There was a significant difference between all the SH lens materials (p<0.05) and there was a significant increase in the release of natamycin from all SH lens materials until 1 hour post soaking (p<0.05), after which the materials reached a plateau (p>0.05). Among the SH lens materials, balafilcon A released significantly higher amounts of natamycin (p<0.05) across all the time points. Similarly, there was a significant difference between all the CH lens types (p<0.05) and there was a significant increase in the release of natamycin from all CH lens materials until 1 hour post soaking in Unisol 4 (p<0.05), and then the materials reached a plateau (p>0.05). No further drug release was observed when the lenses were placed in a fresh solution of Unisol 4. Overall, the release of natamycin was higher in CH lenses than SH lens materials (p<0.001).
Figure 3-3 Natamycin release for balafilcon A (■), lotrafilcon B (●), senofilcon A (▲), comfilcon A (▼), and galyfilcon A (◆) over 24 hours as measured by spectrophotometry. The values plotted are the mean ± standard deviation. Balafilcon A had the highest release of Natamycin amongst the silicone hydrogel lenses after incubation (108 ± 15 µg/lens), followed by galyfilcon A (78.2 ± 5.2 µg/lens). The differences in release amongst all silicone contact lenses were statistically significant (P<0.001).

Figure 3-4 Natamycin release (µg/lens) for omafilcon A (●), etafilcon A (■), vifilcon A (▲), polymacon (▼) over 24 hours as measured by spectrophotometry. The values plotted are the mean ± standard deviation. Polymacon A had the highest release of Natamycin (199.4 ± 29.9 µg/lens). The differences in release amongst all conventional contact lenses were statistically significant (P<0.05).
Table 3-4 Summary of release of natamycin from different lens materials

<table>
<thead>
<tr>
<th>Lens</th>
<th>Microgram of Natamycin release µg/lens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 min</td>
</tr>
<tr>
<td>Balafilcon A</td>
<td>97 ± 13</td>
</tr>
<tr>
<td>Comfilcon A</td>
<td>33.9 ± 4.2</td>
</tr>
<tr>
<td>Galyfilcon A</td>
<td>59.9 ± 7.0</td>
</tr>
<tr>
<td>Lotrafilcon B</td>
<td>19.2 ± 5.3</td>
</tr>
<tr>
<td>Senofilcon A</td>
<td>20.3 ± 2.4</td>
</tr>
<tr>
<td>Etafilcon A</td>
<td>160.2 ± 1.8</td>
</tr>
<tr>
<td>Omafilcon A</td>
<td>161.2 ± 4.3</td>
</tr>
<tr>
<td>Vifilcon A</td>
<td>147.5 ± 7.5</td>
</tr>
<tr>
<td>Polymacon</td>
<td>148.6 ± 25.3</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± standard deviation of 3 lenses.

All the contact lenses in this study remained clear throughout the uptake and release phases of the experiment. One notable observation was the expansion of CH lens materials to approximately 2 to 2.4 times their original size when exposed to DMSO, as shown in Table 3-5 after 24 h. However, upon exposure to Unisol 4 in the release studies, all the CH lenses reverted back to their original sizes within one hour.

Table 3-5 Summary of conventional hydrogel contact lenses diameter expansion

<table>
<thead>
<tr>
<th>Lens</th>
<th>Etafilcon A</th>
<th>Omafilcon A</th>
<th>Polymacon</th>
<th>Vifilcon A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter Original (mm)</td>
<td>14.0</td>
<td>14.1</td>
<td>13.8</td>
<td>14.0</td>
</tr>
<tr>
<td>Diameter in DMSO (mm)</td>
<td>34.0</td>
<td>29.0</td>
<td>34.0</td>
<td>29.0</td>
</tr>
<tr>
<td>Size Increase %</td>
<td>243</td>
<td>200</td>
<td>246</td>
<td>207</td>
</tr>
</tbody>
</table>

3.5 Discussion

To our knowledge, this is the first study that has investigated the uptake and release of the antifungal drug natamycin from commercially available SH and CH contact lens materials. While there are several antifungal drugs available worldwide, natamycin (pimaricin) is currently
the only FDA approved antifungal for the treatment of fungal keratitis.\textsuperscript{19, 34, 35} Natamycin has minimal ocular toxicity, is well tolerated, and effective against fungal pathogens.\textsuperscript{36-38} However, because of its high molecular weight (665.73 g/mol) and its conjugated double bond structure, the drug is poorly soluble in water\textsuperscript{47} and thus is prescribed as a 5\% ophthalmic suspension.\textsuperscript{38} Several solvent systems have been suggested to dissolve natamycin, including methanol, glacial acetic acid and dimethyl sulfoxide (DMSO).\textsuperscript{44, 45} The latter solution was chosen as the appropriate solvent system for this study.

DMSO is a universal solvent which has several beneficial pharmacological properties with regards to ocular drug delivery, including penetration of biological membranes, bacteriostasis, and anti-inflammatory effects.\textsuperscript{48} These properties may be beneficial in aiding the delivery of natamycin into intraocular tissues. Toxicology studies with DMSO in rabbit models showed no long-term toxic effects.\textsuperscript{49} Even a single dose of absolute DMSO (100\%) has minimal ocular toxicity, and has been suggested as a potential vehicle for drug delivery.\textsuperscript{49} Furthermore, toxicology studies in rabbits with intravitreal administration of several antifungals in 100\% DMSO, including ketoconazole, itraconazole, and oxiconazole showed minimal toxic side effects.\textsuperscript{50-52} These reports all suggest that DMSO use with antifungals is relatively non-toxic, and may actually prove to be beneficial in treating severe ocular fungal infections.

Contact lenses placed on the cornea can increase the residence time of the drug in the precorneal tear film, which improves overall drug bioavailability and effectiveness.\textsuperscript{53, 54} Fungal keratitis, which results from an injury to the cornea,\textsuperscript{38} may benefit from the therapeutic use of contact lenses. Contact lenses have been shown to be effective in treating corneal perforation (trauma) by preventing painful contact between the eyelids and the torn cornea, enhancing corneal healing, and preventing further corneal damage and infections.\textsuperscript{27, 55, 56} However, the
therapeutic use of contact lenses with fungal keratitis has not yet been studied in great detail, and will need to be investigated further before it can become acceptable in the treatment of fungal infections.

The uptake and release of several hydrophobic drugs, including ciprofloxacin and dexamethasone, on SH and CH lens materials have been previously reported.\textsuperscript{39, 41} These studies suggest that CH lens materials typically have a higher uptake and release of hydrophobic drugs than SH lens materials.\textsuperscript{39, 41} CH lens materials are primarily composed of co-polymers of poly(2-hydroxyethyl methacrylate) (polyHEMA), which are hydrophilic.\textsuperscript{57} In contrast, SH materials contain various siloxane based monomers and macromers, consequently resulting in a more hydrophobic polymer.\textsuperscript{57} The structure of natamycin is amphipathic, in which the bulk of the molecule is hydrophobic, but the carboxyl group and mycosamine moiety are hydrophilic.\textsuperscript{47} This inherent property would suggest that natamycin could interact with both CH and SH materials. However, because the drug is mainly hydrophobic, it would be expected that it has stronger interactions with SH materials than CH materials. Not surprisingly, in this study CH lens materials released 15-30\% of the drug sorbed, as compared to SH lens materials which released only 2-10\% of the drug sorbed, with the exception of balafilcon A, which released approximately 21\% of the drug sorbed. The reason for the observed partial release of natamycin may be due to its strong interaction with the CL material. An uptake study looking at the interaction between commercially available SH materials with Vitamin E (which is hydrophobic), revealed that even after six months of storage, the amount of vitamin E sorbed by the lenses did not diffuse into the storage buffer (PBS).\textsuperscript{32} A similar study looking at ciprofloxacin uptake with commercial lenses also suggested that some of the drug sorbed was partially irreversible.\textsuperscript{40} Similarly, because of its
hydrophobic nature, there may be a large percentage of the natamycin sorbed within the matrix of the CL material that is irreversibly bound.

The water content of the CL material also has been suggested as a factor influencing drug uptake and release.\textsuperscript{41} With the exception of polymacon, all CH lens materials have a higher water content than SH lens materials.\textsuperscript{57} High drug release is a common feature of high water content CL materials, which could be due to the higher amount of water solvating the drug and transporting it through the CL material.\textsuperscript{41} This transport mechanism may be aided by the relative flexibility of the CL material, and consequently its ability to swell. In this study, CH lens materials swelled up to 2-2.46 times their original sizes when submerged in DMSO, and reverted back to the original sizes when incubated in Unisol 4. This observed flexibility in CH lenses, which is not seen in SH lenses, may facilitate the uptake and release of the drug in CH lenses.

The ionicity of the CL material is also a major determinant of drug uptake and release.\textsuperscript{41} In this study, etafilcon A, vifilcon A, and balafilcon A were charged CL materials. The increase in charge density will result in an increase in effective pore size, as a consequence of the charged repulsion, which will permit higher drug uptake and release.\textsuperscript{58} However, ionicity did not seem to be a major factor in drug uptake and release from CH materials. In contrast, balafilcon A had the highest drug release at 104 ± 18 µg/lens, which was significantly higher than other SH lens materials (Figure 3-3). Lotrafilcon B had the lowest drug release, followed by senofilcon A, comfilcon A, and galyfilcon A (Figure 3-3). A previous study which investigated the uptake and release of ciprofloxacin also found that balafilcon A had the highest drug released amongst the SH lenses.\textsuperscript{39} Similar to other SH lenses in this study, balafilcon A is a low water content lens material. However, unlike other SH materials, it contains a negative charge due to the presence of the carboxylic acid group on the N-vinyl amino acid (see Table 3-1), and is classified as a
group III lens material under the United States Food & Drug Administration.\textsuperscript{57, 58} Based on previous studies, the ionicity of balafilcon A has been suggested as a factor for the observed increase in drug release.\textsuperscript{39, 41} Natamycin is an amino acid, with an isoelectric point of 6.5.\textsuperscript{47} In the uptake phase, neither natamycin nor balafilcon A is charged due to the aprotic nature of DMSO. However, in the release medium (Unisol 4 at pH 7.4), both the drug and the CL material are de-protonated and will have an overall negative net charge. As a consequence, the drug is repelled from the material, resulting in an increase release of the drug. In addition, the increased porosity of the surface and internal network of balafilcon A compared to other SH lens material, may also facilitate the enhanced uptake and release of natamycin.\textsuperscript{59}

The common infectious agents associated with ocular fungal infections are strains of \textit{Fusarium} and \textit{Aspergillus} in tropical regions, and \textit{Candida} in other parts of the world.\textsuperscript{3} Based on previous ocular studies with natamycin, the minimum inhibitory concentration for 90\% of the fungal isolates for \textit{Fusarium spp} are 8 µg/mL, 32 µg/mL for \textit{Aspergillus spp}, and 1 – 4 µg/mL for most \textit{Candida spp}.\textsuperscript{60, 61} In a 2 mL volume, all contact lenses would release sufficient drug to meet the MIC\textsubscript{90} concentrations for both \textit{Candida spp} and \textit{Fusarium spp} within the first 30 minutes. With the exception of lotrafilcon B, comfilcon A, and senofilcon A, the remaining contact lens materials released enough drugs to meet the MIC\textsubscript{90} for the more resistant \textit{Aspergillus spp}. The typical frequency of application for natamycin is one drop at hourly or two-hourly intervals for the first 48 hours depending on the severity of the infection, and may be reduced thereafter. The therapy is generally continued for 6 weeks or until there is a resolution of fungal keratitis.\textsuperscript{62} Thus, to be considered as a viable drug delivery device, the drug-soaked contact lenses should be capable of sustained drug release for a minimum of one day. However, in this study the release of the drug is too rapid, and the tested lens materials released the
maximum amount of drug within the first hour. This burst release profile is typical in uptake and release studies with unmodified commercial contact lens materials; however, it is disadvantageous because it creates a period of drug overdose followed by a period of non-release.39

In conclusion, a total of nine commercially available contact lenses, five SH and four CH lens materials, were tested for their ability to uptake and release natamycin. All lens materials were able to release clinically relevant concentrations of natamycin into the solution after 30 minutes. However, a sustained release profile was not demonstrated and the drug release plateaued after approximately one hour under experimental conditions. CH lens materials had the highest release of natamycin, however, the swelling of these materials in DMSO may not make them practical to be used in this form. Although SH lenses had a much lower release of drug, they are ideal bandage devices because of their high oxygen transmissibility. Among the SH lenses, balafilcon A delivered the highest quantity of drug and showed the most promise as a drug delivery device. This material is also FDA approved for use as a therapeutic lens, and has been shown previously to be effective as a bandage lens.27, 55, 56 Nonetheless, due to the burst release of natamycin observed with all these materials, none of the commercially available contact lens materials in this study are suitable as antifungal ocular drug delivery devices in their current state. Further work is needed to develop a contact lens material which is able to release natamycin at a slow and sustained rate.

3.6 Acknowledgements
This work was funded by the NSERC 20/20 Network for the Development of Advanced Ophthalmic Materials and the Canadian Optometric Education Trust Fund (COETF).
Chapter 4 – In vitro uptake and release of natamycin Dex-b-PLA nanoparticles from model contact lens materials

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</table>
4.1 Overview

4.1.1 Objective

To evaluate the uptake and release of the antifungal agent natamycin encapsulated within poly(D,L-lactide)-dextran nanoparticles (Dex-b-PLA NPs) from model contact lens (CL) materials.

4.1.2 Methods

Six model CL materials (gel 1:poly(hydroxyethyl methacrylate, pHEMA); gel 2:85% pHEMA: 15% [Tris(trimethylsiloxy)silyl]-propyl methacrylate (TRIS); gel 3: 75% pHEMA: 25% TRIS; gel 4: 85% N,N dimethylacrylamide (DMAA): 15% TRIS; gel 5:75% DMAA: 25% TRIS; gel 6: DMAA) were prepared using a photoinitiation procedure. The gels were incubated in: (1) natamycin dissolved in deionized (DI)water, (2) natamycin encapsulated within Dex-b-PLA NPs in dimethylsulfoxide (DMSO)/DI water. Natamycin release from these materials was monitored using UV-Visible spectrophotometry at 304 nm over 7 d.

4.1.3 Results

Natamycin uptake by all model CL materials increased between 1 and 7 d (p<0.001). The uptake of natamycin-NPs was higher than the uptake of the drug alone in DI water (p<0.05). Drug release was higher in materials containing DMAA than pHEMA (p<0.05). All gels loaded with natamycin-NPs also released more drug compared to gels soaked with natamycin in DI water (p<0.001). After 1 h, CL materials loaded with natamycin alone released 28% - 82% of the total drug release. With the exception of gel 6, this burst released was reduced to 21% - 54% for CL materials loaded with natamycin-NPs.

4.1.4 Conclusions

Model CL materials loaded with natamycin-Dex-b-PLA NPs were able to release natamycin for up to 12 h under infinite sink conditions. DMAA-TRIS materials may be more suitable for drug delivery of natamycin due to the higher drug release observed with these materials.
4.2 Introduction

Ocular fungal infections, in particular fungal keratitis, while an uncommon occurrence can lead to vision loss and blindness[1, 2]. These infections are caused by fungal penetration of a compromised corneal epithelium, typically following corneal trauma with vegetable matter[3]. While the prevalence of fungal keratitis is relatively high in tropical regions such as South India, Ghana, and China[4-9], it is generally much lower in temperate regions such as the United Kingdom and the Northern United States[4, 10, 11]. However, there have been numerous case reports of fungal keratitis associated with both therapeutic and daily soft contact lens wear[12-14]. Furthermore, the worldwide outbreak of ocular fungal infections in 2006 associated with the multipurpose contact lens solution ReNu MoistureLoc demonstrated that there is a substantial need for further research in the management of ocular fungal infections[15-17].

The only commercially available and United States Food & Drug Administration (FDA) approved ocular antifungal is natamycin (pimaricin)[18-20]. The drug has low water solubility at physiological pH, and therefore is prescribed as a 5% ophthalmic suspension (Alcon, Fort Worth, TX). However, in this form, it suffers from low drug bioavailability as the drugs are effectively drained out through the naso-lacrimal duct or non-specifically absorbed[21]. As a result, the treatment regimen for ocular fungal infections requires applying drops at hourly or two-hourly intervals for the first 48 hours, and this frequency may be reduced thereafter[22]. This dosing regimen can be very taxing, and patients have to be hospitalized to ensure treatment compliance. As such, a suitable drug delivery platform which can provide sustained drug release will greatly improve the current treatment method.

One strategy to enhance drug delivery to the ocular surface involves the use of contact lenses (CLs). Therapeutic or “bandage” CLs have been used to treat corneal trauma by preventing painful contact between the eyelids and the damaged cornea, enhancing corneal
healing, and preventing further corneal compromise and infections[23-25]. Furthermore, their placement on the eye allows for drug release directly to the cornea, enhancing overall drug bioavailability. The lens polymer can also act as a barrier to slow down drug release to provide sustained drug levels over extended periods, eliminating the need for multiple dosing[26]. However, simple drug loading methods, such as soaking a lens with the drug, often results in rapid drug release[26]. A previous study examining the uptake of natamycin in dimethylsulfoxide (DMSO), and its release from commercial contact lenses indicated that this method resulted in a burst release of the drug from the lens materials within the first hour, followed by a plateau phase[27]. To overcome this problem, a second drug delivery platform using colloidal carriers can be incorporated[28]. Colloidal systems, such as liposomes[29], microemulsions[30], nanosuspension[31], and nanoparticles (NPs)[32]are known to provide selective targeting and sustained drug release. The ladder approach has been shown to be suitable for incorporation with CL materials[26].

Various polymeric materials consisting of poly (D,L-lactide) (PLA), poly(glycolide) (PGA), poly(lactide-co-glycolide) (PLGA), polyethylene glycol (PEG) and poly(caprolactone) (PCL) have been employed for drug delivery using NPs[33, 34]. One of the most commonly used polymers for making biodegradable NPs, PLGA-PEG, produces drug carriers with sizes greater than 150 nm[35]. However, to be effectively incorporated into the lens materials, the NPs should be smaller than the pore size of the desired lens material. For low-water content lens materials, the effective pore size is estimated to be 500 nm and approximately 3500 nm for high-water content materials[36]. Commercially available silicone hydrogel (SH) materials, however, can contain pore sizes well below 150 nm[37]. While the sizes of PLGA-PEG NPs could be made under 100 nm, they tend to suffer from low drug encapsulation and rapid drug release[35].
A recent study has shown that a copolymer consisting of PLA and dextran (Dex-\textit{b}-PLA) can self-assemble into a core-shell structured NP with sizes below 40 nm, and can be precisely fine-tuned between 15-70 nm by altering the molecular weight of the component blocks[38]. These NPs are capable of releasing doxorubicin, a hydrophobic drug, for up to 6 days[38]. We hypothesize that by encapsulating natamycin within nanoparticles, the solubility of the drug can be improved, which will enhance the drug loading into the polymer [39].

The aim of the study was to develop a drug delivery platform which combined the benefits of both a colloidal carrier and CL material for daily treatment of fungal infections. One approach of incorporating NPs into CL materials is to co-polymerize the NP with the lens polymer[26]. However, the synthesis of CL polymers typically involves a photoinitiation or a heating step[26, 40], both of which are not compatible with light or heat sensitive drugs, such as natamycin[41, 42]. Furthermore, by polymerizing the drug-NPs with the lens material, it is likely that the NPs will be trapped within the material. While the drug can still diffuse out of this system, it would be ideal to develop a system in which the NPs could also diffuse out. This will provide more flexibility for future work in regards to modifying the properties of the NPs for improved corneal adhesion and penetration. Here, we develop a method for encapsulating natamycin using Dex-\textit{b}-PLA NPs, and incorporating these NPs into SH lens polymer post lens synthesis. The effectiveness of this drug delivery system in regards to the uptake and release of natamycin from model SH lenses was evaluated.

4.3 Materials and methods

2-hydroxyethyl methacrylate (HEMA), N,N-dimethylacrylamide (DMAA), ethylene glycol dimethacrylate (EGMDA, 3-methacryloxypropyltris(trimethylsiloxy)silane (TRIS), and
dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO). Natamycin was purchased from EMD Millipore (Billerica, MA). NPs were formed using block copolymer Dextran-\textit{b}-poly(\textit{D,L}-lactide), which was synthesized previously[38].

### 4.3.1 Contact lens materials

Model SH materials consisting of HEMA and TRIS were synthesized based on a procedure by van Beek[43]. SH materials consisting of DMAA and TRIS were prepared based on previously published work[44]. The monomer compositions for a 2 mL mixture of the gels are listed in Table 4-1. Additionally, 15 µL of EGDMA (cross-linker) and 9.5 µL of 2-hydroxy-2-methypropiophenone (Irgacure1173, photoinitiator, Sigma-Aldrich) was also added to the 2 mL monomer mixture. The resulting mixture was stirred for 5 minutes before being poured into a 42 mL aluminum weighing mold (Fisher Scientific, Pittsburg, PA). The mold was then placed inside the Dymax Ultraviolet (UV) Curing Chamber (Torrington, CT) and the gel was cured with UV light for 30 minutes (min). The molded gels were hydrated overnight in 100 mL of deionized (DI) water before they were cut into circular discs using a cork borer (1.45cm diameter). The resulting gel discs (1.2mm thickness) were dried overnight before further use.
Table 4-1 Compositions of monomer mixtures (mL) for various gels (15 µL of EGDMA and 9.5 µL 2-hydroxy-2-methylpropiophenone were also added to each composition). Equilibrium water content of model lens materials. The values reported are the mean ± SD (n=3).

<table>
<thead>
<tr>
<th>Composition</th>
<th>HEMA</th>
<th>TRIS</th>
<th>DMAA</th>
<th>Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEL 1 (100% pHEMA)</td>
<td>2.000</td>
<td>-</td>
<td>-</td>
<td>35.2±5.5</td>
</tr>
<tr>
<td>GEL 2 (85% pHEMA/15% TRIS)</td>
<td>1.700</td>
<td>0.300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GEL 3 (75% pHEMA/25% TRIS)</td>
<td>1.500</td>
<td>0.500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GEL 4 (75% DMAA/25% TRIS)</td>
<td>0.300</td>
<td>1.700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GEL 5 (85% DMAA/15% TRIS)</td>
<td>0.500</td>
<td>1.500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GEL 6 (100% DMAA)</td>
<td>-</td>
<td>-</td>
<td>2.000</td>
<td>44.2 ±2.8</td>
</tr>
</tbody>
</table>

4.3.2 Encapsulation of natamycin in Dex-b-PLA NPs via nanoprecipitation

The encapsulation of natamycin in Dex-b-PLA NPs was accomplished using nanoprecipitation as described by Verma et al[38]. Natamycin (1.65 mg/mL) and Dex-b-PLA (6.6 mg/mL) were dissolved in DMSO to form a solution containing 20% drug initial feed. 1 mL of this DMSO solution was added drop-wise into 10 mL of water under stirring for over 5 min. The resulting mixture was allowed to stir for an additional 30 min. The NPs in the water were filtered through a syringe filter (pore size= 200 nm) to remove drug aggregates. The resulting mixture contained approximately 150 µg/mL of natamycin in a 9.1 % DMSO/DI solution. The sizes of the NPs were analyzed using a 90Plus Particle Size Analyzer (Brookhaven, λ = 659 nm at 90°). The volume-averaged multimode size distribution (MSD) mean diameters are reported.

Free drug remaining in the solution was further removed by filtering through Amicon filtration centrifuge tubes (MWCO = 10 kDa, Millipore). The filtered solution was then redissolved in DMSO and the drug loading in the NPs determined by measuring the absorbance of natamycin in the solution using a SpectraMax M5 UV-Vis Spectrophotometer(Molecular Devices, Sunnyvale, CA) at 304 nm. The absorbance was correlated with the concentration of natamycin using the standard calibration curve obtained previously.
4.3.3 Release of natamycin from NPs

Using the procedure described in the previous section, natamycin-encapsulated NPs were prepared and filtered to remove drug aggregates. A purified sample of the NP-drug suspension was collected to measure the maximum absorbance, which was used as the 100% release point. 1 mL of the NP-drug suspension was then injected into a Slide-A-Lyzer Dialysis cassette (MWCO = 20kDA, Fisher Scientific) and dialyzed into 200 mL of the release solution containing phosphate buffered saline (PBS, pH 7.4) at 32±2°C under mild stirring. At specific time intervals $t = 0, 2, 4, 6, 7, 12, 20, \text{and} 24 \text{h}$, 200 µL of the release solution was withdrawn in triplicates, and the drug release was measured using the SpectraMax M5 UV-Vis Spectrophotometer at 304 nm. After the measurement, this solution was then carefully pipetted back into the release medium. All experiments were undertaken in light-minimizing conditions. Two drug NP formulations containing 2.45% and 4.61% drug to nanoparticle weight were tested.

4.3.4 Uptake studies

The model lens materials were incubated in amber vials in two conditions: (1) 20 mL of 30 µg/mL natamycin dissolved in deionized (DI) water, and (2) 4 mL of 150 µg/mL natamycin encapsulated within Dex-b-PLA NPs in 10% dimethylsulfoxide (DMSO)/DI water for 7 days (d) at room temperature on an orbital shaker. Both incubation solutions contained a total amount of 600 µg of natamycin. The uptake of the drug into the lens materials was measured by the depletion of the drug from the solution using the SpectraMax M5 UV-Vis Spectrophotometer at 304 nm. 200 µL was removed from the solution and pipetted into a UV-Star Transparent Plate (Greiner Bio-One, Frickenhausen, Germany) at specific time intervals $t = 0, 1, 2, 3, 4, 5, 6, 7 \text{d}$. The sample was returned to the vial after the absorbance measurement.
4.3.5 Contact lens release studies

After the 7 d uptake period, lenses were removed from the natamycin solution and briefly rinsed with borate buffered saline, pH 7.4 (Unisol 4, Alcon Labs, TX) to remove any residual drug solution not sorbed onto the lens. The lenses were then partially dried on lens paper and placed into amber vials containing fresh 2 mL solution of borate buffered saline. The vials were incubated at 32±1°C with constant rotation over 7 d. The release solution was replenished with a fresh 2 mL of borate buffered saline every 24 h. The release of the drug was monitored using the SpectraMax M5 UV-Vis Spectrophotometer at 304 nm by withdrawing 200 µL from the solution, which was then pipetted into a UV-Star Transparent Plate at specific time intervals t = 0, 1, 30 min, 1, 2, 4, 8, 12, 18, 24 h, 1, 2, 3, 4, 5, 6, 7 d.

4.3.6 Water content

The wet weight (WW) of the lenses was measured using the Sartorius MA 100H (Goettingen, Germany). The lenses were then placed on a piece of lens paper and placed in a microwave for 2 minutes. Thereafter, the dry weight (DW) was measured using the Sartorius MA 100H. The water content (WC) was calculated using the following formula:

\[ WC(\%) = \frac{WW - DW}{WW} \times 100 \]

4.3.7 Statistical analysis

Statistical analysis was performed using Statistica 8 software (StatSoft, Tulsa, OK). All data are reported as mean ± standard deviation, unless otherwise stated. Repeated measures of analysis of variance (RM-ANOVA) were performed to determine the differences across various time points within the same lens material. An ANOVA was conducted to determine the differences between lens materials at each time point. Post-hoc Tukey multiple comparison tests
were used when necessary. In all cases, statistical significance was considered significant for p value of < 0.05. Graphs were plotted using GraphPad Prism 5 software (GraphPad, La Jolla, CA).

4.4 Results

As shown in Figure 4-1a, the encapsulation efficiency of Dex-\textit{b}-PLA NP for 20\% initial drug feed results in NPs containing 2.35\% natamycin, and can be increased to 4.35\% when using 40\% initial drug feed. In contrast, the encapsulation of natamycin for PLGA-PEG is only 0.608\% and 0.697\% under the same conditions. PLGA-PEG encapsulation results in particle sizes between 137 nm to 151.8nm, which is consistent with the literature (Figure 1b)[35]. Dex-\textit{b}-PLA encapsulation produces NPs with particle sizes of 26.1 nm (2.35\% wt) and 26.6 nm (4.61\% wt). Release studies with natamycin-Dex-\textit{b}-PLA NP in phosphate buffered saline (PBS, pH 7.4), show that the NP containing 4.61\% natamycin in weight released more drug than NPs containing 2.35\% natamycin (Figure 4-2). However, both NPs have similar release periods of 12 h before reaching a plateau phase (p<0.05). As a result of these data, the 2.35\% wt NPs were chosen for incubation with the model contact lens materials.

Amongst the model gels, gel 6 was the only one that was brittle. In the hydrated state, DMAA containing gels swelled more than HEMA containing gels. DMAA gels also contain a higher equilibrium water content than HEMA gels (p<0.001) (table 4-1). As shown in Figures 4-3 and 4, the uptake of natamycin within all model lens materials increased between 1 and 7 days (p<0.001). There were no differences in drug uptake between materials containing HEMA (gels 1-3) and DMAA (gels 4-6). However, the uptake of natamycin encapsulated with Dex-\textit{b}-PLA NPs was slightly higher than the uptake of the drug dissolved in DI water (p<0.05). Gel 3 and
Gel 5, which contained the highest amount of TRIS, had the highest amount of natamycin uptake after 7 days for both drug incubation conditions (p<0.05).

As shown in Figures 4-5 and 4-6, the release of the drug was observed to be higher in materials containing DMAA than HEMA (p<0.05). In the first drug loading condition, DMAA gels containing TRIS (gels 4 and 5) released the two highest concentration of drug (p<0.05), 14.2 ± 4.5 µg/lens and 16.1 ± 1.7 µg/lens after 1 day respectively. In the second drug loading condition with NPs, gels 4 and 5 also released the highest drug concentration, 67.2 ± 4.0 µg/lens and 54.5 ± 7.1 µg/lens (p<0.05), respectively. The percentage of drug release was higher for DMAA materials than HEMA materials (p<0.05) after 1 d, as shown in Table 4-2 and 4-3. TRIS containing gels (gels 2-5) also had a lower drug release percentage compared to gels without TRIS (gels 1 and 6) for both drug loading conditions (p<0.05).

Overall, gels loaded using natamycin Dex-b-PLA NPs also released significantly more drug compared to the gels soaked with natamycin in DI water (0<0.001). The percentage of drug release was also higher when the gels were loaded with drug-NPs (p<0.001). After 1 h, CL materials loaded with natamycin alone released 28% - 82% of the total drug release. With the exception of gel 6, this burst release was reduced to 21% - 54% for CL materials loaded with natamycin Dex-b-PLA NPs. In addition, with the exception of gels 2 and 6, the drug equilibration time improved for all gels when incubated with natamycin Dex-b-PLA NPs compared to natamycin in DI water (table 4-2 and 4-3). The drug release within all materials reached an equilibrium within 12 h(p>0.05). However, after replenishing the release solution after every 24 h, gels 1 and 2 incubated with natamycin in DI water continued to release drug for up to 3 days (p<0.05). Gels 2-5 loaded with natamycin Dex-b-PLA NPs continued to release
drugs for up to 4 days (p<0.05), whereas gel 1 released the drug for up to 7 days, when the release solution was replenished every 24 h.

**Figure 4-1** (a) Drug encapsulation efficiency and (b) particle size (nm) of NP

**Figure 4-2** Release of natamycin from ◇ 2.35 wt% and ● 4.61 wt% natamycin-Dex-PLA nanoparticles in 200 mL PBS (pH 7.4)
Figure 4-3 Natamycin uptake (µg/lens) from natamycin in solution for HEMA (gels 1-3) and DMAA gels (gels 4-6) over 7 days as measured by spectrophotometry. The values plotted are the mean ± standard deviation for 3 trials.

Figure 4-4 Natamycin uptake (µg/lens) from natamycin Dex-b-PLA NPs for HEMA (gels 1-3) and DMAA gels (gels 4-6) over 7 days as measured by spectrophotometry. The values plotted are the mean ± standard deviation for 3 trials.
**Figure 4-5** Natamycin release (µg/lens) in Unisol 4 from HEMA (gels 1-3) and DMAA gels (gels 4-6) incubated with natamycin in solution as measured by spectrophotometry. The values plotted are the mean ± standard deviation for 3 trials.

**Figure 4-6** Natamycin release (µg/lens) in Unisol 4 from HEMA (gels 1-3) and DMAA gels (gels 4-6) incubated with natamycin Dex-b-PLA NPs as measured by spectrophotometry. The values plotted are the mean ± standard deviation for 3 trials.
### Table 4-2 Natamycin uptake and release incubated with natamycin dissolved in deionized water. The values reported are the mean ± standard deviation for 3 trials.

<table>
<thead>
<tr>
<th></th>
<th>GEL 1</th>
<th>GEL 2</th>
<th>GEL 3</th>
<th>GEL 4</th>
<th>GEL 5</th>
<th>GEL 6</th>
</tr>
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<tr>
<td>total drug uptake (µg/lens)</td>
<td>57.1 ± 2.3</td>
<td>55.6 ± 2.2</td>
<td>176.9 ± 79.0</td>
<td>103.4 ± 10.3</td>
<td>116.4 ± 4.2</td>
<td>37.9 ± 19.7</td>
</tr>
<tr>
<td>% Drug uptake</td>
<td>9.5 ± 0.3</td>
<td>9.2 ± 0.4</td>
<td>29.5 ± 13.1</td>
<td>17.2 ± 1.7</td>
<td>19.4 ± 0.1</td>
<td>6.3 ± 3.3</td>
</tr>
<tr>
<td>24 h drug release (µg/lens)</td>
<td>6.3 ± 0.5</td>
<td>4.6 ± 0.2</td>
<td>3.5 ± 1.1</td>
<td>14.2 ± 4.5</td>
<td>16.1 ± 1.7</td>
<td>10.6 ± 1.0</td>
</tr>
<tr>
<td>% Release</td>
<td>11.0 ± 0.6</td>
<td>8.3 ± 0.3</td>
<td>2.7 ± 2.2</td>
<td>13.6 ± 3.4</td>
<td>13.8 ± 1.4</td>
<td>32.3 ± 12.7</td>
</tr>
<tr>
<td>Time to equilibrium (h)</td>
<td>6</td>
<td>12</td>
<td>1</td>
<td>0.5</td>
<td>2</td>
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### Table 4-3 Natamycin uptake and release incubated with natamycin Dex-PLA NPs. The values reported are the mean ± standard deviation for 3 trials.

<table>
<thead>
<tr>
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<th>GEL 1</th>
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<th>GEL 4</th>
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<th>GEL 6</th>
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<tr>
<td>total drug uptake condition (µg/lens)</td>
<td>146.3 ± 11.9</td>
<td>88.5 ± 7.7</td>
<td>253.1 ± 56.4</td>
<td>126.6 ± 19.4</td>
<td>152.8 ± 13.5</td>
<td>38.2 ± 8.4</td>
</tr>
<tr>
<td>% Drug uptake</td>
<td>24.4 ± 0.2</td>
<td>14.8 ± 1.2</td>
<td>42.2 ± 9.4</td>
<td>21.1 ± 3.2</td>
<td>25.5 ± 2.3</td>
<td>6.4 ± 1.4</td>
</tr>
<tr>
<td>24h drug release condition (µg/lens)</td>
<td>30.9 ± 2.5</td>
<td>20.6 ± 0.6</td>
<td>23.1 ± 2.4</td>
<td>67.2 ± 5.4</td>
<td>54.5 ± 7.1</td>
<td>27.3 ± 3.1</td>
</tr>
<tr>
<td>% Release</td>
<td>21.3 ± 3.2</td>
<td>23.4 ± 2.6</td>
<td>9.4 ± 1.8</td>
<td>54.3 ± 10.6</td>
<td>35.6 ± 3.2</td>
<td>73.1 ± 13.5</td>
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<td>Time to equilibrium (h)</td>
<td>12</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>0.5</td>
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4.5 Discussion

The first drug loading condition contained model CL materials soaked with natamycin dissolved at its native solubility. The drug uptake into all CL materials in this condition ranged between 6.3% and 29.5%, which are comparable to that of a previous study investigating the uptake and release of natamycin from commercial CL materials[27]. The percentage of natamycin released from these materials after 24 h for SH (2.7%-13.8%) and conventional hydrogel (CH) (11.0%-32.3%) materials are also comparable to results previously reported[27]. The partial release of the drug from the CL material can be attributed to the stronger interaction between the drug and the CL material, in which the equilibrium in an aqueous media highly favours the drug bound to the polymer[27]. Notably, the release time in this study is considerably more favourable, reaching up to 6 and 12 h for gel 1 and gel 2, whereas previously, the drug release reached a plateau for commercial lens materials within 1 h[27]. These differences are likely due to the variations in drug loading protocols, as well as the materials used.

The encapsulation of natamycin with Dex-b-PLA NPs produced particles containing approximately 2.35% natamycin by weight. The PLA chains are suggested to form the hydrophobic core containing the drug, whereas the dextran components are suggested to form the outer hydrophilic layer[38]. As a result, the hydrophobicity of the drug was masked, and the aqueous solubility of natamycin was improved 5-fold. In principle, higher drug concentration in the loading solution will result in higher drug uptake in the hydrogel polymer[45]. Not surprisingly, all model materials (with the exception of gel 6) had a significantly higher drug uptake compared to the first loading condition, when loaded with the drug NPs. Typically higher initial drug loading will consequently lead to an increased drug release. As expected, the gels loaded with natamycin Dex-b-PLA NPs released a greater quantity of drug than the first loading condition.
As shown in Figure 4-2, natamycin encapsulated with Dex-b-PLA NPs can release natamycin over a 12 h period in phosphate buffered saline (PBS, pH 7.4). We hypothesize that over this time frame, the NPs slowly dissociate in solution to release the drug. Since these NPs are less than 30 nm, which is smaller than the pore sizes found in most commercial contact lens materials[37], it is possible that these NPs are absorbed and released from the model CL material without any change in its structural integrity. Similar to the release of other hydrophilic molecules from CL materials, the NPs should equilibrate rapidly in the aqueous release solution[46, 47]. For this reason, we propose that the release mechanism first involves the release of the NPs from the CL material, followed by the slower drug release from the NPs. Since the drug is encapsulated, there should be minimal interaction between the drug and the CL material. The release rate will be primarily dependent on the interaction between the drug and the NP. Thus, the integrity of the released NPs becomes a primary determining factor in sustained drug release. Based on this model, we did not expect any material to have a release period exceeding 12 h. An alternative model would suggest that parts of the NPs could first diffuse from the CL material, and likely act as a surfactant to facilitate the release of the drug from the polymer network.

Upon contact with the CL material, the NPs could dissociate to form undesirable interactions with the polymer. In the case of SH materials, the PLA core of the NP can rearrange to interact with the silicone moieties of the polymer network. As a result, the NPs which are released from these materials may have undergone structural changes, therefore, will release the encapsulated drugs more quickly. Highly hydrophobic gels containing TRIS (gels 3-5) follow this pattern, and release drugs before 12 h. Gel 1(0%TRIS) and gel 2 (15% TRIS) were the only gels able to release the drug for the full 12 h duration. Nonetheless, the release period was
improved for the majority of the gels compared to the first incubation condition. Additionally, we also observed that the percentage of drug release from the polymer also improved.

In this study, we investigated the effects of two common hydrophilic monomers used in soft CL materials, hydroxyethyl methacrylate (HEMA) and dimethylacrylamide (DMAA), on the uptake and release of natamycin-NPs. Notably, HEMA differs from DMAA in that it contains a hydroxyl pendant group, which allows for hydrogen bonding between its monomers. As a result, HEMA materials swelled significantly less than DMAA materials, reflected by the lower equilibrium water content in all model HEMA materials compared to DMAA. While we expected that higher equilibrium water content would correlate to higher drug absorption[46], there were no statistical differences for the uptake of natamycin between DMAA or HEMA gels in both incubation conditions.

The release of the drug-NPs from the CL material initially involves the release of the NPs from the hydrogel network into the aqueous phase of the hydrogel, before they are subsequently released into the surrounding media[45]. Based on this assumption, materials with higher water content should facilitate more drugs released from the polymer[46]. As expected, DMAA containing gels (4-6) released more drug than HEMA containing gels (1-3) for both drug incubation conditions. The percentage of drug released from DMAA materials was also significantly higher than HEMA. However, a high solvent capacity within the hydrogel network would also correlate to a faster drug release period. In both incubation conditions, HEMA containing gels had a longer sustained drug release than DMAA containing gels. When the release solution was replenished after 24 h, HEMA gels could release drugs up to several days. Notably, gel 1 was able to release natamycin up to 7 days when incubated with drug-NPs.
TRIS (3-methacryloxypropyltris(trimethylsiloxy)silane) is a hydrophobic monomer, forming the silicone backbone of the SH materials (gels 2-5) in this study. Interestingly, SH materials containing TRIS were able to uptake more drug-NPs than CH materials. As previously mentioned, the PLA core of the NP can rearrange to interact with the silicone domains of the polymer. This could potentially lead to the drug being exposed, allowing for non-specific interactions between the drug and the lens polymer, such as hydrophobic interaction and hydrogen bonding, between natamycin and the silicone moieties. Consequently, this interaction could result in a slower drug release[39] and a lower percentage of drug release from the polymer. As expected, in both drug loading conditions, CL materials containing TRIS had a lower percentage of drug release, and the quantity of the drug released from model materials were comparable to their CH counterparts. TRIS extended the drug release period only for DMAA gels when loaded with drug-NPs, but not HEMA-containing gels.

In this study, the model CL materials were circular discs, which were approximately 1.2mm thick. This is at least ten times thicker with less surface area than a commercial CL. Thickness is an important parameter in determining drug release, with thicker materials capable of longer extended drug release [47, 48]. With commercial contact lenses, we hypothesize the release of the drug-NPs would occur more quickly. However, since the rate determining step is the release of the drug from the NPs, and not the release of the drug-NPS from the CL, the drug release rate in a CL using these NPs would still be relatively similar.

Dextran-PLA NPs may also be useful in an ophthalmic formulation. However, in the form of an ophthalmic drop, this formulation will not be efficient as it will undergo removal mechanisms including dilution[49-51], dispersion[52], drainage[49, 51], and non-specific absorption[49, 51, 53]. The CL limits drug loss through these routes, while at the same time acts
as a temporary drug-NP reservoir. The post-lens tear film has limited tear mixing [54, 55]. Therefore, the drug released from the CL into this post-lens tear film will have a prolonged contact time with the cornea, leading to enhanced bioavailability [56]. Alternatively, adding mucoadhesive properties to the NPs will also prolong the residence time on the eye.

The delivery of drugs to the ocular surface using CLs have faced considerable challenges and have not yet led to a viable product. While the CL and Dex-b-PLA NPs system proposed in this paper is capable of releasing the drug for up to 12 h, careful consideration in regards to initial release rates should be made. One of the main ongoing challenges for controlled drug delivery using CLs is to obtain zero-order release kinetics, without suffering from the initial burst. Using conventional drug loading methods, CL show burst drug release ranging from 28% - 82% of the total drug release after 1 h. This is in agreement with results found previously in other studies [27, 46, 47]. Although the use of Dex-b-PLA NPs does minimize this burst release to 21% - 54% for CL materials (with the exception of gel 6), the overall burst effect is still present. Nonetheless, burst release followed by a steady release of drug could be considered ideal in regards to corneal infections. The initial burst is aimed at killing the majority of the infectious agents, while the sustained release prevents the growth of the remaining microbes.

The common infectious agents associated with ocular fungal infections are strains of Fusarium and Aspergillus in tropical regions, and Candida in other parts of the world [3]. Based on previous ocular studies with natamycin, the minimum inhibitory concentration for 90% (MIC90) of the fungal isolates for Fusarium spp are 8 µg/mL, 32 µg/mL for Aspergillus spp, and 1-4 µg/mL for Candida spp [57, 58]. Although HEMA gels released drugs slower than DMAA gels, the amount of drug released by these gels in a 2 mL volume can only meet the minimum inhibitory concentration for 90% (MIC90) of the fungal isolates for Candida spp. DMAA gels
released enough drug to meet the MIC for *Fusarium spp* and *Candida spp*, and thus may be more suitable hydrophilic monomers in drug delivery for daily treatment of fungal infections. Nonetheless, the conditions for the release studies are not reflective of ocular conditions. Further in vivo studies need to be conducted in order to determine the true effectiveness of this drug delivery platform in the eye.

4.6 Conclusion

We have demonstrated that the encapsulation of natamycin by Dex-\textit{b}-PLA NPs yields a drug delivery carrier providing extended drug release for up to 12 hours under infinite sink conditions. These drug-NPs can be incorporated into CL materials post lens synthesis for targeted drug release directly to the cornea for daily treatment of fungal infections. This system provides extended natamycin release from CL materials compared to conventional drug loading methods. The delivery system was compatible with HEMA, DMAA, and TRIS containing gels. Overall, materials containing DMAA-TRIS may be more suitable than HEMA-TRIS materials for drug delivery of natamycin due to the higher drug release observed with these materials.

4.7 Acknowledgements

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Chapter 5 – In vitro drug release of natamycin from β-cyclodextrin and 2-hydroxypropyl β-cyclodextrin functionalized contact lens materials

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5.1 Overview

5.1.1 Objective

The antifungal agent natamycin can effectively form inclusion complexes with beta-cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (HP-βCD) to improve the water solubility of natamycin by 16-fold and 152-fold respectively (Koontz, J. Agric. Food.Chem. 2003). The purpose of this study was to develop contact lens (CL) materials functionalized with methacrylated β-CD (MβCD) and methacrylated HP-βCD (MHP-βCD), and to evaluate their ability to deliver natamycin in vitro.

5.1.2 Methods

Model conventional hydrogel (CH) materials were synthesized by adding varying amounts of MβCD and MHP-βCD (0, 0.22, 0.44, 0.65, 0.87, 1.08 % of total monomer weight) to a monomer solution containing 2-hydroxyethyl methacrylate (HEMA). Model silicone hydrogel (SH) materials were synthesized by adding similar concentrations of MβCD and MHP-βCD to N,N-dimethylacrylamide (DMAA)/10% 3-methacryloxypropyltri(trimethylsiloxy)silane (TRIS). The gels were cured with UV light, washed with ethanol and then hydrated for 24 hours (h). The model materials were then incubated with 2 mL of 100 μg/mL of natamycin in phosphate buffered saline (PBS) pH 7.4 for 48 h at room temperature. The release of natamycin from these materials in 2 mL of PBS, pH 7.4 at 32±2°C was monitored using UV-Visible spectrophotometry at 304 nm over 24 h.

5.1.3 Results

For both CH and SH materials, functionalization with MβCD and MHP-βCD improved the total amount of drugs released up to a threshold loading concentration, after which further addition of methacrylated CDs decreased the amount of drugs released (p<0.05). The addition of CDs did not extend the drug release duration; the release of natamycin by all model materials reached a plateau after 12 hours (p<0.05). Overall, DMAA/10% TRIS materials released significantly more drug than HEMA materials (p<0.05). The addition of MHP-βCD had a higher improvement in drug release than MβCD for both HEMA and DMAA/10% TRIS gels (p<0.05).

5.1.4 Conclusions

A high loading concentration of methacrylated CDs decreases overall drug delivery efficiency, which likely results from an unfavourable arrangement of the CDs within the polymer network leading to reduced binding of natamycin to the CDs. HEMA and DMAA/10% TRIS materials functionalized with MHP-βCD are more effective than those functionalized with MβCD to deliver natamycin.
5.2 Introduction

There have been numerous cases of fungal eye infections associated with therapeutic and daily soft contact lens wear.[1-3] These infections occur as a result of fungal penetration of a compromised corneal epithelium,[4] and can lead to vision loss and blindness if left untreated.[5, 6] In 2006, a worldwide outbreak of ocular fungal infections occurred as a result of a multipurpose contact lens solution ReNu MoistureLoc, [7-9] which has prompted further research into the management of ocular fungal infections.

In comparison to bacterial infections, there are few drugs available to treat ocular fungal infections. Fungi are eukaryotic and share similarities with human hosts, which make it difficult to identify unique drug-targets.[10] Currently, natamycin (pimaricin) is the only commercially available and United States Food & Drug Administration (FDA) approved ocular antifungal. [11-13] The drug has low water solubility at physiological pH, and therefore is prescribed as a 5% ophthalmic suspension (Alcon, Fort Worth, TX).

However, in an eye drop form, the drug delivery is inefficient as the drugs are continuously diluted and washed away by tears,[14-16] or dispersed from the eye during blinking,[17] drainage,[14, 16] or non-specific absorption.[14, 16, 18] As a result, it has been estimated that only 1-7% of the medication within an eye drop reaches the target ocular tissue,[17] while the remainder is subjected to systemic absorption.[19] To achieve therapeutic drug concentrations to treat ocular fungal infections, multiple dosing is typically required, sometimes as often as applications at hourly to two-hourly intervals.[20] This can lead to problems relating to patient compliance,[21-23] as well as the potential for drug overdosing.[24]

Drug delivery using contact lenses (CLs) can potentially overcome several of the current limitations associated with eye drops. The post-lens tear film, formed as a result of placing a CL
on the cornea, has limited tear exchange.[25, 26] This is advantageous in regards to drug
delivery, as drugs released from the CL into the tear film will have prolonged contact time with
the cornea.[27] It has been estimated that over 50% of the drugs released from a CL can diffuse
into the cornea, which is at least 35 times more efficient than eye drops.[28] Furthermore, the CL
polymer can also act as a barrier and reservoir to provide sustained drug release over extended
periods, which eliminates the need for multiple dosing.[29] CLs have already been used
therapeutically as ‘bandage’ lenses to treat damaged corneas by preventing painful contact
between the eyelids and the cornea, to enhance corneal healing, and to prevent further corneal
complications. [30-32] Pharmaceuticals, including antibiotics and anti-inflammatory drugs are
typically administered topically in tandem with these CLs.[33] Thus, the application of using
CLs for antifungal ocular drug delivery would be an extension of an already accepted ophthalmic
practice.

However, simple drug soaking with CLs does not produce optimal results, with drug
release occurring rapidly within a few hours.[29] We have previously examined the drug
delivery of natamycin from several commercial CLs, and observed a burst release within the first
hour, followed by a plateau phase. [34] This is not surprising, as commercial CLs are only
designed for refractive error correction, and further material modifications are necessary to
improve drug delivery using these materials. Amongst the various approaches, the synthesis of a
biomimetic material created through molecular imprinting methods have proven to be very
successful in providing sustained drug release.[35] These hydrogels contain recognitive sites,
which can specifically interact with the target drug.[35] However, one major limitation of this
approach is that each material is specific to its target drug, and the same material cannot be used
to provide the same effective delivery for other ophthalmic drugs.
One alternative approach would be to functionalize hydrogels with monomers capable of establishing interactions with a variety of drug molecules. In the pharmaceutical field, cyclodextrins (CDs) have proven to be effective and versatile for a wide range of drug delivery applications, due to their ability to complex with a wide array of drugs.[36] CDs are a family of cyclic oligosaccharides with a hydrophilic outer surface, and a lipophilic central cavity.[36] Commonly used CDs in the pharmaceutical field include α-CD, β-CD, and γ-CD, which are 6-, 7-, 8-membered sugar rings respectively.[36] Their unique chemical structure allows them to be used as complexing agents to increase the aqueous solubility of poorly water-soluble drugs, to increase both drug bioavailability and stability.[37] The use of β-cyclodextrin (βCD) and 2-hydroxypropyl-β-cyclodextrin (HP-βCD) has been suggested to improve the aqueous solubility of natamycin by 16 and 152-fold respectively.[38] Thus, the incorporation of these molecules within a CL may allow for improved interactions between the CL and natamycin, leading to better drug delivery profiles. The purpose of the current study was to develop CL materials functionalized with β-CD and HP-βCD, and evaluate their ability to release natamycin in vitro.

5.3 Materials and methods

2-hydroxyethyl methacrylate (HEMA), N,N-dimethylacrylamide (DMAA), ethylene glycol dimethacrylate (EGMDA, 3-methacroyloxypropyltris(trimethylsiloxy)silane (TRIS) were purchased from Sigma-Aldrich (St. Louis, MO). Natamycin was purchased from EMD Millipore (Billerica, MA). Di-methacrylated β-cyclodextrin (MβCD) and di-methacrylated (2-hydroxypropyl)-β-cyclodextrin (MHP-βCD) were purchased from Specific Polymers (France). The molecular structure of MβCD and MHP-βCD are shown in figure 5-1.
Di-methacrylated β-cyclodextrin (MβCD) (MW=1430 g/mol) and di-methacrylated (2-hydroxypropyl)-β-cyclodextrin (MHP-βCD) (MW=2710 g/mol)

5.3.1 Equilibration of natamycin with MβCD and MHP-βCD

Various concentrations of the MβCD and MHP-βCD were dissolved in deionized (DI) water and vortexed for 10 minutes to determine the maximum water solubility of these compounds. Increasing amounts of natamycin was then added to these solutions, and allowed to equilibrate for 24 h to determine the maximum amount of natamycin that can be equilibrated (when equilibrated, the solution turns from opaque to clear).

5.3.2 Contact lens materials

Model conventional hydrogel (CH) contact lens materials consisting of HEMA were synthesized based on a procedure previously reported by van Beek.[39] Model silicone hydrogel
(SH) materials consisting of DMAA and TRIS were also prepared based on previously published work. [40] MβCD and MHP-βCD were dissolved in DI water (or ethanol) to concentrations of 50, 40, 30, 20, and 10 mg/mL. CH materials were synthesized by adding 400 μL of the above cyclodextrin solution to 1.6 mL of HEMA. For the synthesis of SH materials, due to the immiscibility of water and TRIS, MβCD and MHP-βCD were dissolved in ethanol at similar concentrations, and were added to 1.6 mL of DMAA/10% TRIS. Additionally, 95 μL (5% wt) of EGDMA (cross-linker) and 9.5 μL of 2-hydroxy-2-methypropiophenone (Irgacure1173, photoinitiator, Sigma-Aldrich) were also added to the 2 mL monomer mixture. The resulting mixture was stirred for 5 minutes before being poured into a 42 mL aluminum weighing mold (Fisher Scientific, Pittsburg, PA). The mold was then placed inside the Dymax Ultraviolet (UV) Curing Chamber (Torrington, CT) and the gel was cured with UV light for 10 minutes (min). The molded gels were washed with ethanol, and hydrated overnight in 100 mL of deionized (DI) water before they were cut into circular discs using a cork borer (1.45cm diameter). The resulting gel discs (1.2mm thickness) were dried overnight before further use.

5.3.3 Drug incubation and release

The above CL materials were soaked in a 2 mL suspension (saturated solution) containing 100 mg/mL of natamycin in phosphate buffered saline (PBS), pH 7.4 over 48 h. Due to the turbidity of the suspension, the uptake of the drug into the CL material could not be monitored. After the uptake period, lenses were removed from the natamycin solution and briefly rinsed with PBS to remove any residual drug solution not sorbed onto the lens. The lenses were then partially dried on lens paper and placed into amber vials containing fresh 2 mL solution of PBS. The vials were incubated at 32±1°C with constant rotation over 24 h. The release of the drug was monitored using the SpectraMax M5 UV-Vis Spectrophotometer at 304 nm by
withdrawing 200 µL from the solution, which was then pipetted into a UV-Star Transparent Plate at specific time intervals t = 0, 1, 30 min, 1, 2, 4, 8, 12, 16, 24 h. After each measurement, the 200 µL sample solutions were pipetted back into their respective vials.

5.3.4 Water content

The wet weight (WW) of the lenses was measured using the Sartorius MA 100H (Goettingen, Germany). The lenses were then placed on a piece of lens paper and placed in a microwave for 2 minutes. Thereafter, the dry weight (DW) was measured using the Sartorius MA 100H. The water content (WC) was calculated using the following formula:

\[
WC (\%) = \frac{WW - DW}{WW} \times 100
\]

5.3.5 Statistical analysis

Statistical analysis was performed using Statistica 8 software (StatSoft, Tulsa, OK). All data are reported as mean ± standard deviation, unless otherwise stated. Repeated measures of analysis of variance (RM-ANOVA) was performed to determine the differences across various time points within the same lens material. An ANOVA was conducted to determine the differences between lens materials at each time point. Post-hoc Tukey multiple comparison tests were used when necessary. In all cases, statistical significance was considered significant for p values of < 0.05. Graphs were plotted using GraphPad Prism 5 software (GraphPad, La Jolla, CA).

5.4 Results

Preliminary experiments in our lab with natamycin established that approximately 125 mg/mL of 2-hydroxypropyl-β-cyclodextrin in DI water could equilibrate completely with 2
mg/mL of natamycin after 24 hours (h). This is comparable to results previously reported by Koontz et al.[38] However, the cyclodextrin derivatives, MβCD and MHP-βCD could only be solubilized up to a concentration of 50 mg/mL in DI water. In addition, these CD derivatives could equilibrate only up to 500 μg/mL of natamycin after 48 h; the inclusion complex efficiency of MβCD and MHP-βCD were significantly less than the parent compound.[38]

For all CL materials, the drug release plateaued after 12 hours, with neither MβCD nor MHP-βCD affecting the drug release duration (p<0.05). However, functionalization with cyclodextrin improved the total amount of drugs released for some materials (p<0.05). As shown in figure 5-2, the drug release for HEMA materials containing MβCD followed a trend in which an increase in cyclodextrin beyond 0.22% of total polymer weight resulted in a reduction of drug release (p<0.05). Similarly, HEMA materials containing 0.65% MHP-βCD had the highest drug release, and further increase in cyclodextrin loading led to a decline in drug release (figure 5-2B) (p<0.05). The amount of natamycin released (μg/lens) as a function of time squared (t^{1/2}) for the first hour are plotted in figures 5-2C (MβCD) and D(MHP-βCD).
Figure 5-2 Total natamycin release (µg/lens) from HEMA gels functionalized with (A) MβCD (B) MHP-βCD after 24 h. The relationship between the amount of natamycin released (µg/lens) and $t^{1/2}$ for the first hour are plotted in (C) MβCD and (D) MHP-βCD. The values plotted are the mean ± standard deviation for 3 trials.

For DMAA/10% TRIS materials, the amount of drug release correlated with increasing concentrations MβCD (figure 5-3A) ($p<0.05$), which was an exception to the observed trend. However, the functionalization of MHP-βCD with these materials continued to follow the trend, in which the highest drug release was observed at 0.48% MHP-βCD, and further cyclodextrin addition resulted in a decrease in drug release (figure 5-3B) ($p<0.05$). Figures 5-3C (MβCD) and D (MHP-βCD) show the amount of natamycin released from DMAA/10% TRIS materials as a function of time squared ($t^{1/2}$).
As a general trend, HEMA and DMAA/10% TRIS CL materials functionalized with MHP-βCD produced materials that could release higher amounts of natamycin compared to those functionalized with MβCD (p<0.05). However, as shown in figures 5-4A and B, the percentage of CD in the polymer and the monomer composition also dictate which CD will be more effective. For instance, HEMA gels containing 0.22% MβCD released more drugs than the 0.22% MHP-βCD formulation (p<0.05). DMAA/10% TRIS gels containing MβCD at 1.20% CD of polymer weight released more drugs than the MHP-βCD formulation (p<0.05). Overall, the drug release was higher for DMAA/10% TRIS CL materials than HEMA materials (p<0.01).
Figure 5-4 The relationship between total natamycin released (µg/lens) and the cyclodextrin percent (CD) of total polymer weight for (A) HEMA and (B) DMAA/10% TRIS materials. The vertical bars denote 0.95 confidence intervals.

All model CL materials synthesized in this study were clear by visual inspection. When hydrated, DMAA/10% TRIS materials swelled more than HEMA containing gels. Furthermore, as shown in table 5-1 and 5-2, DMAA/10% TRIS materials also had a higher water content than HEMA gels (p<0.001). The addition of either MβCD or MHP-βCD increased the equilibrium water content (EWC) of the lens material, in which increasing cyclodextrin concentration resulted in higher EWC (p<0.05). Overall, the addition of MβCD resulted in a higher EWC than MHP-βCD at similar concentrations (p<0.001). Tables 5-1 and 5-2 summarize the properties of the CLs, and total amount of drug released by each gel after 8 and 24 h. The highest drug release after 24 h was observed for 0.48% MHP-βCD DMAA/10% TRIS (31.7 ± 1.2 µg after 24 h).
Table 5-1 Total amount of natamycin released after 8 and 24 h in 2 mL of PBS, pH 7.4 for HEMA materials. The values reported are the mean ± SD (n=3).

<table>
<thead>
<tr>
<th>Gel (HEMA)</th>
<th>MβCD (%) by total polymer weight</th>
<th>MHP-βCD (%) by total polymer weight</th>
<th>Water content (%)</th>
<th>Total drug release 8 h (µg/lens)</th>
<th>Total drug release 24 h (µg/lens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>15.5 ± 0.7</td>
<td>10.9 ± 1.3</td>
<td>11.0 ± 1.4</td>
</tr>
<tr>
<td>2</td>
<td>0.22</td>
<td>-</td>
<td>17.7 ± 0.1</td>
<td>20.6 ± 0.2</td>
<td>21.0 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>0.44</td>
<td>-</td>
<td>21.1 ± 4.6</td>
<td>10.1 ± 0.56</td>
<td>10.4 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>0.65</td>
<td>-</td>
<td>22.0 ± 0.5</td>
<td>6.0 ± 0.1</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>0.87</td>
<td>-</td>
<td>23.9 ± 1.0</td>
<td>3.5 ± 0.1</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>1.08</td>
<td>-</td>
<td>24.5 ± 3.0</td>
<td>3.9 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>0.22</td>
<td>15.6 ± 0.3</td>
<td>12.8 ± 0.6</td>
<td>12.8 ± 1.0</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>0.44</td>
<td>16.6 ± 0.6</td>
<td>16.0 ± 0.5</td>
<td>15.3 ± 0.3</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>0.65</td>
<td>20.8 ± 4.0</td>
<td>22.9 ± 1.3</td>
<td>22.8 ± 0.1</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>0.87</td>
<td>20.8 ± 4.0</td>
<td>7.5 ± 0.9</td>
<td>6.4 ± 0.5</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>1.08</td>
<td>18.4 ± 2.1</td>
<td>4.8 ± 0.2</td>
<td>4.5 ± 0.1</td>
</tr>
</tbody>
</table>

Table 5-2 Total amount of natamycin released after 8 and 24 h in 2 mL of PBS, pH 7.4 for DMAA/10% TRIS materials. The values reported are the mean ± SD (n=3).

<table>
<thead>
<tr>
<th>Gel (DMAA/10% TRIS)</th>
<th>MβCD (%) by total polymer weight</th>
<th>MHP-βCD (%) by total polymer weight</th>
<th>Water content (%)</th>
<th>Total drug release 8 h (µg/lens)</th>
<th>Total drug release 24 h (µg/lens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0</td>
<td>0</td>
<td>24.1 ± 3.0</td>
<td>9.5 ± 1.0</td>
<td>11.6 ± 0.2</td>
</tr>
<tr>
<td>13</td>
<td>0.24</td>
<td>-</td>
<td>25.2 ± 0.5</td>
<td>8.6 ± 0.8</td>
<td>10.5 ± 0.1</td>
</tr>
<tr>
<td>14</td>
<td>0.48</td>
<td>-</td>
<td>30.1 ± 0.3</td>
<td>8.8 ± 0.4</td>
<td>10.7 ± 1.1</td>
</tr>
<tr>
<td>15</td>
<td>0.72</td>
<td>-</td>
<td>31.4 ± 1.7</td>
<td>11.4 ± 0.5</td>
<td>15.0 ± 0.6</td>
</tr>
<tr>
<td>16</td>
<td>0.96</td>
<td>-</td>
<td>39.0 ± 2.3</td>
<td>21.0 ± 1.0</td>
<td>22.1 ± 0.4</td>
</tr>
<tr>
<td>17</td>
<td>1.20</td>
<td>-</td>
<td>41.6 ± 2.0</td>
<td>22.6 ± 0.2</td>
<td>27.6 ± 1.0</td>
</tr>
<tr>
<td>18</td>
<td>-</td>
<td>0.24</td>
<td>21.8 ± 1.4</td>
<td>11.4 ± 0.5</td>
<td>16.9 ± 0.5</td>
</tr>
<tr>
<td>19</td>
<td>-</td>
<td>0.48</td>
<td>24.2 ± 2.1</td>
<td>30.2 ± 0.9</td>
<td>31.7 ± 1.2</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>0.72</td>
<td>27.1 ± 0.4</td>
<td>27.5 ± 0.6</td>
<td>28.6 ± 0.3</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>0.96</td>
<td>28.9 ± 1.0</td>
<td>18.4 ± 0.5</td>
<td>21.5 ± 0.7</td>
</tr>
<tr>
<td>22</td>
<td>-</td>
<td>1.20</td>
<td>33.0 ± 1.0</td>
<td>12.0 ± 0.4</td>
<td>15.4 ± 1.1</td>
</tr>
</tbody>
</table>
5.5 Discussion

A previous study by Koontz and colleagues that used natamycin, [38] showed that the solubility of the antifungal can be increased 16-fold, 152-fold, and 73-fold when dissolved at the highest concentration of βCD (1.8% w/v), HP-βCD (50.0% w/v), and γCD (24.6% w/v). It would appear that the most effective CD to complex with natamycin would be HP-βCD, followed by γCD and βCD. However, one important factor to consider is the maximum water solubility of these CD, with βCD only having a maximum solubility at 18 mg/mL compared to HP-βCD at 500 mg/mL.[38] Thus, when CD solubility is also considered, βCD and HP-βCD have very similar inclusion complex efficiency with natamycin. At relatively lower CD concentrations below 20 mM, all three CDs were equally effective at complexing with natamycin.[38] As expected, the addition of two methacrylated chains to βCD and HP-βCD, to produce MβCD and MHP-βCD, resulted in compounds with a water solubility of only 50 mg/mL. This is almost a 10-fold decrease in solubility for HP-βCD, while the solubility for βCD improved by over two-fold. However, as shown in table 5-3, the ability of MβCD and MHP-βCD to complex with natamycin decreased in comparison to the parent compound.[38]

<table>
<thead>
<tr>
<th>Cyclodextrin (CD)</th>
<th>CD Concentration (mg/mL)</th>
<th>Average molecular weight (g/mol)</th>
<th>CD Concentration (mM)</th>
<th>Estimated natamycin solubility (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>βCD</td>
<td>18 (max)</td>
<td>1134.94</td>
<td>15.86</td>
<td>500 [38]</td>
</tr>
<tr>
<td>HP-βCD</td>
<td>50</td>
<td>1375.36</td>
<td>36.35</td>
<td>1250 [38]</td>
</tr>
<tr>
<td>γCD</td>
<td>50</td>
<td>1297.12</td>
<td>38.55</td>
<td>1000 [38]</td>
</tr>
<tr>
<td>MβCD</td>
<td>50</td>
<td>1430.00</td>
<td>35.00</td>
<td>500</td>
</tr>
<tr>
<td>MHP-βCD</td>
<td>50</td>
<td>2710.00</td>
<td>18.50</td>
<td>500</td>
</tr>
</tbody>
</table>

The incorporation of MβCD and MHP-βCD to HEMA and DMAA/10% TRIS model CL materials produced some unexpected results. The highest concentration of CDs initially loaded in the monomer mixture was 10 mg/mL (1.08-1.20% of total polymer weight). At this
concentration, the amount of CDs forming inclusion complexes with natamycin should increase linearly with increasing CD concentration.[38] However, only DMAA/10% TRIS gels incorporated with MβCD followed the expected trend. For HEMA gels incorporated with either MβCD or MHP-βCD above 0.22% and 0.65% of total polymer weight, the amount of natamycin released showed a reduction with increasing loading concentration of CDs. This was also observed for DMAA/10% TRIS materials functionalized with MHP-βCD at concentrations above 0.48%. This effect appears to be dependent on the monomer composition of the material, as well as the type of CD.

The underlying mechanism is not well understood and we propose the following hypothesis. Drugs are released from the CD gels from two sites; (1) non-specific sites formed randomly throughout the free space within the polymer, and (2) specific sites formed by the CD. As the CD concentration increases, there is an increase in specific sites, resulting in increased drug release. However, due to volume constraints, as the number of specific sites increase beyond a threshold concentration, there is also a reduction in the number of non-specific sites. As a result, this offsets any increase in drug release provided by CDs. With a high CD concentration, the arrangement of the CD in the polymer becomes over saturated, in which their complexing centres are hindered by side chains, and become inaccessible to the drug.

We initially expected that the incorporation of CDs within the polymer, which could interact with the drug, would lead to a delayed and extended release of the drug from the polymer. However, all gels within this study released all the drug within the first 12 h, suggesting that time for drugs in CDS to equilibrate with PBS is about 12 h. This release duration is similar to what has been reported for two antifungals, naftifine and terbinafine, from hydrogels functionalized with βCD.[41] The drug release profile from the model CLs in this...
study also suggests a diffusion-controlled process, and the CDs did not significantly affect the rate of drug release. This suggests while the CDs can improve the total amount of drug that can be released, the rate of drug equilibration will remain similar to that of the control material.

In general, the incorporation of MHP-βCD with HEMA and DMAA/10% TRIS gels provides a higher amount of drug release than MβCD. However, the amount of drugs released is also dependent on the percent of CD in the polymer and the monomer composition of the gel. DMAA/10% TRIS materials released more drug than the HEMA-based materials. This trend has been observed previously in another study.[42] The mechanism is not clear, but it has been suggested that DMAA based materials typically contain higher water content than HEMA based materials, which helps facilitate the release of the drug from the polymer.[42]

An important factor in CL synthesis is to ensure a uniform distribution of the individual monomers by minimizing phase separation between monomers.[43] This can be accomplished by reducing the polymerization time via increasing the cross-linking density.[43] It has been reported that a composition of approximately 4% by total weight of a cross-linking agent is most optimal to decrease gelation time, and minimize phase separation effects.[43] In this study, a 5% EGDMA cross-linking density was used to ensure the distribution of CDs throughout the material. However, the typical amount of EGDMA material used in CL synthesis is approximately only 0.5% EGDMA.[42, 44] By increasing the cross-linking density in a fixed volume, the resulting effect is a decrease in equilibrium water content (EWC).[45] Furthermore, EGDMA which is hydrophobic will also increase the overall hydrophobicity of the material. Previous studies report HEMA and DMAA materials with 0.5% EGDMA to contain approximately 30-35% and 44% EWC respectively.[42] By increasing the concentration of EGDMA to 5% in this study, the EWC decreases for both HEMA and DMAA gels to 15.5 ±
0.7% and 24.1 ± 3.0% respectively. The addition of either MβCD or MHP-βCD increased the EWC for all materials, with increasing CD content correlating to increasing EWC. Surprisingly, MβCD provided better improvement in EWC than MHP-βCD, although both of these compounds have similar water solubility, and HP-βCD is more water soluble than βCD. The mechanism as to why MβCD absorbs more water than MHP-βCD is unclear, however, we hypothesize that the additional 2-hydroxypropyl chains on MHP-βCD in a polymer network may occupy and displace water molecules in hydrophilic regions of the polymer.

One important limitation to consider when applying CDs to a CL is the amount of CDs that can be effectively functionalized into the polymer, which correlates to the amount of total drugs that can effectively form inclusion complexes with the material. Based on our results, 50 mg/mL of MβCD or MHP-βCD can effectively equilibrate up to 500 µg/mL of natamycin over 48 h. However, if we take into account the actual volume of CD present in a lens material, the amount of drug that can be complexed with the lens would only be approximately 20 µg per lens. Considering the clinical range where natamycin is effective against various fungi strains, such as Fusarium spp (MIC90=8 µg/mL) and Candida spp (MIC90=1-4 µg/mL), the amount of natamycin that can be complexed with the CD in these CLs may be too little.[46, 47] Nonetheless, all gels in this study released enough drug in a 2 mL volume to meet the MIC90 for Candida spp, and gels 9,16-21 released enough drug to meet the MIC90 for Fusarium spp.

In conclusion, CD functionalized CLs used in this study released more drug than the control CLs, with no significant differences between MβCD and MHP-βCD. When the loading of CDs increases beyond a threshold concentration, the arrangement of the CDs becomes crowded and the CD inclusion site becomes inaccessible to the drug. These CDs improve the EWC of both HEMA and DMAA/10% TRIS materials, with MβCD providing a better
improvement. None of the gels studied released the drug for more than 12 hours, but all model CLs released enough drug to meet the MIC90 for *Candida spp*. The application of MβCD and MHP-βCD could be extended to other hydrogels for the delivery of natamycin, and other antifungal drugs.
Chapter 6 – Development of an in vitro ocular platform to test contact lenses

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2Medella Health, 151 Charles Street West, Kitchener, ON, N2G 1H6

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6.1 Overview

6.1.1 Short abstract
Current \textit{in vitro} models for evaluating contact lenses (CLs) and other eye-related applications are severely limited. The presented ocular platform simulates physiological tear flow, tear volume, air exposure and mechanical wear. This system is highly versatile and can be applied to various \textit{in vitro} analyses with CLs.

6.1.2 Long abstract
Currently, \textit{in vitro} evaluations of contact lenses (CLs) for drug delivery are typically performed in large volume vials,\textsuperscript{1-6} which fail to mimic physiological tear volumes.\textsuperscript{7} The traditional model also lacks the natural tear flow component and the blinking reflex, both of which are defining factors of the ocular environment. The development of a novel model is described in this study, which consists of a unique 2-piece design, eyeball and eyelid piece, capable of mimicking physiological tear volume. The models are created from 3-D printed molds (Polytetrafluoroethylene or Teflon molds), which can be used to generate eye models from various polymers, such as polydimethylsiloxane (PDMS) and agar. Further modifications to the eye pieces, such as the integration of an explanted human or animal cornea or human corneal construct, will permit for more complex \textit{in vitro} ocular studies. A commercial microfluidic syringe pump is integrated with the platform to emulate physiological tear secretion. Air exposure and mechanical wear are achieved using two mechanical actuators, of which one moves the eyelid piece laterally, and the other moves the eyeballeyepiece circularly. The model has been used to evaluate CLs for drug delivery and deposition of tear components on CLs.
6.2 Introduction

Two significant areas of interest within the contact lens (CL) arena include discomfort and the development of novel CL applications. Elucidating the mechanisms underlying CL discomfort is an issue that has eluded the field for decades. The development of novel, functional CLs, such as drug-delivery devices and biosensors, is an area of growing interest, with substantial potential markets. In both circumstances, a sophisticated in vitro model would provide relevant information to assist with selecting appropriate lens materials or design characteristics during the development phase. Unfortunately, current in vitro models for evaluating CLs and other eye related applications are relatively crude and unsophisticated. Traditionally, in vitro CL studies evaluating tear film deposition or drug delivery are performed in static, large volume vials containing a fixed fluid volume, which greatly exceeds physiological amounts. Furthermore, this simple model lacks the natural tear flow component and the blinking reflex, both of which are defining factors of the ocular environment.

The development of a sophisticated, physiologically relevant eye “model” will necessitate a multi-disciplinary approach and require substantial in vivo validation. For these reasons, the fundamental framework for our in vitro eye model is highly versatile, such that the model can be continually improved through future upgrades and modulations. To date, the model is capable of simulating tear volume, tear flow, mechanical wear and air exposure. The aim is to create an in vitro model that will provide meaningful results, which is predictive and complimentary to in vivo and ex vivo observations.

6.3 Protocol

All experiments were completed in accordance and compliance with all relevant guidelines outlined by the University of Waterloo’s animal research ethics committee. The bovine eyes are
generously donated from a local abattoir.

1. Eye model

1.1 Design and production of molds

1.1.1 Design the eye models according to the average physiological dimensions of human adult eyes.

1.1.2 Leave a gap of 250 µm between the eyeball and the eyelid pieces of the eye model. Design the respective molds using computer-aided design (CAD) software.

1.1.3 Create new .cad file or .sldprt file with AutoCAD or Solidworks. Create 3D models of the human eyeball/eyelid. Create molds of the models and save the molds as .stl files.

1.1.4 Import .stl files into 3D printer software (e.g. makeware for replicator2). Specify parameters of the print (location, sparseness, scale, orientation, smoothness, etc.).

1.1.5 Save the file as G-code file for 3D printers to read. Select materials such as PLA (polylactic acid), ABS (acrylonitrile butadiene styrene), PC (polycarbonate), or a combination thereof, to print the molds.

1.1.6 Install desired filament of the material of choice. Import the G-code file into the 3D printer to read. Print the mold.

NOTE: Alternatively, produce the eye molds using a computer numerical controlled (CNC) machine, if a smoother surface on the eye model is desired. For CNC mold production, materials for molds are no longer limited to thermal plastics, but extend to metal, ceramics, and chemically resistive polymers such as Polytetrafluoroethylene.

1.1.7 Open the CNC software interface that is connected to a cutting drill. Construct 3D molds according to front, top, side, and perspective views of the previously-constructed eyeball/eyelid
model molds in control software interface. Select appropriate parameters for the machining (bit size, substrate material, material thickness) and proceed to cut the mold.

1.2 Synthesis of eyepieces using PDMS

1.2.1. Using a syringe, measure 10 mL volume of PDMS (polydimethylsiloxane) base and fill it into a 15 mL – 50 mL centrifuge tube. Add 10% w/v of the elastomer solution by total weight of PDMS. Using a stirring rod, mix the solutions well.

1.2.2. Pour the PDMS solution into the eyeball and eyelid molds. Allow the PDMS to settle at room temperature (RT) overnight (or for at least 12 hours) to start the polymerization and to allow bubbles to dissolve out of the polymer.

NOTE: Ensure that there are no bubbles left in the PDMS that might rise or expand.

1.2.3. Subsequently, put the molds into a 75°C (167°F) oven for 1 h, or 150°C (302°F) for 5 min.

For a softer gel, let the PDMS sit at RT for at least 48 h to completely polymerize.

1.2.4. Put the samples in a freezer for a few minutes; this will shrink the PDMS and simplify the removal of the samples from the molds. Extract the eyepieces from the molds using a thin spatula.

1.2.5. For the delivery of solution into the space between the eyeball and eyelid pieces, connect a 1/16” x 1/8” polytetrafluoroethylene tube with a 1/16” equal leg coupler tube connector and attach it to the eyelid piece at the tubing hole.

1.3 Synthesis of eyeball piece using agarose

NOTE: The eyeball piece can be synthesized using other polymers such as agarose. The following procedure can also be modified to produce eye pieces from a variety of agar types, such as PDA (potato dextrose agar) or SDA (sabouraud dextrose agar).
1.3.1. To produce a 2% (2g/100mL) gel, measure 2 g of agarose and mix with 100 mL of ultrapure water. Bring the solution to a boil (100°C) such that the agarose dissolves completely. Let the solution cool down for 5 min.

1.3.2. Pour the solution into the eyeball mold and allow the solution to cool for 30 minutes at RT. Remove the eyeball pieces with a spatula. Store the eyeball agar in a -20°C freezer for later use. For microbiology studies, sterilize the eyeball molds by autoclaving and/or UV-irradiation.

1.4 Incorporation of bovine cornea on PDMS eyeball

NOTE: This protocol has been adapted from Parekh et al.14

1.4.1. Perform the dissection and incorporation of the bovine corneas in sterile conditions under a laminar flow hood. Acquire the eyes and dissect them on the same day.

1.4.2. Turn the flow hood on for 10 minutes prior to use and sanitize with 70% ethanol alcohol. Ensure that all materials and instruments are sterile by autoclaving at 273°F/133°C for 45 minutes, and positioned no less than 4 inches from the flow hood entrance.

1.4.3. Immerse the bovine eye in a beaker containing 0.5% povidone-iodine solution for 2 minutes. Remove the povidone-iodine solution from the beaker, and rinse eye in a beaker containing phosphate buffered saline (PBS) pH 7.4. Using forceps gently place the eye on a glass petri dish, corneal face up.

1.4.4. Remove the excess muscle and fatty tissue by cutting at the scleral attachment points with blunt end dissection scissors. Dispose of the excess tissue into a sterile beaker designated for animal waste.

1.4.5. Using micro-scissors, remove the conjunctiva from the eye. Wrap the eye with sterile gauze, maintaining a distance of at least 1 cm from the limbus.
1.4.6. Using a scalpel, incise the sclera approximately 2 mm from the limbus region and superficially so as to avoid penetration of the underlying choroid and vitreous body. Carefully extend the incision by 360° using a scalpel or dissection scissors without deforming the cornea from its natural curvature.

1.4.7. With fine forceps, remove the cornea from the eye. Using forceps, carefully remove any adhering uveal tissue and rinse cornea with PBS.

1.4.8. Store the cornea at 31°C in a sterile container with culture medium (such as Medium 199) containing 3% Fetal Bovine Serum to maintain tissue moisture and cell nourishment.

1.4.9. Prior to experimentation, rest the excised cornea on the PDMS eyeball, and clamp the two pieces together with a specialized clip-on.

2. **Blink-platform**

2.1 **Design and production of the blink-platform**

NOTE: The blink-platform is composed of three functional parts: eye model (described in section 1), gear system, and electronic system.

2.1.1. Design and manufacture the blink platform using CAD and 3D printing, similar to that described for the eye model (section 1.1). Design the gear system such that it translates simple rotation of motors into the lateral and rotational motions of the eyepieces.¹⁵

2.1.2. Using the pinion and gear mechanism, translate rotational motion of a stepper motor into the lateral motion of a pinion, which is connected to the eyelid pieces.

2.1.3. Using the conjugate gear system, amplify one rotational motion from a stepper motor into three (or more) rotational motions for three different eyeball pieces.

2.1.4. Align the two gear systems, one for the eyelid and one for eyeball, so that the distance
between the two are constant. Assemble the electronic system with a microcontroller, motor shield, and two motors.

NOTE: Use two stepper motors to provide rotational motors, which is translated by the gear system into a blinking motion.

2.1.5. Connect the two stepper motors with a system consisting of a motor shield stacked on the microcontroller. Connect and configure the electronic components to work with open source software products.

2.1.6. Program the system to control motor parameters such as rounds per minute (RPM), number of rounds forward, number of rounds backward, and turning style. NOTE: Refer to the supplementary “Arduino code file” for details.

2.1.7. Download the system software from the manufacturers’ website.

2.1.8. Install the software and open it. Write the code to control stepper motors in the desired configuration. Connect the system with a source to power the electronic system so that the motors move in the desired manner as defined by the researcher. NOTE: Refer to the supplementary “Arduino code file”.

2.2 Assembly with microfluidics (Artificial Tear Solution)

2.2.1. Take the synthesized eyeball and eyelid pieces and slip them onto their corresponding clip-ons for the eye-model. Connect the tubing that is joined with a syringe and positioned on the microfluidic pump with the eyelid piece (section 1.2.5). Test run the platform and check for consistent movement.

2.2.2. Prime the tubing and check for a steady flow of artificial tear solution (ATS). The recipe for ATS has been previously reported.16
2.2.3. Manually move the eye-model parts together on a level plane, such that the eyeball and the eyelid are in contact. Set the flow rate of the microfluidic pump to desired values. Set physiological flow rates to 1-1.5 µL/min.\(^1\text{7}\)

2.2.4. Start the pump and the actuators to begin experiment. For drug delivery experiments, place the drug-containing contact lens on the eyeball piece.

2.2.5. Allow the flow-through fluid to drip into a 12-well plate. At the desired set time intervals, quantify the analyte or drug concentration using common detection methods such as UV-Vis spectroscopy or fluorescence.\(^1\text{4,18}\)

2.2.6. For studies evaluating deposition of tear components on contact lenses, place the contact lens on the “eyeball” piece. Collect the flow-through fluid, which can be discarded.

2.2.7. After the desired time intervals, remove the contact lens from the eyeball piece and prepare the lens for further analysis such as confocal microscopy.\(^1\text{9}\)

6.4 Results

The synthesized eye molds obtained from the machine shop and from 3-D printing are shown in Figure 6-1. These molds can be used with a variety of polymers, such as PDMS and agarose, to produce eyepieces with the desired properties. The motioned assembly of the eye model platform with a microfluidic syringe pump is shown in Figure 6-2. The platform simulates mechanical wear via the rotation of the eyeball piece, and air exposure through the lateral in and out motion of the eyelid piece. Tear fluid is infused into the eyelid from a microfluidic pump at the desired flow rate, and the flow-through fluid can be collected in a 12-well plate.

The procedure for dissection of a bovine lens, and mounting onto a PDMS eyepiece is depicted in Figure 6-3. The excess tissues are separated from the eye and discarded, followed by
the removal of the conjunctiva. The removal of the cornea begins with an incision into the sclera near the limbus. Figure 6-4 shows the variety of eyepieces that could be used for various in vitro analyses. The mounted eyeball pieces shown are synthesized from PDMS, agar, and an ex-vivo bovine cornea mounted on a PDMS eyeball piece.

Figure 6-5 depicts a study evaluating the release of an antibiotic, moxifloxacin, from CLs.\textsuperscript{18} When measured in the traditional vial model, drug release occurs within the first 2 hours followed by a plateau phase. In contrast, the novel eye model shows drug release to be slow and sustainable for up to 24 hours.\textsuperscript{18} A study evaluating the deposition of cholesterol on CLs is shown in Figure 6-6. The cholesterol in the study was fluorescently tagged in the form of NBD-cholesterol (7-nitrobenz-2-oxa-1,3-diazol-4-yl-cholesterol), and deposition was imaged using laser scanning confocal microscopy. The results indicate that there are substantial differences when the deposition studies are performed in a vial as compared to the eye model.
Figure 6-1 A Eyeball piece mold from machine shop B Eye lid mold from 3-D printing
**Figure 6-2** An *in vitro* ocular platform: 

- **A** Circular motion simulates mechanical wear
- **B** Lateral motion produces intermittent air exposure
- **C** Tear fluid infusion into eyelid
- **D** Collecting well plate

**Figure 6-3** Dissection and incorporation of bovine cornea: 

- **A** Removal of excess tissue
- **B** Removal of conjunctiva
- **C** Incision into the limbus region
- **D** The excised cornea can be stored or mounted on a PDMS eye ball piece
Figure 6-4 Sample eyepieces: Sample of PDMS eye piece with a contact lens, an agar eye piece, and *ex-vivo* bovine cornea mounted eye piece

Figure 6-5 Drug delivery using the *in vitro* ocular platform: Release of moxifloxacin from daily disposable contact lenses from **A** a large volume static vial and **B** the eye model (Re-print with permission from the Association for Research in Vision and Ophthalmology).\(^\text{18}\) All data are reported as mean ± standard deviation.
6.5 Discussion

There are three critical steps within the protocol that require special attention: design and production of molds (section 1.1), platform assembly (section 2.2.1-2.2.3), and monitoring the experimental run (section 2.2.4-2.2.7). In terms of the design and production of molds (section 1.1), the eyeball piece should be designed according to the dimensions of a human cornea. However, it may require multiple prototypes of the mold before an eyeball piece can be created that perfectly fits a commercial contact lens (CL). In addition, the 250 µm needs to be maintained when the eyeball and eyelid piece are in contact to ensure the tear fluid flows smoothly throughout the entire eye model when a CL is present. This distance could be changed in future iterations, but should not be less than 150 µm to allow for enough spacing to fit a CL.

The platform assembly (section 2.2.1-2.2.3) requires careful attention such that the eyeball and eyelid piece come into contact during the blink motion. If the eyepieces are not in perfect contact, then simulation of a closed eyelid and mechanical rubbing fails. The operator should observe the platform in motion for a few cycles to ensure that both the eyeball and eyelid are in contact, and that rubbing occurs as programmed. The current platform is designed to run...
continuously over one month, but an operator should always check on the stability of the system every 24 hours when running an experiment (section 2.2.4-2.2.7). This is important as the current platform does not possess a temperature or humidity control, and fluctuations in these parameters could dry up the CLs. If this occurs, place the eye model within a controlled humidity and temperature chamber. In addition, for drug delivery experiments, the collected flow-through fluid should be analyzed or stored at least every 2 hours to avoid significant evaporation of the sample.

There are currently two limitations of the presented eye model. The first limitation is in regards to exposure to the surrounding environment. Currently, because the eye pieces are not enclosed in a controlled chamber, changes such as temperature and humidity in the work area will influence various aspects of the experiments. For instance, if the environment is too dry, then the CLs dry up quicker and could separate from the eyeball piece, or the flow-through fluid could evaporate. To address this problem, future iterations will house the eye model in a controlled temperature and humidity chamber. The second limitation pertains to the complexity eyeball piece. Currently, the eyepieces are simple, consisting of either PDMS or agarose, neither of which truly represents corneal surface properties. Future work will aim to produce eye models which closer mimics the corneal surface structures.

In vitro ocular research is generally viewed as the preceding testing phase to in vivo research. However, it is important to keep in mind that in vitro research can also be complementary to in vivo data, providing critical insights that otherwise cannot be achieved from in vivo studies alone. Regrettably, the current in vitro models for testing CLs are rudimentary and lack several key components to adequately mimic the in vivo environment. For instance, in vitro CL studies are performed in vials containing 2-5 mL of phosphate buffered saline, which greatly exceeds
physiological tear volumes at 7.0 ± 2 μL. Moreover, two important factors of the ocular environment, natural tear flow and the blinking reflex, are absent from the simple static vial model. The limitations of the conventional vial model have been recognized by researchers, and attempts have been made to create unique *in vitro* eye models simulating the ocular environment, by including a microfluidic tear replenishment component and/or intermittent air exposure. Not surprisingly, the results generated from these experiments are very different than those obtained with the conventional vial model, and may more closely resemble *in vivo* data. Thus, developing an intricate *in vitro* eye model to examine CLs will provide new insights on the interaction of lens materials with the ocular surface, and help facilitate the development of new materials and new applications for CLs in the coming decades.

Arguably, one of the most debated aspects of the *in vitro* eye model is whether the eye resembles an infinite sink, which is particularly important when it comes to drug delivery from CLs. Under infinite sink conditions, the volume of the surrounding solution is significantly higher than the drug saturation volume, such that drug release is not affected by the drug’s solubility. Advocates for the vial as an acceptable eye model argue that the cornea, conjunctiva, and surrounding ocular tissues together function as an infinite sink. While in theory this may be true, the drug must first dissolve into the tear fluid. This rate limiting step is likely not a sink condition, and will be dependent on both tear volume and flow as simulated by our model.

The unique identity of the presented model lies in its ability to emulate the tear film. By adopting a two-piece design, a “corneal/scleral” eyeball section and an “eyelid”, it is possible to create an evenly spread thin layer of tear film across the eyeball piece when both pieces come into contact. To further simulate the ocular surface, mechanical wear and air exposure is incorporated into the model through two mechanical actuators. As the eyelid piece moves
laterally, it simulates the closing of the eye and intermittent air exposure. The rotation of the eyeball simulates the mechanical wear produced during blinking. The system is coupled with a microfluidic pump, which infuses the eye model with tear fluid at a physiological flow rate or any other desired flow rate. The tear film is formed each time the two pieces come into contact, and tear break-up occurs when the two pieces separate.

The aim is to create a universal testing platform to evaluate CLs for various \textit{in vitro} analyses. In order to be versatile, the eyeball pieces can be synthesized from various polymers, such as polydimethylsiloxane (PDMS) or agar. For simple ocular studies, these polymers, which represent hydrophobic and hydrophilic surfaces respectively, will suffice. However, as more complex analyses are required, for example ocular drug penetration or toxicity studies, the eye pieces will need to be further modified. These additional modifications to the model, such as the inclusion of an \textit{ex vivo} cornea as shown, are relatively feasible. However, further validation studies are required, and future work will aim to improve the validity of this model by comparing it with \textit{in vivo} models.
Chapter 7 – Release of fluconazole from contact lenses using a novel in vitro eye model

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7.1 Overview

7.1.1 Objective

The burst release of a drug followed by a plateau phase is a common observation with drug delivery studies using contact lenses (CLs). However, this phenomenon may be attributed to the properties of the release system. The aim of this study was to evaluate and compare the release of fluconazole from seven different commercially available daily disposable (DD) CLs using a conventional vial-based method with a novel *in vitro* eye model.

7.1.2 Methods

An eye model was created using two 3-D printed molds, which were filled with polydimethylsiloxane (PDMS) to obtain an inexpensive model that would mimic the eyeball and eyelid. The model was integrated with a microfluidic syringe pump, and the flow-through was collected in a 12-well microliter plate. Four commercial DD conventional hydrogels (CH) (nelfilon A, omafilcon A, etafilcon A, ocufilcon B) and three silicone hydrogels (SH) (somofilcon A, narafilcon A, delefilcon A) were evaluated. These CLs were incubated with fluconazole for 24 h. The drug release was measured in (1) a vial containing 4.8 mL of PBS and (2) in the PDMS eye model with a 4.8 mL tear flow over 24 h.

7.1.3 Results

Overall, CH CLs had a higher uptake and release of fluconazole than SH CLs (p<0.05). A higher drug release was observed in the vial condition compared to the eye model (p<0.001). As expected, in the vial system, the drugs were rapidly released from the CL within the first 2 h followed by a plateau phase. In contrast, drug release in the eye model was sustained, and did not reach a plateau over 24 h (p<0.05).

7.1.4 Conclusions

Rapid drug release results from using a vial as the release system. Under low tear volume at physiological tear flow, commercial CLs can maintain a sustained drug release profile for up to 24 h.
7.2 Introduction

In the treatment of ocular diseases, eye drops remain the most common method for ocular drug delivery, accounting for 90% of all ophthalmic formulations. There are several advantages of using eye drops, including favorable production cost, formulations are simple to develop, flexibility in dosing regimen, and excellent acceptance by patients. However, eye drops suffer extensively from pre-corneal drug loss resulting from tear dilution, and dispersion from the eye during blinking, drainage, and non-specific absorption. It has been approximated that less than 7% of the medication within an eye drop reaches the target area, while the remainder is routed to systemic absorption. Therefore, to achieve therapeutic drug concentrations to treat these infections, multiple dosing is often required. This in turn leads to problems with patient compliance, and the potential for drug overdosing.

Drug delivery using contact lenses (CLs) can potentially overcome the current limitations associated with eye drops by limiting pre-corneal drug loss. The placement of a CL on the cornea divides the tear film into the pre-lens and post-lens tear film. The post-lens tear film located between the CL and the cornea has very limited tear exchange. In regards to ocular drug delivery, this is advantageous as the drug released from the CL into the post-lens tear film will have prolonged contact time with the cornea. Not surprisingly, modelling studies have predicted that over 50% of the drugs released from a CL diffuses into the cornea, which is over 35 times more effective than eye drops. Drug delivery using CLs can also eliminate the need for multiple dosing by serving as a reservoir and barrier to provide sustained and controlled drug release over extended periods. Consequently, in the past decade, there has been extensive research in developing smart CL materials for drug delivery using techniques such as vitamin E coating, molecular imprinting, nanoparticles, and cyclodextrins.
Although there are no commercial CL products yet available for drug delivery, CLs have already been approved for applications in a similar ophthalmic setting. Currently, CLs can be used therapeutically as ‘bandage’ lenses to treat corneal damage.\textsuperscript{20-22} They act by preventing painful contact between the cornea and the eyelids, enhancing corneal healing, and preventing further complications from secondary infection.\textsuperscript{20-22} In many cases, pharmaceuticals such as antibiotics and anti-inflammatory drugs are administered topically in tandem with these CLs.\textsuperscript{23}

One of the main drawbacks of using CLs as a drug delivery device is the rapid drug release that occurs within the first hour, followed by a plateau phase.\textsuperscript{16, 24-27} To address this problem, the primary focus of research in this field has been dedicated to developing new CL materials capable of providing slow and sustained drug release.\textsuperscript{15, 17, 28-30} However, we theorize that although drug and material properties are both deciding factors for controlling drug release, another important factor that has been overlooked is the property of the release system. For many studies, the \textit{in vitro} system used to study the drug release is performed in a static vial-based system containing 2 – 5 mL of saline buffer.\textsuperscript{16, 24-27} Because drug release is dependent on the drug’s aqueous solubility, in this type of system, it should not be surprising that drug release is rapid and plateaus, as the CL is immediately exposed to a high fluid volume. Of significant relevance is that this static fluid model does not reflect the ocular environment, in which the tear volume is approximately $7.0 \pm 2 \, \mu\text{L}$,\textsuperscript{31} with an average tear flow of 0.95-1.55$\mu\text{L}$/min.\textsuperscript{32} In contrast, at a low tear volume and tear flow, we do not expect CLs to exhibit a burst release or plateau because the amount of fluid that is available to dissolve the drug from the lens is significantly lower.
In this study, the objective was to build an appropriate in vitro eye model to study the release kinetics of drugs from CLs, and use this model to evaluate the release of an antifungal drug (fluconazole) from daily disposable CLs compared to the conventional vial method.

### 7.3 Materials and methods

Four commercially available daily disposable conventional hydrogel (CH) CLs [nelfilcon A (Alcon), omafilcon A (CooperVision), etafilcon A (Johnson & Johnson), ocufilcon B (CooperVision)] and three silicone hydrogel (SH) lenses [somofilcon A (CooperVision), narafilcon A (Johnson & Johnson), delefilcon A (Alcon)] were evaluated in the study. All lenses had a dioptic power of -3.00 and base curve of 8.6mm, obtained from the manufacturer in the original packaging. Tables 7-1 and 7-2 detail the properties of the CH and SH disposable CLs respectively.
Table 7-1 Properties of conventional hydrogels (CH) used in the study

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<tr>
<th>United States adopted name (USAN)</th>
<th>BioMedics 1Day</th>
<th>1-DAY ACUVUE MOIST</th>
<th>Proclear 1 Day</th>
<th>DAILIES AquaComfort Plus</th>
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<td>Manufacturer</td>
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<td>Johnson &amp; Johnson</td>
<td>CooperVision</td>
<td>Alcon</td>
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<td>58%</td>
<td>60%</td>
<td>69%</td>
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<tr>
<td>FDA group</td>
<td>IV</td>
<td>IV</td>
<td>II</td>
<td>II</td>
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<tr>
<td>Centre thickness (mm)</td>
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<td>0.08</td>
<td>0.09</td>
<td>0.10</td>
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<tr>
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<td>33</td>
<td>26</td>
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<tr>
<td>Oxygen transmissibility (x10^9)</td>
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<td>25.5</td>
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<td>26.0</td>
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<td>Principal monomers</td>
<td>HEMA, PVP, MA</td>
<td>HEMA, PVP, MA</td>
<td>HEMA, MA, PC, EGDMA</td>
<td>PVA, FMA, HPMC, PEG</td>
</tr>
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EGDMA, ethyleneglycol dimethacrylate; FMA, N-formylmethyl acrylamide; HEMA, hydroxyethyl methacrylate; HPMC, hydroxypropylmethylcellulose; MA, methacrylic acid; PC, phosphorylcholine; PEG, polyethylene glycol; PVA, polyvinyl alcohol; PVP, polyvinyl pyrrolidone;

Table 7-2 Properties of silicone hydrogels (SH) used in the study

<table>
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<tr>
<th>United States adopted name (USAN)</th>
<th>DAILIES TOTAL1®</th>
<th>1-DAY ACUVUE® TruEye®</th>
<th>clariti™ 1day</th>
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DMA, N,N-dimethylacrylamide; HEMA, hydroxyethyl methacrylate; MPDMS, monofuncional polydimethylsiloxane; PVP, polyvinyl pyrrolidone; TEGDMA, tetraethylenglycol dimethacrylate;
7.3.1 Eye model fabrication and set up

An eye model was created using two 3-D printed molds (eyeball and eyelid), designed with Solid Works 2013. Depending on the required application, the molds can be filled with the desired polymer. In this study, the molds were filled with polydimethylsiloxane (PDMS) to obtain an inexpensive two-piece product representing the eyeball and the eyelid. This particular 2-piece design was chosen to mimic the eyeball and the eyelid, and when they are placed together and when fluid flows through those two pieces, it would simulate physiological tear volume by minimizing the amount of tear volume that comes in contact with the CLs (figure 7-1). The set up for the eye model is shown in figure 7-1. To emulate tear secretion and tear flow in the human eye, a microfluidic syringe pump (PHD Ultra™, Harvard Apparatus) is integrated with the eye model. Also, unique to this model is the vertical orientation of the model, which correctly simulates the natural eye position during the day, and utilizes gravity to generate a natural flow. The limitations of the eye model will be discussed in the discussion section. The flow-through is collected in a standard 12-well microliter plate. This work was undertaken in collaboration with Medella Health (Kitchener, ONT).
Figure 7.1 Schematic of the two-piece eye model

7.3.2 Uptake studies

Six lenses of each type were incubated in 1.0 mg/mL fluconazole (VWR International, Mississauga, ONT) in PBS, pH 7.4 over 24 h. The absorbance of fluconazole in this experiment was measured at 259 nm using the SpectraMax M5 UV-Vis Spectrophotometer (Molecular Devices, Sunnyvale, CA), which is similar to the absorbance maxima of fluconazole reported in the literature.\textsuperscript{33} The initial and final absorbances were measured at 0 minutes and after 24 h. The difference in absorbance was calculated and converted to the amount of drug uptake for each lens.
7.3.3 Release studies

Vial

After the 24 h drug incubation, the lenses were removed from the drug solution and partially blotted on lens paper. The lenses were then placed in a vial containing 4.8 mL solution of PBS, pH 7.4. At specific time intervals, t = 0, 1, 5, 15, 30 min, 1, 2, 4, 8, 12, 16, and 24 h, 200 µL of the sample was withdrawn from the vial and pipetted into a UV-Star transparent plate (Greiner Bio-One). After each absorbance measurement at 259 nm, the sample solutions were pipetted back into their respective vials.

Eye model

Each lens was placed in the eye model, and the model was set up as shown in figure 7-1B. The flow rate was controlled using a syringe pump at a rate of 200 µL/ h (4.8 mL/ day), and the flow through fluid was collected in the 12-well plate. At specified time intervals, t = 0, 1, 2, 3, 4, 5, 6, 8, 12, 16, and 24 h, 200 µL of this solution was withdrawn and pipetted into a UV-Star transparent plate and measured.

7.3.4 Statistical analysis

Statistical analysis was performed using Statistica 8 software (StatSoft, Tulsa, OK). All data are reported as mean ± standard deviation, unless otherwise stated. Repeated measures of analysis of variance (RM-ANOVA) were performed to determine the differences across various time points within the same lens material. An ANOVA was conducted to determine the differences between lens materials at each time point. Post-hoc Tukey multiple comparison tests were used when necessary. In all cases, statistical significance was considered significant for p
value of < 0.05. Graphs were plotted using GraphPad Prism 5 software (GraphPad, La Jolla, CA).

7.4 Results

The uptake and release of fluconazole after 24 h for the seven daily disposable CLs are summarized in table 7-3. The drug uptake was higher than the amount of drug released in either the vial or the eye model eye (p<0.05). Overall, CH lens materials had a higher uptake of fluconazole than SH CLs (p<0.05). Consequently, CH CLs also released statistically significantly more drug than SH CLs (p<0.05).
Table 7-3 Uptake and release (µg/lens) of fluconazole after 24 h from conventional hydrogel (CH) and silicone hydrogel (SH) daily disposable contact lenses

<table>
<thead>
<tr>
<th>Commercial name</th>
<th>Material</th>
<th>FDA Group</th>
<th>Water Content (%)</th>
<th>Drug uptake µg/lens</th>
<th>Drug release in vial(µg/lens)</th>
<th>Drug release in eye model (µg/lens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH Dailies Aqua Comfort Plus</td>
<td>nelfilcon A</td>
<td>II</td>
<td>59</td>
<td>165.3±16.0</td>
<td>47.8±14.6</td>
<td>75.6±9.70</td>
</tr>
<tr>
<td>CH Proclear 1 Day</td>
<td>omafilcon A</td>
<td>II</td>
<td>62</td>
<td>202.3±18.7</td>
<td>128.5±20.9</td>
<td>128.1±7.64</td>
</tr>
<tr>
<td>CH 1-Day Acuvue Moist</td>
<td>etafilcon A</td>
<td>IV</td>
<td>58</td>
<td>203.0±11.7</td>
<td>155.5±29.9</td>
<td>101.6±17.5</td>
</tr>
<tr>
<td>CH Biomedics 1 Day</td>
<td>ocufilcon B</td>
<td>IV</td>
<td>52</td>
<td>257.3±17.7</td>
<td>191.9±15.0</td>
<td>137.5±13.8</td>
</tr>
<tr>
<td>SH Clariti 1 Day</td>
<td>somofilcon A</td>
<td>V</td>
<td>56</td>
<td>74.2±9.3</td>
<td>86.2±6.0</td>
<td>88.9±16.6</td>
</tr>
<tr>
<td>SH 1-Day Acuvue TruEye</td>
<td>narafilcon A</td>
<td>V</td>
<td>46</td>
<td>83.7±28.0</td>
<td>80.14±1.2</td>
<td>45.2±3.75</td>
</tr>
<tr>
<td>SH Dailies Total 1</td>
<td>delefilcon A</td>
<td>V</td>
<td>33</td>
<td>182.3±65.9</td>
<td>148.9±6.0</td>
<td>82.05±21.8</td>
</tr>
</tbody>
</table>

As a general trend, there was a higher quantity of drug released in the vial condition than the eye model, as seen with etafilcon A, ocufilcon B, and delefilcon A (p<0.001). However, there were no differences in the amount of drug released after 24 h for nelfilcon A, omafilcon A, somofilcon A, and narafilcon A (p>0.05). In the vial model, the majority of the drug release occurred within the first 2-4 hours (p<0.05), followed by a plateau phase, as shown in figure 7-2. In contrast, as depicted in figure 7-3, the drug released in the eye model was sustained and did not reach plateau within the 24 h time period (p<0.05). However, although the release profiles over 24 h are significantly different between these two release models, the overall trend in drug release for each material is fairly similar. Ocufilcon B (Biomedics 1 Day) had the highest amount of drugs released in both the vial and the eye model. Similarly, the lowest drug release was observed for nelfilcon A (Dailies Aqua Comfort Plus) and narafilcon A (1-Day Acuvue True Eye) in both systems. All lenses were virtually clear throughout all phases of the study. The eye model was washed with 400 µL of PBS to wash off any residual fluconazole on the eye model. However, there was no detectable sorption of fluconazole on the eye model.
### Figure 7-2
Release of fluconazole (µg/lens) from daily disposable commercial contact lenses in 4.8 mL of PBS. The values plotted are the mean ± standard deviation for 3 trials.

### Figure 7-3
Release of fluconazole (µg/lens) from eye model with a flow rate of 4.8 mL over 24 h. The values plotted are the mean ± standard deviation for 3 trials.

### 7.5 Discussion
In the United States, the only approved drug for the topical treatment of fungal keratitis is natamycin.\(^{34-36}\) However, this drug has very low water solubility at physiological pH, and
therefore has to be prescribed as a 5% ophthalmic suspension. While currently not FDA-approved, fluconazole has been suggested as a potential ocular antifungal agent for the treatment of fungal keratitis. As a member of the azole class of antifungal compounds, it exhibits its action by limiting ergosterol synthesis via inhibition of P450-dependent enzyme 14-α-demethylase. Azoles are considered fungistatic at low concentrations, but can become fungicidal at higher doses. Unlike natamycin, fluconazole is stable, water-soluble, and has a low molecular mass, which results in high bioavailability and low toxicity.

Due to costs and convenience, the majority of in vitro studies with CLs have used the static vial as an accepted model for the eye. Studies observing in vitro deposition of protein and lipid are typically performed by incubating CLs in a vial containing 1-2 mL of incubation fluid. Similarly, for drug delivery using CLs, the release studies are performed in vials containing 2 – 5 mL of release buffer. Under these circumstances, it should not be surprising that rapid drug release and plateau would occur, as the CL is rapidly exposed to a static and high fluid reservoir. Nonetheless, this phenomenon has been reported and accepted as a drawback of drug delivery using CLs, although it is more plausible that these observations are due to the system used to study the drug release.

The approximated 2 mL volume used in the vial method is based on the average physiological tear flow, 0.95-1.55μL/min, which in a 24 h period accumulates into 1.4-2.2 mL of fluid. However, we would expect that exposing a CL to a static 2 mL volume compared to 2 mL over a 24h period should yield significantly different release profiles. As seen in this study, the release of fluconazole from commercial CLs in a vial follows the typical burst release pattern within the first 2 hours, followed by a plateau phase. However, using our eye model, in conjunction with a microfluidic pump, we show that the release of fluconazole from CLs is slow.
and sustained over this time frame, and does not exhibit a plateau effect. This release profile is similar to those of other studies observing the release of other drugs from CLs using a microfluidic system. This confirms that our model produces results that are in agreement with other studies using microfluidic systems.

However, more important than sustained drug release is the rate of drug release, which determines the overall efficacy and toxicity of the drug delivery device, and consequently the therapeutic outcome. Unfortunately, as seen in this study, the release rates obtained in vitro will be highly dependent on the parameters of the release system, such as tear flow rate and tear volume. Other parameters, such as temperature, pH, proteins, lipids, drug sorption, drug elution, and drug penetration can also affect the rate of drug release. This is a fundamental drawback of in vitro models, and thus we should be cautious when making absolute conclusions in terms of device efficacy based on the release rates obtained from in vitro studies. For this reason, although all CLs are capable of sustained release, without further investigation it is difficult to state if any of these CLs could effectively kill fungi based solely on the release rates. Nonetheless, these values still serve as very useful predictors and indicators of materials which could perform well in vivo. For example, ocufilcon B, which released the most drug in this study, could be the most effective material amongst the seven CLs for ocular drug delivery of fluconazole. However, we should note that the ideal release kinetic profile for drug delivering contact lenses has not yet been established, and further in vivo studies are required to answer this question.

An important topic in modelling drug delivery on the eye is the notion of “perfect sink” conditions. A perfect sink condition is defined when the volume of the release medium is at least three to ten times higher than the drug saturation volume. Under these conditions, drug release
is not affected by the saturation of the drug in solution. For most drugs, the conventional vial incubation methods represents perfect sink conditions. Part of the acceptance of the vial as an appropriate model is the thought that the corneal epithelium, the conjunctiva, and the surrounding ocular tissues could potentially act as an infinite sink. However, in order for drugs to be absorbed by these ocular tissues, they must first be solubilized by the tear film. As a result, the initial drug dissolution would be primarily dependent on the amount of tears exposed to the contact lens matrix, which is very small. Thus, it is our opinion that it is more likely that a model of a non-sink condition dictates drug release from a lens on the eye, as simulated by our eye model.

Despite significant differences in the release patterns and the amounts of drug released between the vial method and the eye model, the overall trends in drug delivery efficacy from these CL materials are similar. For instance, the amount of drug released was observed to be generally higher in CH than the SH material, similar to previously reported results, regardless of what system was used to measure the release.24, 25 Furthermore, the CLs that released the highest (ocufilcon B) and lowest (nelfilcon A, narafilcon A) amount of drug were also similar in both release conditions. These results suggest that the vial system can still be a viable method to determine the relative efficacy of CL materials in drug delivery, such as which material is better in delivering a particular drug. However, for the field of ocular drug delivery with CLs to further advance, it becomes increasingly important to have a relevant eye model to help close the gap between in vitro and in vivo results.

The ocular microenvironment is highly complex, with a multitude of ocular factors that can influence how a CL behaves. However, to replicate all these factors in an in vitro model would be expensive and unfeasible. As such, researchers have identified key elements, such as
blinking\textsuperscript{52} and physiological tear flow,\textsuperscript{41-44, 53} as important factors to include in their \textit{in vitro} eye models. However, these current eye models are still relatively expensive to set up and maintain, which poses barriers to adopting these eye models into regular practice.

To address these issues, our eye model platform is designed to emulate normal physiological tear flow in normal eyes, while still maintaining relatively low cost for set up and production. Unlike previous models, the two piece design in our eye model, consisting of an eyeball piece and an eyelid piece, allows for high precision control of the thin film of tears found on the eye. In addition, our model is positioned vertically to simulate the natural waking position, and to utilize gravity to help move the fluid across the eyeball. To reduce the overall production costs, 3D printing technology is utilized in the manufacturing process. Furthermore, our model is designed for ease of integration with any microfluidic system and inexpensive flow-through collecting unit.

There are several important shortcomings of our model that will need to be improved upon for future studies. Firstly, the current model only simulates a closed eye environment and lacks a proper blink mechanism. The effect of blinking has been show to facilitate drug release from hydrogels.\textsuperscript{54} Secondly, both the eyelid and corneal eye pieces are synthesized from polydimethylsiloxane (PDMS), a highly hydrophobic material. The hydrophobicity and surface roughness of PDMS could affect drug release. Thirdly, 3D printing does not produce a smooth surface that mimics the ocular surface. At the stage when we need to mimic the smoothness of the ocular surface, the eye models can be created using Teflon molds. Fourthly, the current eye models are chemically inert and release is evaluated in the absence of other interactions. However, in an \textit{in vivo} settings, the released drugs can interact with several tear components, as well as be absorbed by the surrounding ocular tissues. Thus, the amount of drugs released using
the current model may be an overestimation. Future iterations would (ideally) include a cellular interface consisting of corneal epithelial cells and include a mechanism to mimic the action of blinking, and include some ability to mimic the inter-blink drying period by exposing the lens/tear/drug to the atmosphere. Previous studies have shown that incorporating such a drying phase increases lipid adsorption\textsuperscript{52} and thus the interaction of hydrophobic drugs with the material would likely be impacted.

The drug release profiles observed in the eye model assumes physiological tear flow. However, for eye infections such as fungal keratitis, increased tearing\textsuperscript{55, 56} may occur which could significantly accelerate the release of drugs from CLs. For this reason, drug release would be highly variable between patients depending on their individual tear flow rates. In the case of excessive tearing, all the drugs could be released within 2 hours, as shown under infinite sink conditions. In an animal study by Hui et al., control lenses which have a similar release profile as that of the vial in this study, were unsuccessful in an \textit{in vivo} keratitis model.\textsuperscript{17} This may suggest that these animals may have increased tearing that rendered the control lenses ineffective. Future studies will examine the effects of flow rate using this system on drug release profiles from CLs.

In order for the CL drug delivery field to move forward, \textit{in vivo} studies are necessary to validate that these devices are superior to conventional eye drops. However, to date, there are a limited number of animal studies (in dogs and rabbits), that have shown the effectiveness of a drug delivering CL.\textsuperscript{17, 57} The aim of our eye model development is to provide a reliable \textit{in vitro} platform that will help facilitate studies in the \textit{in vitro} phase, prior to moving ahead with \textit{in vivo} studies. With future iterations of the model, we hope to provide a complementary model to \textit{in vivo} animal studies to reduce the amount of animal testing required.
In this study, we show that the release profile from CLs using a large volume, static vial-based model is significantly different than *in vitro* eye model that mimics physiological tear flow and volume. CLs in the physiological flow-based eye model show sustained release of fluconazole over 24 h, while in the vial, the release reaches a plateau within 2 h. These results indicate that the parameters of the release system also need to be taken into consideration when making conclusions about the properties of a CL material. Future work aims to improve this eye model and incorporate other mechanical elements such as blinking motion, as well as extend this model to evaluate other contact lens applications.
Chapter 8 – Effects of antifungal soaked silicone hydrogel contact lenses on Candida albicans in an agar eye model

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8.1 Overview

8.1.1 Objective
To evaluate the effects of two commercial silicone hydrogel contact lenses (CLs) soaked with natamycin (NA) or fluconazole (FL) on the growth of Candida albicans in an in vitro eye model.

8.1.2 Methods
3-D printed molds were used as a cast for making eye shaped models comprised of potato dextrose agar (PDA). Senofilcon A (SA) and lotrafilcon B (LB) CLs were incubated with either 2 mL of NA or FL at a concentration of 1 mg/mL for 24 h. To simulate a fungal infection, the eye models were coated with C. albicans. The drug-soaked lenses were placed on top of the eye models. Seven experimental conditions were examined: (1) NA-SA (2) NA-LB (3) FL-SA (4) FL-LB (5) SA (6) LB (7) control - no lens. At specified time points (t=1, 8, 16, 24, 48 h), the agar eyes from each experimental condition were removed from the incubator and photographed. The yeast cells from the 24 and 48 h time point were also analyzed using light microscopy.

8.1.3 Results
At 24 and 48 h, there was considerable growth observed for all conditions except for the NA-SA and NA-LB conditions. When observed under the microscope at 24 and 48 h, the morphology of the yeast cells in the FL-SA and SA condition were similar to that of the control (oval shaped). There was limited hyphae growth observed for LB and significant visible hyphae growth for the NA-LB group. For NA-SA, NA-LB, and FL-LB groups, the cells were significantly smaller compared to the control.

8.1.4 Conclusions
For NA-SA and NA-LB, there was limited growth of C. albicans observed on the eye models even after 48 h. Under the microscope, the cell morphology differs noticeably between each testing condition, and is dependent on drug-lens combinations.
8.2 Introduction

Fungal keratitis is a major cause of vision loss and blindness in the world. These infections attracted substantial attention in 2006 when several cases of fungal keratitis were linked to a multipurpose contact lens (CL) solution. Unfortunately, treatment for ocular mycoses are inadequate. The only antifungal FDA-approved for topical applications is natamycin, formulated as an ophthalmic suspension. Treatment for fungal keratitis requires multiple dosing as often as hourly intervals, and patients often have to be hospitalized to ensure compliance. Thus, an improved method of drug delivery to reduce the need for multiple dosing would be invaluable in the management of fungal eye infections.

In the past decade, there has been considerable interest in developing CLs for ocular drug delivery. Treating microbial and fungal infections have been suggested as potential applications for these devices. A CL, when placed on the cornea, partitions the tear film into pre- and post-lens layers. In regards to ocular drug delivery, the post-lens tear film is of great importance due to the limited amount of tear mixing and exchange within this partition. As a result, drugs released from a CL into the post-lens tear film have extended contact with the cornea in comparison to topical administration with eye drops. The CL also provides another advantage by functioning as a drug-reservoir, which could be modified to provide slow and sustained drug release.

One of the major barriers to commercializing a CL as an ocular drug delivery device is the limited amount of in vivo data to support the claims of this approach. To date, there are only three published studies on animals, which have shown that CLs are more effective drug delivery vehicles than eye drops. The lack of in vivo studies can be attributed to current in vitro models used to evaluate drug release from CLs, which are rudimentary and fail to adequately simulate the ocular environment. For instance, the typical in vitro model consists of testing drug
release from a CL in a 2-5 mL vial,\textsuperscript{18,21-24} which neither mimics the on-eye tear volume nor the tear flow. Furthermore, measuring the quantity of drugs released from a CL over time does not necessarily correlate with the inhibition or killing of microbes. Thus, a more suitable \textit{in vitro} eye model is needed to determine the anti-microbial efficacy of drug releasing CLs, prior to their receiving clearance for \textit{in vivo} studies.

The aim of this study is to develop an \textit{in vitro} agar-based eye model that can be used to qualitatively assess the growth of \textit{Candida albicans} when exposed to a drug delivering CL. To model drug delivery from CLs, two commercial silicone hydrogel lenses, senofilcon A and lotrafilcon B, were doped with natamycin and fluconazole, and their effects on yeast-containing agar eye models were evaluated.

\textbf{8.3 Materials and methods}

\textbf{8.3.1 Materials}

Senofilcon A (SA) (Johnson & Johnson) and lotrafilcon B (LB) (CIBA Vision) silicone hydrogel contact lenses (CLs) were selected as the model drug delivery CLs in this study. The agar eye models and the corresponding lid pieces (see figure 8-1) were casted from novel 3-D printed molds (polycarbonate-acrylonitrile-butadiene-styrene) using potato dextrose agar (PDA). Each eye model measured 24 mm in diameter and 12 mm in height.

\textbf{8.3.2 Drug incubation solution}

Natamycin (NA) (EMD Millipore, Billerica, MA, USA) was dissolved in phosphate buffered saline (PBS), pH 7.4 as a 1 mg/mL suspension. Fluconazole (FL) (VWR International, Mississauga, ONT) was dissolved completely in PBS, pH 7.4 at 1 mg/mL. 10 CLs of each type
were incubated in 2 mL of the natamycin suspension, and another 10 CLs were incubated with 2 mL of fluconazole for 24 h at room temperature. The drug incubation with natamycin was performed in light minimizing conditions.

8.3.3 Experimental setup for visual observations

*Candida albicans* (*C. albicans*) ATCC 10231 (ATCC Rockville, MD, USA) were regrown from frozen stocks onto potato dextrose agar (EMD Millipore, MA, USA), and incubated at 37 °C for 24 h. The organism was harvested with 10 mL of PBS, placed in a centrifuge tube, and centrifuged for 10 minutes at 500 x g. After centrifugation, the supernatant was discarded and the pellet was re-suspended in 10 mL of PBS. With the aid of the SpectraMax M5 UV-Vis Spectrophotometer (Molecular Devices, Sunnyvale, CA), the fungal cell concentration was then adjusted to 1.0 x 10^8 CFU/mL. For this instrument, the optical density (OD) equivalent to this cell concentration is 0.3.

After the 24 h drug incubation period, each eye model was coated with 70 µL of a solution containing approximately 3.3x10^7 CFU/mL (2.3x10^6 CFU) of *C. albicans*. The agar eye models were then placed in a petri dish and divided into seven experimental conditions: (1) NA-SA (2) NA-LB (3) FL-SA (4) FL-LB (5) SA (6) LB (7) control - no lens. The drug-containing lenses were blot dried on lens paper before being placed on top of the agar eye models. Two independent experiments were performed for each condition to confirm the results (n=2). To prevent evaporation, the corresponding lid pieces were placed on top of each eye model. The petri dish was then inverted and incubated at 37°C. At specified time points, t=1, 8, 16, 24, and 48 h, the eye models from each experimental condition were removed from the incubator and
photographed. The amount of yeast growth was qualitatively characterized based on the relative amount of beige colonies covering the surface of the eye model and the contact lens.

Figure 8-1 Outline of the experimental procedure for qualitative analysis of agar eye models

8.3.4 Morphology analysis

The agar eye models and their corresponding lids at 24 h and 48 h were ground and homogenized in 10 mL of PBS using the PT10/35 homogenizer (Kinematica GmbH, Switzerland) for 3 minutes. Between each grinding and homogenization step, the homogenizer was rinsed with 70% ethanol and milliQ water to prevent cross-contamination of the samples. 2 mL of the homogenate was centrifuged at 3500 RPM for 30 seconds to separate the agar. The resulting solution was diluted to $10^0$, $10^{-1}$, $10^{-2}$ and $10^{-3}$, and 100 µL of each dilution was spread plated on PDA. Yeast cells from the PDA plates with a count between 30 colony forming units (CFU) and 300 CFU were transferred to a microscope slide using a culture swab and analyzed under a microscope. The slides were analyzed at 400X and 1000X magnification, and the morphology of *C. albicans* for each experimental condition was noted.
8.4 Results

As shown in figure 8-2, there were no visual signs of *C. albicans* growth observed at 1 h or 8 h for any condition. At 16 h, considerable growth was observed for the control (7), SA (5), and LB (6) conditions. Medium amount of growth was apparent for FL-SA (3), and limited growth for FL-LB (4). From 24 h onwards, there was considerable growth on the agar eye models for all conditions except NA-SA (1) and NA-LB (2), which had very limited amounts of growth. For these conditions, only a small amount of growth was visually detected on the periphery of the eye model; the CL and the corresponding lid component did not have any visual signs of growth. These observations suggest that NA-CLs but not FL-CLs can effectively inhibit the growth of yeasts on the agar eye models for 48 h.
Figure 8-2 Growth of *Candida albicans* on the agar eye models over 48 h for natamycin (NA) or fluconazole (FL) containing lenses: (1) NA-SA (senofilcon A) (2) NA-LB (lotrafilcon B) (3) FL-SA A (4) FL-LB (5) SA (6) LB (7) control no lens. The corresponding lid pieces are also shown for the 48 h time point (far right). NA conditions show very limited amount of yeast growth at all time points.

At the 24 h and 48 h time points, the yeast cells were transferred to a microscope slide and examined at 400 X and 1000X magnification. As the images were identical for both these time points, only the 24 h images are shown in figure 8-3. Of interest was the colony morphology observed for the NA groups, which were smaller and more translucent, compared to the other experimental conditions. When observed under the light microscope, the morphology
of the yeast cells in the FL-SA and SA groups were similar to the control, which were oval-shaped. For the LB condition, there was presence of hyphal growth. There were significant amounts of hyphal growth for the NA-LB condition, although the hyphae were smaller in size. No hyphae were observed in the FL-LB condition. The morphology of cells in NA-SA, and FL-LB were significantly smaller and rounder compared to the control.

![Figure 8-3](image)

Candida albicans under the light microscope at 400X and 1000X magnification for natamycin (NA) or fluconazole (FL) containing lenses: (1) NA-SA (senofilcon A) (2) NA-LB (lotrafilcon B) (3) FL-SA (4) FL-LB (5) SA (6) LB (7) control no lens at 24 h. The circled area shows (A) cells which are significantly smaller and rounder in size, (B) small hyphae, (C) hyphae, and (D) typical oval cell shape of Candida albicans.

8.5 Discussion

Drug delivery using contact lenses (CLs) provides an interesting approach to address the problems associated with ocular drug delivery. However, while this concept appears viable in theory, currently there are only a limited number of studies to validate its efficacy in vivo.\textsuperscript{17, 19, 20} Furthermore, previous in vitro studies only provided an understanding of drug release kinetics.\textsuperscript{18, 21-24} In developing a drug delivery device for ocular mycoses, it is also important to show that...
these CLs are effective in inhibiting microbial growth. Thus, in this study an agar-based eye model was developed and used to evaluate the growth of *C. albicans* when exposed to an antifungal containing CL.

The two antifungals, natamycin (NA) and fluconazole (FL), were selected based on their differing drug properties. NA, a polyene antifungal, inhibits fungi by binding directly to ergosterol, a sterol present only in fungal cytoplasmic membranes. The drug has a high molecular weight and is highly hydrophobic. In contrast, FL, an azole antifungal, impedes ergosterol synthesis by inhibiting 14-α-demethylase. FL also has a lower molecular mass and a higher water solubility as compared to NA. Another important difference is that azoles, such as fluconazole are fungistatic at low concentrations and are fungicidal only at high concentrations.

The two silicone hydrogels, senofilcon A (SA) and lotrafilcon B (LB), were selected due to their unique material properties. Notably, SA contains an internal wetting agent, whereas lotrafilcon B is coated with a 25 nm plasma coating. In comparison to conventional hydrogels, silicone hydrogel contact lenses with their superior oxygen permeability, can be worn for extended periods without adverse hypoxic effects. Thus, silicone hydrogels would be better candidates than conventional hydrogel lenses for drug delivery, and SA is already approved for therapeutic use.

As yeast cells are able to grow inside the agar, the agar eye models were homogenized and re-plated on PDA plates. Surprisingly, even though the eye models appeared clear for the NA-SA and NA-LB conditions, the plates yielded colony forming units (CFU) counts similar to other experimental conditions and the control. While the underlying mechanism is unclear, we hypothesize that natamycin, which is hydrophobic, does not readily diffuse into the hydrophilic agar. As a result, the observed inhibition is superficial and the yeast cells can continue growing.
inside the agar. Further work is necessary to develop a protocol to reliably quantify the CFU
using this eye model.

The morphology of the yeast cells was analyzed using light microscopy after 24 h and 48h. Interestingly, the morphology of the cells were dependent on both the CL material and drug
properties. As shown in figure 8-3, the control cells of *C. albicans* are oval shaped. This cell
morphology was also observed for the FL-SA and SA conditions. A small amount of hyphae
growth was observed for the LB condition, and a significant amount was observed for the
NA-LB group. Morphogenesis to hyphae in *C. albicans* is controlled by multiple pathways,
which respond to various conditions present in the environment.\(^{36}\) While the mechanism for
hyphae growth is not entirely clear, it may be attributed to the 25 nm plasma surface
modification present on LB, but not SA.\(^ {31,32}\) Hyphae growth also appears to be dependent on
drug-lens combination, as hyphae were not observed for the FL-LB condition, while substantial
growth was observed for the NA-LB group. The general acceptance is that hyphae growth is a
sign of virulence, as it grants fungal cells the ability to penetrate host tissues.\(^ {36}\)

The fungal cells in the NA-SA and NA-LB conditions were significantly smaller and rounder
compared to the control. These observations are similar to what has been previously reported
with an azole antifungal, sertaconazole.\(^ {37}\) In the presence of sub lethal doses of antifungals, the
yeast cells retain the capacity for budding, however, newer cells do not reach maturity and
separate prematurely from the parent.\(^ {37}\) As a consequence, the number of cells increases, while
their sizes are significantly reduced. This effect appears to be more pronounced for NA than FL,
and may be attributed to their mechanism of inhibition.

The *in vitro* uptake and release of NA from SA and LB have been previously reported to be
comparable.\(^ {18}\) The release of FL from these lenses also have been reported to be similar.\(^ {24}\) In
both studies, the \textit{in vitro} release of the drugs is rapid within the first hour, followed by a plateau phase.\textsuperscript{18, 24} This suggests that observed differences in yeast inhibition and morphologies between lens types are not likely due to the amount of drugs sorbed or released.

One of the limitations of the current eye model is the absence of ocular cells or immune cells. As such, the yeasts are not subjected to several growth pressures, such as those from the immune system, which would otherwise be present in an \textit{in vivo} environment.\textsuperscript{38} Furthermore, the current model only provides a qualitative assessment of yeast growth in response to an antifungal delivering CL. Future work will aim to develop a methodology using this eye model to quantitatively analyze yeast growth. Nonetheless, the current agar-based eye model provides a convenient method to visually assess the effects of an antifungal containing CL on the growth of \textit{C. albicans}. 
Chapter 9 – General Discussion

For over half a century, the notion of using contact lenses (CL) for drug delivery was but a dream.\textsuperscript{1,2} However, within the last few decades, significant progress has been made with biomaterials, and specifically CL materials, which have addressed the problems associated with corneal hypoxia.\textsuperscript{3,4} As a result, extended CL wear was made possible, and various medical applications for CL have become a possibility.\textsuperscript{5-11} This breakthrough, combined with an increased understanding of the interactions of CLs with biomolecules\textsuperscript{12-22} and therapeutic agents,\textsuperscript{23-28} have been pivotal in setting the stage for research towards CL drug delivery.

As mentioned in Chapter 2, fungal keratitis is an ideal candidate for ocular drug delivery with CLs. These infections, often overlooked due to their low prevalence in North America,\textsuperscript{29-34} can lead to severe vision loss and blindness.\textsuperscript{35,36} Treatment for fungal keratitis requires an intensive dosing regimen, which can be extremely taxing for the patient.\textsuperscript{37} A simple yet effective treatment approach for ocular mycoses is therefore in high demand.
9.1 Key factors affecting drug release

In developing new materials for ocular drug delivery, two important elements need to be considered: (1) the total quantity of drugs released and (2) the rate at which such drugs are released. The work in the previous chapters have described the development of novel CL materials for antifungal drug delivery and a unique in vitro model to test this concept. In light of this work, we have furthered our understanding of the factors influencing the quantity and rate of drug release from CLs. These findings, in conjunction with the published literature (Table 9-1), can be summed up in Figure 9-1 as the key factors influencing general drug delivery from CLs. The three key components, (1) material, (2) drug, and (3) system dynamically interact with one another to influence the uptake and release of drugs from CLs. It is important to note that many properties are inter-related; for example increasing silicone content in a material reduces water content and swelling size.
<table>
<thead>
<tr>
<th>Material</th>
<th>Drug</th>
<th>System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomer composition(^{38})</td>
<td>Solubility(^{27, 39, 40})</td>
<td>Drug release faster in artificial tear solution than in water(^{41})</td>
</tr>
<tr>
<td>Cross-link density(^{42})</td>
<td>Hydrophobicity/</td>
<td>Drug release faster at higher temperatures(^{43})</td>
</tr>
<tr>
<td></td>
<td>Hydrophilicity(^{39, 40})</td>
<td></td>
</tr>
<tr>
<td>Thickness(^{27, 44})</td>
<td>Molecular diffusivity(^{27, 40, 44})</td>
<td>Drug release follows zero-order release rate under microfluidic flow(^{45})</td>
</tr>
<tr>
<td>Silicone content(^{40})</td>
<td>Molecular size(^{39, 40})</td>
<td>Drug release in a vial is rapid within the first few hours, followed by a plateau(^{27, 40, 42, 46-49})</td>
</tr>
<tr>
<td>Water content(^{40})</td>
<td>Chemical structure(^{39, 40})</td>
<td>Drug release higher in infinite sink conditions compared to a microfluidic flow system(^{45})</td>
</tr>
<tr>
<td>Swelling size(^{39})</td>
<td>Drug loading concentration(^{40, 50})</td>
<td>Drug release is faster with rubbing (unpublished Ocuflow data)</td>
</tr>
<tr>
<td>Ionicity(^{40})</td>
<td></td>
<td>Drug release is affected by air exposure (unpublished Ocuflow data)</td>
</tr>
<tr>
<td>Drug loading capacity(^{38, 51, 52})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 9-1 Summary of key factors which can potentially influence release kinetics of drugs from contact lenses
9.2 Material-drug interactions

There are several important material-drug interactions generally observed throughout this thesis. One key finding is that materials with higher water content typically release more drugs, irrespective of the drug’s properties, than materials with lower water content.\textsuperscript{46, 53, 54} This is in part also related to the amount of silicone content within the material, where higher silicone content negatively impacts water content and material swelling. As a result, conventional hydrogels (CH), such as pHEMA-based lenses, will release more drugs than their silicone hydrogel (SH) counterparts.\textsuperscript{40, 46, 47, 53-55} The predicted trends for drug uptake and release from a CL, based on our studies and published literature, are summarized in Table 9-2.

<table>
<thead>
<tr>
<th></th>
<th>Hydrophobic drug</th>
<th>Hydrophilic drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional hydrogels</td>
<td>high uptake\textsuperscript{46, 47}</td>
<td>high uptake\textsuperscript{40}</td>
</tr>
<tr>
<td></td>
<td>high release\textsuperscript{40, 46, 47, 53-55}</td>
<td>high release\textsuperscript{40, 55}</td>
</tr>
<tr>
<td>Silicone hydrogels</td>
<td>high uptake\textsuperscript{46, 47}</td>
<td>high uptake\textsuperscript{40}</td>
</tr>
<tr>
<td></td>
<td>low release\textsuperscript{40, 46, 47, 53-55}</td>
<td>low release\textsuperscript{40, 55}</td>
</tr>
</tbody>
</table>

Table 9-2 trends for drug uptake and release from conventional (CH) and silicone hydrogels (SH). The terms high vs. low are meant as a “relative comparison” between CH vs. SH. There are some exceptions to this trend.

One assumption would be that lower drug release in SH materials would also be correlated to a lower amount of drug uptake in these materials. Surprisingly, drug sorption in SH
lenses is similar to that seen with CH lenses.\textsuperscript{40,46} This important finding suggests that drugs can irreversibly bind to the CL polymer, or be immobilized in regions where drug elution is not possible. This observation was most pronounced for SH materials with natamycin, which is hydrophobic, where the majority of drugs sorbed are not released.\textsuperscript{46} However, based on the hydrophobic-hydrophobic interaction principle,\textsuperscript{56} this is not surprising, as a hydrophobic drug will prefer a hydrophobic substrate over the surrounding aqueous environment. Previous studies have also reported that partial drug release from CLs is a common phenomenon.\textsuperscript{40,47} To understand this important material-drug interaction, a schematic has been proposed in figure 9-2 which illustrates the general mechanisms underlying drug uptake and release from materials.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9-2.png}
\caption{Schematic for general drug uptake and release from conventional hydrogel (CH) and silicone hydrogel (SH) materials. CH materials have increased swelling and water content compared to SH, leading to a higher quantity and rate of drugs released. For hydrophobic drugs, irreversible drug binding to the polymer could occur at the silicone moieties.}
\end{figure}

Since drug release is primarily a diffusion process, increasing material thickness will extend the time of drug release.\textsuperscript{27,44} However, what was not as apparent was that thickness also plays a crucial role in determining the overall effectiveness of a bulk polymer modification approach. Our strategy to modify the lens chemistry with the incorporation of cyclodextrin (CD)
moieties to interact with natamycin did not yield a significant increase in drug release time. The CDs were shown to complex effectively with natamycin in solution, but when incorporated into a gel, their effects were ambiguous. Increasing the concentration of CDs within the material improved drug release up to a threshold concentration, after which further increases in CDs had the opposite effect. One proposed mechanism is that beyond a critical concentration, due to the dimensional constraints of a CL, the arrangements of the CDs become crowded and the drug interaction sites become inaccessible. These CDs then occupy the space that would otherwise have been available for drug binding. So while thicker gels could benefit significantly from a bulk polymer modification approach, when the same strategies are applied to a CL (with a thickness of less than 100 µm), the effects will be less pronounced.

Considering the ratio of thickness (~ 100 µm) to surface area (~ 14 mm diameter) of a CL, it should be apparent that CL surface area plays a substantial role in determining the rate at which drugs are released. We hypothesize that the majority of the drugs released from a CL are in fact bound on the surface of the lens, rather than within the bulk of the material. The observed burst release profiles of drugs within the first few hours from our studies, as well as from other published papers, support this hypothesis. That being said, modifications to the lens surface to act as a diffusion barrier or interact with the target drugs to some extent, should have the most impact on extending drug delivery time. To this end, we had considerable success in extending the release duration of natamycin by coating lens materials with natamycin-dex-PLA nanoparticles (chapter 4).
9.3 Material-drug-system interactions

The importance of the testing system, although a critical component in developing any drug delivery device, has often been overlooked. It is somewhat odd that CLs designed for external ocular drug delivery are tested under conditions that contrast markedly with the ocular environment. These results are then extrapolated to predict the performance of the device in vivo, without considering the parameters of the system used to test the device.

The ideal testing system for a drug delivering CL is a human being. Understandably, this is currently not possible and therefore we must rely on other systems, both in vivo and in vitro, to predict the performance of these CLs on a human eye (figure 9-3). In vivo results from animal studies will provide useful data on toxicology and efficacy. On the other hand, in vitro results will help elucidate the “how” and “why” a CL behaves, and this information will facilitate the development of better materials. In both cases, the parameters of the testing system need to be considered carefully to avoid making erroneous predictions about the behavior of these devices on the eye.

Figure 9-3 In vitro and in vivo models complement each other to help make predictions on how a drug delivering CL would behave on the human eye.
One of the missing links that a simple fixed volume vial model cannot provide are the release rates \textit{in vivo}. The rates of drug release from CLs are highly dependent on the system used to test them. For the majority of the studies performed in-vial for commercial CLs, the reported release rates have followed a ‘burst’ release profile followed by a plateau.\textsuperscript{27, 40, 46, 47, 53, 54} From these studies, there are no marked differences between hydrophobic and hydrophilic drugs; both types of drugs are released rapidly. This is puzzling, as hydrophobic drugs should elute much slower into an aqueous environment. With the developed Ocuflow model, we were able to show that the elution of a hydrophilic drug (moxifloxacin) does happen much faster than the release of a hydrophobic drug (ciprofloxacin).\textsuperscript{55}

Arguably, one the most debated topics in ocular drug delivery from CLs is whether the eye resembles an infinite (perfect) sink. Under infinite sink conditions, the volume of the release media is significantly higher than the drug saturation volume, by a factor of at least 3-10 times more.\textsuperscript{58} As a result, the elution or dissolution of the drug from the CL to the surrounding system is not affected by its solubility. Proponents for using the vial as a representative model argue that the corneal epithelium, conjunctiva, and surrounding ocular tissues act as perfect sinks. In theory, this may be true for hydrophilic drugs, which dissolve readily in low tear volume. However, for more hydrophobic drugs, the drugs first must be solubilized by the tear film before they can be absorbed by the ocular tissues. The initial drug dissolution is dependent on the volume of tears exposed to the contact lens matrix, which is significantly less than the 2 mL volume used for vial studies. In this case, the vial is a poor model for drug release studies. Our hypothesis is that although the eye as a whole is an infinite sink, the rate limiting step, the dissolution of the drug into the tear film, represents a non-sink condition. For this reason, as simulated by our developed eye model, the rate of drug release from a CL is significantly
dependent on tear volume and tear flow. Nonetheless, until \textit{in vitro} models become even more sophisticated, judgment should be reserved on which model is truly representative of the human eye.

One of the biggest limitations of all \textit{in vitro} models thus far, including our Ocuflow model, is that they only measure drug release from CLs as a whole. There is no distinction between the amounts of drug released to the pre-lens tear film from the anterior CL surface, and the quantity of drugs released to the post-lens tear film from the posterior CL side. This distinction is important, as drugs released to the pre-lens tear film are effectively subjected to the ocular removal mechanisms discussed previously. Furthermore, even drugs which are released into the posterior-lens tear film are not guaranteed to be absorbed by the cornea. As a result, the quantity of drugs released from CLs measured using the current \textit{in vitro} models do not predict true efficacy of the device. A more sophisticated model, in which the parameters for corneal drug absorption are also taken into account, will be able to provide better \textit{in vivo} predictions.

\textbf{Figure 9-4} drugs released from the CL into the pre-lens tear film are subjected to ocular removal mechanisms; even drugs released to the post-lens tear film are not guaranteed to be absorbed by the cornea.
9.4 Barriers to commercialization

While this thesis has elucidated the majority of the mechanisms for drug release from CLs, there are still several hurdles that need to be overcome before commercialization of such a device will be possible. These challenges are highlighted in table 9-2, and will need to be addressed before these devices will gain acceptance from the CL industry, clinicians, and patients. Some of these concerns warrant further attention and will be discussed below.

One of the key challenges is determining whether continuous drug release is ideal. Firstly, because the release is strictly diffusion-controlled, the majority of the drugs are released immediately, followed by a decreasing release rate over time. If consistent dosing is required to treat fungal keratitis, then this type of non-zero-order release kinetic is undesirable and will need to be addressed. Secondly, it is not certain whether continuous release is beneficial over pulsed release, such as in eye drops, when it relates to treating fungal keratitis. Thirdly, continuous drug release could also result in a variety of clinical complications, such as ocular irritation or increased microbial resistance. The drugs released from CLs can be trapped underneath the post-lens tear film\(^{59}\), which is less than 4 microns thick\(^{60,61}\). As a consequence, even minute amounts of drugs released in this micro environment over time can significantly increase drug concentrations to toxic levels.

The second main challenge is developing materials which are compatible with current manufacturing processes. Current strategies to chemically modify the CL polymer are still exploratory, and thus do not follow industry standards for CL manufacturing. However, in order
### Table 9-3 Key challenges that need to be addressed before commercialization for a drug delivering contact lens

<table>
<thead>
<tr>
<th>Technical</th>
<th>Clinical</th>
<th>Regulatory</th>
<th>Market</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of drug release continuously decreases over time</td>
<td>A drug delivering CL might be an improvement over one eye drop instillation, but is it able to outperform multiple eye drops?</td>
<td>Classified as FDA class III medical device – requires highest level of safety and control</td>
<td>More expensive than eye drop treatment</td>
</tr>
<tr>
<td>System is confined to continuous release - may be that pulse release is more beneficial</td>
<td>May not be safe for patients with microbial infections to wear contact lenses</td>
<td>Requires enormous investment in time and money</td>
<td>Patients willingness to wear contact lenses as a medical device</td>
</tr>
<tr>
<td>Current chemical modifications to CL polymer are not compatible with the industry’s manufacturing processes</td>
<td>Continuous drug release could lead to ocular irritation or microbial resistance</td>
<td>Significant financial risks may deter industry</td>
<td>Unattractive technology for non-contact lens wearers</td>
</tr>
<tr>
<td>Maintaining lens properties (water content, transparency, oxygen permeability) limits amounts of drugs that can be loaded</td>
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</table>
for the industry to adopt this technology, the methods to produce these medical devices must be in parallel with industry manufacturing processes.

The third challenge, and the most difficult to overcome, is to pass the regulatory hurdle. To enter the North American market, the device must gain Food and Drug Administration (FDA) approval. Under FDA regulations, a drug delivering CL would be classified as a class III medical device, which requires the highest level of control and safety assurance. This regulatory process requires an enormous investment in time and money. Since no device of its kind has been released, the financial risks are exceedingly high and therefore many companies will shy away from being the first to take on this venture.

9.5 Addressing barriers

Maintaining lens properties, notably water content, transparency, and oxygen permeability, significantly limits the amount of drug that can be loaded into a CL. To address this issue, the device can be designed with the option of reloading the drugs on a daily basis. In this manner, the patient wears the device during the day, and reloads the device with drugs during the night. These drugs could be incorporated within the CL cleaning solution, in a dropper bottle that is added to the cleaning solution during the overnight soak or in a dropper bottle that is dripped over the lens prior to sleep (in the case of an extended wear lens).

The development of a CL drug delivery device should be done with the industry’s manufacturing process in mind. This likely means that chemical changes to the main polymer structures may not be a practical approach. It may be worthwhile to devote future efforts in developing novel coatings, such as drug-nanoparticles, for commercial CLs. Furthermore, with
this approach, new CL materials do not need to be developed, which considerably simplifies the FDA approval process.

To de-risk this technology and make it more attractive to industry partners, alternative CL drug delivery approaches should be explored. For example, CLs could also be used to release comfort agents or anti-allergy drugs. This type of CL release device would have a much better chance for FDA approval, and its entry to the market will significantly help open doors for its future drug-delivery successors.

9.6 Conclusion

This thesis has attempted to explore and develop CLs for antifungal ocular drug delivery. Several important findings have been reported, and the shortcomings also have been discussed in this chapter. In light of this, with the right approach, an antifungal ocular drug delivery CL is a commercial possibility, but not in the near future.
References

Chapter 1 references


Chapter 2 references


Chapter 3 references


Chapter 4 references


Chapter 5 references


Chapter 6 references


Chapter 7 references


Chapter 8 references


Chapter 9 references


15. Walther H, Subbaraman L, Jones LW. In Vitro Cholesterol Deposition on Daily Disposable Contact Lens Materials. Optom Vis Sci 2015; accepted for publication.


Appendix A – Copyright permissions

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Figure 1-1

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<th>Chau-Minh Phan</th>
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Chapter 1 - Contact lenses for antifungal ocular drug delivery: a review
Chapter 3 - In vitro uptake and release of natamycin from conventional and silicone hydrogel contact lens materials
Chapter 4 - In vitro uptake and release of natamycin Dex-b-PLA nanoparticles from contact lens materials
Chapter 5 - In vitro drug release of natamycin from β-cyclodextrin and 2-hydroxypropyl β-cyclodextrin functionalized contact lens materials
Chapter 6 – Development of an in vitro ocular platform to test contact lenses

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**Author:** Chau-Minh Phan, Magdalena Bajgrowicz, Huayi Gao, et al

**Publication:** Optometry and Vision Science

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