In Vitro Cornea Models in Contact Lens Based Ocular Drug Delivery

by

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Saman Mohammadi

Abstract

The development of suitable *in vitro* release and diffusion models for contact lens-based drug delivery may lead to the establishment of *in vitro/in vivo* correlations. It is evident that an *in vitro* model that can reproduce the *in vivo* results would prevent the disqualification of an otherwise effective drug delivery material or method due to poor release results obtained using unsuitable release models. Conversely, it would also aid in discarding materials with poor properties more efficiently.

An overwhelming amount of research has shown that both drug-soaked silicone hydrogel and conventional hydrogel materials are unable to continuously release medication at therapeutic levels. For example, studies have shown that commercially available contact lenses can reach extended drug release for up to 24 hours *in vivo* while significantly shorter release of the same drug from similar contact lens materials have been observed when tested using current *in vitro* release models. The lack of appropriate *in vitro* release and diffusion models that are more representative of the physiological conditions in the eye has provided the impetus for this thesis.

The guiding objective of this thesis was to investigate the several well-recognized passive/static *in vitro* ocular drug release models and to introduce novel dynamic *in vitro* drug release and diffusion experimental models which may help in explaining the discrepancies between *in vitro* and *in vivo* results.

To effectively recreate the human eye environment in drug delivery studies, the model needs to consider the limited volume and replenishment of the tear as well as the incorporation of corneal cells. By comparing three *in vitro* models (i.e., fixed volume, dynamic, and cell), the results presented in this thesis aim to offer a robust, reliable and cost effective testing platform more suitable for assessing the drug releasing capabilities of hydrogel contact lenses. The release results prove the importance of a dynamic release platform for testing contact lens materials by identifying interactions between the drug and lens material that would otherwise be disregarded in the fixed volume model.

While interactions between lens and material are important to consider during developement and testing in the experimental model, another important parameter to consider

is drug interactions with cells. Prodrugs such as prostaglandins belong to a large group of ophthalmic drugs. These compounds are precursors to ophthalmic drugs that are specifically designed to improve the drug residence time in tear film and enhance the drug uptake by the cell membrane. Within corneal cells, these prodrugs can be metabolized through different pathways into the active form of the drug, before reaching the targeted tissue. By using an *in vitro* cornea model, it was demonstrated that the 24-hour release of prostaglandin prodrug from a pre-soaked silicone hydrogel was comparable to the daily dose of that ophthalmic drug delivered as an eye-drop. These results emphasized the importance of the presence of cells when characterizing the release of drug-delivery materials, and demonstrated how experimental *in vitro* models have a significant impact on the outcomes of testing ophthalmic drug delivery materials.

It was also further hypothesized in this thesis that a cell-based *in vitro* cornea model combined with a tear replenishment method to study drug release from a contact lens is better suited for release studies from ophthalmic materials. Through modeling the microfluidics of *in vivo* tear replenishment and using a curved surface to grow cells, a tear replenishment cell culture system was developed as an *in vitro* testing platform for ocular drug delivery. Via continuous replenishment of a tear solution analogue over the surface of the cell culture model, results from this work demonstrated yet another important role that a dynamic release model will have in predicting the amount of drug loss from a contact lens into the tear film/lacrimal system.

Recreating the geometry of the ocular surface as well as the microfluidics of tear replenishment combined with the incorporation of human corneal epithelial cells in this research proved the potential of drug eluting contact lenses when tested under more realistic conditions. The results and the new models developed in this research project may also help to explain or provide means to investigate the discrepancies between *in vitro* and *in vivo* studies.

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Dedicated to my parents

for teaching me how to fail,
how to learn from my failures,
and allowed me to define my success.

Dedicated to Maud

for providing:
a safe environment to fail,
an opportunity to learn and grow,
and an unparalleled example of ***leadership****.*

Dedicated to those who

struggle to free the enslaved minds,
and pursue the prosperity of the living.

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Chapter 1

Ocular Drug Delivery and the Case for *in Vitro* Studies of Corneal Drug Diffusion

Topical eye-drops are the most common drug delivery method and are used in the treatment of more than 90% of ophthalmic diseases. Eye-drops are characterized as pulse drug delivery methods whereby they deliver a high concentration of a medication over a short period of time, followed by a long period of underdosing before the next the eye-drop is administered. Fig. 1.1 shows the drug concentration profile in the tear film for various modes of drug delivery. Because of the inefficiency of eye-drops, frequent administration of drops is necessary, which in turn may lead to the patient's noncompliance with the therapy. The relatively short drug residence in the tear film, combined with the essential function of the cornea as a barrier against xenobiotics, limits the drug absorption to the eye and results in the low bioavailability of the ophthalmic medications. For example, a study with latanoprost[1] in rabbits showed that only 1% of the ophthalmic medication applied as a topical droplet was actually taken up into the eye [1].

Soft contact lenses were introduced by Otto Wichterle in 1965 [2, 3], and since then extensive research has been performed to study their potential as a drug delivery device to

[1] An ophthalmic drug used to treat glaucoma.

replace conventional methods of ocular drug delivery, such as eye-drops and ointments [4].

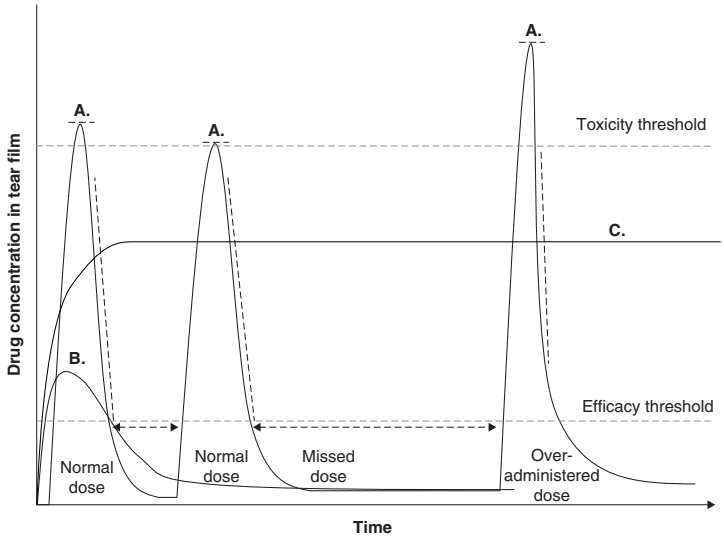


Figure 1.1: Tear film drug concentration profile for eye-drop delivery method. A) Drug concentration in tear film reaches its maximum immediately after the application then drops quickly below the efficacy threshold due to lacrimation and drainage and absorbance into the cornea, until the application of the next dose. A late or missed dose results in a prolonged period of insufficient drug levels. An over-administered dose does not increase drug residence time in the tear film, and may result in a drug concentration above the toxicity threshold. B) It is believed that drug-soaked contact lenses, as a drug delivery device, release insufficient amounts of a drug over a short period of time before they are depleted. C) An ideal drug delivery device to the eye will deliver an adequate amount of medication to the eye, with accurate targeted dosing at a sustained and controlled fashion to increase bioavailability of the drug. The picture is reprinted with permission from ref. [5].

To overcome low bioavailability of topical eye-drops and ointments as well as patient noncompliance, soft contact lenses may be used as drug delivery systems [6]. In a mathematical modeling of the drug diffusion, Li and Chauhan have shown that the drug delivery efficacy can be improved up to 50% when using soft contact lenses [7]. Although *in vivo* studies are required to prove efficacy, *in vitro* experiments can be used initially to compare release profiles and identify the most promising candidates for *in vivo* studies. Drug delivery studies using contact lenses are generally performed in fixed volume release solutions in the absence of cells. Human corneal epithelial models have been used extensively to investigate biocompatibility of various ophthalmic materials [8–10]. Although these experimental models have been used to measure drug permeation, they have not been used as *in vitro* models to study drug delivery using a contact lens. This research aimed to investigate how new experimental models can support the development of drug delivery devices.

In this thesis, a brief review of the human eye anatomy and glaucoma disease (Sections 1.1 and 1.2) are covered. In section 1.3, a review of ongoing research in the field of contact lens based drug delivery is presented. Section 1.4.1 introduces *in vitro* cornea constructs and their application in the field of biocompatibility studies of contact lens materials or other ophthalmic compounds. Finally, the corneal drug permeation/diffusion studies using excised corneas, as well as cornea constructs, are discussed in section 1.4.1. At the end of this chapter, a case for the *in vitro* study of corneal drug diffusion using contact lenses is made.

1.1 Human Eye Anatomy

The eye is a sophisticated organ surrounded by a bony protective orbit. While extraocular muscles help move and rotate the globe, the ciliary muscles contract and dilate to adjust the power of the lens to focus on an object. The eye is comprised of several layers and components and only the structures relevant to this thesis research project will be introduced.

As shown in Fig. 1.2, the inside of the eye is divided into the anterior and posterior segments. The posterior segment is located behind the lens and consists of the vitreous, the posterior sclera, and the retina. The anterior segment (AS) of the eye is in front of the posterior segment and is further divided into two chambers. The iris, the pigmented part of the eye, separates the anterior chamber (AC) from the posterior chamber.

The cornea is the window of the eye and with assistance from the tear film is the strongest refractive entity in the visual system and responsible for two-thirds of the eye's focusing power. The cornea, with its five layers, is a strong barrier against bacterial and viral infections and xenobiotics, such as pathogens, drugs, or small particles. The cornea in normal conditions is avascular, and nourished through capillaries in the conjunctiva, episclera, and sclera as well as through the aqueous humor as mentioned above. It is oxygenated from the atmosphere via the tear film.

Each of the five layers of the cornea has a distinct function. The cornea's layers, shown

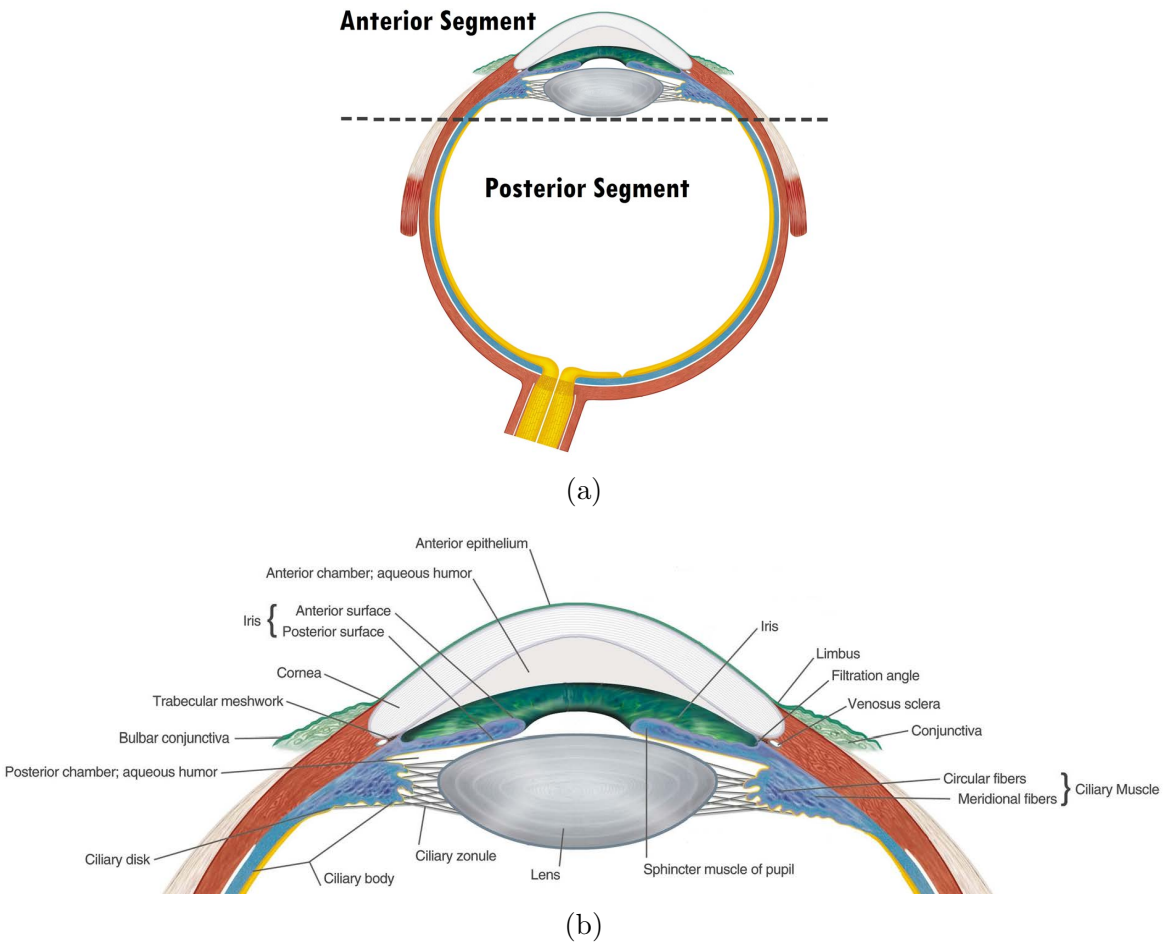


Figure 1.2: a) Segments of the eye, b) Anterior segment of the normal eye. [Images were retrieved from `http://bumseku.hol.es/anatomy-eye`, July 2015]

in Fig.1.3, from the outside to the inside are (a) the epithelium, (b) Bowman's membrane, (c) the stroma, (d) Descemet's membrane, and (e) the endothelium. The epithelium, as the outer-most layer of the cornea, protects the stroma from external injuries and provides a smooth refractive surface. The epithelium acts as the primary barrier against transcorneal permeation (illustrated in Fig.1.3(f)&(g)) [11, 12]. The center of the epithelium has five to six layers of cells (also known as stratified epithelium). The single internal layer of basal cells is responsible for the reproduction of corneal cells. The basal cells migrate to the front and become part of the two to three layers of the wing cells (polygonal cells). The wing cells' migration continues and forms the two layers of flattened epithelial cells before eventually shedding. The limbus (Fig.1.2b), separating the cornea from the sclera is also active as an additional source of new epithelial basal cells that migrate into the corneal

epithelium [13]. The tight junctions present between the superficial cells in the corneal epithelium prevent the paracellular passage of ophthalmic drugs [14].

Bowman's membrane is composed of uniform collagen fibers and protects the eye against microorganisms. Bowman's membrane has a smooth anterior surface and a less defined posterior surface that merges into the stroma. The stroma consists of 200 to 250 transparent lamellae and comprises 90% of the entire cornea. The relatively dehydrated state of the stroma[2] and the non-interweaving lamellae laid down at right angles to each other contribute to the clarity of the stroma. Several cell types, such as keratocytes and fibroblasts, are present in the stroma and play a role in corneal healing and inflammation. The membrane made up of fine collagen fibers at the posterior side of the stroma is Descemet's membrane, and acts as a basement membrane for the endothelium cells. The endothelium is a flattened honey-comb like single layer of cells. The corneal endothelium acts as a water pump and contributes to the dehydration of the stroma. The interlocked plasma membranes of the cells are hydrophobic and provide a barrier against the aqueous humor.

During every blink, the epithelium is bathed with tears, and excess tear is pushed toward the nose and leaves the eye through a drainage system. The lacrimal system provides lubrication to the eye along with protecting the eye from trauma and too much light. The tear film lubricates the cornea and the external globe and also provides oxygen and nutrients to the cornea. The tear film was previously described as having three layers (the inner mucin layer, the intermediate aqueous layer, and the outer lipid layer) [13]. Recent research has proposed a new model whereby the tear film lipid layer is composed of 2 layers. The nonpolar outer layer is in contact with air and retards water evaporation, but it is thermodynamically unstable as a thin film and collapses into lipid droplets. The inner polar lipid sublayer is believed to create a substrate to stabilize the upper lipid sublayer [15].

Due to the hydrophobic nature of the corneal epithelium, the cornea cannot be moistened properly by an aqueous layer. The mucoid (composed of transmembrane glycoproteins

[2]As a hydrogel, a fully hydrated stroma (100% saturation) would be opaque, the relativly dehydrated state refers to the saturation of 75%.

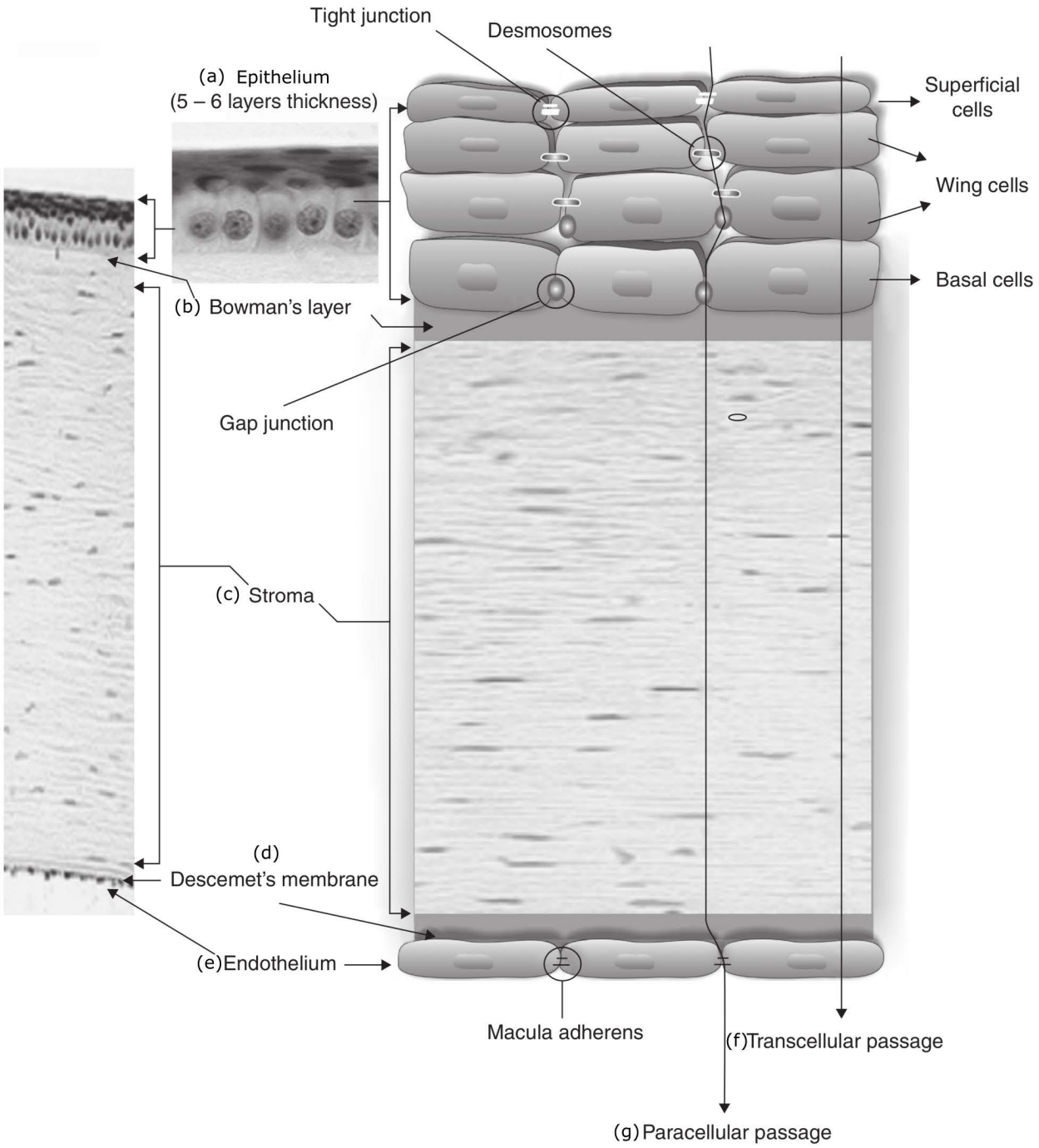


Figure 1.3: Structure of the normal cornea covered with tear film, from top to bottom: (a) the epithelium, (b) Bowman's membrane, (c) the stroma, (d) Descemet's membrane, (e) the endothelium. (f) transcellular, and (g) paracellular pathways that make up the two possible transcorneal drug diffusion routes. The picture is reprinted with permission from ref. [14]

and mucins) coats the corneal epithelium by binding to the microvilli[3] that covers the epithelial surface, thereby improving hydrophilicity of the epithelium. The aqueous layer is the major component of the tear film. The outer lipid layer slows the evaporation of the aqueous component of the tear film.

1.2 Intraocular Pressure and Glaucoma

The space between the cornea and the crystalline lens is filled with aqueous humor, which is a clear gelatinous fluid similar to blood plasma but lower in proteins. The aqueous humor provides nutrients to the lens and the cornea and drains metabolites. It is produced by the ciliary processes in the posterior chamber at a rate of $2 - 2.5\mu l/min$ and then circulates around the pupil into the anterior chamber. The aqueous humor leaves the AC through the trabecular meshwork (TM) that lies within the angle and then it is collected in the venosus scler (also called the canal of Schlemm). The angle is the actual anatomical angle created by the anterior surface of the iris and the arch of the cornea [13]. A cross section of the angle and ciliary body is shown in Fig. 1.4. The dynamics of aqueous humor production at the ciliary processes and its drainage through the TM creates the intraocular pressure (IOP). The normal physiologic balance between the inflow and outflow is reached when the IOP ranges from 10 to 21 $mmHg$ in adults. People whose IOP stays over 21 $mmHg$ are more likely to develop glaucoma.

1.2.1 Glaucoma

Glaucoma is a group of disorders that results in irreversible damage to the optic nerve and loss of vision. Ocular hypertension (OHT), or high intraocular pressure, is considered a major risk factor for glaucoma, even though in some populations, damage to the optic nerve occurs despite normal IOP [13]. Age is the next main risk factor for this disease [17]. According to the US centers for disease control and prevention (CDC), more than 60% of affected people are 50 or above (with the average age of the affected population being 67

[3]Microscopic cellular membrane protrusions

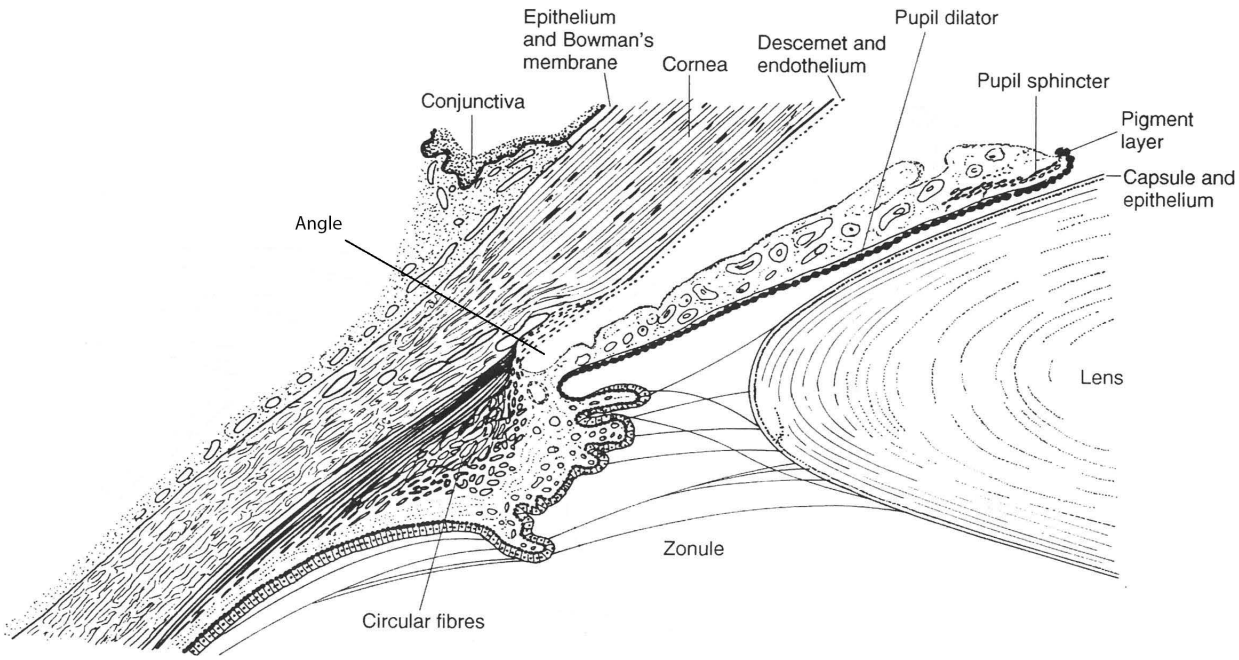


Figure 1.4: Anterior chamber of the human eye. Reprinted with permission from ref. [16].

years old). After cataract (39%) and uncorrected refractive errors (18%), glaucoma (10%) represents the third cause of blindness [18]. When compared to cataracts, the greater challenge of glaucoma to the public health lies in the fact that the damage caused by this disease is irreversible [19].

Glaucoma is classified into various primary types: open-angle glaucoma (OAG), angle-closure glaucoma (ACG), normal-tension glaucoma, and congenital glaucoma with variations of OAG and ACG. Open-angle glaucoma is the most common form of glaucoma. It occurs when the trabecular meshwork that drains aqueous humor becomes blocked, leading to rise in intraocular pressure. Angle-closure glaucoma is less common and the buildup of fluid is caused by blockage of the angle structure in the anterior chamber. OAG is an insidious disease with no apparent symptoms for the patient until vision loss appears. In ACG, the onset is sudden and the symptoms are headache, blurred vision and pain in the eye [19]. Due to the acute nature of ACG, invasive surgical techniques are often used to treat the disease. In OAG, the disease is managed by medication to lower the IOP.

Apart from rare cases of congenital glaucoma, the disorder affects the elderly most, and as the world's population ages, it is becoming an increasing cause of blindness [19].

The World Health Organization's study in 2007 shows that in 2010, the number of people projected to be blind from open-angle and angle-closure glaucoma would be 8.3 million out of the 60.5 million suffering from glaucoma. By the year 2020, they predicted that if no action was taken, this would increase by 30% [17]. The lack of awareness about glaucoma and its treatment as well as the lack of early diagnosis methods limit prevention of vision loss. Furthermore, once diagnosed, patient compliance with daily, lifelong use of eye-drops remains the primary challenge [17]. In a survey of ophthalmologists in the United States, lack of patient compliance for glaucoma medications was attributed first to patients' forgetfulness and second to high costs of medication [20]. Therefore improving patient compliance can have a major impact on lowering the number of blind people.

Several groups of drugs in the form of topical droplet are used to manage ocular hypertension including prostaglandin $F_{2\alpha}$ analogues, β-blockers, α-agonists, and combinations of the two drug types. Although all of these drugs are used to treat glaucoma, the prostaglandin $F_{2\alpha}$ analogues are the most effective and first-line standards of care [21]. In 2010, the three prostaglandin $F_{2\alpha}$ analogues, Xalatan (Latanoprost), Lumigan (Bimatoprost), and Travatan Z (Travoprost), accounted for 60% of the $2.2 billion generated revenue of prescription pharmaceuticals to treat OAG [22]. For most patients, glaucoma medications effectively control IOP to target levels [22]. However, for ACG and OAG, at least one-third of patients are prescribed two or more medications due to declining efficacy or inadequate responses to therapy [20, 22].

Reports of side effects of using prostaglandins to lower IOP date back to 1965 [23]. Early studies showed that sustained lowering of IOP for more than 24 hours was also accompanied by severe conjunctival hyperemia and discomfort [24]. Prostaglandins, being carboxylic acids, also have a limited permeation through the cornea [25]. Therefore, the more lipophilic esterified or amide prodrugs of the carboxylic acids were developed to better penetrate through the cornea [26]. Less polar analogues of the prostaglandin $F_{2\alpha}$ such as Latanoprost, Travoprost, Bimatoprost, and Tafluprost, have shown minimal discomfort and increased corneal penetration [27].

The esterified or amidified prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogues that are currently

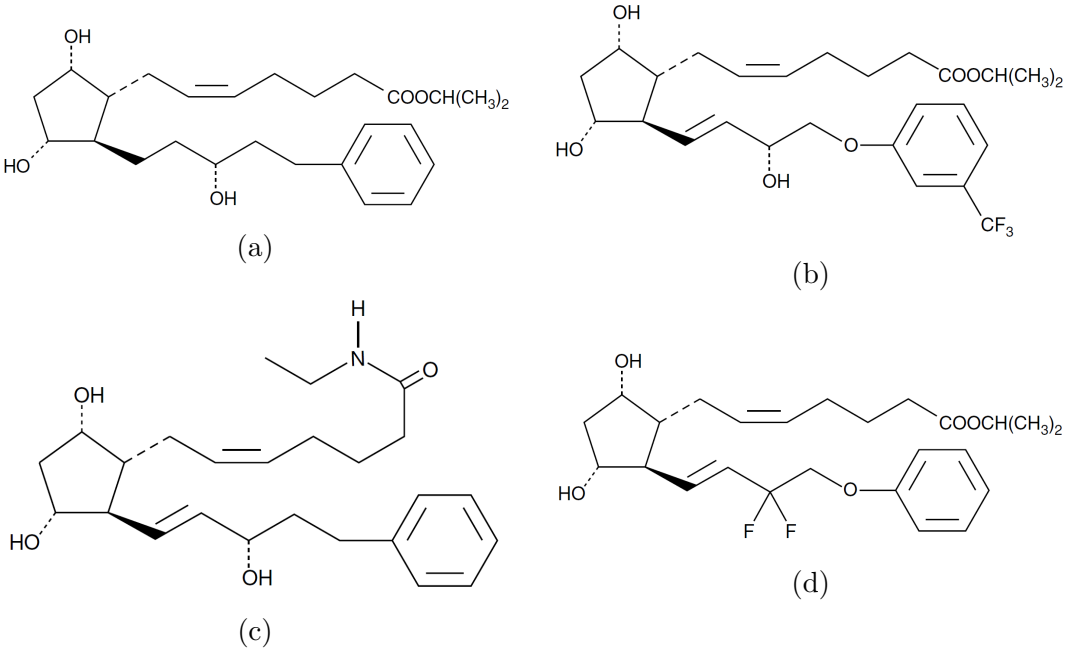


Figure 1.5: Chemical structure of prostaglandin $F_{2\alpha}$ analogues: a) Latanoprost b) Travoprost c) Bimatoprost, d) Tafluprost [Retrieved from Cayman Chemical: www.caymanchem.com, July 2015].

prescribed are presented in Fig. 1.5. All $PGF_{2\alpha}$ analogues have more or less similar pharmacokinetics [27] and have shown consistently a significant increase in aqueous outflow [21]. $PGF_{2\alpha}$ analogues enhance outflow through mechanisms including changing the shape of cells, widening the connective tissue-filled spaces and remodeling of the extracellular matrix in the trabecular meshwork [21]. Prostaglandins break into their free-acid form by hydrolysis facilitated by the enzymes available in corneal cells [28]. Studies have shown instantaneous hydrolysis of some $PGF_{2\alpha}$ analogues [29–32], while bimatoprost has been reported to have a slower conversion rate of approximately $25\mu g/24h$ [33].

1.3 Ocular Drug Delivery

Ocular drug delivery is either intended to target the ocular surface to manage superficial conditions such as dry eye, microbial keratitis and conjunctivitis, or to treat intraocular disorders such as glaucoma, and age-related macular degeneration. Eye-drops, followed by ointments and gels are still the most common ocular drug delivery method [34]. Eye-drop

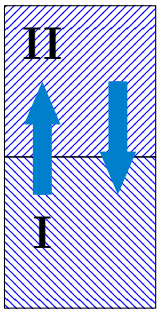
medications are applied topically to the eye in the form of either a solution or suspension in water [6]. The aqueous eye-drop is rapidly diluted in the tear film and most of it is drained through the lacrimal system, therefore requiring frequent applications [35].

Studies show that only about 1 to 5% of the applied dose penetrates the cornea [1], and that due to the relatively fast turnover rate of the aqueous layer of the tear film, the residence time of hydrophilic medications is around 2 to 5 minutes [36]. The relatively slow turnover rate of the tear film lipid layer results in increased residence time for lipophilic drugs, which reside in this layer, and consequently leads to an increased uptake into the eye. The purpose of topical ophthalmic drug delivery devices is to deliver an adequate amount of medication to the anterior segment of the eye, with accurate targeted dosing at a sustained and controlled rate to increase bioavailability of the drug. Several commercial ocular delivery devices are currently available, including surface-located inserts [37], degradable or non-degradable implants [38], and *in situ* forming gels [39]. Despite almost 50 years of research being conducted on the potential use of soft contact lenses to deliver topical ophthalmic drugs [4], no drug delivery contact lens has yet been commercialized [7].

1.3.1 Contact Lens Drug Uptake Mechanism

When it comes to soaking a contact lens in a drug solution, the governing physics can be described by the partitioning mechanism as long as the drug solution remains diluted and uptake is to the bulk of the contact lens material as opposed to adsorbed on the surface of the lens. Beyond the dilute solution region, the solute-solute interactions can not be neglected and therefore the partition law may not apply. The *Partition Law* states that: *"If a third substance be added to a system of two immiscible liquids, the added component will tend to distribute itself between the two solvents until, at equilibrium, the ratio of the concentrations (or mole fractions) of the distributed substance will attain a certain value"* [40].

This ratio, as shown in Fig. 1.6, is generally insensitive to pressure, temperature and concentration changes. The partition law is a phenomenon that is observed from measurements and applies to two immiscible liquids but it is not limited to immiscible liquids [41].



$$C_{ii} = [X]_{II}$$

$$P = \frac{[X]_I}{[X]_{II}}$$

$$C_i = [X]_I$$

Figure 1.6: Partition ratio of the compound X in solutes I and II is the ratio of that compound's concentration in the solutes; figure adapted from ref. [40]

A hydrogel contact lens is not a liquid, but due to its high water content, it holds properties of a liquid solvent[4]. Therefore, it is assumed that the partition law applies and for a drug soluble in water and a soft contact lens, the partition ratio would reach a constant value at the equilibrium. If a drug is ionic, associates or dissociates in a certain solvent, the *"distribution ratio"* can be used instead [40]. Also in case of a self-associating drug or a drug that can be adsorbed on the surface of a contact lens due to *hydrodynamic*[5] attributes, modifications should be made to the mathematical uptake model to encompass all uptake venues.

A hydrogel contact lens (silicone and conventional) mainly consists of cross-linked polymers/co-polymers and water. Fig. 1.7 illustrates a simplified representation of a hydrogel contact lens material soaking in a drug solution. It can be contemplated that the larger concentration gradient in the solution drives the drug into the aqueous component of the hydrogel. It has been suggested that the drug may then interact with the polymer matrix through charge-charge, charge-dipole, hydrogen bonding, weaker polar or hydrophobic mechanisms [43]. The amount of the drug dissolved in the water content of the polymer can be calculated at the equilibrium using the drug solubility in aqueous solution. It has been shown that the ratio of dissolved drug in water content to the total drug taken up by the lens is negligible for relatively hydrophobic drugs (i.e., partition ratio, $P > 0$). Therefore, the contact lens material could be considered a homogeneous material where

[4]Soft contact lenses are highly saturated polymer networks which may contain up to 75% of water. Assuming the contact lens material as a solvent, immiscible in water, is commonly used in contact lens research.

[5]*hydrodynamic* refers to all non-lipophilic and interactive modes with the contact lens surface (*e.g.* hydrophilic, ionic, and van der Waals types of interaction) [42]

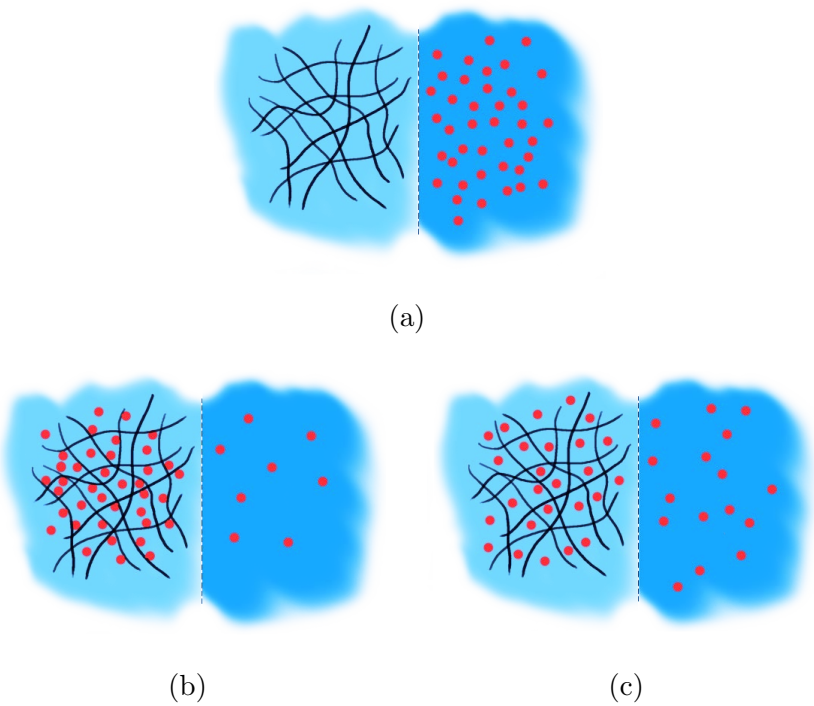


Figure 1.7: Illustration of the drug uptake into a soft contact lens in drug solution; a) before equilibrium is reached, b) after equilibrium is reached in case of a drug with large partition ratio vs. c) a drug with small partition ratio. Lighter shade (left of the dashed line) represents the aqueous component of the contact lens material.

there is a partition ratio between the contact lens material and the soaking solution for a certain hydrophobic drug or compound. For hydrophilic species, it would be important to adjust for the fraction of the drug held in the aqueous portion of the hydrogel.

It can be concluded that for drugs with high partition ratio (hydrophobic drugs), as illustrated in Fig. 1.7(b), a significant amount of drug will be taken up through association with the polymer network of the hydrogel. Also, in the case of a significantly smaller partition ratio, as shown in Fig. 1.7(c), although most of the drug may or may not be taken up by the hydrogel, a greater portion of the drug would remain in the aqueous solution (represented by both the solvent and the water content of the contact lens material).

The drug uptake through adsorption on the surface of the hydrogel, in case of a surface treated contact lenses, will be affected by the surface charge and the hydrodynamic attributes. The other important factor would be the size of the drug molecule. Due to the compact structure of the contact lens polymer network, larger drug molecules, such as

polypeptides or proteins, may not be loaded effectively by simply soaking the hydrogel in the drug solution. Therefore, loading a drug into a contact lens through soaking, as the most likely method to be used in the commercial production of therapeutic contact lenses, would reasonably be limited to low molecular weight drugs (< 500Da).

Furthermore in an extensive study of ophthalmological drugs loaded into commercially available contact lenses, Tabuchi *et al.* have shown that the hydrophobic interactions of the actives (i.e., drugs or other compounds) with the contact lens material is the primary governing factor in the adsorption of those compounds [44]. During the soaking of the contact lens in a drug solution, drugs that showed higher affinity toward the contact lens material adsorbed significantly more on and into contact lens material compared to hydrophilic drugs [44]. This is also consistent with the observations of Mahomed *et al.* with hydrophobic surrogate drugs (see below, [43, 45]).

When considering drug uptake, the effects of the edge in a drug eluting polymeric device can be ignored, if the aspect ratio of the polymer diameter is greater than 10 times the thickness [46]. However, the thickness of the lens will affect the total volume and thus the uptake. Contact lens thickness varies depending on the prescription. Therefore, to eliminate variations in total lens material volume, it is necessary to maintain the prescription the same in drug release experiments.

1.3.2 *In Vitro* Contact Lens Drug Release Models

In pharmaceutical research, solute release from polymeric devices is most often performed under *perfect sink conditions*. Under such conditions, the fractional solute release ratio is defined as $^{M_t}/_{M_\infty}$, where M_t is the amount of drug released at time t, and M_∞ is the total drug release, or drug release as time reaches infinity. It has been shown that this ratio can be approximated by a simple exponential relation $^{M_t}/_{M_\infty} = kt^n$, where k is a constant, and $0.43 < n < 0.5$ depending on the geometry of the polymeric device (i.e., slab, disk, cylinder, or sphere) [47]. The sink condition is maintained by testing the drug releasing device in $500mL$ to $1000mL$ of solution. The concentration of the solute in the release media should remain below 10% of its solubility to retain near sink conditions. The

direct implication of infinite sink assumption is that the most of the drug if not all will be released from the contact lens, since the concentration gradient will drive the drug out of the polymer network. While drug releasing contact lenses are generally tested similarly to a drug eluting polymeric devices under sink conditions, it is clear that this assumption is far from the limited tear volume conditions in the front of the eye. The fractional solute release ratio provides relevant information about certain characteristics of the drug releasing polymeric devices and enables comparison of different polymers and their drug releasing capabilities. However, the lack of sink condition in the eye, both in terms of the volume of the release solution and the assumption of drug concentrations well below drug solubility, limits drawing conclusions on actual ocular drug release profile based on those characteristics.

Drug releasing ophthalmic materials have used a range of experimental models to study the drug-polymer interactions and efficacy of the drug release. Over 80% of drug release studies from contact lens materials has been conducted in passive release models. These models use less than 10 mL volume of deionized (DI) water, or Phosphate Buffered Saline (PBS), creating a small sink condition [48]. In these studies, the drug-eluting contact lens material is placed in a vial with a specific volume of the release solution, and either samples are collected from the solution at various time points or the entire solution is refreshed to maintain the sink condition. In the fixed volume release studies, parameters such as the release medium and its volume, as well as mixing condition are important [49].

Passive release studies of ocular drug delivery devices have given rise to the popular belief that simple "soaking" of a contact lens in a topical drug solution may be insufficient for adequate elution on the ocular surface. Therefore, such a method has been considered to have a low potential for success [49–51]. Thus, a variety of research efforts are attempting to increase the drug uptake and/or release rates. These have included prolonged (up to 2 weeks) soaking [52], soaking the lenses in super-critical drug solutions [53], soaking the dehydrated contact lenses in drug solutions [54], molecular imprinting [55–57], and using vitamin E as a barrier to decrease diffusion of the drugs [52, 58, 59]. To improve drug release from contact lens materials, two approaches have been used. First, attempts have

been made to add diffusion barriers such as vitamin E into the lens as well as molecular imprinting which successfully slowed down release of the loaded drug [52, 59]. In a second approach to improve release, researchers have attempted to increase the amount of drug uptake, which has appeared to have minimal to no effect on the elution time and release kinetics [49].

Due to their low cost and ease of quantification, the use of ophthalmic fluorescent dyes as surrogate drugs has been recognized as an appropriate approach when designing high-throughput assays to characterize and identify desired polymeric compounds for drug delivery applications [60]. In a series of publications, Mahomed *et al.* investigated the interactions of hydrophilic and hydrophobic surrogate drugs with commercial contact lens materials in order to identify the role of physicochemical properties (such as molecular weight, charge, partition coefficients) and the influence of parameters such as uptake solution concentration and pH [43,45]. They have shown that the extraction media volume and the trigger mechanisms such as mechanical agitation also play a role in the amount of the drug released [45]. Mahomed *et al.* demonstrated that under passive release conditions for the range of surrogate drugs and contact lens materials studied, the release kinetic maintained a first order release kinetics, which followed the Fickian diffusional release[6] from a thin slab of polymer [43]. This first order release kinetic results in an initial burst of drug, followed by a slow release. Unfortunately, this does not meet the requirement of the ocular drug delivery devices, which is to achieve a zero-order release to deliver a sustained and adequate amount of the drug to the eye.

The effects of temperature, release volume, and mixing condition has been studied extensively by Tieppo *et al.* Using four ophthalmic drugs loaded on synthesized conventional and silicone hydrogel materials, these studies showed that poor mixing conditions as well as inadequate release media volume will affect the release kinetics dramatically: the equilibrium is reached significantly faster, thus reducing the continued release of the retained drug in the contact lens matrix [48].

It has become evident from the studies by Tieppo *et al.* and Mahomed *et al.* that

[6]Diffusive flux is proportional to the concentration gradient under steady state condition, $J = -D\frac{\partial C}{\partial x}$.

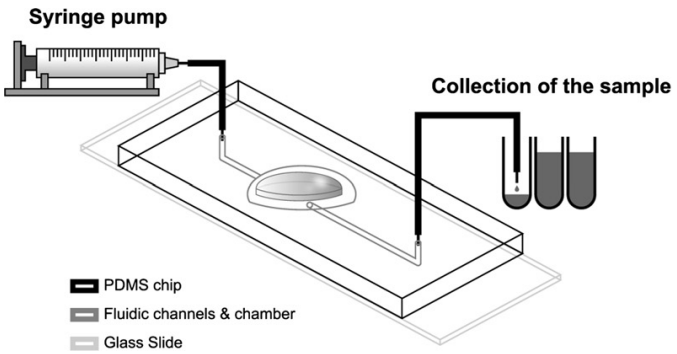


Figure 1.8: Schematic of an experimental microfluidic set-up for contact lens drug delivery evaluation reproducing the physiological flow rate of the eye. The picture is reprinted with permission from ref. [46]

the drug release profile can be significantly affected by the experimental set-up [43, 45, 48]. Before this was actually recognized, an early attempt by Ali *et al.* using physiological flow conditions ($3\mu L/min$) suggested that the release rate of ophthalmic drugs could be slower and more linear when compared to the small sink model often used to study release profiles [61]. As a continuation of that work, Tieppo *et al.* introduced a microfluidic device, as depicted in Fig. 1.8, which could provide a more representative release model of the ocular environment by reproducing physiological flow rates to study the release from ophthalmic drug delivery materials. The results of this study showed a zero-order release for approximately 48 hours [46]. In the physiological flow model, an overall reduced release was also observed which was attributed to the concentration boundary layer resulting from the limited liquid volume surrounding the contact lens material [46]. However, considering the material used for the microfluidic device was polydimethylsiloxane (PDMS), also a major component of silicone hydrogel contact lenses, adsorption of the drug into the chamber material may also have contributed to the reduced release.

In two recent studies, Phan *et al.* and Bajgrowicz *et al.* used a two piece design to model the geometric and microfluidic conditions of the ocular surface [62, 63]. This design allows vertical placement of the contact lens on a convex piece, modeling the cornea, and creating a cavity for the flow of the release media by encasing the contact lens between a secondary concave piece, modeling the eyelid (see Fig. 1.9). The release results using the model showed a sustained release over time when compared to the burst release of the

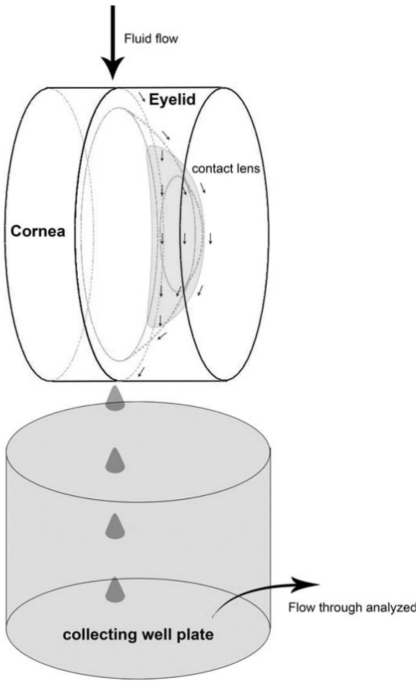


Figure 1.9: Schematic of a two-piece microfluidic ocular surface model for contact lens drug delivery evaluation reproducing the geometry and physiological flow rate of the eye. The picture is reprinted with permission from ref. [62].

tested drug in a passive fixed volume release model. The two-piece design in this model allows incorporation of mechanical stimulation by means of moving the eyelid piece.

There has also been efforts to produce artificial tear solution by adding proteins such as albumin, lysozyme, mucin, lactoferin, immunoglobulin G, and various lipids to a buffered solution [64]. Artificial tear solutions have been used to investigate the deposition of tear film components on ophthalmic materials [65]. The use of artificial tear solution in drug release studies from contact lens materials may prove valuable due to the barrier effect as a result of protein deposition on contact lens. However, there are no current reports of using such solution in drug release studies, most likely due to the added complexity to the drug release measurements.

While in the past few years new experimental models have been introduced to study mechanisms of release from drug delivery devices under dynamic conditions, these models do not take the drug interactions with the cells into consideration.

1.4 Drug Diffusion through the Cornea

The conjunctiva and the sclera have been reported to play a small role in drug delivery to the anterior chamber [13]. Therefore, the cornea remains the most important tissue to study in drug interactions with the ocular surface [14, 66]. As mentioned previously, the human cornea consists of three main cellular layers, the epithelium, the fibroblastic stroma, and the endothelium. The primary drug permeation route to the front of the eye is through transcorneal pathways, which includes paracellular and transcellular pathways [25]. The lipid bilayer cell membrane retards the permeation of hydrophilic compounds. The paracellular passage (the pathway through gaps between cells) is the main pathway for hydrophilic actives [14]: the corneal epithelium and the tight junctions control the transport of the hydrophilic drugs through the cornea (see Fig. 1.3) [67–69]. The corneal epithelium is considered to be the rate-limiting factor in the transcorneal permeation of most ophthalmic drugs [12], especially for hydrophilic drugs [67, 68]. The transcellular passage is the major pathway for hydrophobic compounds. Therefore, the uptake of a topically applied hydrophobic drug in the cornea is dependent on the lipophilicity of the compound. However, the hydrophilic nature of the stroma will generate a resistance toward the permeation of hydrophobic drugs. Through expression of membrane transporters and specific enzymes present in the epithelial cells, the cornea is thought to be involved in metabolism and transport of prodrugs and their active forms [29–32, 70]. In a recent study, Kidron *et al* used a statistical model to identify correlation of the corneal permeability of 58 ophthalmic drugs with parameters such as molecular weight, molecular volume, polar surface area (PSA), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), total number of putative hydrogen bond (HB_{tot}), partition ratio as well as distribution ratio at various pH levels (LogP & LogD). The results of this study challenge the role that membrane transporters are believed to play in ophthalmic drug permeation [71].

1.4.1 *In Vitro* Cornea Models

With the increasing interest in using contact lens as drug delivery devices and the desire to understand the complex interactions between lens cleaning solutions and lenses, there has been an increasing need for *in vitro* cornea models that can better assess biocompatibility and identify promising candidates for animal study. While *in vivo* studies enable the assessment within the physiological systems, they are often very costly, involve the sacrifice of animals, and may be difficult to interpret given both interspecies variation and animal-to-animal inconsistency [72–75]. A classic failure of animal models has been that the bioavailability of oral drugs determined from animal data may not be extrapolated to human [76].

Human cornea *in vitro* models may offer a cost effective and more standardizable substitute [66]. Reconstructed cornea equivalents as well as cell culture models of the human corneal epithelium (HCE) are being used to study *in vitro* ocular toxicity and permeability [77–82]. Reconstructed cornea equivalents which consist of all layers are expected to correlate well with *in vivo* results [25]. However, it is recognized that the epithelium acts as the primary barrier against transcorneal permeation which can in turn be correlated to the electrical resistance of the cornea. The epithelium is responsible for 99% of corneal electrical resistance [83, 84]. Thus, the epithelium alone can also be considered for an *in vitro* model as it would reduce cell culture cost and time, and allow for higher throughput testing of biomaterial interaction and drug permeation [80]. Primary corneal epithelial cells can be isolated from human donor corneas. The isolation protocol is lengthy and contamination with other cells may occur. Primary cells also have a limited lifespan and corneal epithelial cells can only be cultured for up to five passages prior to changes in cell phenotype. While primary corneal epithelial cells are commercially available (which reduces the risk of contamination), the cost associated with using primary cells is significant due to their limited lifespan. Due to these difficulties with primary cultures of human corneal epithelial cells (HCEC), two cell lines with properties similar to normal corneal epithelial cells were established [85, 86]. Both simian virus 40 (SV-40) immortalized HCEC and human corneal epithelial cells transfected with human papillomavirus (HPV) 16 E6/E7 genes

[7] can achieve an extended lifespan. They can also be grown into a stratified cornea model that can form tight junctions similar to the human corneal epithelium. While monolayers of these cell lines have been used to study toxicity and biocompatibility [78, 87, 88], stratified cultures have been shown to better mimic the human cornea [89]. The stratification[8] is achieved through the creation of an air-liquid interface which promotes the formation of tight-junctions and is an essential characteristic of the cornea's barrier function [90]. During stratification, up to five layers of cells have been observed [78].

The main challenges in developing *in vitro* models lie in being able to replicate the relevant factors of the *in vivo* environment and in understanding the limitations of the model's capabilities. Generally, *in vitro* studies using monolayers of corneal epithelial cells show lower tolerance compared to *in vivo* studies [89]. A human eye holds approximately $7 - 30\mu L$ of tear film with a turnover rate of $0.5 - 2.2\mu L/min$ [84, 91–94]. Spontaneous blinking happens at the rate of 6-15 times/min and helps to mechanically spread the tear film evenly across the ocular surface and to remove any foreign objects in contact with the eye [84]. Maintaining an air-liquid interface in the *in vitro* cornea models helps cell cultures to retain their epithelial characteristics. However, it is also necessary to keep the surface moist or the corneal epithelium will undergo an abnormal, squamous (skin-like), differentiation [82].

1.4.2 *In Vitro* Cornea Models for Corneal Drug Diffusion

In vitro cell models with different levels of complexity are used in the evaluation of drug delivery systems as well as new drugs [95]. However, the use of HCEC models has been limited to exploring the effects of metabolic conversion and active drug transport in corneal permeability assessments [25, 81, 96–98]. In these experiments, permeability of cornea constructs are studied in static diffusion cells (Franz cells) or dynamic diffusion cells (Bronaugh cells) as shown in Fig.1.10. In a Franz cell, the drug solution diffuses from the donor cham-

[7] Epithelial cells infected with an amphotropic recombinant retrovirus containing HPV-16 E6/E7 genes to extend their lifespan.

[8] The process of cells growing in layers, expressing certain phenotypes based on their position in the multilayer structure.

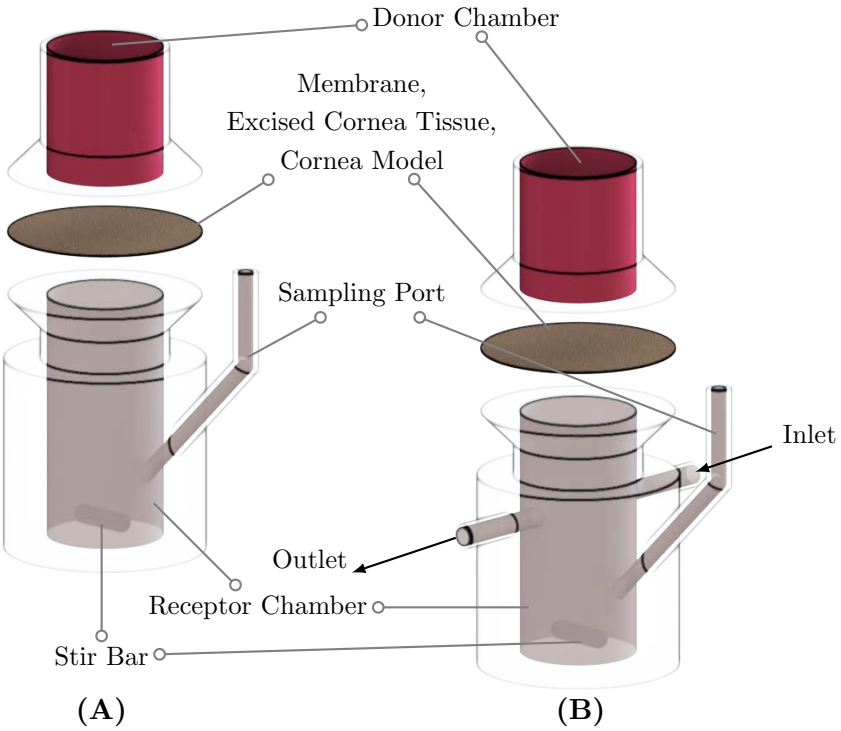


Figure 1.10: Existing *in vitro* cornea constructs are used to assess permeability of the tissue using A) static (Franz) diffusion cell, and B) dynamic (Bronaugh) diffusion cell. The drug solution diffuses from the donor chamber through the membrane into the receptor chamber where drug samples are collected at regular intervals. In a Bronaugh cell, the flow-through system maintains sink conditions and allows continuous collection of samples.

ber through the excised corneal tissue or *in vitro* cornea model into the receptor chamber where samples of the drug are collected. The stirring prevents formation of a stagnant boundary transfer layer. The diffusion cells were used traditionally with skin models as well as porcine or rabbit excised cornea to measure permeability of drugs. Later, they were adopted for cornea constructs [81, 96, 99–101].

Steady-state permeability experiments enable the understanding of corneal permeability, metabolism and active transport. However, it does not account for the rapid elimination of the eye-drops in the tear film. Understanding the pre-corneal elimination kinetics, as well as distribution and elimination in the anterior chamber of the eye, when combined with the permeability studies may lead to the successful simulation of *in vivo* drug diffusion through the cornea [102]. Following the measurement of the mentioned parameters, Ranta *et al.* mathematically simulated the concentration of timolol in rabbit aqueous humor and

showed that it matches experimental *in vivo* results [103].

Currently, to keep a cornea model hydrated, corneal epithelial cells are immersed in cell culture medium or artificial tear liquid. However, the immersion cell culture system poorly models the tear replenishment occurring in the human eye via the lacrimal system. Artificial tear liquids have been utilized to hydrate the surface of corneal epithelial models by incorporating a moisture holding substance [82]. To simulate blinking, a rocker mechanism has also previously been used to generate intermittent flow on top of a cell culture model [104]. These methods try to address inadequacies of conventional tear replenishment models, but lack modeling of the physiological environment parameters of the eye such as constant fluid exchange and limited tear volume.

1.5 A Case for an *in Vitro* Study of Corneal Drug Diffusion

In the field of contact lens drug delivery, the inadequacies of current *in vitro* release models has limited progress mostly by giving rise to false kinetics of release where the reported behavior cannot be recreated in the physiologic environment. Table 1.1 compares the amount of drug released from commercial contact lenses loaded with the same drug and released in either a fixed volume solution or an *in vivo* model. The amount of release and elution time is consistently smaller in the fixed volume versus the *in vivo* environments. In the fixed volume conditions, the drug release mechanism is governed by Fickian diffusion, where concentration gradients generate the driving force and the ratio of the concentration between the contact lens and the medium is dictated by the partition ratio. The fixed volume environment does not represent the eye environment where there is a limited amount of tear liquid with a relatively fast tear turnover. The composition of the release medium also plays an important role in release studies. While a certain contact lens material may present optimal release in deionized-water (DI), their performance might be reduced dramatically in the presence of ions or surfactants [55].

The contribution of enzyme and transport activities such as the esterase activity of

Table 1.1: Drug Uptake and Release from Contact Lens Materials - Release Medium Comparison

Drug	MW [$\log P^{\dagger}$]	Contact Lens	Loaded Drug Released $\mu g/_{lens}$	Release Time (Medium)	Ref.
Acetazolamide	222.245 [-0.26]	balafilcon A	100	$30min$ ($5mL$ saline)	[53]
		sauflon PW	not reported	Up to 7.5h (*in vivo* Leporine)	[105]
Gentamicin	477.59 [-3.1]	etafilcon A	186	$20-30min$ ($3mL$ saline)	[106]
		sauflon 85	$10-30\mu g/mL$	Up to 2h (*in vivo* human)	[107]
Ketotifen	309.425 [2.2]	Various	$105-227$	$\leq$ 1h ($2mL$ saline)	[51]
		SiHy($100\mu m$ thickness)	$1200\mu g/mL$	15-24h (*in vivo* Leporine)	[108]
Lomefloxacin	351.348 [2.8]	etafilcon A	150	1h	[109]
		etafilcon A	750	8h (*in vivo* Leporine)	[110]

Adapted from ref. [49]. All contact lens materials were loaded through lens soak in drug solution. The amount of uptake has not been reported, however the amount of release varies between *in vivo* and fixed volume release medium. Larger partition ratios show a larger difference in release amount and elution time. SiHy, Silicone Hydrogel; † Drug information has been retrieved from DrugBank Canada database (Version 3.0). Partition ratios are experimental values, and if not available, the predicted values of the active ingredient are reported.

the corneal epithelium has been utilized in the design of ophthalmic prodrugs such as prostaglandin $F_{2\alpha}$ analogues [111]. The lipophilicity as the result of esterification or amidification in prodrugs facilitates the penetration through the cornea. Hydrolysis of prodrugs such as prostaglandin analogue into the relatively hydrophilic acid form inside the epithelial cells allows permeation through the stroma [112] and thus, increases the bioavailability of the active substance in the interior of the eye [113]. Despite extensive studies on the role of cells in drug permeation and metabolism, their role has largely been ignored in ocular drug release studies. This could be attributed to the complexity and cost associated with cell culture models. In the case of prostaglandin $F_{2\alpha}$ analogues as a major group of medications administered to control the progression of glaucoma, the pharmacokinetics of these drugs has been extensively studied *in vivo* [1, 72]. However, there has been very limited *in vitro* studies of these prodrugs. Moreover, a recent study of a latanoprost eluting contact lens has shown inconsistent release results between an infinite sink release model and *in vivo* (rabbit model) [114]. Such an inconsistency is likely due to the lack of cells in the *in vitro* model. Given the cells' contribution to hydrolysis of latanoprost, the absence of cells most likely led to inconsistency of the *in vitro* results.

Given the current state of *in vitro* models for drug delivery, we may conclude that there is a need to establish an *in vitro* cornea model capable of replicating the corneal surface physiology, and that may help provide better *in vitro-in vivo* correlations in ocular drug delivery experiments.

Chapter 2

Thesis Scope and Hypotheses

In release studies of ophthalmic drug delivery materials, researchers rely on maintaining sink condition as a means to maximize release and obtain extended release from the contact lens material. However, the prevailing situation in the eye is the exact opposite, where a constantly replenished, limited tear volume is retained. To translate release studies into effective therapies, understanding the cellular and molecular mechanisms of the corneal tissue and the physiological conditions of the front of the eye is required. The need for an organotypic model that can better recreate the dynamic microfluidic conditions of the ocular surface is evident. Also, considering that the primary drug permeation route to the front of the eye is through transcorneal pathways, it is important to consider the role of the cornea in drug release studies.

This thesis aims to demonstrate that a cell-based *in vitro* cornea model combined with a tear replenishment method would further enhance the study of drug release from ophthalmic materials targeted toward the front of the eye when compared to the fixed volume release model.

Given the limitations of current drug release test platforms in recreating the tear replenishment, three goals were pursued. First to design and implement an acellular dynamic drug release model (DDRM) to recreate the limited tear volume as well as the continuous tear replenishment of the human eye. This model then was used to investigate the uptake and release of ophthalmic dyes as surrogates for ophthalmic drugs from commercially

available contact lenses. Comparisons of the release results obtained using the dynamic system and conventional models, presented in Chapter 3, provided unique insights into the release mechanisms that could be used in interpreting discrepancies seen between *in vitro* and *in vivo* results. Using surrogate drugs in an acellular ocular surface model also allowed investigating interactions of those surrogate drugs with the contact lens materials while offsetting the high cost of ophthalmic drugs and the cost associated with their measurements.

The cornea, through expression of membrane transporters as well as the presence of enzymes in the epithelial cells, is involved in metabolism and transport of prodrugs and their active forms. Another objective of this research was to investigate the role o corneal epithelial cells in the release and hydrolysis of an ophthalmic prodrug from contact lens materials. The results, presented in Chapter 4 and published as a journal paper [115], demonstrated the impact of the presence of live cells on the release mechanisms when compared to the current acellular models.

While the interactions of the drug and lens material with cells could be studied in static models such as the immersion *in vitro* cornea model, the immersion cell culture system poorly mimics the tear replenishment occurring in the human eye. The lack of tear replenishment in existing *in vitro* human cornea models limits their ability to accurately simulate the physiological environment of the human cornea. The third objective of this thesis, the culminating point in this research, was to design and implement the tear replenishment system (TRS) as a platform to incorporate a dynamic liquid exchange to the existing *in vitro* cornea models. Integration of the tear replenishment system with a well characterized cell culture system allowed investigation of release of ophthalmic drugs from contact lens materials in a robust and reliable fashion. The results, presented in Chapter 5, helped to understand interactions among cells-lens-replenishment parameters that would otherwise be neglected or dismissed.

Chapter 3

In Vitro Drug Release from Contact Lens

3.1 Overview

Since the development of hydrogel contact lenses in 1965, their potential use as a drug delivery platform has been studied [4]. Different mechanisms have been utilized to load a drug into a soft contact lens, from soaking them in a drug solution to molecular imprinting, and carrier mediated release [49]. Because of its simplicity, the drug-soaked contact lens remains the most popular and practical drug loading mechanism and it is most likely to be adopted for commercial ophthalmic drug delivery devices. However, previous studies have suggested that for most ophthalmic drugs and commercially available contact lens materials, low drug loading and elution times ranging from few minutes to two hours prevent a prolonged and controlled release.

It was hypothesized that the lack of relevant ocular surface test method for drug release studies may have contributed to underestimating the drug delivery capabilities of contact lenses. To test this hypothesis, the study presented in this chapter investigated the uptake and release of three surrogate drugs from commercially available contact lenses using three *in vitro* models. A dynamic drug release model (DDRM) was developed to recreate the limited tear film volume as well as the continuous tear replenishment that occurs on the

ocular surface. Using the DDRM, the release of several surrogate drugs from five contact lens materials was studied. The release results were then compared to a cell based *in vitro* cornea model and a conventional small sink release method.

3.2 Design and Implementation of the Dynamic Drug Release Model

The key purpose of designing the dynamic drug release model is to offer an affordable and simple release model that sufficiently recreates the release conditions on the surface of the eye. While a cell model can more closely simulate the eye's physiological environment, the requirement of a cell culture facility will be prohibitive for most researchers who focus on the contact lens material or study the drug-lens interactions. This section focuses on the design and implementation of a dynamic drug release model with the goal of recreating the microfluidic conditions of the ocular surface.

3.2.1 Tear Film and Tear Flow, Merits of an Intermittent Fluid Flow

Blinking in the human eye replenishes the tear film and spreads it over the surface of the eye. The microfluidic interactions of a drug releasing contact lens with the tear film can be simplified to a contact lens encased in a chamber with a limited volume of available release media representing tear film, whereby the release media is replenished intermittently with the frequency of human blinking representing tear flow. Therefore, an intermittent fluid flow would be preferable over a continuous laminar flow which is the basis of the design discussed in this section.

3.2.2 Design Constraints and Criteria

The key constraints that must be met by the instrument's design are:

1. providing a suitable surface geometry for contact lenses to be placed (similar to Byrne's model [46]),
2. using inert materials that will not interact with the drug or the contact lens material,
3. creating the limited release volume of the tear film accurately and repeatably,
4. delivering a reproducible and adequately small volume of release media intermittently,
5. providing an even distribution of fluid flow over the contact lens material for the duration of the release,
6. allowing the testing of multiple (i.e., 4) contact lenses at a time,
7. minimizing the loss of the release media through evaporation.

A secondary objective would be to design a cost efficient, open hardware/ open software instrument that can be adopted, modified and improved by other researchers for the purpose of dynamic drug release studies from drug eluting materials.

3.2.3 DDRM Design

The design of the desired microfluidic system can be classified according to the mechanism that drives the flow, and the approach employed to control the rate of the flow. The liquid displacement can be generated using the "flow concept", such is the case with a syringe pump whereby the liquid movement is the result of the movement of an electromechanical actuator. The liquid displacement can also be generated using the "effort concept" whereby a pressure gradient is the driving force behind the movement of the liquid. While both design concepts have their own merits, using the flow concept in resolving the situation at hand would require as many syringes as there are test chambers. Using a single syringe

would result in an uneven flow rate between the chambers, while using multiple syringes in a syringe pump would limit the control of the flow independently. Thus, the effort concept is utilized whereby a pressure gradient is generated through pumping a tear solution analogue through a pressure relief valve before closing the loop of the system as illustrated in Fig. 3.1.

The controlled flow rate can be achieved by blocking the liquid flow either upstream or downstream using a series of isolation valves (shut-off valve). The upstream approach, as would be the case with the syringe pump design, would require the release chamber and the contact lens to be subjected to atmospheric pressure. The major drawback of such a design would be the inability to create a uniform flow over the contact lens within the chamber. On the other hand, limiting the flow downstream would allow a controlled discharge of the fluid, while maintaining a uniform flow regime within the chamber. The secondary benefit of a downstream-controlled flow would be the ability of the pressure gradient in removing air bubbles from the chamber. Eliminating the air bubbles from the release chamber is necessary to achieve a uniform release from the entire contact lens material, since a bubble would partly cover the surface of the lens and prevent release from that region of the lens.

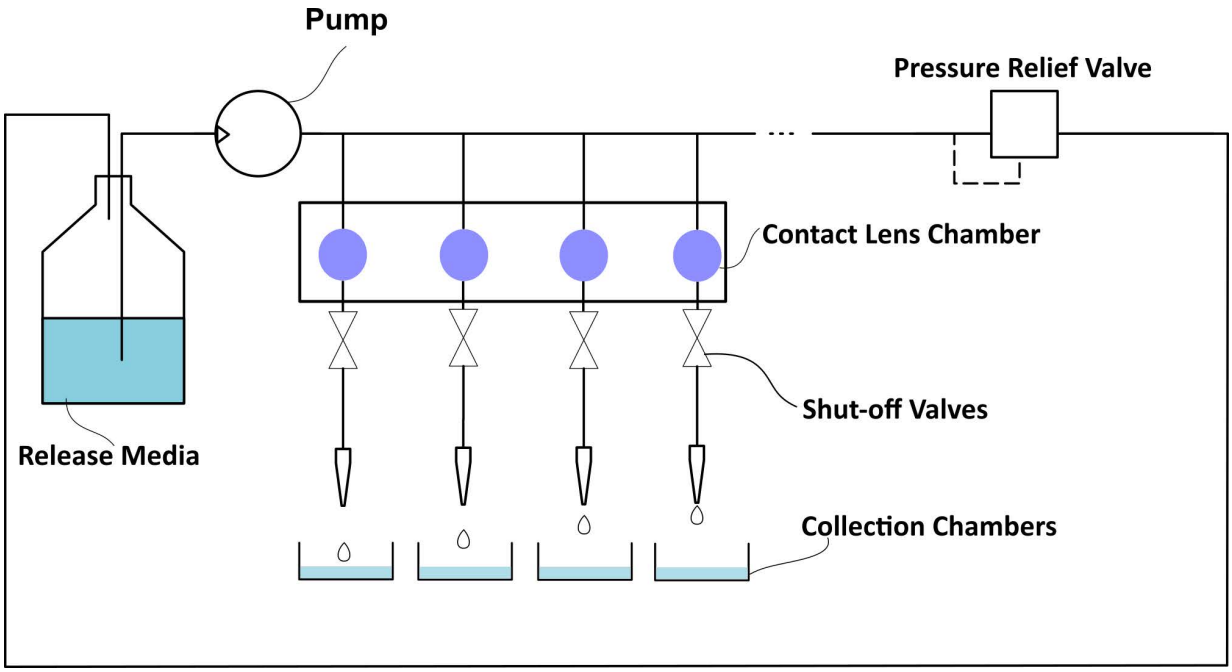


Figure 3.1: Design schematic of the dynamic drug release model: the solenoid pump provides enough back pressure to a series of isolation valves to control the flow rate as well as the duty cycle of the intermittent flow.

Tear flow rate in the human eye is approximately $100\mu L/hr$ with a tear volume of $7 - 30\mu L$ [45, 84, 91–94]. The volume of a contact lens depending on the radius, base curvature, and the prescription may vary between 140 to $170\mu L$. While the prescription of the contact lenses used for the experiments can be fixed, not all contact lens manufacturers produce lenses with the same base curve and radius. To accommodate a wide range of contact lenses, the curved chamber encasing the contact lenses was designed to have a cavity volume of $210\mu L$, while the estimated average volume of the contact lenses used for the experiments was $160\mu L$. The remaining volume is within the same order of magnitude of the human tear film. The rate of flow can be adjusted by the duty cycle[1] of the isolation valves.

3.2.4 Electromechanical System

The electromechanical components of the designed instruments include: a solenoid operated micro-pump from Bio-Chem Fluidics™, which is used to pump the tear solution analogue (here PBS) through PTFE[2] tubing (Bio-Chem Fluidics, NJ, USA) passing through a pressure relief valve preset to $20psi$ before looping back to the release media container (as illustrated in Fig. 3.2). The two pieces of manifold that encased the lenses and are used for mounting the solenoid valves were machined out of Polyacrylic and PTFE sheets because of their excellent inert chemical properties; these parts are depicted in Fig. 3.3a. Contact lenses are placed in each chamber (the cross section is shown in Fig. 3.3b), and the release flow aliquots are collected in a 12-well plate (4x6 wells), with each well collecting the release over a 2h period. The valves, pump, and the motor that moves the 12-well plate are controlled and driven using a small board computer that is installed inside the enclosure as depicted in Fig. 3.3c. The enclosed chamber and fluid channels eliminate loss of release flow through evaporation.

[1] The percentage of one period where the valve is open.

[2] Polytetrafluoroethylene, a bio-inert synthetic fluoropolymer with excellent chemical resistance

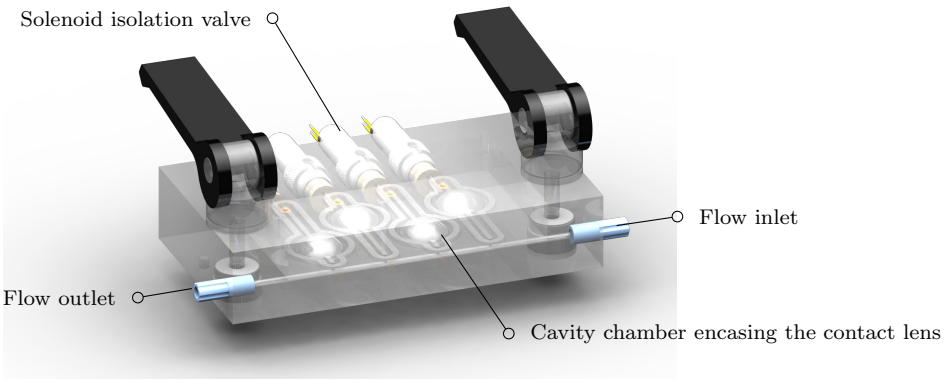
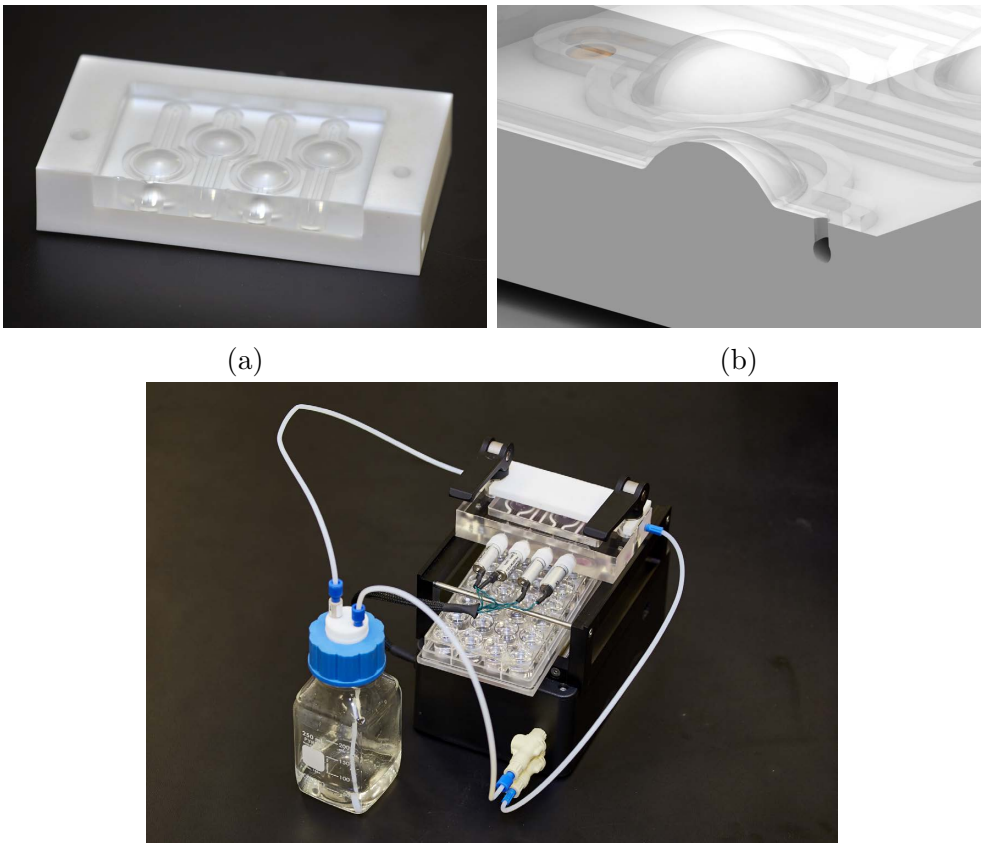


Figure 3.2: Dynamic Drug Release Model's cavity design. The tear solution analogue is circulated through the manifold, while solenoid isolation valves control the flow rate downstream.



(a) (b)

(c)

Figure 3.3: Dynamic Drug Release Model. a) Polycarbonate manifold and the contact lens cavity chambers. b) cross-section of the lens chamber. c) The final design of the DDRM. DDRM can easily fit inside an incubated shaker, or any small environmental chamber.

3.3 Materials and Methods

Corneal epithelial cells were grown on a flat cell culture insert to study the drug release from a contact lens and its diffusion through the corneal model. The drug release from contact lenses, as it has been discussed before, has been studied mostly in a fixed volume of a chosen solution. In this section, the procedure for release studies of three surrogate drugs from five commercial contact lens materials using a fixed-volume, an *in vitro* cell culture as well as the dynamic release models are described.

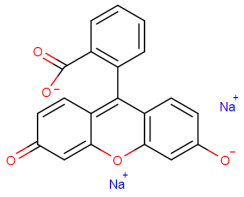
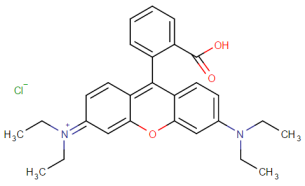
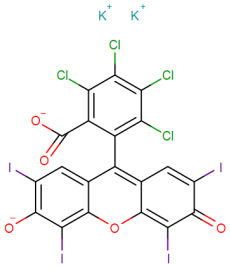
3.3.1 Preparation of Surrogate Drug Solutions

In this study, three structurally similar ophthalmic dyes were selected to investigate the various factors and their effects (see below) on the uptake and release mechanisms from commercially available contact lens materials. The ophthalmic dyes fluorescein sodium salt (FlNa), rhodamine B (RhB), and rose bengal (RsB) share a similar backbone while having different molecular weight and partition coefficient. They all have polar surface area[3] (PSA) of less than 120-140$\mathring{A}^2$ which is a critical property for ophthalmic drugs that use paracellular or transcellular routes. Table 3.1 presents the properties of these ophthalmic dyes which will act as the surrogate drugs in this study.

The surrogate drug solutions were prepared by dissolving fluorescein sodium salt, rhodamine B, and rose bengal (Sigma-Aldrich Chemical Co., St. Louis, MO), in Phosphate Buffered Saline (Lonza, Walkersville, MD). Various drug concentrations were tested in a cell toxicity study to determine the maximum permissible drug concentration, thus eliminating any unwanted drug-cell cytotoxic interactions. Except for rose bengal, the surrogate drug concentration of $10\mu g/mL$ was selected as experiments determined them to have no cytotoxicity effect (see results section). Due to the toxicity of rose bengal, drug release studies in the cell model were limited to fluorescein sodium and rhodamine B.

[3]The polar surface area of a molecule is defined as the surface sum over all polar atoms, primarily oxygen and nitrogen, also including their attached hydrogens [`https://en.wikipedia.org/wiki/Polar_surface_area`, retrieved July 2016].

Table 3.1: Properties of the Ophthalmic Dyes Used as Surrogate Drugs

Surrogate Drug	**Fluorescein Sodium**	**Rhodamine B**	**Rose Bengal**
Molecular Weight $[g/mol]$	376.27	479.01	1049.85
$LogP$ (Hydrophobicity)	2.65	1.78	8.78
Polar Surface Area† [Å^2]	89.49	52.78	89.49
Maximum Absorbance Wavelength (nm)	490	550	548
Molecule			

Chemical properties have been retrieved from Chemicalize.org®. †Cell permeability of drug molecules with a polar surface area greater than 140Å^2 is less than 10% and therefore PSA of 120-140Å^2 is the upper limit PSA for a drug design that uses paracellular or transcellular routes.

3.3.2 Contact Lens Materials

Soft contact lens materials are categorized into 4 groups according to their water content and ionicity, with the addition of silicone hydrogels as the fifth group (silicone hydrogels may also be classified in the low water content, ionic and nonionic groups). To represent the majority of common contact lens material categories, five commercially available contact lenses, etafilcon A, senofilcon A, hilafilcon B, polymacon, and balafilcon A, were used. Senofilcon A and balafilcon A represent non-ionic and ionic silicone hydrogel contact lens materials respectively. The majority of silicone hydrogel materials have low water content ($< 50\%$). Polymacon is a conventional non-ionic lens material with low water content (Group I). The ionic etafilcon A and non-ionic hilafilcon B are high water content hydrogel lens materials (Groups IV and II respectively). The properties of the five lens types used in this study are presented in Table 3.2. Dk value, which describes the intrinsic ability of the material to transport oxygen, are not reported as this property does not affect the uptake and release. To ensure consistency in lens material thickness, all lenses had a back vertex power of -3.00 diopter.

3.3.3 *In Vitro* Cell Culture

HPV immortalized human corneal epithelial cells [4] were cultured in keratinocyte serum free medium (KSFM) supplemented with bovine pituitary extract, recombinant epidermal growth factor, and Penicillin/Streptomycin (Pen/Strep) [5] (ScienCell, Carlsbad, California) at $37°C$ and 5% carbon dioxide (CO_2). Fresh KM was added every other day and cells were grown to 90% confluency in tissue culture treated flasks. Adherent cells were removed using TryplExpress (Life Technologies, Burlington, Ontario, Canada) dissociation solution. Cells were routinely observed for any morphological changes and were used to prepare *in vitro* cornea models.

[4] Epithelial cells infected with an amphotropic recombinant retrovirus containing HPV-16 E6/E7 genes to extend their lifespan.

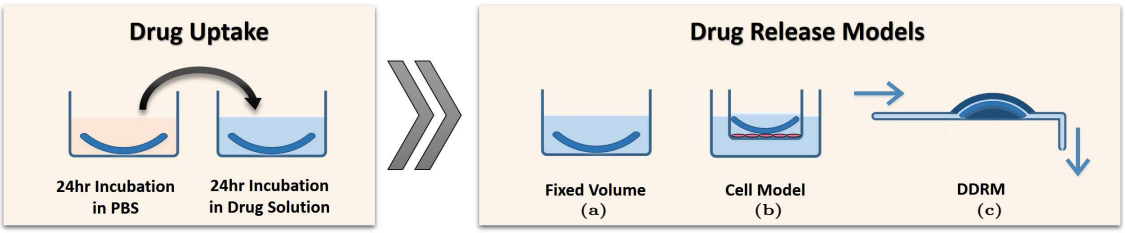
[5] This supplemented medium will be referred to as KM

Table 3.2: Properties of the Contact Lens Hydrogel Materials Used in Drug Delivery Studies

Commercial name (US adopted name)	Acuvue 2 etafilcon A (EA)	Acuvue Oasys[†] senofilcon A (SA)	SofLens 38 polymacon (Pm)	SofLens Daily Disposable hilafilcon B (HB)	PureVision 2[‡] balafilcon A (BA)
Manufacturer	Johnson & Johnson	Johnson & Johnson	Bausch & Lomb	Bausch & Lomb	Bausch & Lomb
Water content (WC)	58	38	38	59	36
Principal Monomer	HEMA + MA	mPDMS + DMA + HEMA + siloxane macromer + TEGDMA + PVP	HEMA	HEMA	NVP + TPVC + NVA + PBVC
FDA group[*]	IV High WC Ionic	V(I) Low WC Non-ionic	I Low WC Non-ionic	II High WC Non-ionic	V(III) Low WC Ionic

[*] FDA (Food and Drug Administration) categorizes all silicone hydrogel contact lenses as group V, however it is more practical to use groups for conventional hydrogels to better understand their material properties. [†] Internal wetting agent (PVP) has been used to compensate for the low wettability of silicone hydrogels. [‡] Plasma oxidation process has been used as the surface treatment to increase wettability.

HEMA, HydroxyEthyl MethaAcrylate; MA, Methacrylic Acid; mPDMS, monofunctional PolyDiMethylSiloxane; DMA, DiMethAcrylate; TEGDMA, Tetra-EthyleneGlycol DiMethAcrylate; PVP, Polyvinylpyrrolidone; NVP, N-Vinylpyrrolidone; TPVC, Tris(trimethylsiloxysilyl) Propyvinyl Carbamate; NVA, N-Vinyl Aminobutyric Acid; PBVC, Poly(dimethysiloxy)di (silylbutanol) Bis(Vinyl Carbamate



(A) **(B)**

Figure 3.4: Uptake and Release Models. A) Individual contact lenses were washed in PBS overnight before soaking in $1.5mL$ of surrogate drug solution for 24 hours. B) Drug soaked lenses were tested in three drug release models. The release in static models was measured for 48 hours. a) Fixed volume release studies conducted in $1.5mL$ of PBS, b) In the cell model, the lenses were immersed in $0.5mL$ of KM on top of a monolayer of human corneal cells grown on a cell culture insert (PET filter). Samples were aliquoted from $1.0mL$ of KM on the bottom and later were measured using UV-VIS spectrophotometry. c) In the dynamic drug release model, an intermittent liquid flow at the rate of $1.2mL/hr$ is discharged after passing over the lens encased in the chamber. The aliquots were collected over the period of 2h in a 6-well plate.

3.3.4 Drug Uptake

Lenses were first incubated for 24 hours in $3mL$ of phosphate buffer saline (Lonza, Allendale, New Jersey) to remove any remnants of their packing solution. Preliminary experiments showed that a competitive equilibrium between the drug doping solutions ($1.5ml$ of the $10\mu g/mL$) and all contact lens materials were achieved after few hours. However, the lenses were incubated for 24 hours to ensure a consistent uptake. The incubation of lenses in low drug concentrations as well as small volume of doping solution could be representative of the concentrations of common ophthalmic drugs (i.e, $50\mu g/mL$ for latanoprost and $40\mu g/mL$ for travoprost) as well as the limited volume of lens packaging solution (typically ranging from $1mL$ to $2.5mL$). To determine the uptake amount by the contact lenses, samples from the original drug doping solution and the solution after soaking the lenses were aliquoted to measure the uptake of drug into each lens material. Fig. 3.4A illustrates the uptake procedure.

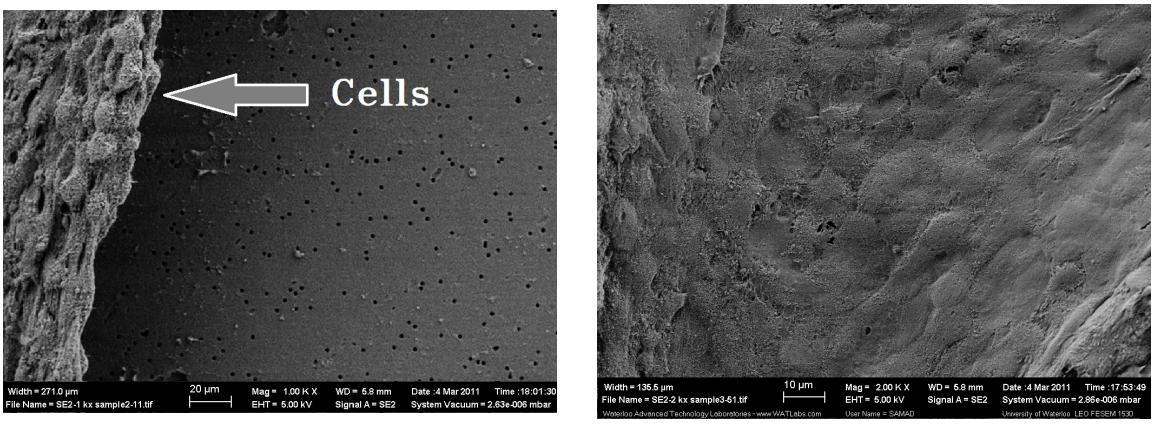
3.3.5 *In Vitro* Drug Release Models

The *in vitro* cornea model was prepared by growing a monolayer of human corneal epithelial cells on a Millicell PET membrane (hanging cell culture insert) with a $1.0\mu m$ pore size. The inserts were seeded with 10^5 cells/insert. The cornea models were fed fresh KM on each of the basal and apical sides of the insert for seven days, with medium being exchanged every other day; they were then ready for experimentation. The cell culture inserts as well as the grown monolayer were examined by scanning electron microscopy, as shown in Fig. 3.5.

The three *in vitro* models used to assess drug release from the contact lenses are summarized in Fig. 3.4B. In the fixed volume model, drug release into $1.5mL$ of PBS was measured by taking aliquots of $100\mu L$ and replacing by fresh PBS. In the *in vitro* cornea model, the contact lens was immersed in $0.5mL$ of cell culture media. The drug released into the media on top of the cell monolayer and then diffused through the corneal cells and the polyethylene terephthalate (PET) filter (also referred to as culture inserts or culture membrane) to the bottom of the well containing $1.0mL$ of media. Aliquots of $100\mu L$ were taken from the bottom of the *in vitro* models and replaced by fresh culture medium. For both the fixed volume and cornea model release experiments, samples were taken at 2, 6, 24 and 48 hours. In the dynamic release experiments using DDRM, PBS was used as the release media. The liquid was discharged from each chamber at a rate of $20\mu L/min$, which is higher than the physiological tear replenishment rate of $2\mu L/min$. The flow rate was primarily selected to ensure adequate release media was collected. The flow rate can be adjusted for much smaller flow rates, possibly for future studies. The release experiments were conducted for 24 hours, with the discharged liquid from the chambers being collected over periods of 2-hour in separate wells.

3.3.6 Cell Viability Assay

After 48 release studies in the cell model, lenses and medium were removed. To obtain a measure of the cellular viability, the MTT assay was performed. Dimethyl thiazoyl blue tetrazolium bromide ($0.5mg/mL$, MTT, Sigma Aldrich, Oakville, ON, Canada) was added



(a) (b)

Figure 3.5: a) Microstructure of Millicell PET filter, b) Tight monolayer of human corneal cells grown on a PET filter

to the apical and basal sides of the cell culture insert and was incubated for 3 hours at $37°C$ and 5% CO_2. The MTT solution was then removed and isopropanol was added to both the apical and basal sides of the insert and plates were agitated for 2 hours. The solutions in the apical and basal sides were mixed together and samples were read in a UV-Vis spectrophotometer at an optical density of 595 nm with a reference at 650 nm. All results are expressed as the relative viability compared to control cells: cells incubated in the absence of a contact lens.

3.3.7 Drug Concentration Measurements and Data Analysis

To measure the concentration of the surrogate drugs, the optical density of each sample was read in a UV-Vis spectrophotometer at the maximum absorbance wavelength of each of the surrogate drug compounds as listed in Table 3.1.

Results reported in this study represent the mean of at least three experiments ± standard deviation. Experiments were performed on different days. To evaluate the significance of the differences between various contact lens materials, *in vitro* models and across the time points, an analysis of variance (ANOVA) was performed, followed by multiple pair-wise comparisons using the Holm-Sidak test and a pairwise comparison using T tests according to the Sidak correction of Bonferroni inequality in SigmaPlot™.

3.4 Results and Discussions

3.4.1 Surrogate Drugs' Interaction with Cells

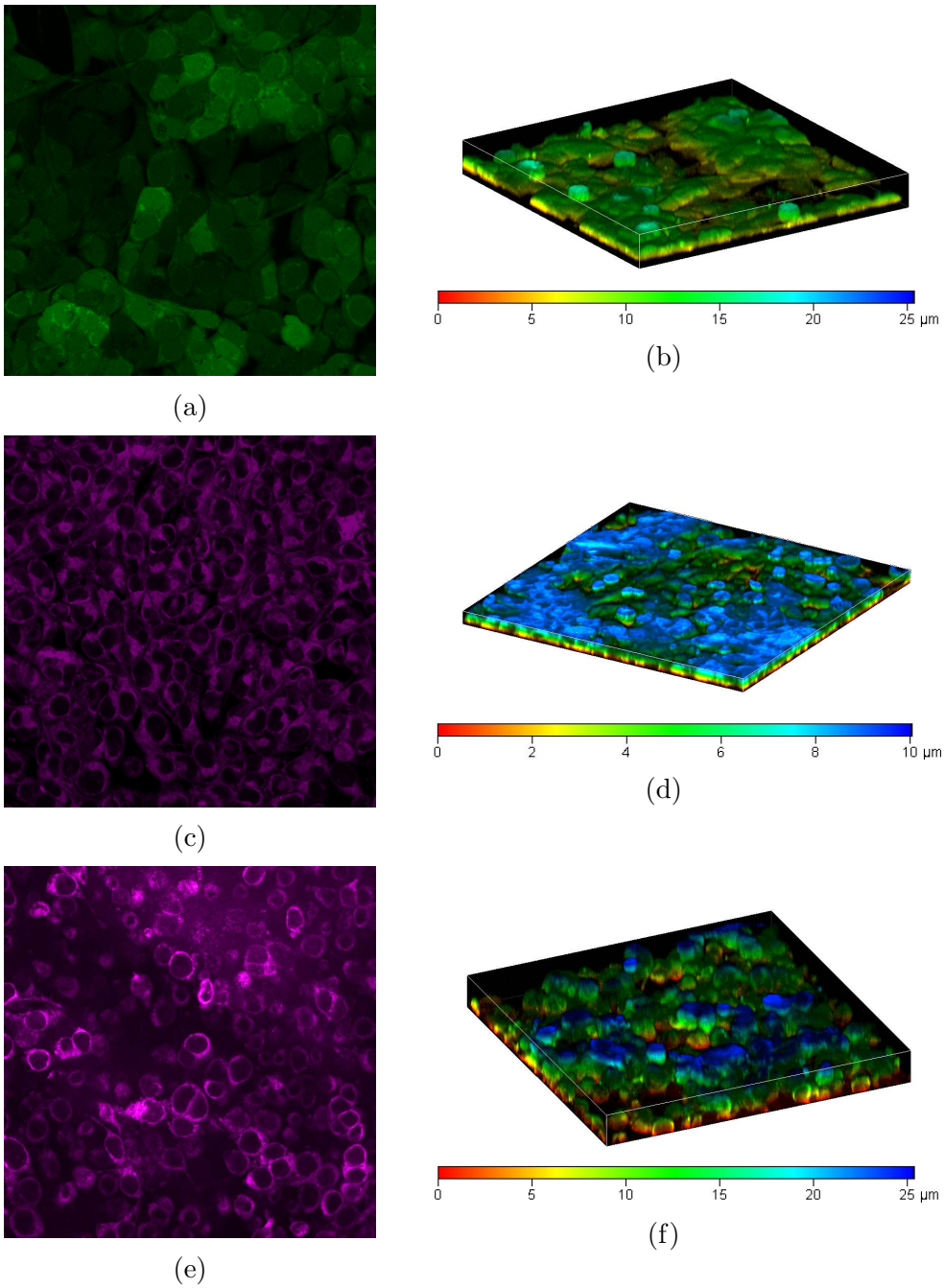
It has been suggested that the ophthalmic fluorescent dyes can be used as surrogate drugs to characterize and identify desired polymeric compounds for drug delivery applications [60]. Due to their low cost and ease of measurement (i.e., UV-VIS spectrophotometry), these compounds were used in this study to compare the uptake and release from contact lens materials in the three studied models. The use of ophthalmic dyes as surrogate drugs also allows direct observation of uptake, release, and the interactions of those actives with the cells and the release model, in addition to the advantage of allowing spectrophotometric assays for accurate measurements of uptake and release. To assess the effect of surrogate drugs on cells, specifically the effects on cell morphology, monolayers of HCEC grown for 7 days on a collagen treated glass-bottom culture dish were incubated with $10\mu g/mL$ of surrogate drug solutions for 2 hours, then imaged using a confocal laser scanning microscope after rinsing the cells to remove residual dye.

The reconstructed 3-dimensional monolayer image indicated no damage to the cell layer. The uptake of the ophthalmic surrogate drugs/dyes by cells are shown in the confocal laser scanning electron micrographs (Fig. 3.6). After 2 hours of incubation, rhodamine B showed no uptake by the cell cytoplasm but binding to the cell membrane, while fluorescein was observed both on the cell membrane and in the cytoplasm. An uneven uptake of fluorescein was noticed, whereby some cells took up more while some appeared to have almost no fluorescein in their cytoplasm. The dark areas in both in Fig. 3.6a and Fig. 3.6b are not indicative of holes in the monolayer, but rather indicates no fluorescence despite having viable cells as part of the monolayer. This is consistent with previous *in vitro* and *in vivo* results [78, 116, 117] showing inconsistent fluorescein uptake by epithelial cells. For rose bengal, in the 3D stacked image of the cell monolayer, a severely compromised monolayer of cells can be identified by the presence of round cells and damaged cell membranes (Fig. 3.6f). The impact of rose bengal on corneal cell integrity was significant enough to warrant further investigation into the cytotoxicity of the surrogate drugs in the *in vitro*

cell model.

3.4.2 Cell Viability Assay of Surrogate Drugs

Cell viability studies were conducted to assess the toxicity of the surrogate drugs *in vitro*. Exposing cells to cytotoxic concentration levels of these surrogate drugs would invalidate the release results obtained from the cell studies as it would affect the barrier and metabolic functions of the cells. To this end, solutions of the surrogate drugs were prepared in cell culture media at $5, 10, 20$, and $50\mu g/mL$. The *in vitro* cell models were incubated for 24 hours with $500\mu L$ of the surrogate drugs at various concentrations added to the top only. The *in vitro* monolayer was fed with $1.0mL$ of fresh KM on the bottom. The experiment was repeated 5 times on different days. The viability results are presented in Fig. 3.7. These results confirmed the confocal microscopy observations. Rose bengal even at low concentrations significantly damaged cells ($p < 0.001$) as shown by a viability of less than 30% regardless of the concentration tested. Based on the viability results, drug concentration of $10\mu g/mL$ was selected for drug doping solutions since at this concentration, neither fluorescein sodium nor rhodamine B were found to induce any cytotoxicity in the *in vitro* cell model.



(a) (b) (c) (d) (e) (f)

Figure 3.6: Representative confocal laser scanning micrographs showing the uptake of each surrogate drug into the cell cytoplasm or on the membrane and the effect of surrogate drugs on cell morphology. The rainbow color spectrum represents the height of the cell layer. a) HCEC monolayer stained with fluorescein sodium. b) 3D reconstructed image ($25\mu m$ in thickness) shows a $10\mu m$ thick cell layer. Note that the dark area in (a) and (b) do not indicate the lack of cells, but the fact that cells did not uptake fluorescein. c)HCEC monolayer stained with rhodamine B, only cell membrane is stained. d) 3D stacked image shows a $10\mu m$ thick impervious cell layer. e) HCEC monolayer stained with rose bengal, and f) 3D reconstructed image ($25\mu m$ in thickness) of the monolayer shows damaged membranes and cells with different morphology.

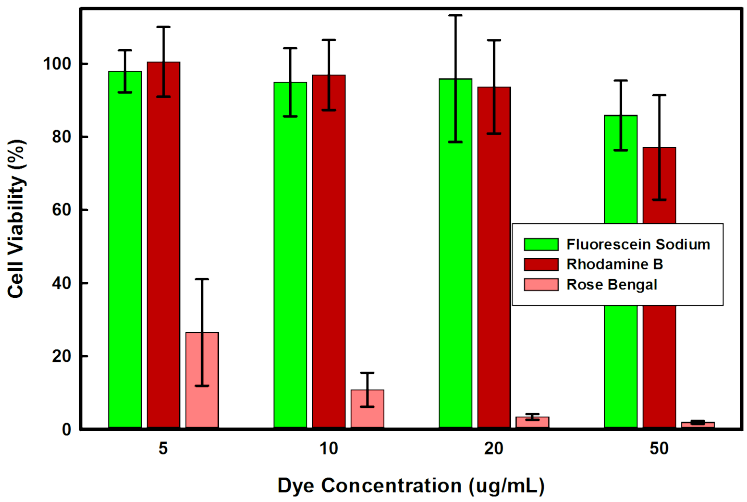


Figure 3.7: The effect of surrogate drug concentration on cell viability. Viability was measured by MTT assay and is expressed as a percentage relative to cells without treatment. Rose bengal showed a significant toxicity at all concentrations ($p < 0.001$) (n=5 mean $\pm$ standard deviation).

3.4.3 Uptake Studies

The uptake results are presented in Fig. 3.8; $15\mu g$ of drug was available in the doping solution and it can be observed that up to $14\mu g$ of rose bengal was taken up in contact lens materials.

It was previously discussed, in Chapter 1, that both water content and polymer matrix of a soft contact lens hydrogel can affect the uptake of hydrated species such as drugs or ophthalmic dyes. These compounds, which have a wide range of hydrophobicity, are able to dissolve in aqueous solutions through association/dissociation[6]. However, the interactions between the polymer network and the dye depend on the surface charge and bulk properties of the polymer, as well as the partition ratio ($logP$) and the distribution ratio ($logD$) of the ophthalmic dye/surrogate drug. In the case of rhodamine B and rose bengal, where at neutral pH there is only one dominant specie, the uptake mechanism is driven by the partition ratio and the properties of the polymer network, which explains the difference in the uptake of these two actives within each polymer network. Thus in case of silicone

[6]The process whereby an ionic compound breaks into two ions, i.e, fluorescein sodium is dissociated into sodium and fluorescein.

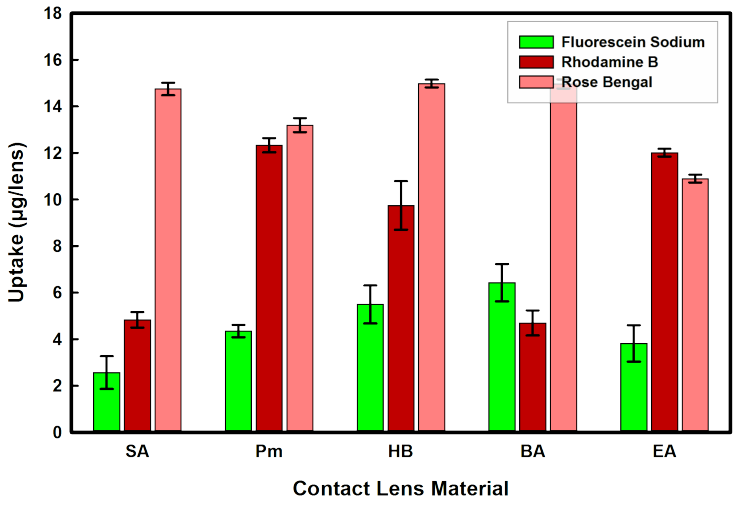


Figure 3.8: Uptake data for the three surrogate drugs and five contact lens materials: senofilcon A (SA), Polymacon (Pm), hilafilcon B (HB), balafilcon A (BA), etafilcon A (EA). Lenses were soaked in PBS for 24 hours before an incubation in $1.5mL$ of doping solution ($10\mu g/mL$). The uptake is calculated based the residual concentration in the solution (n=6, mean $\pm$ standard deviation).

hydrogels (senofilcon A and balafilcon A) with hydrophobic regions, significantly higher uptake of rose bengal compared to rhodamine B was observed ($p < 0.001$). But in case of etafilcon A, a high water content charged hydrogel the rose bengal uptake was smaller that rhodamine B.

Fluorescein sodium at neutral pH is dissociated into two major species with highly hydrophilic properties. This is reflected in the distribution ratio of this compound ($logD \approx -1$, negative $logD$ values imply the tendency of a solute to remain ionized in aqueous environment). This would explain the low uptake of fluorescein sodium compared to the other drugs. However, in the case of balafilcon A, a silicone hydrogel with a strong surface charge and hydrophobic bulk properties, these properties directly contribute to an increased fluorescein uptake, while the relatively high and positive distribution ratio of rhodamine B ($logD = 2.8$) consequently results in lower uptake.

The uptake results are in agreement with previously reported studies conducted by Mahomed *et al.* in which uptake of several ophthalmic dyes into a wide range of contact lens hydrogels at 10 and $100\mu g/mL$ concentrations were investigated [43].

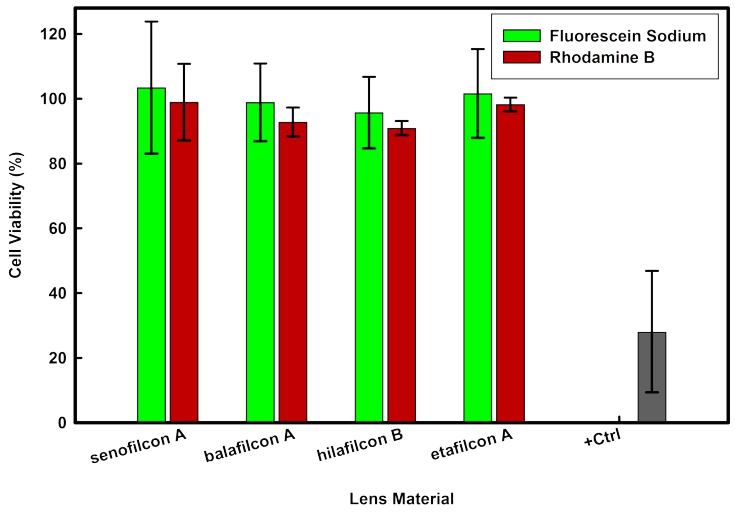


Figure 3.9: Cell viability after 48 hours of surrogate drug release. Viability was measured by MTT assay and is expressed as a percentage relative to cells with no lens. The results represent the mean of three experiments (n=3 ± standard deviation). A sterile ophthalmic solution of benzalkonium chloride (BAK) was used as the positive control: BAK is a preservative used in several commercially available eye drop formulations and is a well-known irritant [89].

3.4.4 Viability Analysis

Cell viability was determined on the *in vitro* cell models after 48 hours of release studies. Based on the intensity and homogeneous coloration of the monolayer with MTT, there was no visible damage to the monolayer. MTT viability assay further confirmed visual observation and indicated that minimal changes in cell viability and metabolism occurred following 48-hr exposure to fluorescein and rhodamine B, as shown in Fig. 3.9. The confirmation of the absence of significant cell damage allow the use of the drug release results (i.e, significant cell damage would invalidate the results of a cell model due to the disruption in the integrity of the cell barrier function).

3.4.5 Release Results

The uptake results demonstrated the significance of the interactions between dyes and contact lens materials. While it is logical to investigate the effect of these two in release

experiments, it was hypothesized that the model itself would also affect the release results. First it is important to understand the release in a small sink, since it is the most commonly used method to study drug eluting contact lenses. In a fixed volume release, similar to the equilibrium reached during the uptake, the concentration of the drug in the solution will reach an equilibrium plateau.

It should be emphasized that hydrogels are highly saturated polymer networks. Therefore, "hydrophobicity" is a relative term, which in case of certain hydrogels such as silicone hydrogels or co-polymers may refer to hydrophobic regions in the polymer network of such materials. If one were to compare a highly hydrophilic hydrogel to a relatively hydrophobic hydrogel, two different mechanisms may occur:

(1) in case of a highly water-soluble compound (with distinct hydrophilic properties such as fluorescein sodium), the uptake into a hydrophilic or charged hydrogel would be higher than a relatively hydrophobic hydrogel (i.e, non-ionic silicone hydrogel). The release of such a compound from the polymer network would be relatively high in both cases of hydrophilic/ relatively hydrophobic polymer networks. However, due to the higher uptake in hydrophilic hydrogels, both higher rate (initial burst) and higher total release (release to uptake ratio) can be expected.

(2) in case of a highly hydrophobic compound (such as rose bengal), it can be expected to have a relatively lower uptake into a hydrophilic/ charged hydrogel, while a significantly higher uptake into a relatively hydrophobic/ neutral hydrogel (i.e, silicone or HEMA hydrogels). In contrast to the former mechanism, the release of a highly hydrophobic compound would be relatively higher in hydrophilic/ charged hydrogels when compared to the hydrophobic/ neutral hydrogels.

These two mechanisms would be an over-simplification of the more complex behavior that might define the interactions of a certain compound with a hydrogel. The release for different polymer matrix and drug combinations will depend on the properties of the polymer and interactions of the drug with the polymer and the release environment. The release of a compound from a contact lens material in the fixed volume model creates a baseline to compare the release from these same contact lens and drug combinations in

the cell and the dynamic release models. Noteworthy is also the fact that the release in a fixed volume might be very different from infinite sink models, where a significantly larger volume of release media is refreshed after a certain period (typically every hour) to maximize and extend the release from a contact lens, therefore hindering the concentration of the drug to reach an equilibrium.

While the release using fixed volume and cell models were conducted for 48h, the release results presented in this section are limited to the first 24h to allow for easier comparison with the studies performed with DDRM. The release results for 48h are presented in Appendix C.

In the fixed volume model as expected, regardless of the dye and lens material, a burst release occurred. For the passive release results of fluorescein sodium in fixed volume, depicted in Fig. 3.10a, release profiles (early burst followed by a plateau) were similar for all lens material, but were observed at two different levels (lower release below $1000ng$, higher release above $2000ng$). The two lenses with the highest amount of release, balafilcon A and hilafilcon B, also showed the highest levels of the uptake (suggesting uptake/release mechanism # 1, $p < 0.001$ compared to all other lenses). As mentioned before, fluorescein sodium through dissociation in aqueous environment becomes very soluble. In the case of balafilcon A and hilafilcon B, fluorescein sodium was released at significantly higher levels compared to the other lenses ($p < 0.001$), either due to its high solubility in the water phase of the hydrogel, as most likely is the case with high water content of the neutral hilafilcon B, or through charge-charge interactions of the dissociated fluorescein species with the hydrogel, as might be the case with the ionic silicone hydrogel balafilcon A. The release of fluorescein sodium as a ratio of its uptake into each contact lens material using the fixed volume release method is shown in Fig. 3.11a. Analysis of the release to uptake ratio showed the two lenses with significantly higher uptakes (BA and HB), released more of the drug taken up by the lens material ($\sim$ 40%; $p < 0.001$), while polymacon and etafilcon A showed the least release ($\sim$ 20%). While senofilcon A showed the least uptake of fluorescein sodium among all tested lenses most likely due to its hydrophobic polymer matrix, as expected, this lens showed moderately higher release to uptake ratio, placing it

between the groups discussed previously.

The passive release of rhodamine B from the studied contact lenses pointed towards the role of the partition ratio between the hydrogel material and the drug, as illustrated in Fig. 3.10b. The high uptake of a drug into a polymer network would imply a relatively high partition ratio between the aqueous solution and the polymer matrix. As suggested by mechanism #2, the release rate of a hydrophobic compound would decrease in such cases. In case of polymacon, etafilcon A and to some extent hilafilcon B materials which had the highest uptake ($p < 0.001$) due to the high affinity of rhodamine B toward their polymer network, these materials also showed less release compared to balafilcon A and senofilcon A ($p < 0.001$). An opposite behavior was observed for systems with low partition ratio as was the case for balafilcon A and senofilcon A hydrogels. Rhodamine B is not a highly hydrophobic compound ($LogP = 1.78$ as opposed to 7.78 in the case of rose bengal). Therefore it should be noted that while the partition ratio was a driving factor in uptake and release of this surrogate drug, other effects such as charge-dipole and hydrogen bonding between the drug and polymer network might also play a secondary role. Analysis of the release to uptake ratio results as shown Fig. 3.11b confirmed a significantly different behavior ($p < 0.001$) between the lenses whereby more than 60% of the drug uptake was released from balafilcon A and senofilcon A, while polymacon and etafilcon A showed less than 20% of the uptake being released. Hilafilcon B, showed marginally higher release to uptake ratio as a result of its moderately less uptake when compared to polymacon and etafilcon A.

Due to the high hydrophobicity of rose bengal ($LogP_{oct/wat} = 8.78$), the effect of the partition ratio in release was even more dominant. Except for etafilcon A, all contact lens materials used in this study either have a neutral polymer network (hilafilcon B and polymacon with their HEMA polymer network), or have neutral regions (silicone hydrogels senofilcon A and balafilcon A). Etafilcon A has a charged polymer network of co-polymerized HEMA-MA (methacrylic acid), which contributed to the charged properties of this hydrogel. While neutral contact lens materials showed significantly more uptake of rose bengal, etafilcon A showed the smallest uptake of rose bengal ($p < 0.001$), with an

uptake levels even smaller than that of rhodamine B. Based on material and dye properties, it was expected that etafilcon A would release the highest amount among the tested materials, which was confirmed by the passive release results (Fig. 3.10c). While polymacon is a low water content HEMA based hydrogel, HEMA lenses also have some levels of residual methacrylic acid in their network [43]. The residual MA in the polymer network of polymacon might shift the properties of an otherwise neutral polymer toward a charged polymer such as etafilcon A. The rose bengal release results in a fixed volume seemed to confirm this shift in behavior, whereby it can be seen that polymacon releases over $1000ng$ of rose bengal ($p < 0.001$ compared to the other contact lenses), while other neutral hydrogels (SA, BA, and HB) barely release any. The effect of a drug with high partition ratio in drug/lens material interactions is clearly illustrated when looking at release to uptake ratio as shown in Fig. 3.11c. Over 24h, polymacon released 10% of the drug taken up, while etafilcon A released above 20%. The other lens materials released less than 5% in fixed volume model. This is in clear contrast with fluorescein sodium and rhodamine B.

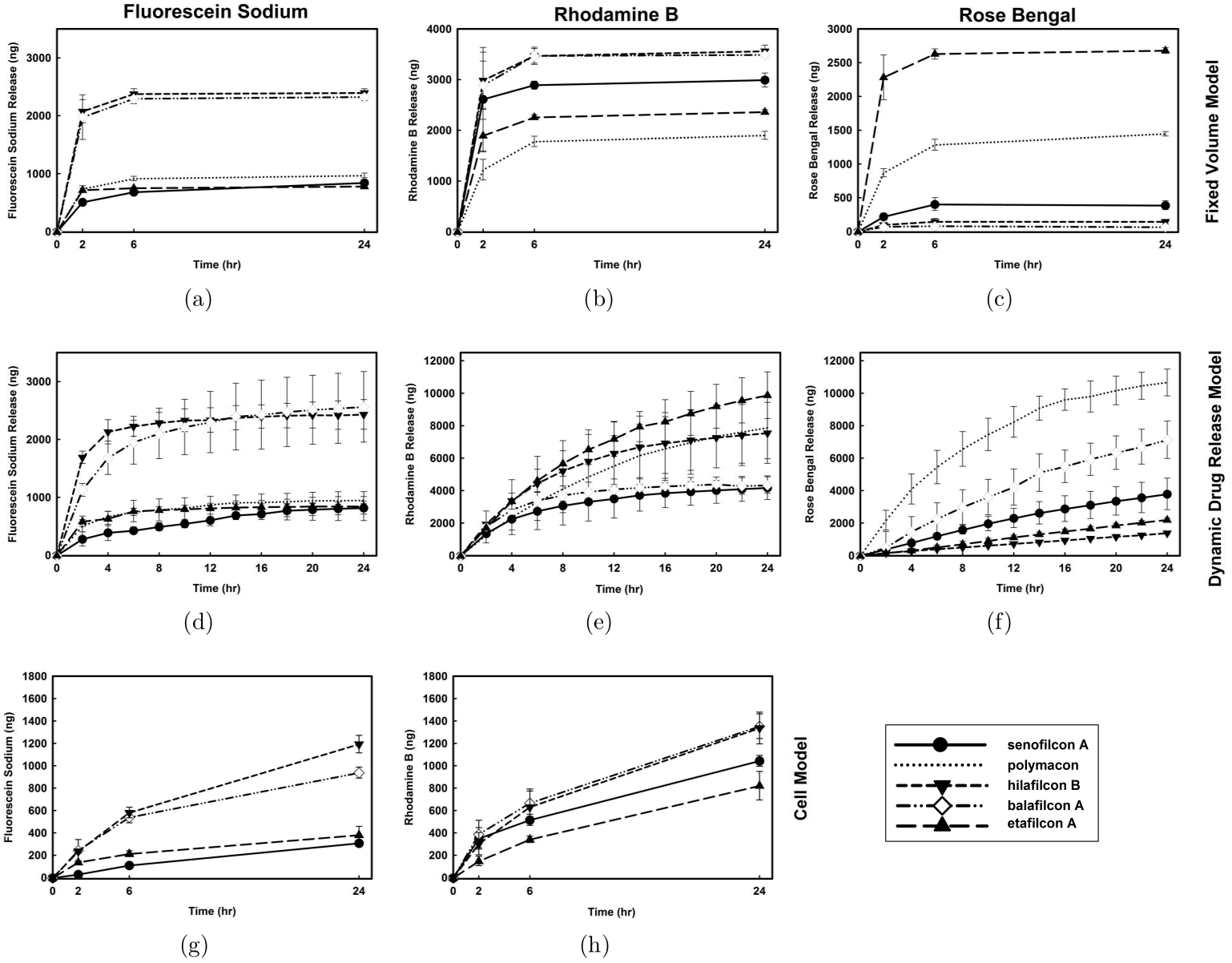


Figure 3.10: Release results comparison among the contact lens materials, surrogate drugs, using various release models. (a), (b), and (c) Fixed Volume Model: release of surrogate drugs from contact lenses in the fixed volume model. (d), (e), and (f) DDRM: release of the drugs in dynamic drug release model. (g) and (h) Cell Model: release of fluorescein sodium and rhodamine B in *in vitro* cell model. No experiments were performed with rose bengal due to cytotoxicity.

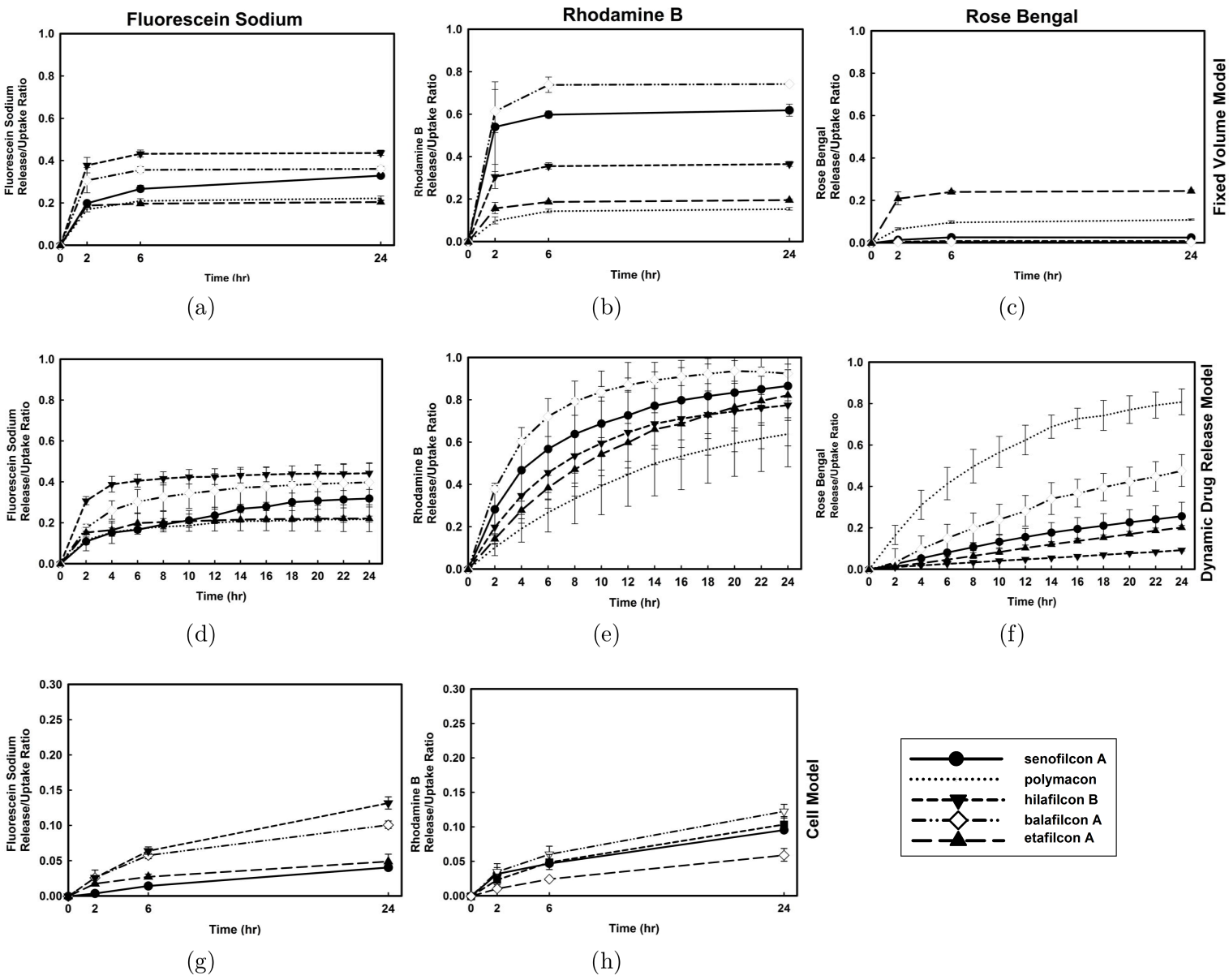


Figure 3.11: Release to uptake ratio results. Comparison among the contact lens materials, surrogate drugs, in three release models. (a), (b), and (c) Fixed Volume Model: release of surrogate drugs from contact lenses in the fixed volume model. (d), (e), and (f) DDRM: release of the drugs in dynamic drug release model. (g) and (h) Cell Model: release of fluorescein sodium and rhodamine B in *in vitro* cell model.

Passive release studies demonstrated that significant quantities of drug can be retained in the contact lens after a short burst in release. The release in the dynamic environment of the ocular surface where mechanical action of the eyelid and the constant tear replenishment combined with the effects of the proteins present in tear film will certainly be different. The objective with DDRM was to allow investigation of the effects of the tear replenishment on drug release from contact lens. Thus the DDRM was designed to model the tear replenishment effect on the surface of the eye, through providing a limited release volume with constant replenishment. The flow rate used in the dynamic drug release experiments was significantly higher than the tear replenishment in the eye ($20\mu L/ml$ vs. $2\mu L/min$). This most likely resulted in an increased total release of the drug from the contact lens materials. However, this allowed, in some cases, depletion of the drugs from the contact lens material, which otherwise would not have been possible. It should also be noted that the release experiments in the dynamic environment were performed for 24 hours.

The release results from fluorescein sodium in DDRM are illustrated in Fig. 3.10d. Similar to the release in the fixed volume, two distinct release levels were present, however, while no major change in release kinetics can be observed, a minor shift towards a slower kinetic and a plateau being reached at a later time was observed. This decline in release rate, most apparent in case of senofilcon A was the direct effect of the dynamic release environment. Recognizing the significantly smaller uptake of fluorescein sodium by the contact lens materials, and the higher affinity of the dissociated surrogate drug species toward aqueous solution, a relatively fast release was expected. However, the slowed initial release rate would imply the limited volume in the DDRM was reducing the rate at which fluorescein sodium can be released from the contact lens. Again, similar to the fixed volume method, the release to uptake ratio showed release between $20 - 40\%$ of the drug uptake, with marginally slowed initial release rate (Fig. 3.11d).

The release of rhodamine B in the dynamic environment resulted in a more contrasting behavior. The release results as shown in Fig. 3.10e, demonstrated a significantly slower release kinetic, and significantly higher total drug release ($p < 0.001$). While the release of

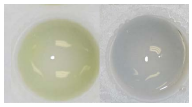
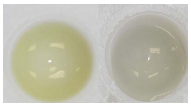
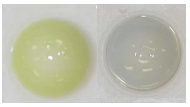
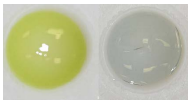
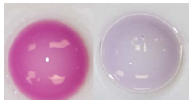
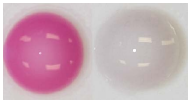
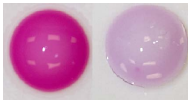
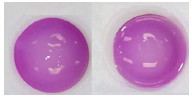
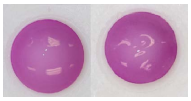
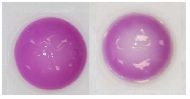
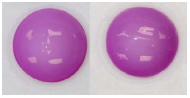
rhodamine B from all lens materials was significantly higher in DDRM compared to fixed volume model ($p < 0.001$), major difference was observed in the release from etafilcon A and polymacon. In fixed volume release, the release of rhodamine B from these two materials was curtailed due to the high affinity of rhodamine B toward their polymer network (as suggested by the high uptake ratio). In the dynamic release model, due to constant replenishment of the release media, the release of the drug was facilitated (Fig. 3.12 illustrates this difference). The effects of the dynamic release model were further amplified with the release of rose bengal. Comparing Fig. 3.11c and Fig. 3.11f, it was evident that considerably higher quantity of the drug was released ($p < 0.001$) from the contact lens materials at significantly slower rates resulting in a continuous release over time. The release kinetics also shifted from a first-order release to a zero-order[7] release. While polymacon released most of the loaded drug, etafilcon A retained most of the drug (Fig. 3.11f). Even when compared to the fixed volume model, etafilcon A only released slightly more drug.

One compelling argument for using ophthalmic dyes as surrogate drugs is the ability to visualize release through color change. Table 3.3 provides a visual assessment of drug loaded contact lenses, before and after 24 hours release in the dynamic drug release model. The surrogate drug/ lens material interactions ranged from high uptake (polymacon, rose bengal), to low uptake (fluorescein sodium), significantly less release ratios (rose bengal), and high release ratios (rhodamine B release from senofilcon A and balafilcon A). The other benefit of using such surrogate drugs was a means to verify the uniform distribution of flow regime inside the cavity chamber of the model. This had particular relevance to the design of the flow system where a nonuniform color pattern on the lens would imply an irregular flow and thus an unsuitable flow chamber design. Last but not least, the use of ophthalmic dyes helped identify any component in the device where a significant quantity of the drug might be absorbed.

In drug release experiments, when an ocular drug delivery device is intended to deliver an ophthalmic compound inside the eye, and notably behind the cornea, it is important to

[7]In a zero-order release kinetic, the rate of the release is constant, where in a first-order release, the rate of release was dependent on the concentration gradient of the releasing compound.

Table 3.3: Visualization of the uptake (left) and release (right) of surrogate drugs from various contact lens materials tested in the DDRM.

	senofilcon A	**balafilcon A**	**polymacon**	**hilafilcon B**
Fluorescein Sodium LogP = 2.65 LogD = -1				
Rhodamine B LogP=1.78 LogD = 3				
Rose Bengal LogP=8.78 LogD = 4.2				
Water content (WC)	38	38	59	36
FDA group*	V(I) Low WC Non-ionic	I Low WC Non-ionic	II High WC Non-ionic	V(III) Low WC Ionic

Chemical properties have been retrieved from Chemicalize.org®. Each pair represents the same contact lens after 24 hours in doping solution (uptake) and 24 hours inside the DDRM (release).

consider the barrier effect of the cornea. To investigate the role of the cornea, simplified *in vitro* cornea models were prepared by growing human corneal epithelial cells in a cell culture insert as described in Section. 3.3.5. The concentration of the diffused drug through a cell layer, after the release on the apical side of the cornea model are reported. This model allowed to consider interactions with the cells to complement investigation of the surrogate drugs and lens material interactions assessed using both passive and dynamic models.

As discussed in Section. 3.4.2, due to the cytotoxicity of rose bengal, only release studies with fluorescein sodium and rhodamine B were performed in the cell model, furthermore only four contact lens materials were tested as minimal differences were observed between etafilcon A and polymacon. The release results for both dyes indicated a slower kinetic, as well as a slower release rate (as shown in Fig. 3.10g and 3.10h). The total amount of the drug that was released from the lens, then diffused through the cell model, was significantly smaller than in the fixed volume method, even after 24 hours, i.e, $200ng$ versus $700ng$ for fluorescein sodium ($p < 0.001$). When comparing the release of the dyes in the cell model to the fixed volume model, the cornea model seemed to be functioning only as a barrier against the diffusion of the these drugs. Also, in the cell model, the contact lens was submerged in a noticeably smaller volume of the release media ($500\mu L$), resulting in a seemingly accelerated equilibrium. The combination of the two mechanisms could explain the shift toward slower release kinetics in the cell model. While one may conclude that the cell model provides a very limited benefit when assessing the release from contact lenses in case of the tested ophthalmic dyes, it must be noted that the same barrier effect exist within the human eye whereby the rate of diffusion of the released drug will decline due to this barrier effect.

Fig. 3.12 presents a direct comparison of the release of rhodamine B from etafilcon A and hilafilcon B in the three models presented in this research. This figure highlights the extended release results that may be obtained using the dynamic model, as well as the barrier effect of the cell model when compared to the fixed volume method.

The release results of the three ophthalmic dyes at 24 hour using the three *in vitro* release models are compiled and compared to provide a better perspective (Figures. 3.13,

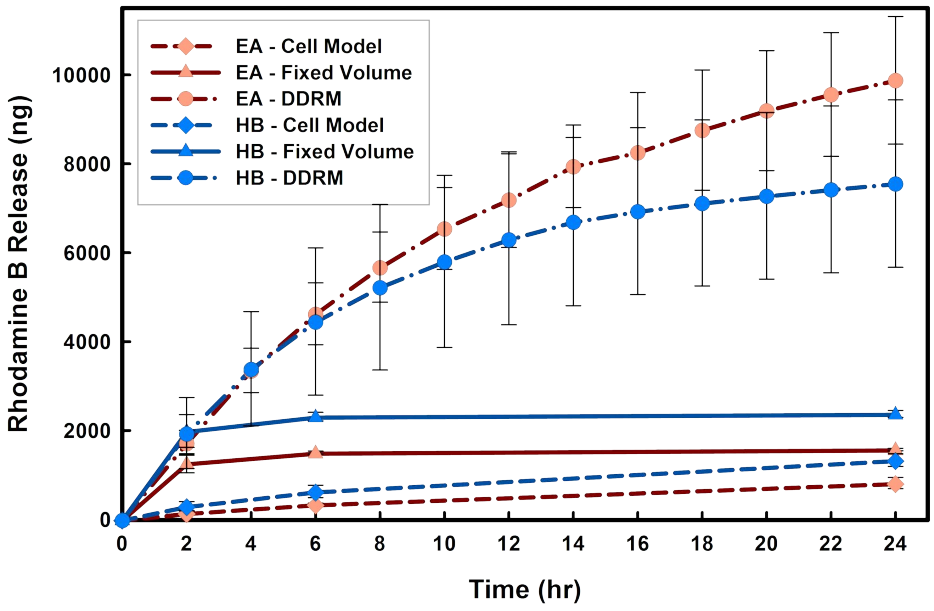


Figure 3.12: Comparison of the release of rhodamine B from etafilcon A and hilafilcon B in three release models. While the release in the fixed volume (solid lines) showed an initial burst, the release from the same contact lens materials in the cell model (dashed lines) showed a slower release rate due the barrier effect of the cell layer. The release in the dynamic release model(dash-dot lines) showed a significantly higher total release amount, significantly different from cell model ($p < 0.001$) and fixed volume model ($p < 0.001$)

3.14, and 3.15). In all three graphs, the two lighter shade of colors represents the uptake of the surrogate drug into each contact lens material, and the remaining surrogate drug in the doping solution. Within each contact lens material, the release after 24 hours is presented in bar graph. This allowed an easier comparison among release models used in these experiments. As discussed previously, the uptake of fluorescein sodium is relatively smaller compared to the other surrogate drugs. The release of fluorescein sodium at 24 hour time point for all contact lens materials shows the barrier effect of the cell layer resulting in significantly less drug released ($p < 0.001$) (Fig. 3.13). While the release of fluorescein sodium in the DDRM showed an increase compared to the other two models ($p < 0.001$), the role of a dynamic model was more prominent in case of rhodamine B and rose bengal as shown in Fig. 3.14 and 3.15. These results suggested that the release of rhodamine B from balafilcon A and senofilcon A may continue until near depletion, as corroborated by the observing the lens before and after release shown in Table. 3.3. Similar observations can be made regarding the release of rose bengal from polymacon lens material. Fig. 3.15 also highlights the relatively high uptake-low release in case of rose bengal surrogate drug.

3.5 Conclusion

To effectively model the human eye environment and further characterize ophthalmic drug delivery devices, it is important that experimental studies consider the limited volume and the replenishment of the tear as well as the presence of cells. By comparing three *in vitro* models (fixed volume, dynamic and cell), the results presented in this chapter aimed to introduce a robust, reliable and cost effective testing platform to asses the drug releasing capabilities of hydrogel contact lenses. The release results demonstrated the importance of a dynamic release model for testing contact lens materials.

It should be noted that the use of ophthalmic dyes not only allowed the utilization of UV-VIS spectrophotometry to accurately measure uptake and release, but also enabled a visual qualitative estimation of the uptake and release. This unique characteristic of the ophthalmic dyes can be exploited in facilitating rapid assessment of suitable synthesized

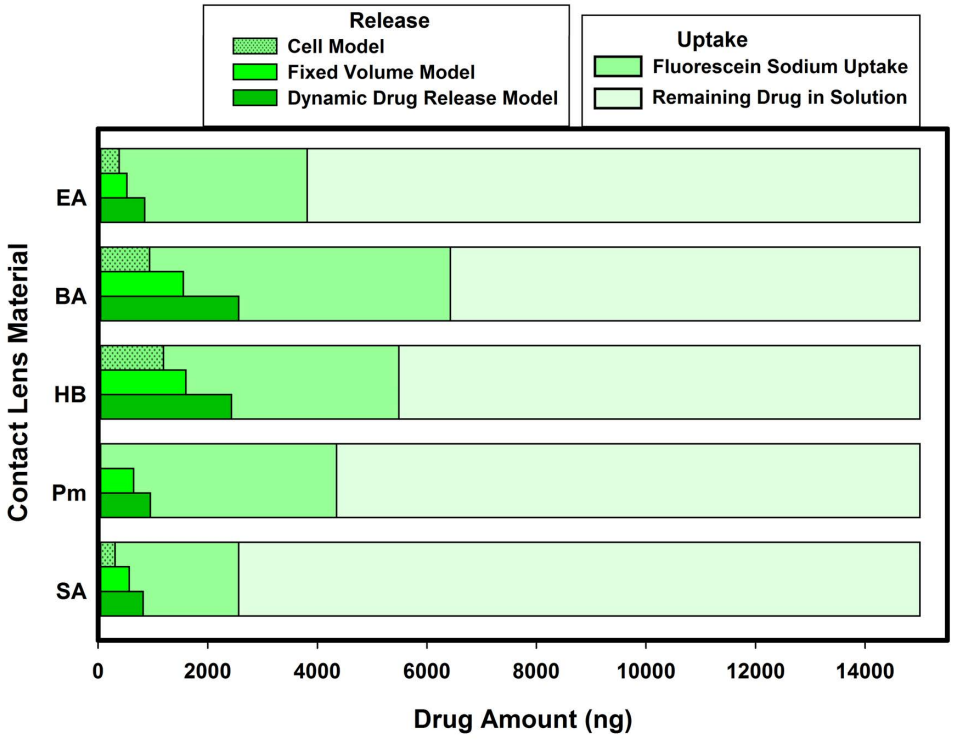


Figure 3.13: Comparison of the uptake and release of fluorescein sodium in various release models. The drug loaded into each lens material and the remaining drug in doping solution is presented in lighter shades of color. The release in cell, fixed volume, and dynamic models are presented for each lens material and compared to the uptake results.

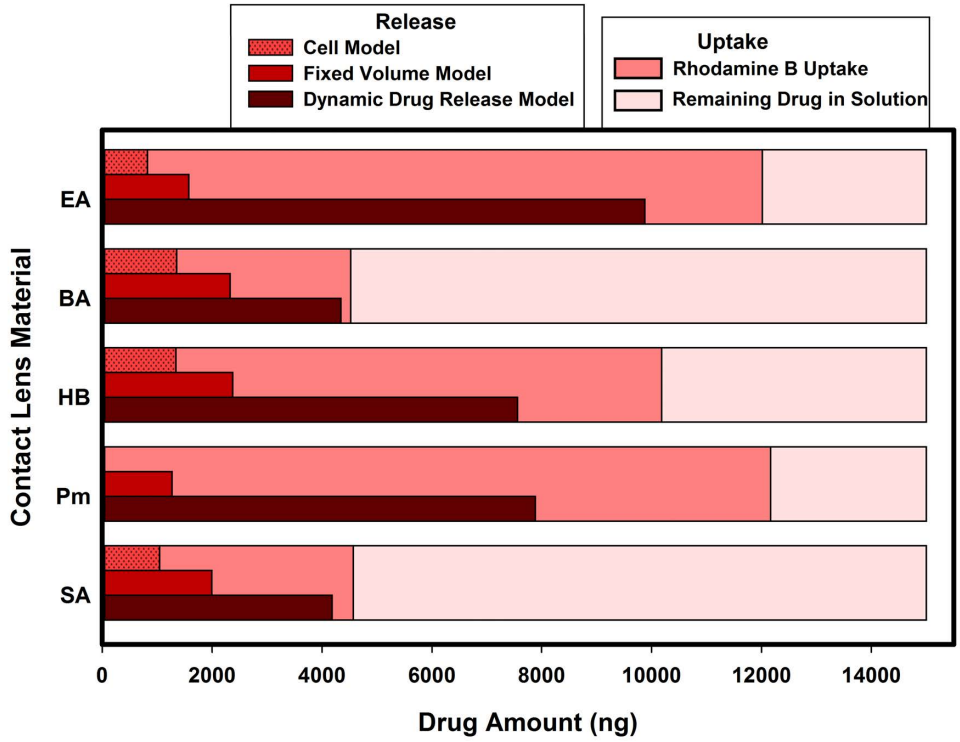


Figure 3.14: Comparison of the uptake and release of rhodamin B in various release models. The drug loaded into each lens material and the remaining drug in doping solution is presented in lighter shades of color. The release in cell, fixed volume, and dynamic models are presented for each lens material and compared to the uptake results.

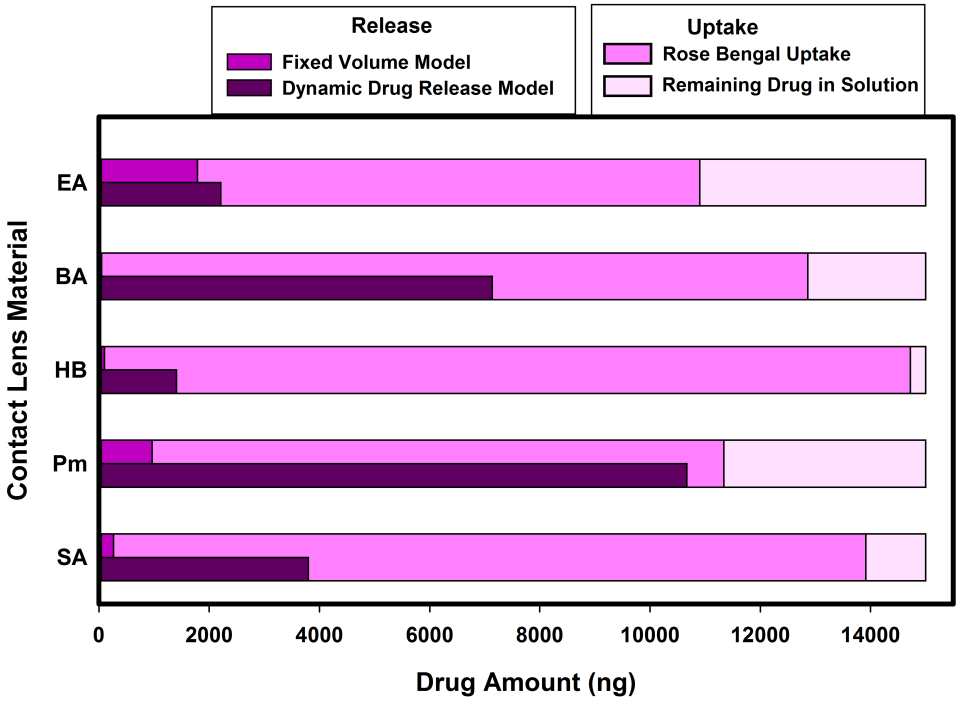


Figure 3.15: Comparison of the uptake and release of rose bengal in various release models. The drug loaded into each lens material and the remaining drug in doping solution is presented in lighter shades of color. The release in fixed volume and dynamic models are presented for each lens material and compared to the uptake results. No cell experiments were performed due to cytotoxicity of rose bengal.

hydrogel polymers for ocular drug delivery purposes.

It is evident that modeling the microfluidic environment of the ocular surface highlighted interactions that would otherwise be disregarded in the fixed volume model. On the other hand, the release results in the cell model, while they confirmed the barrier role of the cornea, did not provide any insight into any possible interactions between the drug eluting contact lens and the cells.

The ophthalmic dyes, unlike a large group of ophthalmic prodrugs, are not processed in any parts of the cell metabolism pathways or the enzymes present in the cells. Prodrugs are precursors to ophthalmic drugs that are designed to improve the drug residence time in tear film and enhance the uptake by the cell membrane. These prodrugs through different pathways can be metabolized into active form of the drug, inside the corneal cells, before reaching the targeted tissue. It can be concluded that understanding the role of the corneal cells in drug delivery studies requires investigating the release of such actives from contact lens materials in the presence of cells.

Chapter 4

Extended Latanoprost Release from Commercial Contact Lenses: *In Vitro* Studies Using cornea models

4.1 Introduction

Ocular drug delivery is used in the treatment of glaucoma, an intraocular disorder. Eye-drops remains the most common drug delivery method but studies show that only about 1 to 5% of the applied dose penetrates the cornea [1]. Contact lenses have been considered as a means to deliver topical ophthalmic drugs [4] and as discussed in Chapter 1, experimental models to study drug release tend to be designed to characterize the mechanisms of release due to polymer-drug interactions and often do not take into account cell-drug interactions. It is well recognized that the primary drug permeation route to the front of the eye is through the transcorneal pathways, and thus it is also important to consider the role of corneal cells in drug release studies [25]. The lipid bilayer cell membrane retards the permeation of hydrophilic compounds. Furthermore, the presence of cell membrane transporters and enzymes in corneal epithelial cells actively contributes to the transport and metabolization of prodrugs [29–32]. Using surrogate drugs loaded onto contact lenses, the studies in Chapter 3 highlighted the barrier effect exerted by cells, however the use of

ophthalmic dyes did not adequately represent the active interactions (transport, metabolization) that occur with prodrugs. Latanoprost is used in eye-drops to treat glaucoma and is a $432Da$ prostaglandin F_{2_α} analogue drug that is metabolized to its free-acid form by enzymes available in corneal cells [29]. Studies have also suggested that active transport using OATP2A1 transporters contributes to latanoprost permeability across the cornea [32].

It was therefore hypothesized that the presence of corneal epithelial cells may have an impact when assessing drug-delivery materials in vitro. In this chapter, which is published as a standalone paper [115], we investigated for the first time, the release of latanoprost from commercially available contact lenses using *in vitro* models containing corneal epithelial cells.

4.2 Materials and Methods

4.2.1 Preparation of Drug Doping Solutions

The lens doping solution was prepared by dissolving latanoprost and latanoprost free-acid (solution in methyl acetate, Cayman Chemical, Ann Arbor, MI) in PBS (Lonza, Walkersville, MD). The concentration of the stock drug solution was $131\mu g/mL$.

4.2.2 Preparation of Contact Lenses

Four commercially available contact lens materials, galyfilcon A, senofilcon A, omafilcon A, and balafilcon A were used. The properties of the four lens types are presented in Table 4.1. All lenses had a back vertex power of -3.00 diopter. Lenses were incubated for 24 hours in PBS (Lonza, Allendale, New Jersey) to remove any remnants of their packaging solutions, before incubation in $1.5mL$ of the drug solution for 24 hours.

Table 4.1: Properties of the Contact Lens Hydrogel Materials [118]

Commercial name	Acuvue Advance	Acuvue Oasys	ProClear	PureVision
(US adopted name)	galyfilcon A	senofilcon A	omafilcon A	balafilcon A
Manufacturer	Johnson & Johnson	Johnson & Johnson	Coopervision	Bausch & Lomb
Water content	47	38	60	36
Principal Monomer	mPDMS + DMA + HEMA+ siloxane macromer+ EGDMA + PVP	mPDMS + DMA + HEMA + siloxane macromer + TEGDMA + PVP	HEMA + PC	NVP + TPVC + NVA + PBVC
FDA group*	V(I) Low water Non-ionic	V(I) Low water Non-ionic	II High water Non-ionic	V(III) Low water Ionic

* FDA (Food and Drug Administration) categorizes all silicone hydrogel contact lenses as group V, however it is more practical to use groups for conventional hydrogels to better understand their material properties. HEMA, Hydroxyethyl Methacrylate; PC, Phosphotidylcholine; NVP, N-Vinylpyrrolidone; TPVC, Tris(trimethylsiloxysilyl) Propyvinyl Carbamate; NVA, N-Vinyl Aminobutyric Acid; PBVC, Poly(dimethysiloxy)di (silylbutanol) Bis(Vinyl Carbamate); mPDMS, monofunctional Polydimethylsiloxane;DMA, N,N-Dimethylacrylamide; EGDMA, Ethyleneglycol Dimethacrylate; PVP, Polyvinyl Pyrrolidone; TEGDMA, Tetra-Ethyleneglycol Dimethacrylate

4.2.3 *In Vitro* Cell Culture

HPV-immortalized human corneal epithelial cells, a generous gift from Dr. May Griffith (Integrative Regenerative Medicine (IGEN) Centre, Linköping University, Sweden) [79] were cultured in keratinocyte serum free medium (KSFM) supplemented with bovine pituitary extract, recombinant epidermal growth factor, and penicillin/ streptomycin (Pen/Strep) (ScienCell, Carlsbad, California, USA) at $37°C$ and 5% carbon dioxide (CO_2). Fresh medium was added every other day and cells were grown to 90% confluency in tissue culture treated flasks. Adherent cells were removed using TryplExpress (Life Technologies, Burlington, Ontario, Canada) dissociation solution. Cells were routinely observed for any morphological changes and were used before their eleventh passage.

4.2.4 *In Vitro* Drug Release Models

Three *in vitro* models were used to assess drug release from commercially available contact lenses including diffusion through a) a Polyethylene Terephthalate (PET) membrane (Millicell PET membrane with a $1.0\mu m$ pore size, also referred to as culture inserts, Millipore, MA, USA) with no-cells; b) a PET membrane with a monolayer of human corneal epithelial cells (HCECs) and c) a PET membrane with a multilayer of HCECs (stratified culture). For the two latter models, the PET membranes were seeded with 10^5 cells. The corneal epithelium models were fed with KSFM on each of the basal and apical sides of the cells layer for five days, with medium being exchanged every other day. After five days, for the multilayer models, cell differentiation was induced by exposing the monolayer to an air-liquid interface. Cells were fed only on the basal side with 2% fetal bovine serum (FBS, Invitrogen, Burlington, ON, Canada) in 1:1 Dulbecco's minimum essential medium (DMEM, Invitrogen) in Hams F12 nutrient medium (DMEM/F12, Invitrogen); the medium was exchanged daily [78]. The cells grew under these conditions for seven days and were then ready for experimentation.

4.2.5 Measuring Drug Concentrations

Aliquots of $100\mu L$ (10 % of the total volume of the medium present in the bottom) were taken from the bottom of the *in vitro* models and replaced by fresh culture medium. For the latanaprost experiments, samples were taken at 1, 3, 6, 12, 18, 24 and 48 hours. For latanoprost free-acid experiments, samples were collected at 1, 3, 6, and 24 hours.

Collected samples were analyzed by an enzyme immuno-assay (EIA) for latanoprost (Cayman Chemical, Ann Arbor, MI, USA). Following the EIA kit instructions, each collected sample was analyzed in duplicate and at two different dilutions. To determine the uptake amount by the contact lenses, samples were also aliquoted from the original drug stock solution as well as the remaining drug solutions after soaking the lenses.

The release results represent the concentration of the drug on the other side of the PET membrane, meaning that the drug has been released from the contact lens material on top of the membrane, then diffused through the cells, if present, and the culture membrane. Note that the EIA kit does not distinguish between the free-acid form and ester form of the drug.

4.2.6 Drug Concentration Calculations

As mentioned above, to measure the amount of released drug, samples were taken from the bottom of the wells and replaced by fresh solution at each time point. Refreshing a fraction of the medium in the bottom at each time point affects measurements. Therefore, it is necessary to account for the dilution effect and adjust the measured concentrations to provide an accurate measure of the concentration without the dilution effect.

Assuming the fraction of total volume of medium in the bottom which is being aliquoted is "k", the mass balance principle can be used to estimate for the actual concentration at each time point.

$$m_i = C_i V_b \tag{4.1}$$

$$m_{i,a} = C_i V_b + k \sum_{j=1}^{i-1} C_j V_b \tag{4.2}$$

In Eq.(4.1), m_i refers to the amount of drug at *i-th* time point (t_i), C_i refers to the measured concentration at time t_i, and V_b is the volume of the liquid in the bottom. An estimate of the actual amount of drug diffused through the insert adjusted for the dilution effect, $m_{a,i}$, can be calculated using Eq.(4.2). This equation can be obtained as below by calculating the accumulated drug amount in the medium by adding the removed amount in previous steps to the amount of the drug available in medium at each step.

$$\begin{aligned}
&m_{a,1} = m_1 = V_b C_1 && \Delta m_1 = k C_1 V_b \\
&m_{a,2} = m_2 + \Delta m_1 = C_2 V_b + k V_b C_1 && \Delta m_2 = k C_2 V_b \\
&m_{a,3} = m_3 + \Delta m_2 + \Delta m_1 = C_3 V_b + k(C_1 + C_2) V_b && \Delta m_3 = k C_3 V_b \\
&\vdots
\end{aligned} \tag{4.3}$$

The adjusted concentration, $C_{a,i}$ at *i-th* step can be found as below:

$$C_{a,i} = C_i + k \sum_{j=1}^{i-1} C_j \tag{4.4}$$

The proposed method to estimate adjusted concentrations neglects the effect that dilution might have on the diffusion rate. However, for small difference between calculated and measured concentrations, the change in diffusion rate will be insignificant.

4.2.7 Data Analysis

Results are presented as the mean of six experiments for latanoprost and three experiments for latanoprost free-acid ± standard deviation. All experiments were performed on different days. To evaluate the significance of the differences between various contact lens materials, *in vitro* cornea models and time points, an analysis of variance (ANOVA) was performed, followed by multiple pair-wise comparisons using the Holm-Sidak test (SigmaPlot™, San Jose, California, USA).

4.3 Results

Preliminary studies showed that there was no decay of latanoprost and latanoprost free-acid in the culture medium or buffered solution used in the current research (results not presented), thus enabling the use of the enzyme immuno-assay method to measure drug concentrations in both solutions for up to 48 hours. All the results presented have also been adjusted according to Eq. (4.4), to take into account the small dilution that may occur as samples are taken out and fresh medium is added.

The uptake analysis showed that 95% of the dissolved latanoprost was taken up by the galyfilcon A and senofilcon A silicone hydrogels and 98% by the balafilcon A (thus approximately $185\mu g/lens$) and nearly 25% of the latanoprost solution was taken up into omalfilcon A ($50\mu g/lens$).

4.3.1 Release in the absence of cells

In the no-cells model, release was first measured in KSFM to allow for comparisons between all *in vitro* models. As shown in Fig. 4.1, an initial burst in the first 6 hours was observed, followed by saturation, when no more drug was released, despite the available drug in the contact lens material.

The effects of the release medium were also assessed with the three silicone hydrogel contact lens materials for 24 hours, where the cell culture medium (KSFM) was substituted with PBS. When compared to KSFM, the latanoprost release decreased significantly in the no-cell model in PBS ($p < 0.001$), Fig. 4.2.

4.3.2 Release in the presence of a monolayer or multilayer model

Performing the contact lens release experiments in the presence of corneal epithelial cells resulted in significant changes. As illustrated in Fig. 4.3, the amount of latanoprost released from senofilcon A was dependent on the presence of cells in the *in vitro* models; a significantly higher amount of latanoprost was released in the monolayer and multilayer

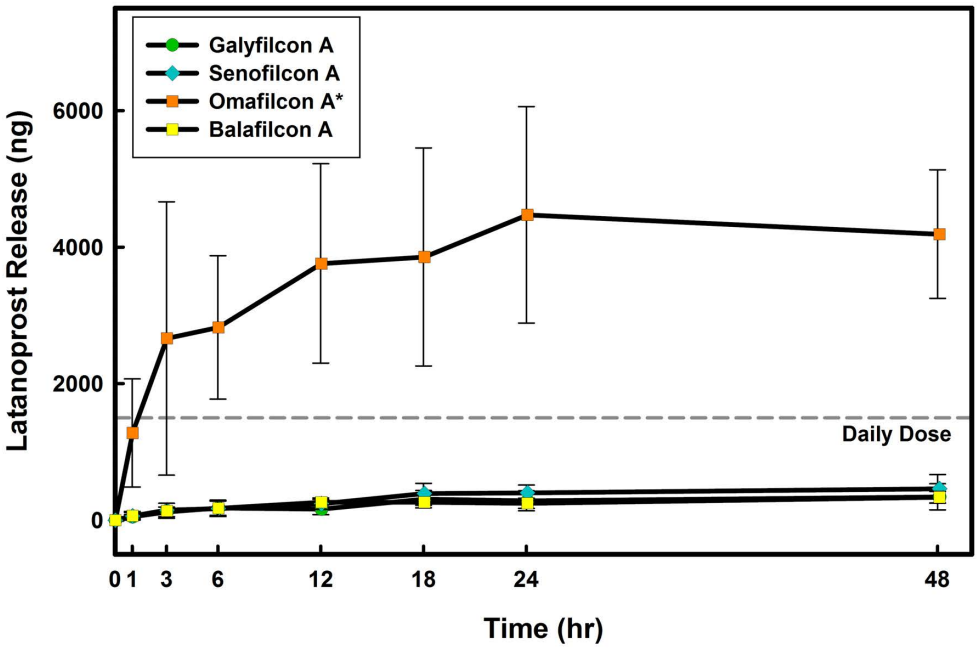


Figure 4.1: Time course of latanoprost release from four contact lens materials through the no-cells model. Lenses were soaked for 24 hours in drug solution ($131 \mu g/mL$) and then overlayed on the model for 24 hours. Aliquots were taken at specific times from the lower compartment and concentrations was measured using EIA. Daily dose line represents the amount of the administered latanoprost for a glaucoma patient [27]. *Significantly different from silicone hydrogel contact lens materials ($p < 0.001$). (n=6 Mean $\pm$ SD).

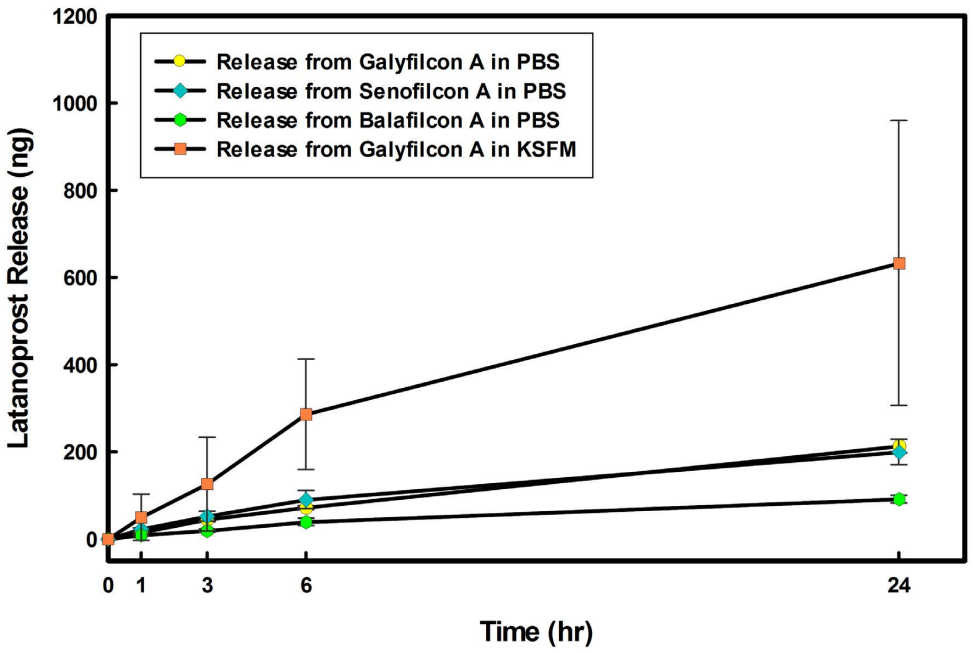


Figure 4.2: Comparison of latanoprost release from silicone hydrogels in no-cells model. Release from three silicone hydrogel contact lens materials in PBS as well as release from galyfilcon A in KSFM (Keratinocyte Serum Free Medium) is shown. Lenses were soaked for 24 hours in drug solution ($131\mu g/mL$) and then overlayed on the model for 24 hours. Aliquots were taken at specific times from the lower compartment and concentrations were measured using EIA. (n=3 Mean $\pm$ SD).

models ($p < 0.001$) when compared to the no-cell model. Furthermore, while in the no-cell model, no significant difference in release was observed over time, for the monolayer and multilayer models, there was a significant increase in the amount released at 1, 3, 12, 18, 24 and 48 hrs ($p < 0.05$). For all contact lens materials studied, in the monolayer and multilayer *in vitro* cornea models, an extended release of drug was observed over time (Fig. 4.3). The improved release profiles from latanoprost-soaked contact lenses was similar between the monolayer and multilayer models ($p = 0.678$).

The release results for all tested commercial contact lenses are summarized in Table 4.2. While the amount of drug released fell within potential therapeutic ranges, only 2% of the amount of the drug sorbed into silicone hydrogel contact lens material was released after 24 hours (Table 4.2). A significantly higher amount (between 10 to 17% depending on the model used) was released from the high water content hydrogel material, omafilcon A. The high release of latanoprost from omafilcon A (Fig. 4.4) is in spite of the lower drug uptake, which results in a significantly higher release percentage ($p < 0.001$, Table 4.2). Latanoprost release from galyfilcon A and senofilcon A were not significantly different ($p = 0.736$) and neither were they different from the release observed with balafilcon A ($p > 0.3$).

4.3.3 Release of Latanoprost Free-Acid

Since in the absence of cells, the hydrolysis of latanoprost into its free-acid form cannot happen, the release of latanoprost free-acid from contact lens materials was studied to determine if latanoprost free-acid may be used as a substitute to latanoprost in a no-cell model. To allow for a more complete comparison between models and drug forms, release of latanoprost free-acid was also tested with the same *in vitro* models.

With latanoprost free-acid, contrary to what was observed with the ester form of the drug, a significantly lower release occurred in the presence of cells when compared to no-cells (Fig. 4.5). Table 4.3 presents the release of latanoprost free-acid from tested commercial contact lenses after 24 hours for each of the *in vitro* models. When comparing the amount of drug release at 24 hours in the monolayer model, the latanoprost free-acid

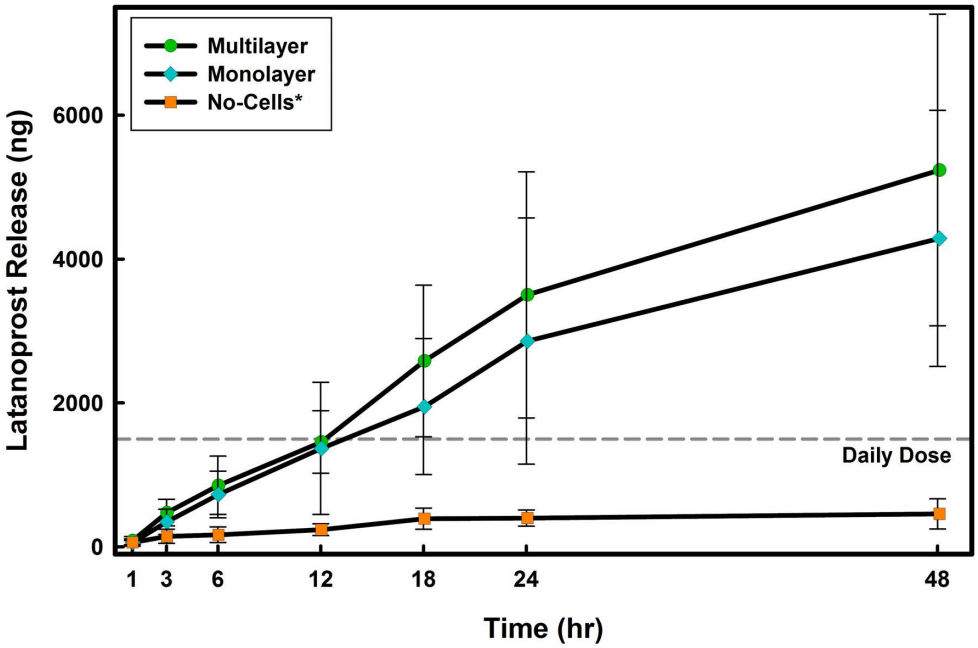


Figure 4.3: Time course of latanoprost release from senofilcon A in the three *in vitro* models. Lenses were soaked for 24 hours in drug solution ($131\mu g/mL$) and then overlayed on the model for 24 hours. Aliquots were taken at specific times from the lower compartment and concentrations were measured using EIA. Daily dose line represents the amount of the administered latanoprost for a glaucoma patient [27]. No-Cells Model: Cell culture inserts (PET membrane) without cells, Monolayer Model: PET insert with a monolayer of human corneal epithelial cells, Multilayer Model: PET insert with a multilayer of human corneal epithelial cells (stratified culture). *Significantly different from *in vitro* models with cells ($p < 0.001$). (n=6 Mean $\pm$ SD).

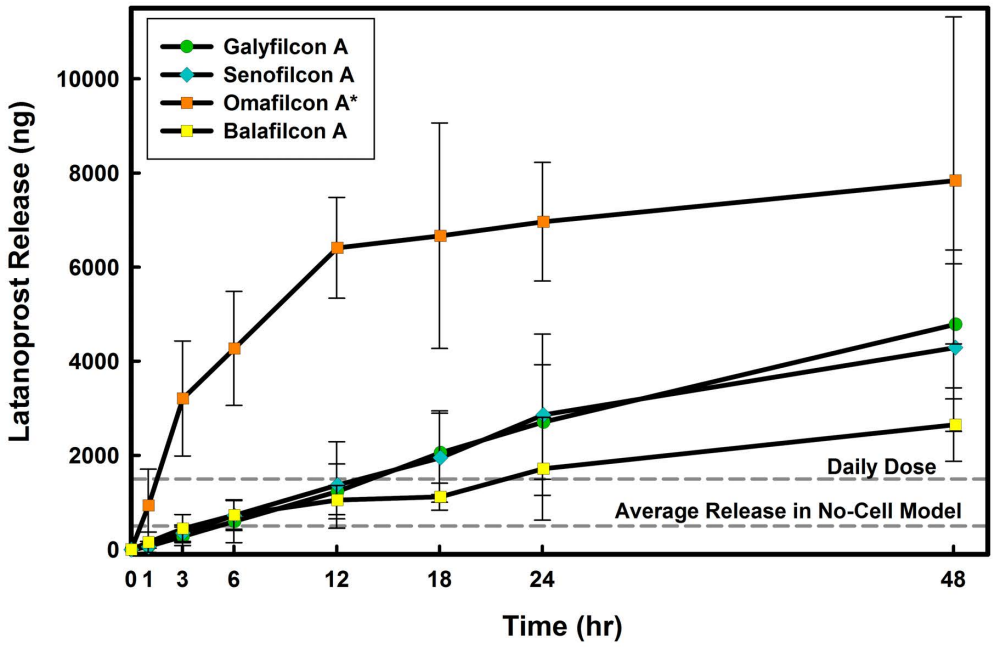


Figure 4.4: Time course of latanoprost release from four contact lens materials through the monolayer model. Lenses were soaked for 24 hours in drug solution ($131 \mu g/mL$) and then overlayed on the model for 24 hours. Aliquots were taken at specific times from the lower compartment and concentrations were measured using EIA. Daily dose line represents the amount of the administered latanoprost for a glaucoma patient [27]. Monolayer Model: PET insert with a monolayer of human corneal epithelial cells. *Significantly different from *in vitro* models with cells ($p < 0.001$). (n=6 Mean ± SD).

Table 4.2: Latanoprost Release and Release Percentage of Latanoprost from Tested Commercial Contact Lenses after 24 Hours

Contact Lens Material	No-Cells Model		Monolayer Model		Multilayer Model	
	Release $[\mu g/lens]$	Percentage of Release $^{\dagger}(\%)$	Release $[\mu g/lens]$	Percentage of Release $^{\dagger}(\%)$	Release $[\mu g/lens]$	Percentage of Release $^{\dagger}(\%)$
Galyfilcon A	0.28 ± 0.14	0.15 ± 0.08	$2.71 \pm 1.21^{*}$	$1.47 \pm 0.66^{*}$	$2.79 \pm 0.96^{*}$	$1.51 \pm 0.52^{*}$
Senofilcon A	0.40 ± 0.11	0.22 ± 0.06	$2.86 \pm 1.71^{*}$	$1.55 \pm 0.93^{*}$	$3.50 \pm 1.71^{*}$	$1.90 \pm 0.93^{*}$
Omafilcon A	$4.47 \pm 1.58^{\#}$	$10.13 \pm 3.59^{\#}$	$6.96 \pm 1.26^{\#*}$	$15.77 \pm 2.85^{\#*}$	$7.30 \pm 1.88^{\#*}$	$16.53 \pm 4.25^{\#*}$
Balafilcon A	0.25 ± 0.08	0.13 ± 0.04	$1.71 \pm 1.09^{*}$	$0.90 \pm 0.57^{*}$	$1.93 \pm 0.84^{*}$	$1.01 \pm 0.44^{*}$

n = 6, Mean ± Standard Deviation. Concentration of latanoprost were measured using EIA.
No-Cells Model: Cell culture inserts (PET membrane) without cells, Monolayer Model: PET insert with a monolayer of human corneal epithelial cells, Multilayer Model: PET insert with a multilayer of human corneal epithelial cells (stratified culture).
† The release as a percentage of uptake has been calculated based on the ratio of the released concentration over the sorbed amount. $^{\#}$Significantly different from other contact lens materials (silicone hydrogel) ($p < 0.001$). *Significantly different from the amount released by respective materials in the no-cells model ($p < 0.001$).

Table 4.3: Latanoprost Free-Acid Release from Tested Commercial Contact Lenses after 24 Hours

Contact Lens Material	Release Model [$\mu g/lens$]		
	No-Cells	Monolayer	Multilayer
Galyfilcon A	3.76 ± 1.32	3.27 ± 1.10	2.56 ± 0.46
Senofilcon A	3.06 ± 0.99	1.94 ± 0.56	2.27 ± 0.66
Omafilcon A	2.94 ± 1.73	3.14 ± 1.62	2.05 ± 1.42
Balafilcon A	$5.45 \pm 1.76^{\$}$	$3.65 \pm 0.27^{\$}$	$6.26 \pm 2.71^{\$}$

n=3, Mean ± Standard Deviation. Concentration of latanoprost free-acid were measured using EIA.
No-Cells Model: Cell culture inserts (PET membrane) without cells, Monolayer Model: PET insert with a monolayer of human corneal epithelial cells, Multilayer Model: PET insert with a multilayer of human corneal epithelial cells (stratified culture).
$^{\$}$Significantly different from other lens materials ($p \leq 0.025$).

results show a significant decrease (approximately 30%) in the amounts of the drug that has been released from galyfilcon A and senofilcon A silicone hydrogels (Table 4.2).

4.3.4 The Role of Live Cells

To study the importance of metabolically active cells, which not only provide a physical barrier to drug permeation, but also are able to transfer and metabolize the drug, a set of experiments was designed to compare latanoprost release from the galyfilcon A silicone hydrogel material through a fixed and a live monolayer cornea model. In the fixed monolayer, cells are dead and thus metabolism of the drug cannot occur.

As shown in Fig. 4.6, in the presence of fixed (dead) cells, the amount of latanoprost that was released from the soaked galyfilcon A lens and diffused through the monolayer was lower in the presence of paraformaldehyde-fixed cells when compared to metabolically-active cells. These results clearly highlight the importance of the metabolism and transport in *in vitro* model of drug releasing materials.

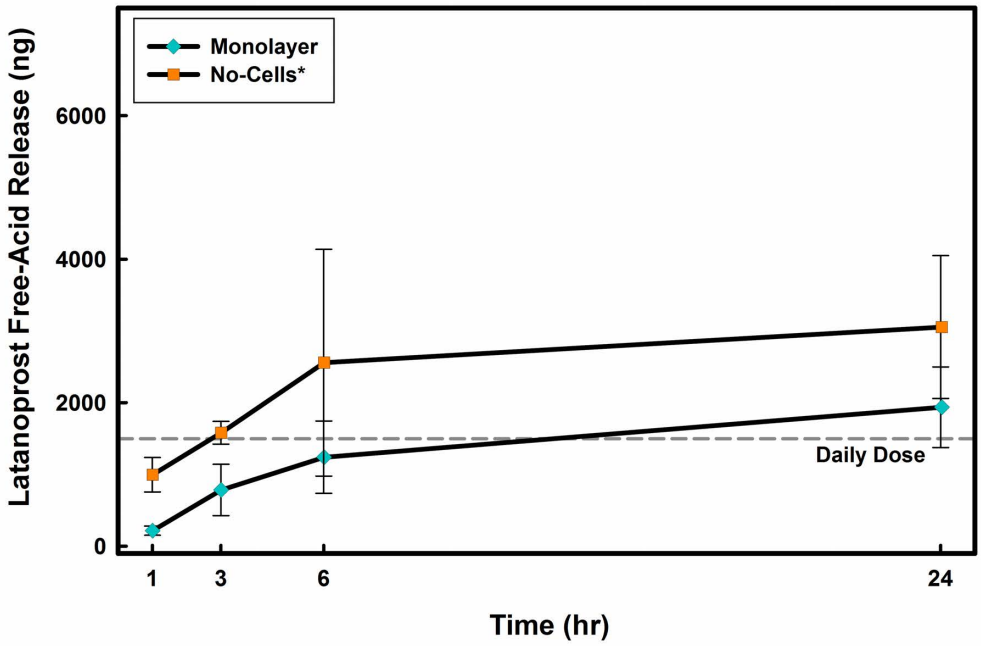


Figure 4.5: Time course of latanoprost free-acid release from senofilcon A in No-Cell and Monolayer *in vitro* models. Lenses were soaked for 24 hours in drug solution ($131\mu g/mL$) and then overlayed on the model for 24 hours. Aliquots were taken at specific times from the lower compartment and concentrations were measured using EIA. No-Cells Model: Cell culture inserts (PET membrane) without cells, Monolayer Model: PET insert with a monolayer of human corneal epithelial cells. Daily dose line represents the amount of the administered latanoprost for a glaucoma patient [27]. *Significantly different from *in vitro* models with cells ($p < 0.001$). (n=3 Mean ± SD).

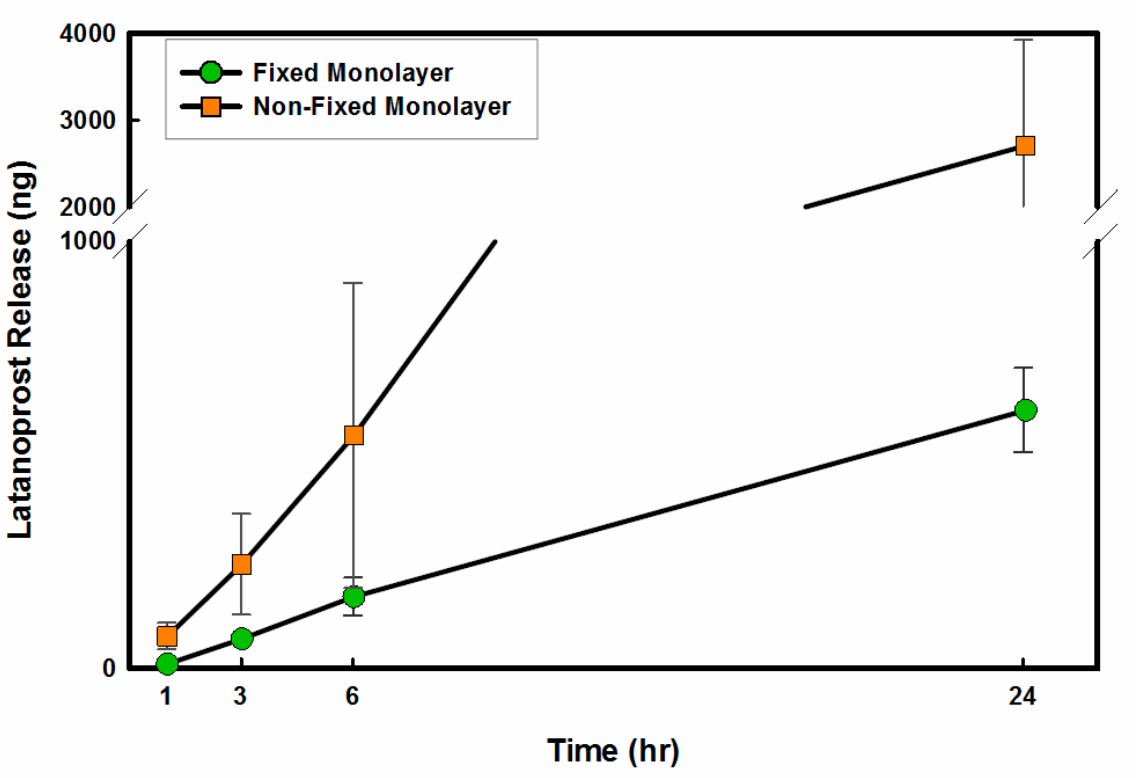


Figure 4.6: Time course of latanoprost release from galyfilcon A contact lens through live and dead monolayer models. Cells were killed by fixing in paraformaldehyde. Lenses were soaked for 24 hours in drug solution ($131\mu g/mL$) and then overlayed on the model for 24 hours. Release experiments through fixed monolayer were conducted on separate days (n=2, Mean $\pm$ SD). The results were compared to release through monolayer models, (n=6 Mean $\pm$ SD).

4.4 Discussion

This study was undertaken to determine the impact of the presence of cells in *in vitro* models of drug releasing materials. The cells used in these experiments, HPV-immortalized corneal epithelial cells have been used previously by Griffith *et. al.* for corneal constructs and have been shown to exhibit key physiological functions and biochemical marker expression of corneal epithelial cells [79].

Our initial release experiments with drug soaked contact lens material in the absence of cells provided results similar to many others [49, 51, 105, 107, 109], showing a mechanism of a first order release. The limited amount of drug that was released in our fixed volume model is likely the result of the high partition ratios of the latanoprost between the contact lens material and the aqueous solutions. Furthermore, our results from the no-cells model suggest that latanoprost has a lower affinity toward PBS compared to KSFM. The better solubility of latanoprost in KSFM compared to PBS is likely due to the difference in composition, such as the presence of growth factors and other ionic compounds in the culture medium which are absent in the buffered saline solution. While, in our experiments, the nature of the medium was found to have a statistically significant impact on release in the no-cell model, the actual improvement in drug release is actually insignificant when compared to the *in vitro* models with cells.

Significantly higher drug release and diffusion were observed in the presence of cells. Due to their hydrophobicity, ester prostaglandin analogues, such as the latanoprost prodrug, have a greater chance of diffusion through the hydrophobic corneal epithelium [111]. Furthermore, enzymatic activity will also play a role in the presence of live (metabolically active) cells, since the hydrolysis of latanoprost prodrug is expected by cells [27, 30–32] before diffusion through the cell layer. The product of hydrolysis, the latanoprost free-acid, has a smaller partition ratio (3.06 vs 3.98) almost 10 times more soluble in aqueous solution when compared to latanoprost. Therefore, the presence of cells will improve the drug diffusion rate. A layer of cells will also improve drug release from the contact lens material by maintaining the concentration gradient between the lens and the solution above the cells through metabolism of the latanoprost.

A recent study showed that the ester form of latanoprost contributed to only 4% of the total drug diffused through an *in vitro* cornea model and that no detectable amount of ester form of the latanoprost was observed in an *ex vivo* model [25]. We may thus assume that the majority of the diffused drug through the *in vitro* cornea models with cells is the free-acid form.

Different latanoprost release profiles were observed among the hydrogel contact lens materials tested. Compared to the silicone hydrogel materials, the high release of latanoprost from omafilcon A in spite of the lower drug uptake may be explained by the low affinity of the latanoprost (an hydrophobic compound) toward the omafilcon A contact lens material, which is a high water content hydrogel. The large partition ratio results in a low uptake by this material when soaking in aqueous solution of hydrophobic drugs, as well as relatively fast release rates in the release solution.

Using latanoprost esterified form, i.e. the active drug compound, latanoprost free-acid, affected results in all models. Higher water solubility of the latanoprost free-acid led to higher amounts of drug being released from the silicone hydrogel lens materials in the no-cell model. As a more polar molecule, latanoprost free-acid has a lower partition ratio between the hydrophobic silicone hydrogel contact lens materials and the aqueous solution when compared to latanoprost. While higher amounts of latanoprost free-acid were released in the no-cell model, lower releases were observed in the presence of cells. With latanoprost free-acid, epithelial cells now act as a barrier against the diffusion of the latanoprost free-acid, and hence limit the diffusion of the hydrophilic drug.

As one compares the latanoprost and latanoprost free-acid release results, it becomes evident that similar drug release profiles cannot be obtained by replacing the prodrug with the drug, even in the no-cell model. Not only are the amounts released significantly different by an order of magnitude, but while all silicone hydrogel materials released similar amounts of latanoprost, balafilcon A released significantly more latanoprost free-acid compared to the other two silicone hydrogels. The balafilcon A/latanoprost free-acid results are likely due to the fact that balafilcon A material has an overall net negative charge due to the incorporation of some acidic material components [27] and its surface charge increases the

hydrodynamic attributes of the material [118], therefore increasing the role of adsorption of the hydrophilic drug on the surface of the contact lens during the uptake process and its subsequent release in solution. Nevertheless, taken together, our latanoprost free-acid results highlight the relevance of using *in vitro* models with cells when studying release of a prodrug that will undergo hydrolysis before diffusion through the tissue to the site of treatment.

Due to the lack of previous *in vitro* studies on prostaglandin analogues, our results can only be compared to the release of drugs from the contact lens materials with similar size and hydrophobicity. Previous *in vivo* studies have shown a prolonged release of relatively hydrophobic drugs such as ketotifen [108] and lomefloxacin [110], however such release profiles could not be replicated *in vitro* using a fixed volume release model [51, 109]. The extended release of latanoprost observed in the monolayer and multilayer *in vitro* models correlates well with the extended release profiles of the hydrophobic drugs observed *in vivo* [108, 110]. The release results of latanoprost in the no-cells model is also comparable to the release results of hydrophobic compounds in fixed volume solution [51, 109]. The significant role of cell metabolism and transport was further demonstrated using fixed (metabolically inactive) cells. Taken together, our results suggest that the absence of cells in *in vitro* models of drug release likely contributes to the contradiction between these *in vitro* and *in vivo* studies [51, 108–110].

4.5 Conclusion

Poor release results from commercially available contact lens materials soaked in hydrophobic compounds such as latanoprost have been obtained with fixed volume release models similar to the no-cells model used here. However, we have demonstrated, using drug-soaked silicone hydrogel materials, that the amount of drug diffusing through an *in vitro* cornea model is in the order of $2-3\mu g$ over a period of 24 hours, which is comparable to the $1.5\mu g$ of drug in every drop of the commercial latanoprost. Our results emphasize the importance of the presence of cells when characterizing the release of drug-delivery materials and

demonstrate how experimental *in vitro* models have a significant impact on the outcomes of testing ophthalmic drug delivery materials. Our *in vitro* study suggests that silicone hydrogels have the potential to deliver latanoprost effectively over an extended period of time. Fig. 4.7 presents a graphical abstract of the key results and the drug uptake and release model used in this study.

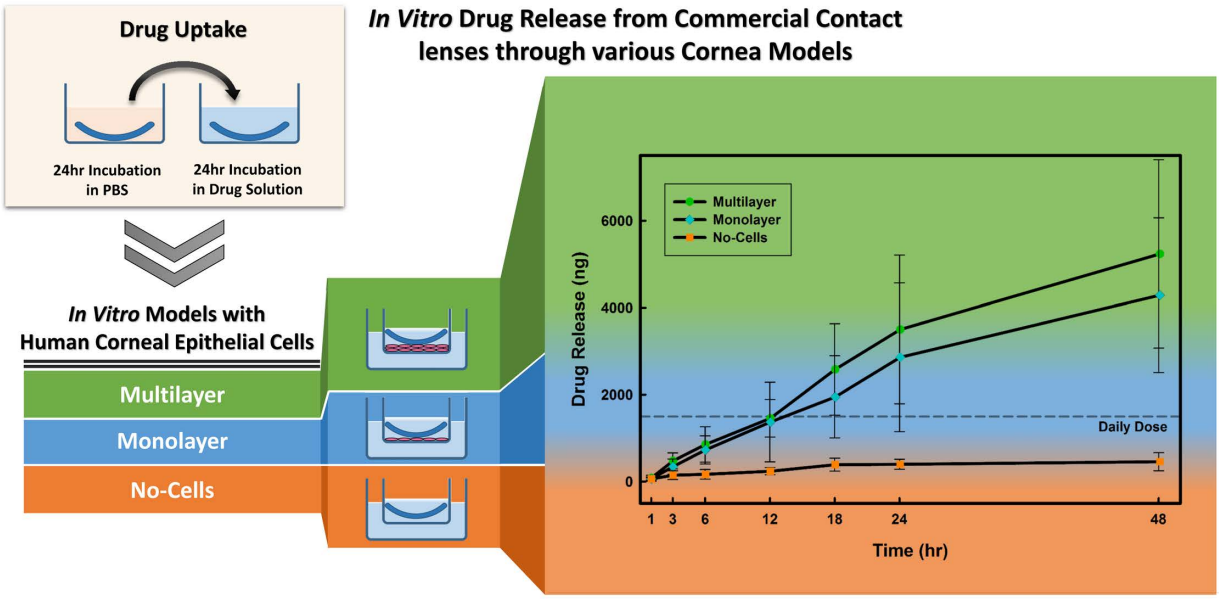


Figure 4.7: Impact of corneal epithelial cells on latanoprost drug release from a contact lens material. Commercial contact lenses were washed in PBS overnight, before the soaking in drug solution for 24hrs. The drug release from contact lenses were measured for 24 to 48 hours in various release models.

Chapter 5

Tear Replenishment System:

A More Realistic Approach to Ocular Surface Biocompatibility Studies

5.1 Overview

While there is a constant exchange of fluid over the ocular surface, conventional *in vitro* cell models poorly mimic this physiological condition/environment. Most *in vitro* models currently do not remove/replenish nutrients at the air-liquid interface during incubation, and thus the cells or ophthalmic materials are exposed to the same concentration over time. While a monolayer of corneal epithelial cells is capable of forming tight-junctions, in a stratified cornea model, differentiation of epithelial cells and formation of a superficial epithelial cell layer that forms tight-junctions is achieved through the creation of an air-liquid interface. The tight-junctions are an essential characteristic of the cornea's barrier function [82].

In order to reproduce tear replenishment *in vitro*, a method and a device are needed to deliver artificial/non-artificial tear liquid to an *in vitro* ocular cell culture model and the ophthalmic material interacting with the ocular cell model, while maintaining the air-liquid interface. In this chapter, this device and method hereinafter referred to as "Tear Replenishment System (TRS)" will be put forth as a solution.

To assess the value of the TRS in studying ophthalmic drug delivery devices, the release of latanoprost from three commercial contact lenses under replenished conditions was investigated. The results, presented in this chapter, were compared to the release from the same contact lenses using the conventional immersion model. To this end, curved cornea models (CCM) were prepared and are fully described in the following section. It should be noted that the viability studies using TRS covered in this chapter are published [119].

5.2 *In Vitro* Curved Cornea Models

Cornea models have shown good *in vitro/in vivo* correlation in drug permeation studies when compared to *ex vivo* models [25]. This makes them a more standardizable and cost effective substitute for animal studies. Monolayers and multilayers of immortalized human corneal epithelial cell grown on cell culture inserts are used as *in vitro* cornea models for a variety of cytotoxicity and biocompatibility tests [87, 89]. None of the commercially available models such as HCE by SkinEthic, EpiOcular by MatTek, and Clonetics by Lonza are suitable for contact lens studies due to their limited surface area (the well size of these models is between 6 and 8 mm compared to the 13-16 mm diameter of a contact lens). To overcome the limitations of these models, a curved cornea model was developed at the *Materials Interactions with Biological Systems* (MIBS) lab to study the interactions of contact lens materials and multipurpose solutions [78]. The curved cornea models used in this research project followed those protocols with some modifications as described in this section.

5.2.1 *In Vitro* Cell Culture

HPV-immortalized human corneal epithelial cells were cultured in keratinocyte serum free medium (KSFM) supplemented with bovine pituitary extract, recombinant epidermal growth factor, and Penicillin/Streptomycin (Pen/Strep) (ScienCell, Carlsbad, California) at $37°C$ and 5% carbon dioxide (CO_2). Fresh medium was added every other day and cells were grown to 90% confluency in tissue culture treated flasks. Adherent cells were removed

using TryplExpress (Life Technologies, Burlington, Ontario, Canada) dissociation solution. Cells were routinely observed for any morphological changes and were used before their eleventh passage.

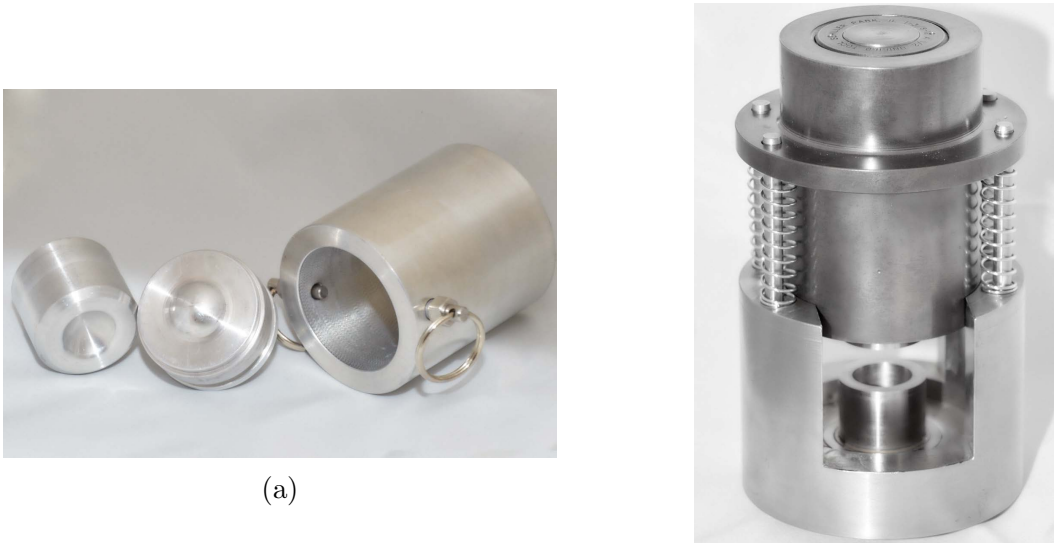
5.2.2 Preparing Curved Cornea Models

The curved cornea models (CCMs) (monolayer and stratified) were grown on a Millicell-HA (mixed cellulose esters) membrane (Millipore, Billerica, MA, USA) with a $0.45\mu m$ pore size. The 30 mm diameter filters were first formed using a custom-shaped curved forming mold (Fig. 5.2a) as shown in Fig. 5.1. Silicone rings were punched into rings (inner diameter of 15.9 mm and an outer diameter of 23 mm) from Press-to-Seal sheets with adhesive (Life Technologies, Burlington, Ontario, Canada) using an in-house fabricated punch die (Fig. 5.2b). Once cut, the rings were disinfected with 70% ethanol and placed on top of the curved filters. The assembled inserts were then UV sterilized. After sterilization, inserts were coated with collagen type I ($0.05mg/ml$ - $30min$ at $37°C$). The inserts were then rinsed with PBS before adding cells.

The curved inserts were seeded with 6×10^5 cells/insert. The CCMs were fed with KSFM on each of the basal and apical sides of the curve for the first seven days, with medium being exchanged every other day. The monolayer CCMs were ready for experimentation at this point. In case of stratified CCMs, after day five of KSFM feeding, cell differentiation was induced by exposing the monolayer to an air-liquid interface. Cells were fed only on the basal side with 2% Fetal Bovine Serum (FBS) in 1:1 Dulbecco's Minimum Essential Medium (DMEM) in Ham's F12 nutrient medium (DMEM/F12) and medium was exchanged daily. The cells grew under these conditions for seven days and were then ready for experimentation. The scanning electron microscopy as well as the cross-section of the stratified corneal epithelium are presented in Fig. 5.3. The fixed CCMs (in 10% neutral buffered formalin) were sectioned and stained with hematoxylin and eosin (H&E). The H&E staining results demonstrated the integrity of the stratified corneal cells firmly attached to the cellulose filter. Also, as shown in SEM micrographs of the mixed cellulose ester filters, Fig. 5.4, the microstructure of the filters, which plays an essential role in cell

(a) (b) (c)

Figure 5.1: Mixed Cellulose Ester Filters: a) before and b) after deforming the filters c) and with a silicon ring



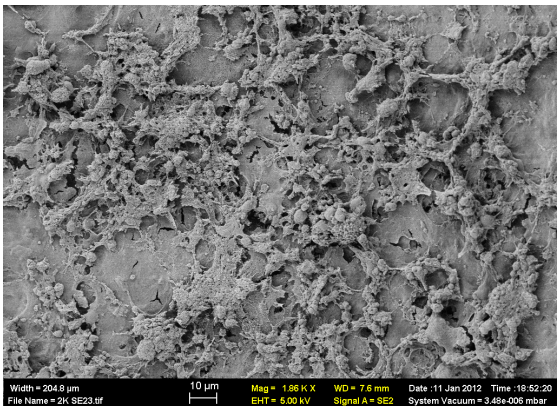
(a) (b)

Figure 5.2: a) The forming mold to custom-shape the cellulose ester filters and, b) The punch die to cut silicone Press-to-Seal sheets that limit the cell growth to the curved area of the filters in curved cornea models

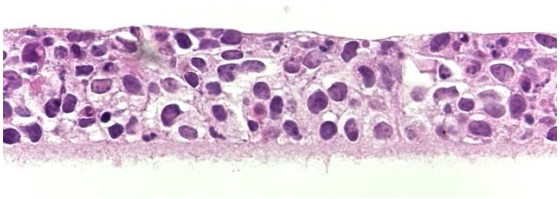
adhesion to the surface, was retained after the curve-forming process.

5.3 A Microfluidic Approach to Tear Replenishment

Mimicking tear replenishment in the eye required the delivery of a tear analogue to the surface of the *in vitro* cornea model at a rate within the range of a physiological tear flow rate, in a recurring fashion. The objective was to cover the surface of the cornea model with a small amount of artificial tear liquid sufficient to hydrate the surface of the model and maintain the air-liquid interface. Designing a fully operational microfluidic device

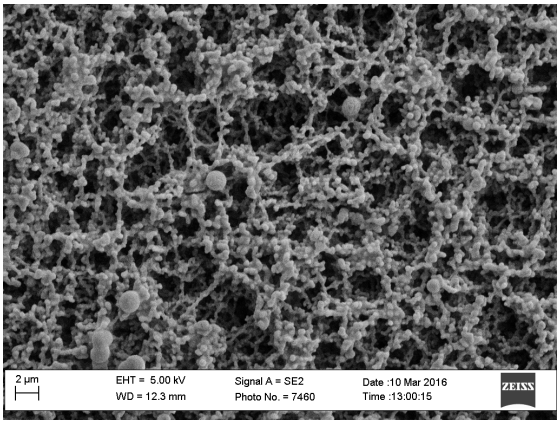


(a)



(b)

Figure 5.3: a) Scanning electron microscopy analysis, and b) H&E staining of a multilayer curved cornea model. In (a) loosely attached cells on top of a compact stratified corneal epithelial cells can be observed. Due to the lack of blinking, cornea models grown *in vitro* lack the ability to properly discard naturally occurring cell shedding. H&E staining shows the integrity of the multilayer corneal cells firmly attached to the cellulose filter.



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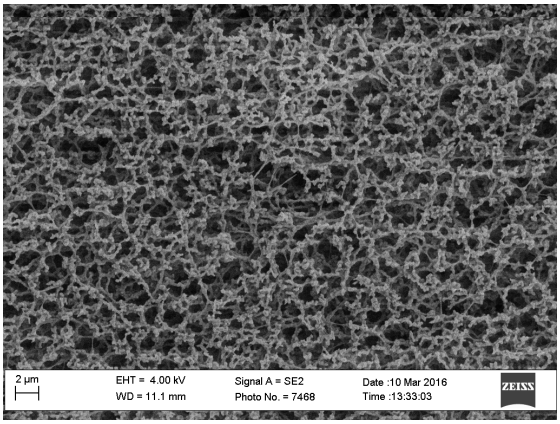


(b)

Figure 5.4: a) Microstructure of cellulose ester filter a) before, and b) after forming, using the forming mold. The filter maintains its porous micro-structure which is essential for cell adherence and cell growth promotion.

required multiple iterations around the dimensional parameters as well as the type of fluid flow of the system. The final design is a reliable platform that allows control of experiment parameters through an interactive interface.

5.3.1 Generating an Intermittent Micro Flow

To bring artificial tear liquid to the surface of the *in vitro* cornea model, injection of a liquid through a needle or a nozzle could be considered. A spectrum of flows, from a slow drip, to a laminar jet, to a spray, can be achieved based on the flow parameters of the jet. The laminar flow does not adequately mimic the intermittent nature of the tear replenishment on the surface of the *in vitro* cornea model. Therefore, it is necessary to use either a dripping flow or an atomized/aerosolized flow to model the tear replenishment.

In a dripping flow, a droplet forms when the force of gravity on the liquid exceeds the surface tension, therefore the liquid is pulled away from its attachment. To achieve a physiological tear flow rate of approximately $100\mu L/hr$ [45], it can be shown that the internal diameter of the needle needs to be smaller than $10\mu m$. Due to the complexities of using such a needle, mostly because of clogging by floating particles in artificial tear liquid, such a needle is not suitable for the tear replenishment system. Considering that the nature of the flow is intermittent, larger diameters for shorter intervals can be used.

Spraying a specific amount of liquid with the frequency of human blinking can also be utilized to cover the surface of the *in vitro* cornea model in order to recreate the tear replenishing effect. *"Spraying occurs when the cohesive and disruptive forces applied to the surface of a cylindrical jet of liquid generates small perturbations that can amplify under favorable conditions to disintegrate the jet into small droplets"* [120]. In the following section the design based on these types of flow will be discussed.

5.3.2 Design Constraints and Criteria

Any microfluidic device that models tear replenishment of the human eye *in vitro* is required to:

1. deliver a reproducible and adequately small volume of tear solution analogue intermittently to the surface of the cornea models,
2. deliver sterile tear solution under aseptic conditions through biocompatible microfluidic components with minimal interaction between liquid and the system to minimize the risk of contamination or leaching of any components that may interact with cells,
3. achieve sterility for the device components through readily accessible means,
4. integrate within a cell culture incubator,
5. accommodate the readily available cell culture plates and inserts.

Two types of fluid flow were used in the design of the TRS. In the initial design, properties of an atomized jet were exploited to hydrate the surface of the CCMs. The initial design proved that the microfluidic approach could be employed successfully to model the tear replenishment *in vitro*. However, the impediment to using the spray jet was the damage caused by the fast fluid jet to the integrity of the *in vitro* cornea model.

Subsequent to the first complete design/implementation of the TRS, significant improvements in every aspect of the system were made in the second design. The second iteration of the TRS took advantage of a dripping flow to deliver a tear solution analogue, while allowing individual collection of supernatant liquid (excess "tear" collected on the CCMs), and closer monitoring of the performance of the system using a more robust and flexible circuitry. As a first step, computer-aided numerical simulations were used to validate the microfluidic system's design. Also a closed system approach to the microfluidic system allowed for maintaining the sterility of the system. Corrosion in the initial design, due the acidic environment of the incubator, was addressed by encasing most components in

Table 5.1: Tear Solution Properties

Symbol	Parameter	value
ρ	tear liquid density	1 g/cm^3
μ	tear liquid viscosity	4.4-8.3 mPa.s [121]
σ	surface tension against the air	0.043 N/m [122]

a sealed stainless steel enclosure, and relocating electronic circuits to outside of the incubator. The most significant improvement in the final design was adopting modular design concepts. This enabled optimization of the individual subsystems thus minimizing the required troubleshooting effort as well as the rate of failure. Further details of the designs are provided in the following sections.

5.3.3 Atomized Liquid Jet Design (TRS v1.0)

The classification of modes of disintegration of a jet as well as the idealized and actual breakup of a low velocity jet is illustrated in Fig. 5.5. At small flow rates, the liquid jet will breakup after traveling the distance L, referred to as the "Rayleigh varicose breakup length", according to the empirical equation shown below:

$$L = 19.5 d_0 (1 + 3Oh)^{0.85} \sqrt{We} \tag{5.1}$$

in which Oh, Ohnesorge number is defined as:

$$Oh = \frac{\mu}{\sqrt{\rho \sigma d_0}} = \frac{\sqrt{We}}{Re} \tag{5.2}$$

where We and Re are the Weber and Reynolds numbers.

Table 5.1 presents some of the properties of the tear solution, although properties of an artificial tear solution [64] will be slightly different from human tears.

For the physiological tear flow rates and fluid properties shown in Table 5.1, using Eq.(5.1), the Rayleigh varicose break-up length will be as short as a few micrometers for a nozzle exit diameter of $50\mu m$, which implies that the liquid jet will atomize upon exiting

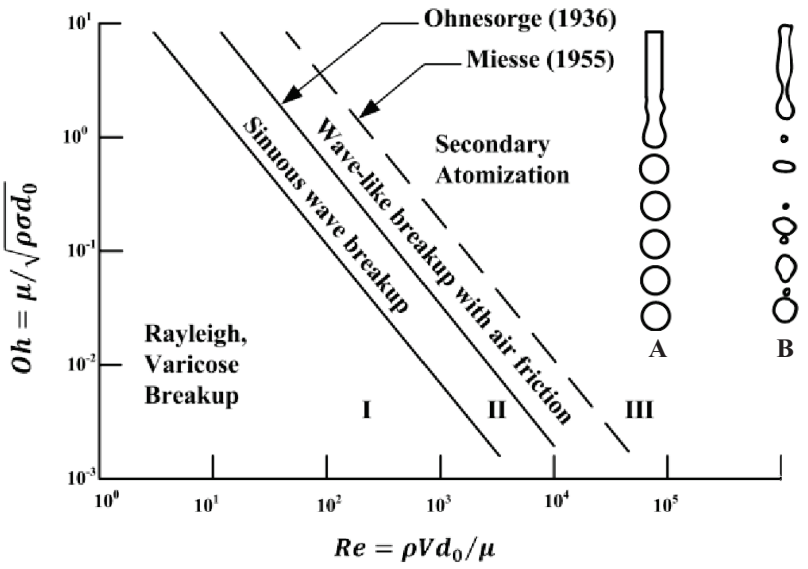


Figure 5.5: Classification of modes of disintegration of a jet. A) Ideal and, B) Actual breakup of a low velocity jet. Diagram is adapted from [120]

the nozzle.

Based on these calculations, the microfluidic system shown in Fig. 5.6 is proposed for use as a tear replenishment system. The proposed system uses a pressurized supply line from which a tear solution analogue is sprayed through a nozzle onto the surface of the *in vitro* cornea model. The amount of sprayed solution is controlled through a series of solenoid isolation valves by adjusting the fraction of time that each valve is open. The air-liquid interface is maintained by collecting the sprayed solution and draining it from each well.

To assess the feasibility of the microfluidic system's design, LMS Imagine.Lab AMESim© was used for modeling the proposed system. The numerical modeling results showed an adjustable flow rate within the desired range could be achieved for the duty cycle of $25-100ms$ in the isolation valves. The total accumulative volume transferred to each well over a period of one min was calculated to be $2.2\mu L$ as depicted in Fig. 5.7.

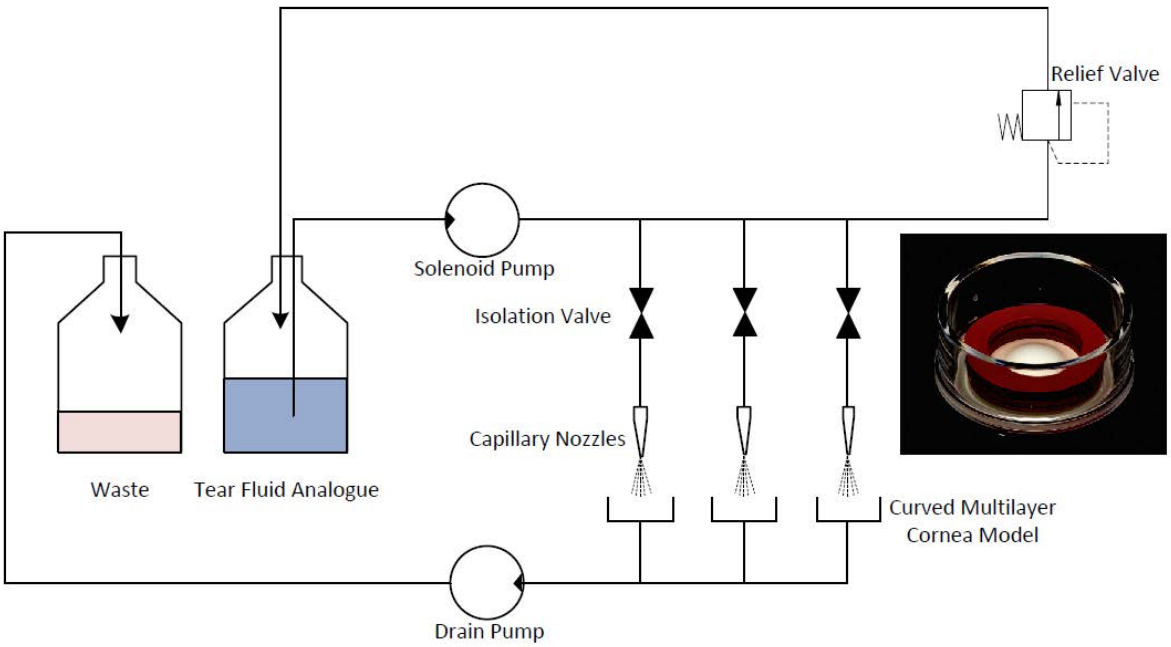


Figure 5.6: Schematic of the "atomized jet based" tear replenishment system: the solenoid pump provides enough back pressure to a series of isolation valves to control the flow rate as well as the duty cycle of the tear analogue spray over the curved cornea model. The vacuum pump drains the supernatant liquid from the top the CCMs into the waste container.

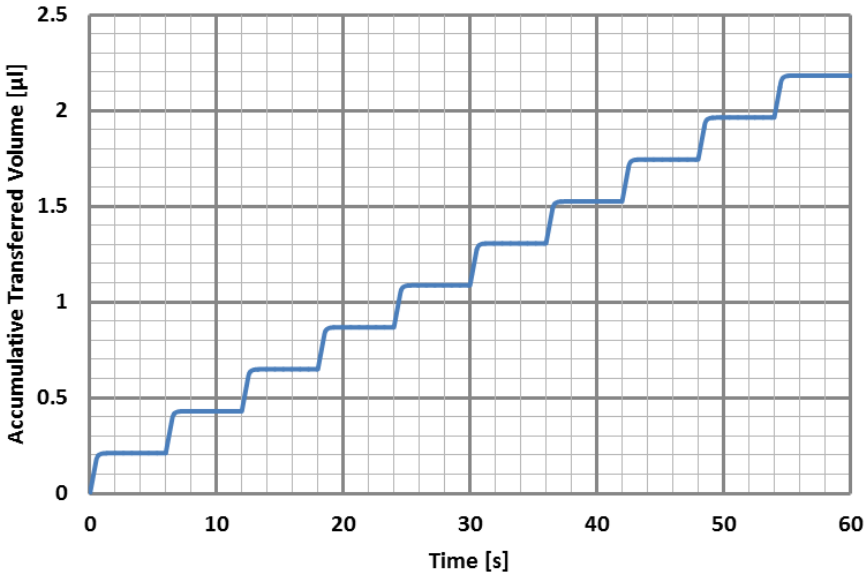


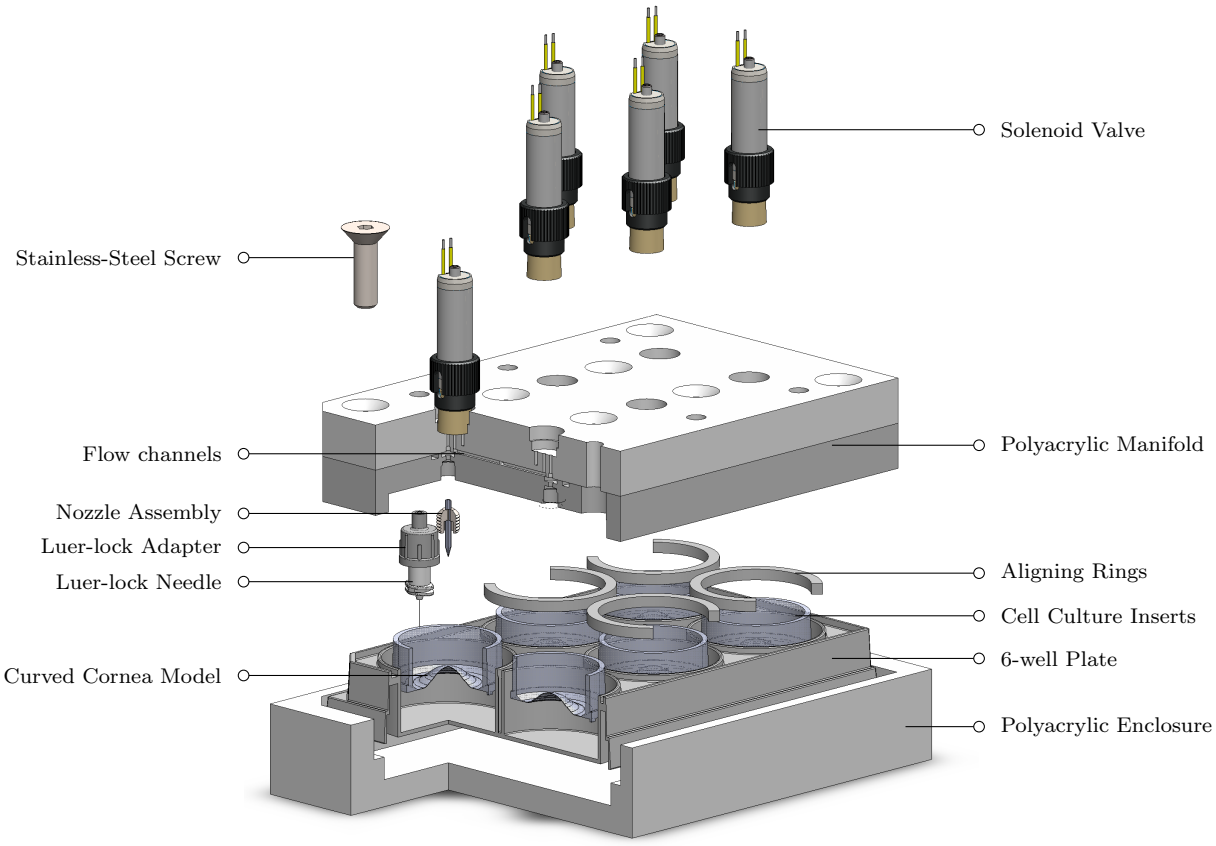
Figure 5.7: The accumulative transferred volume in each well over one minute period using a 250ms opening time of the isolation valve.

5.3.3.1 Mechanical Design

The situation that must be resolved via this design is one of a microfluidics device which can spray tear analogue on the surface of an *in vitro* curved cornea model. The tear in the human eye is produced in the rate of 0.5-2.2 $\mu L/min$, which is spread over the eye through blinking nearly 10 times every minute [93, 94]. Therefore the designed system requires providing an adjustable amount of tear fluid within the range of $1 - 4\mu L/min$, 10 times in a minute. A solenoid operated micro-pump from Bio-Chem Fluidics™ was used to pump the tear analogue through PTFE tubing (Bio-Chem Fluidics, NJ, USA). All the components used in the device are sterilizable and biologically inert. The sterile tear analogue solution passes through solenoid operated isolation valves (Bio-Chem Fluidics, NJ, USA) and ceramic nozzles (CoorsTek®, USA) before being sprayed on the surface of the cornea model and then drained through another solenoid operated micro-pump. Fig. 5.8 shows the designed device. The implemented tear replenishment system and the interior of the device are presented in Fig. 5.9.

5.3.4 Dripping Flow Design (TRS v2.0)

While the goal in the "atomized jet design" was to deliver an adjustable rate of $1-4\mu L/min$ to the surface of CCMs, as it will be discussed in the following sections, it significantly damaged the integrity of the cornea model. Changing the flow regime into dripping was favored over other flow types as it would generate an intermittent flow. The atomization in the initial design occurred due to the significantly higher fluid velocity while exiting the nozzle. To generate a dripping flow, a needle could be used instead. However, to maintain similar flow rate to the initial design, the needle diameter requires to be less than $10\mu m$. Such needle does not exist nor can it be reliably utilized for delivery of the tear analogue that may contain small particles. To overcome this limitation, a needle with an internal diameter of $50\mu m$ was adopted. The droplet size is governed by the balance between the gravity on the liquid and the surface tension force. The larger diameter needle impacts the droplet size. To retain the frequency at which the tear solution is delivered to the surface of the CCMs, the total flow rate of the TRS increases to $1mL/hr$ which is 10 times larger



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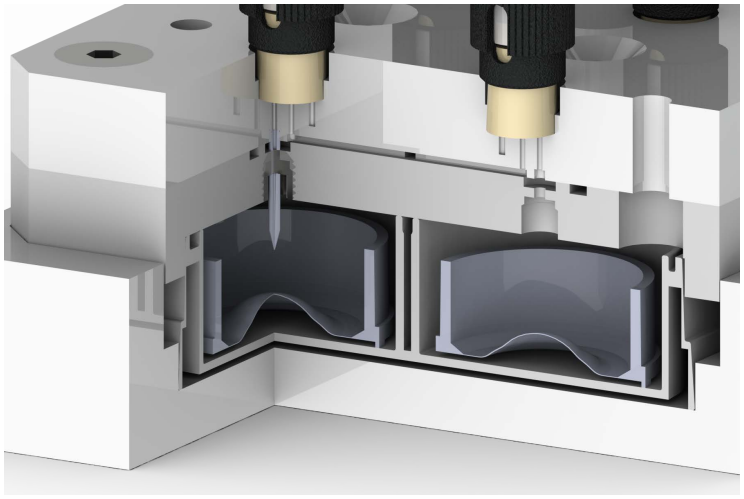
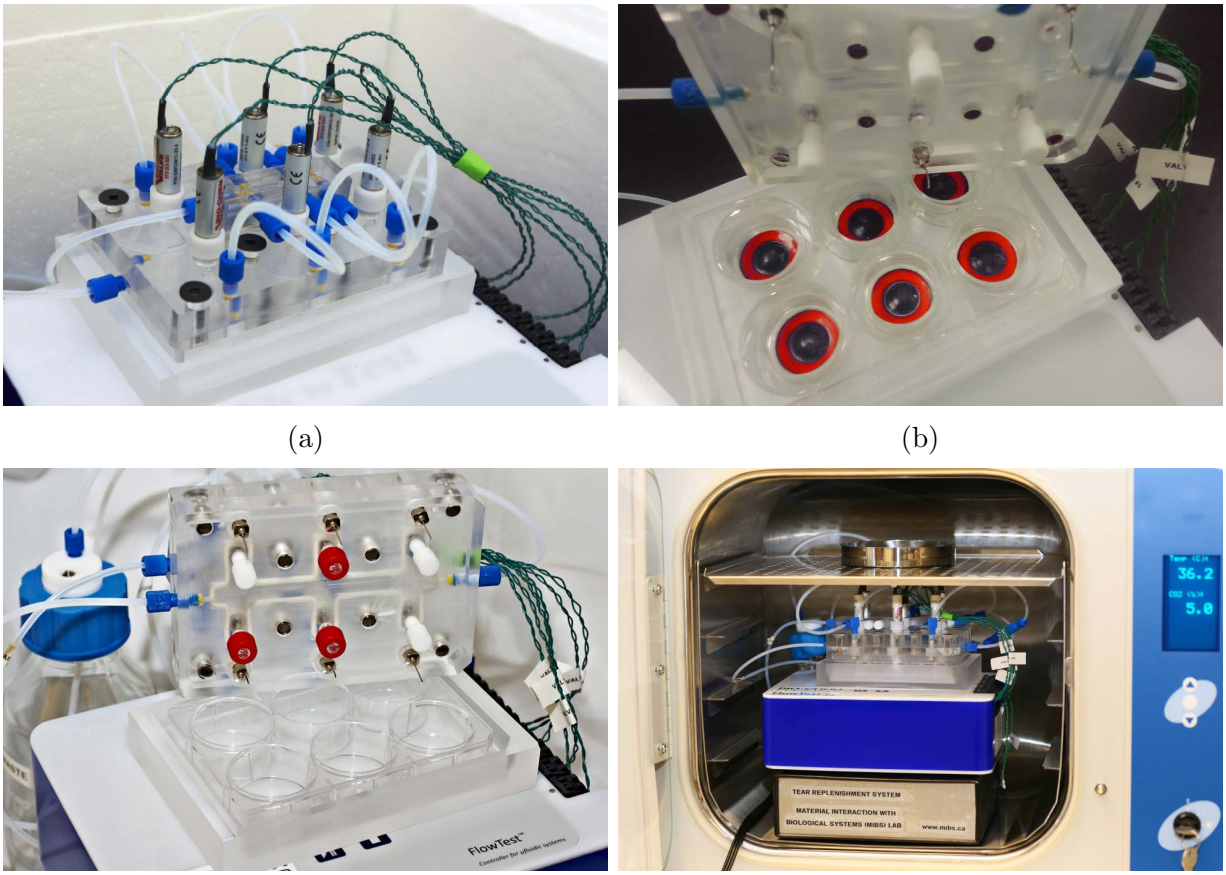


(b)

Figure 5.8: a) Exploded view of the TRS v1.0 and its various components. b) Schematic cross section of the TRS with the nozzle assembly and the curved cornea model.



(a) (b)

(c) (d)

Figure 5.9: The Tear Replenishment System v1.0. a) and b) Polyacrylic manifold, nozzles and culture plate can be identified. A cell culture plate with *in vitro* cornea models, the cornea models appear dark purple as they have been stained with a metabolic dye following experiments in the TRS; c) Closed TRS: the system is built to ensure that testing with the *in vitro* cornea model is performed under aseptic conditions. d) The TRS is designed to fit in a cell culture incubator so that cells are exposed to the proper growing environment.

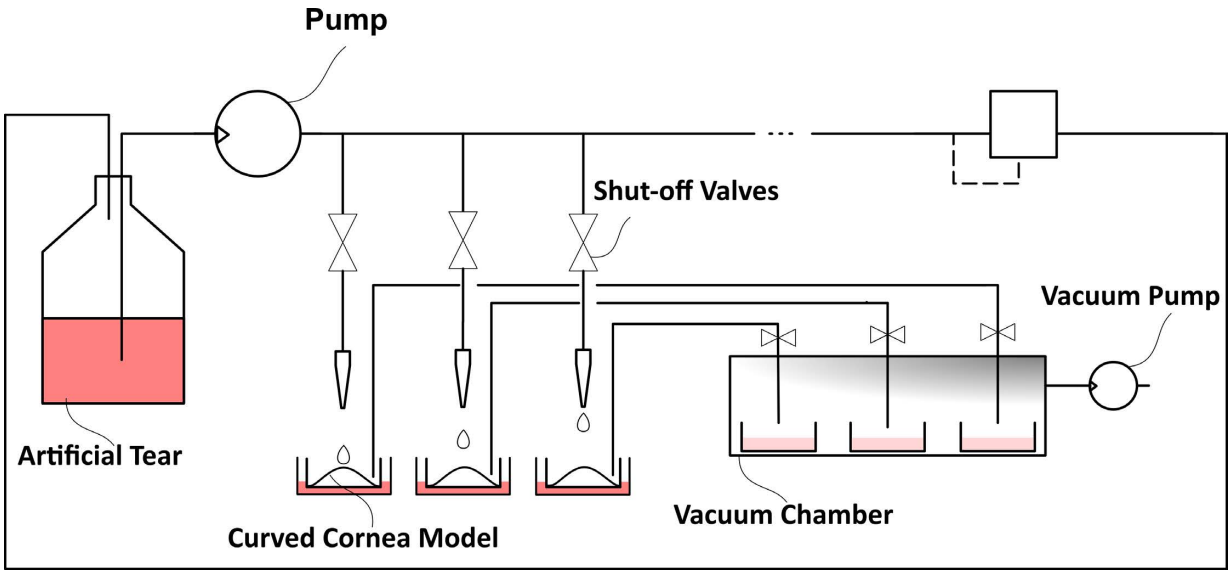


Figure 5.10: Schematic of the "dripping flow based" tear replenishment system, v2.0. A solenoid pump provides enough back pressure to a series of isolation valves to control the flow rate as well as the duty cycle of the tear spray over the curved cornea model. An air pump creates a relative vacuum which drains the supernatant liquid from the top of each CCM and collects them individually.

than physiological tear replenishment rate. While significantly larger, the flow rate is still sufficiently small to mimic the effect of tear replenishment *in vitro*.

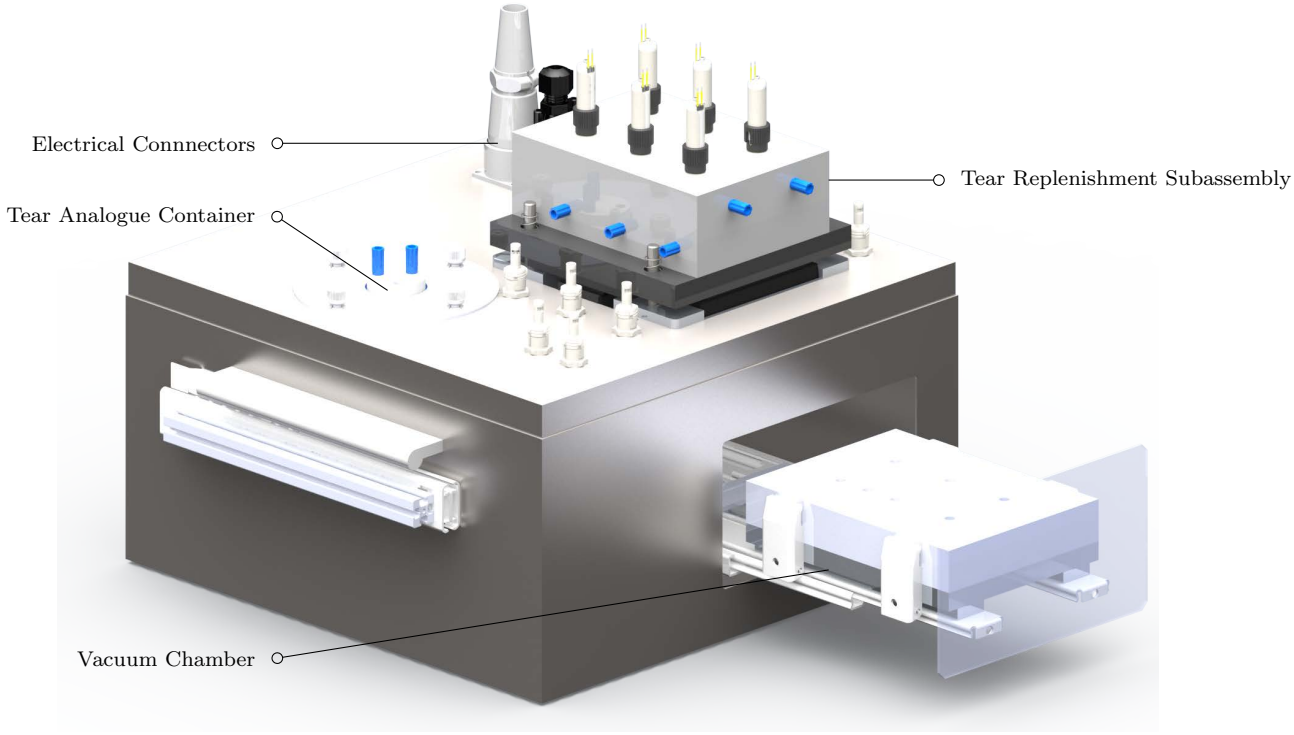
The final design of the microfluidic system, as shown in Fig. 5.10, improves upon the initial design. The proposed system uses a pressurized supply line; however, the tear analogue is transferred through a needle above the surface of the *in vitro* cornea model before dripping onto the surface. The amount of delivered solution is controlled through a series of solenoid isolation valves similar to the former design. The air-liquid interface is again retained by collecting the supernatant solution and draining it from each well into containers corresponding to each individual CCM.

5.3.4.1 Mechanical & Electrical Design

An air jacketed CO_2 mini incubator (VWR International, Mississauga, Canada) was adapted to enclose the tear replenishment system and provide ideal cell culture environment (100% humidity, $37°C$ temperature , and 5% CO_2). The mechanical components of the TRS is divided into "tear replenishment module" and "supernatant drainage module". While the

tear replenishment module requires to be accessible to place/remove the cell culture plate containing the CCMs, the supernatant module, along with the rest of electro-mechanical components are encased in a stainless-steel enclosure. The same solenoid operated micro-pump from Bio-Chem Fluidics™ is used to pump the tear analogue through PTFE tubing (Bio-Chem Fluidics, NJ, USA). All the electrical components are located in the electrical panel of the incubator. The electro-mechanical components are passed through the incubator compartment using a water-proof, chemically resistant connector. All the components used in the device are sterilizable and biologically inert. The sterile tear analogue solution passes through solenoid operated isolation valves (Bio-Chem Fluidics, NJ, USA) and stainless-steel needles (Hamilton Company®, Nevada, USA) before dripping on the surface of the CCM and then drain through another solenoid operated pinchvalve into a 6-well plate encased in the vacuum chamber as part of the supernatant drainage module. Relocating electrical components also eliminates an extra heat source that would destabilize the incubation temperature. Fig. 5.11 shows the designed system and the two modules. The implemented tear replenishment system as well as the interior of the device are presented in Fig. 5.12.

The electro-mechanical components of the system are driven and controlled using a small board computer (SBC) running Linux which provides a user-interface through the touchscreen enabled lcd on the front of the incubator panel.



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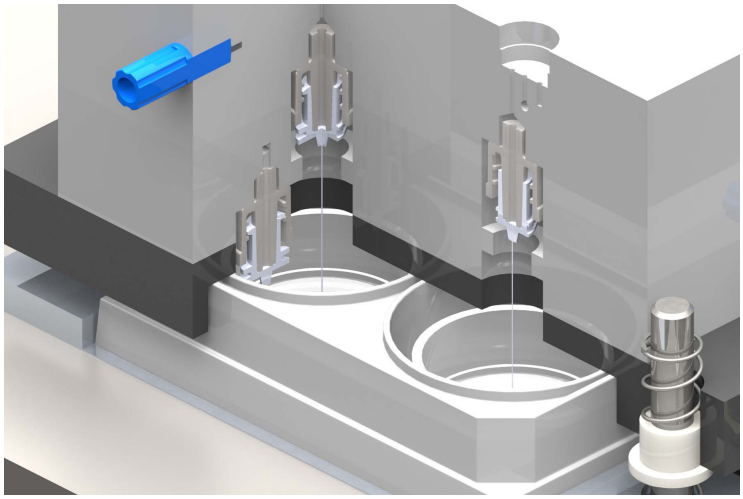
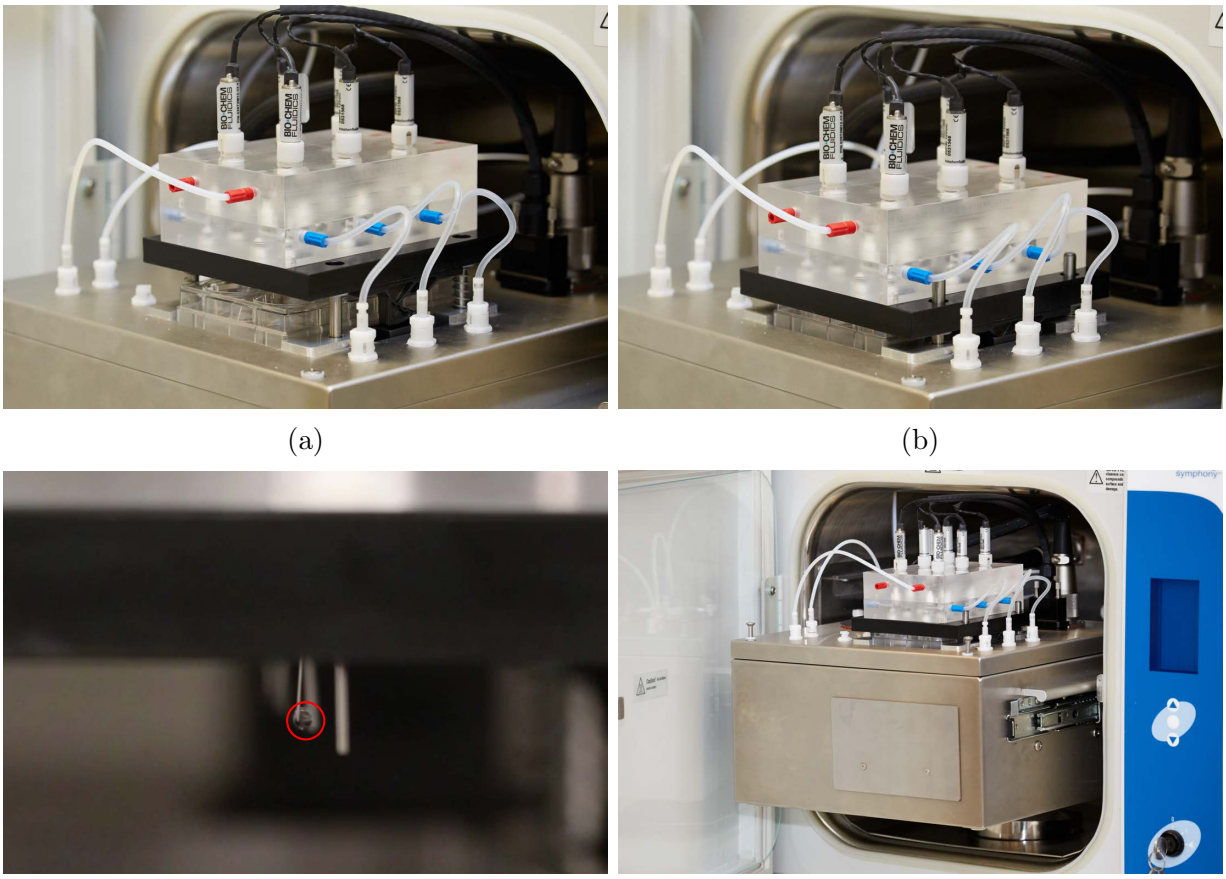


(b)

Figure 5.11: a) Exploded view of the TRS v2.0 and its various components. b) Schematic cross section of the TRS v2.0 with the nozzle assembly and the stratified curved cornea model.



(a) (b)

(c) (d)

Figure 5.12: Tear Replenishment System v2.0, "Dripping Flow Based" Design. A 6-well plate with 6 CCMs is placed inside the replenishment subassembly a) before, b) after clamping the subassembly to seal the 6-well plate; c) Tear analogue dripping from microneedle d) The TRS is designed to fit in a cell culture incubator so that cells are exposed to the proper growing environment.

5.4 Materials and Methods

5.4.1 Preparation of The Tear Replenishment System

The microfluidics system was sterilized by running 70% Ethanol through the device and then PBS. The device awas then required to stabilize near $37°C$ to eliminate temperature fluctuations as well as condensation on the device components during the experiment. Afterwards, six curved cornea models were placed in the device under aseptic conditions, then the entire device was transferred back to the cell culture incubator and experiments were performed for 12 hours.

5.4.2 Viability Studies: Preparation of Contact Lenses

Two commercially available contact lens materials, balafilcon A, and etafilcon A were selected for this study and their properties are listed in Table 5.2. Lenses had back vertex power of -3.00. Phosphate buffered saline, recognized to be biocompatible [88], was used as the negative control lens solution. A sterile ophthalmic solution of benzalkonium chloride (BAK) was used as the positive control: BAK is a preservative used in several commercially available eye-drop formulations and is a well-known irritant [89]. The commercially available Moisture Eyes (ME; Bausch & Lomb, Rochester, NY, USA) has a BAK concentration of 0.01% w/v. Contact lenses were removed from their packaging and soaked for 24 hours either in Phosphate Buffered Saline (PBS, Lonza, Allendale, New Jersey) or in the undiluted BAK-containing solution. Each lens was totally immersed in the solutions in a sterile 12-well polystyrene plate and all procedures were performed under sterile conditions.

5.4.3 Viability Studies: Experiments Using TRS v1.0

To ensure that the *in vitro* TRS system was a viable platform to model the ocular surface, the ability of the *in vitro* tear replenishment device to hydrate the topical surface of a cornea epithelial model with and without contact lenses was tested and compared to the conventional immersion method.

The contact lenses used in this study were soaked either in PBS or in a benzalkonium chloride containing solution, Moisture Eyes. For lens placement, cells were fed with fresh KSFM on the basal side of the cell culture inserts. Apically, $500\mu L$ of KSFM was added to wet the surface. Solution-soaked and PBS-soaked contact lenses were then removed from their respective incubation solution and were placed concave-side down on top of the curve. $300\mu L$ of KSFM was then applied to the top of the wells that were not subjected to spray (no-replenish condition) to a total of $800\mu L$ of KSFM. The rest of the wells were subject to spray (replenished condition). The contact lenses, onlayed on the curved cornea models, covered the entire surface of the stratified CCMs.

In the experimental design, there were two different lenses and two different solutions tested for 2 and 6 hours. Each of the 8 combinations were tested, and all combinations including controls were subjected to both replenish and no-replenish conditions.

5.4.4 Cell Viability Assay

After 2, 6 and 12 hours incubation (2 and 6 hours for viability studies and 12 hours for drug release studies, respectively), lenses and medium were removed. To obtain a measure of the cellular viability, the MTT assay was performed. Dimethyl thiazoyl blue tetrazolium bromide ($0.5mg/mL$, MTT, Sigma Aldrich, Oakville, ON, Canada) was added to the apical and basal sides of the cell culture insert and was incubated for 3 hours at $37°C$ and 5% CO_2. The MTT solution was then removed and isopropanol was added to both the apical and basal sides of the insert and plates were agitated for 2 hours. The solutions in the apical and basal sides were mixed together and samples were read in a UV-Vis spectrophotometer at an optical density of 595 nm with a reference at 650 nm. All results are expressed as the relative viability compared to control cells: cells incubated in the absence of a contact lens and without replenishment (immersion model).

5.4.5 Drug Release Studies: Drug Doping Solution

The lens doping solution was prepared by dissolving latanoprost (solution in methyl acetate, Cayman Chemical, Ann Arbor, MI) in PBS (Lonza, Walkersville, MD). The concentration of the stock drug solution was measured at $123\mu g/mL$.

5.4.6 Drug Release Studies: Preparation of Contact Lenses

Based results previously obtained [115], three commercially available contact lens materials, senofilcon A, etafilcon A, and balafilcon A were selected. The properties of the three lens types are presented in Table 5.2. All lenses had a back vertex power of -3.00 diopter. Lenses were incubated for 24 hours in PBS (Lonza, Allendale, New Jersey) to remove any remnants of their packaging solutions, before incubation in $1.0mL$ of the drug solution for 24 hours.

5.4.7 Drug Release Studies: *In Vitro* Drug Release Models

The *in vitro* curved cornea models were used to assess drug release from commercially available contact lenses. The curved cornea models were grown on a Millicell-HA (mixed cellulose esters) membrane (Millipore, Billerica, MA, USA) with a $0.45\mu m$ pore size. The curved inserts were seeded with 6×10^5 cells/insert. The CCMs were fed with KSFM on each of the basal and apical sides of the curve for seven days, with medium being exchanged every other day. A confluent monolayer with formed tight junctions grew under these conditions and was then ready for experimentation at day 7.

5.4.8 Drug Release Studies: Measuring Drug Concentrations

Aliquots of $200\mu L$ (10 % of the total volume of the medium present in the bottom) were taken from the bottom of the *in vitro* models and replaced by fresh culture medium for both replenish and no-replenish models. The samples were taken at 1, 4, 8, and 12 hours, as well as an aliquot of the supernatant solution.

Table 5.2: Properties of the Contact Lens Hydrogel Materials Used in the Tear Replenishment System

Commercial name (**US adopted name**)	Acuvue 2 etafilcon A	Acuvue Oasys† senofilcon A	PureVision 2‡ balafilcon A
Manufacturer	Johnson & Johnson	Johnson & Johnson	Bausch & Lomb
Water content (WC)	58	38	36
Principal Monomer	HEMA + MA	mPDMS + DMA + HEMA + siloxane macromer + TEGDMA + PVP	NVP + TPVC + NVA + PBVC
FDA group*	IV High WC Ionic	V(I) Low WC Non-ionic	V(III) Low WC Ionic

* FDA (Food and Drug Administration) categorizes all silicone hydrogel contact lenses as group V, however it is more practical to use groups for conventional hydrogels to better understand their material properties. † Internal wetting agent (PVP) has been used to compensate for the low wettability of silicone hydrogels. ‡ Plasma oxidation process has been used as the surface treatment to increase wettability.
HEMA, HydroxyEthyl MethaAcrylate; MA, Methacrylic Acid; mPDMS, monofunctional Poly-DiMethylSiloxane; DMA, DiMethAcrylate; TEGDMA, Tetra-EthyleneGlycol DiMethAcrylate; PVP, Polyvinylpyrrolidone; NVP, N-Vinylpyrrolidone; TPVC, Tris(trimethylsiloxysilyl) Propyvinyl Carbamate; NVA, N-Vinyl Aminobutyric Acid; PBVC, Poly(dimethysiloxy)di (silylbutanol) Bis(Vinyl Carbamate)

Collected samples were analyzed by an enzyme immuno-assay (EIA) for latanoprost (Cayman Chemical, Ann Arbor, MI, USA). Following the EIA kit instructions, each collected sample was analyzed at four different dilutions. To determine the uptake amount by the contact lenses, samples were also aliquoted from the original drug stock solution as well as the remaining drug solutions after soaking the lenses.

The release results represent the concentration of the drug on the basal side of the CCMs, meaning that the drug has been released from the contact lens material on top of the membrane, then diffused through the cells and the cell culture insert's membrane. It should be noted that the EIA kit does not distinguish between the free-acid form and ester form of the drug.

5.4.9 Data Analysis

For all studies, a minimum of three experiments were performed on different dates. All viability results are expressed as relative viability compared to control cells, cells grown in the absence of a contact lens under no-replenish condition. Results are reported as the mean of at least three experiments ± standard deviation. To evaluate the significance of the differences in cell viability, an analysis of variance (ANOVA) was performed, followed by multiple pair-wise comparisons using the Holm-Sidak test and a pairwise comparison using T tests according to Sidak correction of Bonferroni inequality in SigmaPlot™.

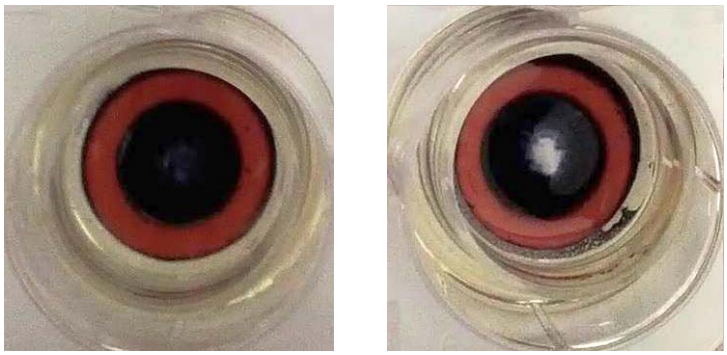
5.5 Results & Discussions

5.5.1 TRS 1.0 - Viability Studies

Initial experiments using the tear replenishment system v1.0 showed damaged to the *in vitro* cornea model due to the strong focused jet, which appeared to be washing cells from the membrane, as shown in Fig. 5.13. However, after increasing the nozzle diameter to $150\mu m$, exposing the stratified CCMs to the tear replenishment system did not result in any visible cell damage when compared to the immersion model (see Fig. 5.14).

This was further confirmed by the cell viability results; following exposure to replenishment conditions for up to 6 hours, in the absence of a lens, cell viability of the CCM was not affected as shown by a viability of $96 \pm 11\%$ compared to control (no lens, no-replenishment) ($p > 0.5$). Furthermore, no difference in viability was observed with the multilayer exposed to PBS-soaked lenses with or without replenishment (Fig. 5.15).

As a means to identify a positive control and to gain a better understanding of the potential effect of replenishment, BAK-soaked lenses were tested and used with the TRS to determine if the release of a cytotoxic compound from a contact lens on the stratified cornea model would be affected by the replenishment conditions. While no significant differences were observed at 2 h (Table 5.3), following a 6 h exposure, a significant reduction in viability was observed with BAK-soaked etafilcon A under both replenishment and static



(a) (b)

Figure 5.13: Initial results from Tear Replenishment System v1.0 with damaged cornea models a) tear replenishment in the presence of a contact lens, b) tear replenishment in the absence of contact lens.

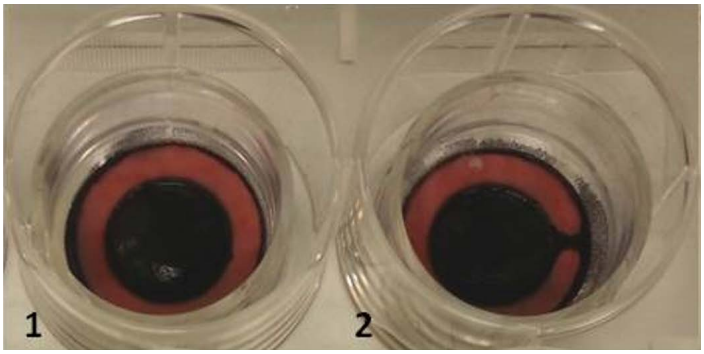


Figure 5.14: Visual confirmation of cell viability for "atomized jet based" TRS after modification to flow regime. CCMs stained with metabolic dye (MTT) following 2 hour incubation in the presence of tear replenishment (well 1) and in the absence of replenishment (well 2, well entirely immersed in solution). No damage to the multilayer can be observed following exposure to solution being sprayed on the surface intermittently.

immersion conditions (no replenishment, Fig. 5.15). Furthermore, tear replenishment had a significant effect on viability whereby exposure to BAK-soaked etafilcon A in a dynamic fluid-exchange system resulted in a significantly lower viability when compared to static/no replenishment conditions ($p = 0.03$). With BAK-soaked balafilcon A, a reduction in cell viability, albeit not statistically significant when compared to the no-lens and PBS-soaked balafilcon A control ($p > 0.1$) was observed and the tear replenishment had no effect ($p > 0.75$).

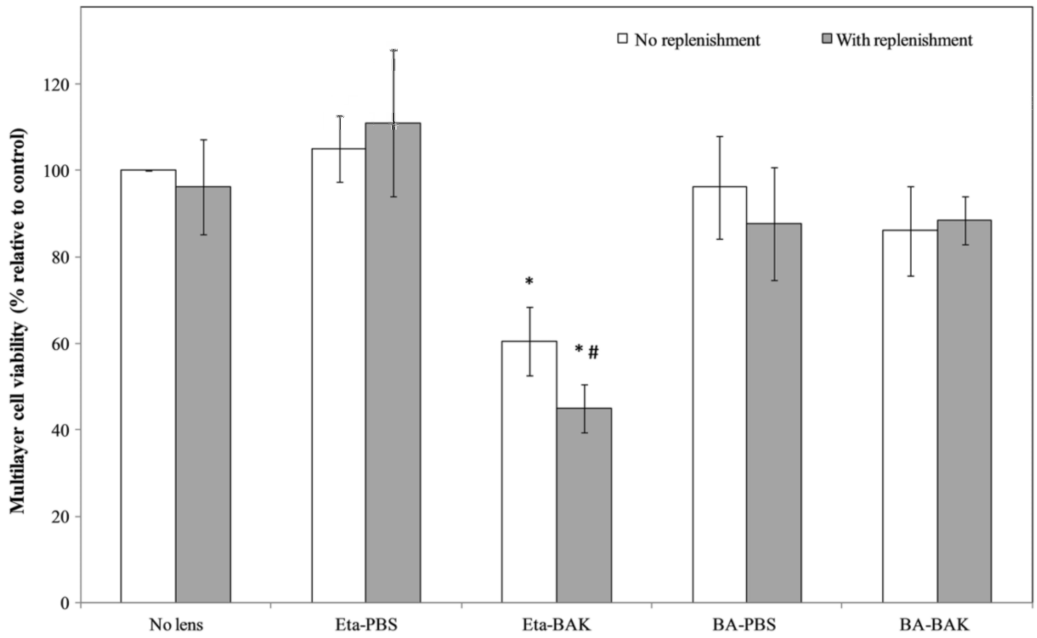


Figure 5.15: Effect of tear replenishment vs. immersion conditions (no replenishment) on corneal epithelial cell viability after 6 h incubation in TRS device. Viability was measured by MTT assay and is expressed as a percentage relative to *in vitro* cornea model without contact lens and spray. (n=3-4 ± standard deviation). *Significantly different from no lens and PBS-soaked lens control, ($p < 0.01$); #Significantly different from no replenishment sample, ($p = 0.03$). PBS, phosphate buffered-solution; BAK, benzalkonium chloride

5.5.2 TRS 1.0 - Viability Studies: Discussion

Results from both the no-lens control samples and the PBS-soaked lenses demonstrated that exposure to the tear flow from the TRS did not damage the stratified CCM and thus the TRS can be safely used to assess lens-cell interactions in a dynamic system. While BAK toxicity has been reported before, *in vitro* models have mostly been used to investigate the cytotoxicity of BAK solution alone [123, 124]. To our knowledge, this is the first time that HCEC were exposed to BAK-releasing materials. The lack of toxicity observed with BAK-soaked balafilcon A lenses compared to BAK-soaked etafilcon A highlights the difference in material properties and the ensuing difference in the mechanisms of BAK uptake and release. Similar results have been observed previously with differential uptake and release profiles of lens care solutions with different contact lens materials [42, 88].

In a dynamic system, if one were to compare with a static incubation *in vitro* model, two different mechanisms may occur: (1) the *in vitro* cytotoxic effect may not be as pronounced

Table 5.3: Effect of Tear Replenishment on Viability of Cells Exposed to BAK-soaked Lenses for 2h.

	No-Replenish† Viability (%)	Replenish Viability (%)	Test for with vs. without replenishment, p value
balafilcon A-PBS	106 ± 5	100 ± 10	1.00
balafilcon A-BAK	86 ± 10	86 ± 5	0.99
etafilcon A-PBS	101 ± 14	113 ± 15	1.00
etafilcon A-BAK	80 ± 15	76 ± 18	0.54

n=3-4 mean ± standard deviation

† cells-contact lens system was fully immersed in solution for 2 hours.

compared to the static incubation model; this would be due to the dilution of the toxic substance, not accumulating, and being washed away and removed by the TRS, as it would be in tears, or (2) cytotoxicity may increase due to an increase in concentration of the toxic substance being released into a much smaller residual tear liquid, especially between the contact lens and the corneal cells, as it might be the case in tear film between the epithelium and the contact lens. The latter mechanism was confirmed by results from a 6h exposure to BAK-soaked etafilcon A whereby a statistically significant difference in cell viability between replenishment vs. immersion model was observed. Interestingly, the BAK-soaked balafilcon A lens did not act as a strong cytotoxic stimulus to cells, which in turn made it difficult to identify differences between dynamic vs. static conditions. The surface treatment of balafilcon A associated with the amphiphilicity of BAK is likely responsible for the limited uptake (and then release of BAK) [42, 118], which makes this BAK-lens combination of limited use when investigating the effects of tear replenishment on cytotoxicity.

The experiments demonstrated that the implemented design to mimic tear replenishment can deliver tear fluid to the stratified curved cornea models at a flow rate and frequency that are similar to the human eye. Due to the static nature of the current *in vitro* models, accumulation of the toxic agents in the culture medium may occur; thus, the release profile may also be affected by the lack of replenishment. In the dynamic sys-

tem presented with the tear replenishment, we demonstrated that replenishment can be achieved without damaging the integrity of the stratified cell culture, that the replenished conditions allowed the contact lenses to remain moist at all times without requiring immersion in solution and that tear replenishment may have significant effect on measured outcomes (here cytotoxicity).

5.5.3 TRS 2.0 - Drug Release Studies

The total drug uptake was calculated by measuring the remaining drug in the doping solution and the drug doping solution. The stock solution concentration was measured at $123.4 \pm 12.73 \mu g/mL$. It is important to note that the intended concentration of of doping solution was $50 \mu g/mL$ to match the prescribed dose of commercial eye-drops. However, the reported drug amount by the supplier (Cayman Chemical, Ann Arbor, MI), which was used as the basis for doping solution preparation, deemed to be incorrect when measuring the stock solution concentration. Table 5.4 details the uptake results. While the concentration of drug doping solution used for the tear replenishment experiments was comparable to the release studies conducted in the previous chapter, the smaller doping solution volume ($1mL$ vs. $1.5mL$) resulted in lower total drug available for uptake.

Table 5.4: Latanoprost Uptake Results into Three Commercial Contact Lens Materials

Contact Lens Material	**senofilcon A**	**balafilcon A**	**etafilcon A**
Drug remaining in doping solution (μg)	0.163 ± 0.048	0.178 ± 0.048	3.713 ± 0.846
Drug Uptake[$\mu g/lens$]	123.24	123.23	119.7
Uptake as a percentage of available drug(%)	99.9	99.9	97.0

n=4, Mean ± Standard Deviation.
Lenses were soaked for 24 hours in $1mL$ of drug doping solution ($123.4 \mu g/mL$). Latanoprost concentrations were measured using EIA.

5.5.3.1 TRS v2.0 - Cell Viability

Cell viability studies were conducted to verify the integrity of the monolayer CCMs after the drug release experiments. A significantly damaged cornea model would invalidate the obtained release results. No visible damage to the cornea models was observed after 12h of drug release experiments. This was confirmed by the viability assay (see Fig. 5.16). The viability remained above 80% in all cases. No significant difference in viability was observed among the lens materials ($p > 0.4$). While the viability was significantly less under replenish conditions compared to no-replenish ($p = 0.002$), this was likely due to the removal of loosely attached cells under dynamic flow conditions.

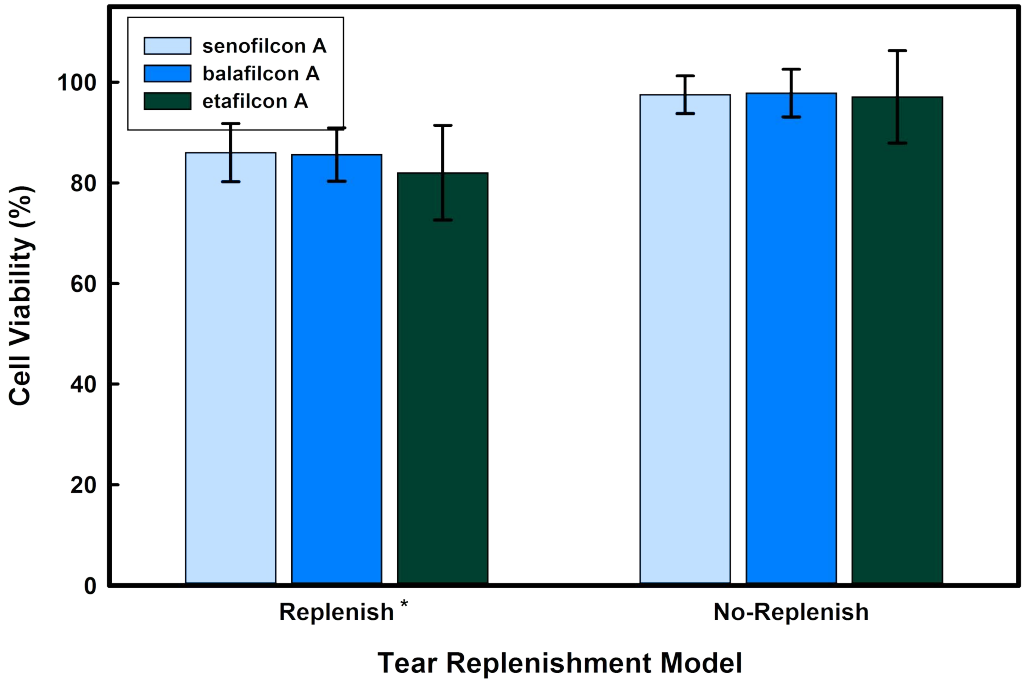


Figure 5.16: Effect of replenishment on corneal epithelial cell viability after 12 hour release study with and without tear replenishment. Viability was measured by MTT assay and is expressed as a percentage relative to *in vitro* CCM without replenishment. The results represent the mean of three experiments (n=3 ± standard deviation). * Significantly different from no-replenish ($p = 0.002$). PBS, phosphate buffered-solution; BAK, benzalkonium chloride

5.5.3.2 TRS 2.0 - Latanoprost Apical Release

The apical release of the drug in the immersion model is defined as the amount of drug released from the contact lens into the supernatant tear solution on the apical side of the CCM ($2.5mL$). In the replenish model, the apical release is defined as the amount of the drug released from the contact lens and collected in the supernatant drainage module ($12mL$ total volume). In the human eye, the drug released from an ophthalmic material is first dissolved in the tear film with a limited volume before it diffuses through the cornea. Comparing the apical release between replenish and immersion (no-replenish) models allowed to gain a better understanding of the role that tear replenishment might play in the human eye. The apical release results, as depicted in Fig. 5.17, showed that significantly more latanoprost was dissolved in the supernatant in the immersion model compared to the replenish model ($p < 0.001$), despite significantly smaller supernatant liquid volume ($2.5mL$).

Furthermore, the apical release of latanoprost from etafilcon A was significantly higher compared to the silicone hydrogels ($p < 0.005$), while there was no difference between silicone hydrogels. This is consistent with the results from the previous chapter, where a significantly higher amount of drug release was observed from high water content hydrogel contact lenses such is the case for etafilcon A. This can be attributed to the bulk properties of such materials and their relatively lower affinity towards highly hydrophobic compounds such as latanoprost (see discussion).

5.5.3.3 TRS 2.0 - Release Results

Previous studies, presented in Chapter 4, proved the crucial role of live cells in the latanoprost's release from contact lens material and its diffusion through the cornea model. Those studies were performed utilizing the immersion model. Drug release studies using the TRS allowed us to gain an insight into the role of tear replenishment in drug release on the basal side of the CCMs. The release results, as illustrated in Fig. 5.18, showed a linear increase for all contact lens materials on the basal side in both immersion and replenish models. The drug concentration on the basal side was similar in both models ($p > 0.2$),

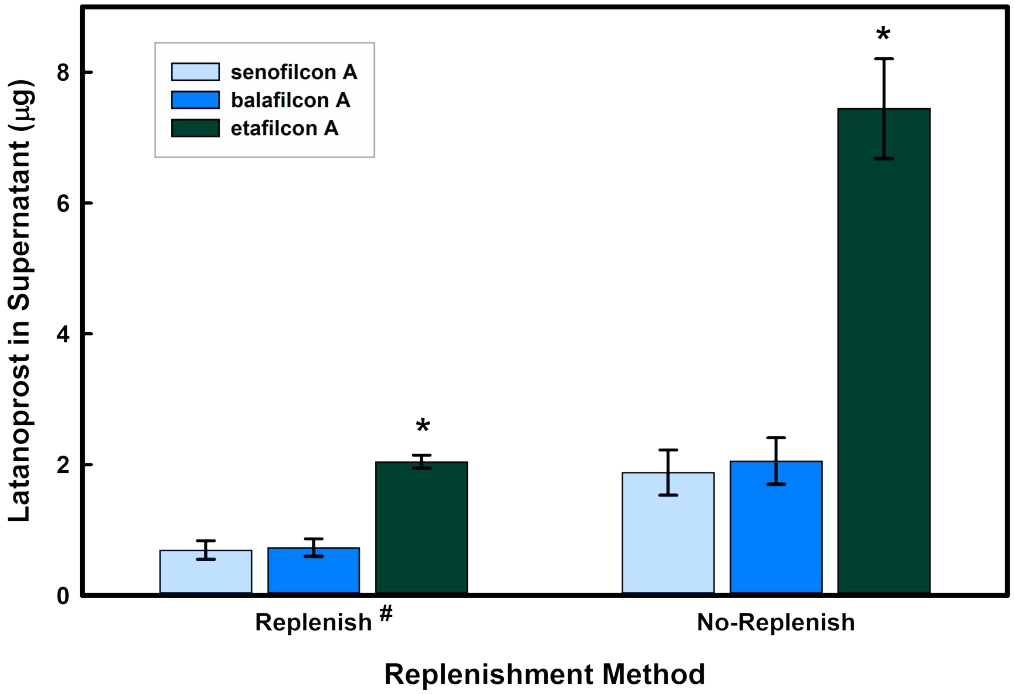


Figure 5.17: Effect of replenishment on the latanoprost release from three contact lens materials in the monolayer CCMs. Lenses were soaked for 24 hours in drug solution ($123.4\mu g/mL$) and then onlayed on the CCM for 12 hours. Aliquots were taken at the end of the experiment from the apical side of the models without replenishment and from the supernatant container for the replenished models. Then concentrations were measured using EIA. # Significantly different from no-replenish model ($p < 0.001$). *Significantly different from silicone hydrogels ($p < 0.005$). (n=3, mean ± standard deviation).

and the amount of latanaprost on the basal side was comparable to the prescribed dosage. The different release profiles for the various contact lens materials were consistent with our previous results [115].

5.5.4 TRS 2.0 - Discussion

This study aimed to assess the impact of modeling tear replenishment *in vitro* for drug delivery studies from ophthalmic materials. The curved cornea models used in these experiments have proven to be an effective platform for understanding biocompatibility of contact lens material and lens cleaning solutions [78, 88, 119], which can further be used with a dynamic replenishment model. The results highlight the importance of *in vitro*

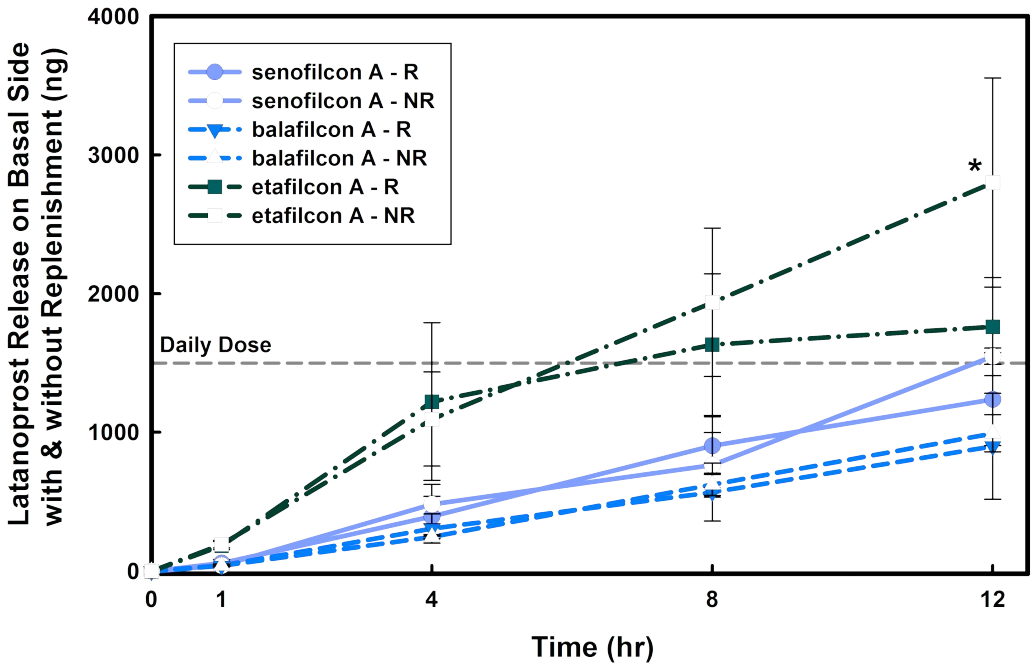


Figure 5.18: Effect of replenishment on the latanoprost release from three contact lens materials through the CCMs on the basal side. Lenses were soaked for 24 hours in drug solution ($123\mu g/mL$) and then onlayed on the CCM for 12 hours. Aliquots were taken at specific times from the basal side and concentrations were measured using EIA. Daily dose line represents the amount of the administered latanoprost for a glaucoma patient [27]. No significant difference was observed when comparing the latanoprost release from the contact lens material and diffusion through the model to the basal side between replenish (R) and no-replenish (NR, immersion) models ($p > 0.2$). *Release from etafilcon A in no-replenish model was significantly higher at 12 h ($p < 0.001$). (n=3, Mean ± SD).

experimental cornea models allowing continuous replenishment at the surface of the cell culture model for ocular drug delivery platforms in predicting the release profile and total amount of intraocular drug diffusion under physiological conditions.

It is important to note that the drug release studies were performed on a monolayer CCM rather than a stratified model. "Natural" cell sloughing in a dynamic replenished environment is promoted, whereby in a stratified model, the underlying cell layer will contribute to the regeneration of the superficial layer. However, in a monolayer model, due to lack of a basal cell layer, this cannot happen, which will then result in a slightly reduced viability. Thus, the observed decrease in viability, while statistically significant, is unlikely

to be biologically significant for this study.

In case of drug release studies in a dynamic system, in comparison to the immersion *in vitro* model, two different mechanisms may occur:

(1) the *in vitro* drug release may be elevated compared to the immersion model; this would be due to the high solubility of the drug in the tear solution, and being washed away and drained by the TRS, as it would be in tears through the lacrimal system, or

(2) the drug release may decrease due to the low solubility of the drug in the tear solution resulting in lower concentration of the drug being released into a much smaller residual tear liquid, especially between the contact lens and the corneal cells, as it might be the case in the tear film between the epithelium and the contact lens.

The first mechanism may be the more dominant effect for hydrophilic ophthalmic drugs while the second mechanism would be the predominant effect for hydrophobic compounds such as latanoprost.

To provide a better comparison between the apical and and the basal release results at 12 hour, for the three commercial contact lenses, the results were normalized to the total drug uptake into each lens material. This analysis is presented in Fig. 5.19, and shows that the total drug release from silicone hydrogels (balafilcon A and senofilcon A) only accounts for less than 2% in the replenish model and less than 3% in the immersion model. The considerably high drug uptake and low drug release from the silicone hydrogel lenses can be attributed to the higher affinity of the hydrophobic latanoprost to the hydrophobic silicone hydrogel material (as would be the case with mechanism #2). Comparing the results obtained using the two models showed the dominant effect of the hydrophobic-hydrophobic interactions between the drug and the contact lens material resulting in a reduced apical drug loss into the supernatant. Etafilcon A showed marginally less affinity toward latanoprost when compared to the silicone hydrogels (see Table. 5.4). This resulted in increased drug release on the apical and diffusion into the basal sides.

The significantly elevated latanoprost release on the apical side in the immersion model is in spite of the considerably lower volume of supernatant fluid in the immersion model ($2.5mL$ vs. $12mL$). This phenomenon can be understood more extensively if one were to

consider the role that live cells play in hydrolyzing the prodrug. Due to the hydrophobicity of the latanoprost, it may easily penetrate through the transcellular pathways into corneal cells where it will be hydrolyzed into the free-acid form. The free-acid drug may leave the cell membrane to either the apical or basal side. The limited post contact lens tear film volume in the replenished model would inhibit the apical diffusion of the latanoprost free-acid, while in the immersion model, the apical release would be promoted.

The latanoprost prodrug will be hydrolyzed upon entry into the cell due to the abundance of esterase enzymes. While latanoprost may diffuse to either side of the cell layer, the diffusion to the basal side may slightly decrease due to both the cell layer and the cell culture membrane. This may explain the higher apical release in the no-replenish model. While small differences might be justified by this mechanism, the significantly higher apical release in etafilcon A requires further investigation. As discussed in section 3.4.5, the highly hydrophobic rose bengal showed a significantly larger release. Latanoprost is also a highly hydrophobic drug, that could easily release from etafilcon A and dissolve in the apical solution in the ester form. This might explain the elevated apical release of latanoprost from etafilcon A in no-replenish model. However as mentioned before, the EIA kit used in these studies does not distinguish between the two drug forms and thus it will be difficult to verify such a hypothesis.

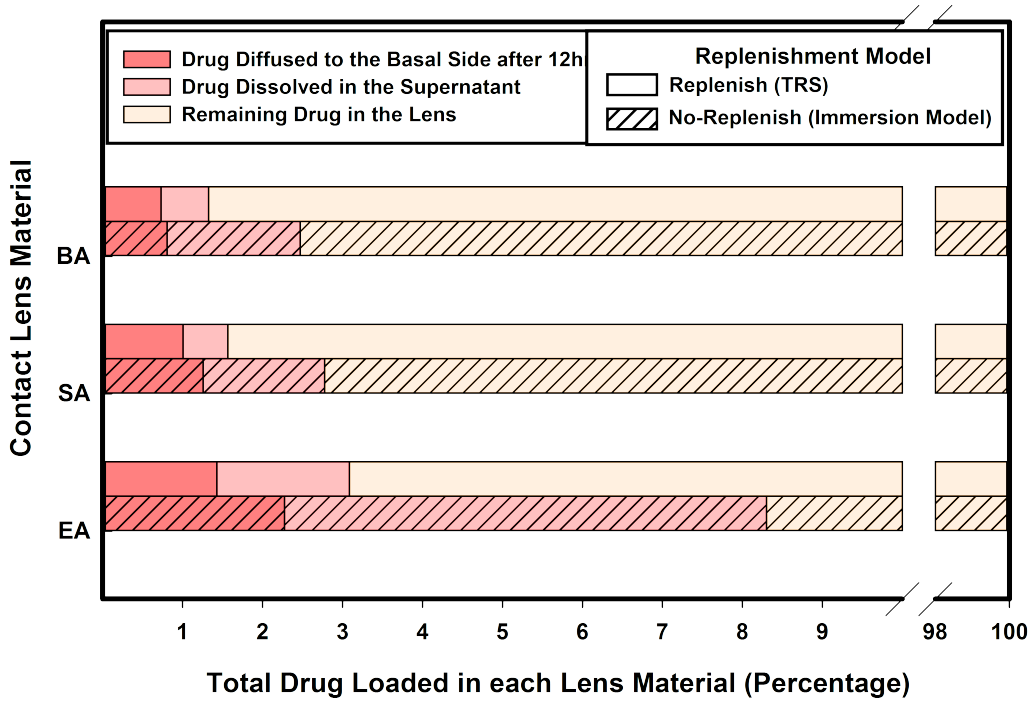


Figure 5.19: Effect of replenishment on the latanoprost release from three contact lens materials on both basal and apical sides of the monolayer CCMs. The ratios for each contact lens material were calculated based on the total drug uptake into each lens. No significant difference was observed when comparing the latanoprost release from the contact lens material and diffusion through the model to the basal side between replenish and no-replenish model. However, etafilcon A showed more latanoprost release to the basal side when compared to the two silicone hydrogel materials ($p < 0.001$). (n=3, Mean ± SD).

5.6 Conclusion

Through modeling the microfluidics of the tear replenishment *in vitro*, the TRS offers an ocular drug delivery testing platform with a reliable and continuous replenishment of the surface of the cell culture model that can contribute to a better understanding of corneal cell-lens interactions *in vitro*. The latanoprost release studies, as discussed in chapter 4, further confirmed the significant role that cells play in the release of an ocular prodrug from a contact lens material. The results presented in this chapter demonstrated yet another important role that a dynamic release model will have in predicting the amount of drug that can be lost from a contact lens into tear film/lacrimal system.

The *in vivo* dynamic environment provided by tear replenishment impacts ocular in-

flammation, drug delivery to the eye, and lens cleaning solution biocompatibility. While in the current study, the tear replenishment system was used to investigate drug eluting contact lenses, a more physiologically relevant *in vitro* ocular surface model can prove valuable in biocompatibility studies for future drugs and ophthalmic materials. This model provides one of the first approaches to study these effects in the presence of cells.

Chapter 6

Contributions and Future Work

6.1 Scientific Contributions

A need for an organotypic culture system that can better model the physiological conditions of the front of the eye was identified. This thesis aimed to answer three specific questions:

1. Does recreating the limited tear volume as well as the continuous tear replenishment in studies of drug eluting contact lenses provide any unique insights to the release mechanisms, that might be otherwise neglected or overlooked?

2. Does incorporating human corneal cells into an *in vitro* drug release model create a meaningful impact in predicting the release of the drug from a contact lens material, that could offset the high complexity and cost associated with operating a cell culture facility?

3. And last but not least, if the answer to the questions above is yes, does integration of a dynamic fluid exchange, modeling the tear replenishment in the human eye, with a cell culture system, modeling the human cornea, allow investigation of release of ophthalmic drugs from contact lens materials in a robust and reliable fashion?

The methods and results presented in Chapters 3, 4 and 5 of this thesis provided compelling evidence and ample support to answer each of those questions positively. In

relation to the questions this thesis aimed to answer, the specific contributions of this research can be described as follows.

1. The Dynamic Drug Release Model (DDRM) is a novel testing platform, specifically designed to test drug delivery contact lens. With its ability to test four contact lenses simultaneously in a reusable and bio-inert chamber, the DDRM provides a unique, cost-efficient and acellular drug release testing platform, capable of reproducing the microfluidic environment of the ocular surface reliably. The systematic approach to the design of the platform not only improves reliability but also enables the use of this platform by researchers to perform studies on both commercially available and custom-made contact lenses. Surrogate drug release experiments performed using the DDRM validated the suitability of this testing platform for the study of release from contact lenses. Using ophthalmic dyes as surrogate drugs with silicone and conventional hydrogel lenses, this study is the first to directly compare drug release under passive (fixed volume), passive with cells and dynamic (DDRM) conditions.

2. While neither stratified cornea models nor the idea of using latanoprost release from a contact lens are novel, no ophthalmic drug release study had yet investigated the impact that the presence of cells would have on drug release. The latanoprost release study, published in PLOS ONE in 2014 [115], was performed using *in vitro* cell models and demonstrated for the first time the significant role that cells play in drug delivery study of ophthalmic materials, especially in the case of prodrugs.

3. The curved cornea model, developed by the MIBS lab, closely mimics the geometry and physiology of the corneal epithelium, and is uniquely positioned to be integrated into a tear replenishment system. The first prototype of the tear replenishment system (TRS) was the first proof of concept which demonstrated that a curved stratified corneal epithelial cell culture could (1) reliably be exposed to intermittent flow, mimicking the tear replenishment, and (2) wear a whole contact lens that would remain hydrated -due to the presence of intermittent flow - for at least 6 hours. Results from this study published in the Annals of Biomedical Engineering in 2014 also highlighted

the fact that tear replenishment affects release and hence potential cytotoxicity of ophthalmic solutions [119]. In a second iteration, the TRS was further improved to allow for longer incubation time and separate collection of the supernatant/apical drug release from each well. The new TRS design continues to preserve the air-liquid interface of the stratified culture while mimicking tear replenishment conditions; this is a novel and unique ocular cell model. Using contact lenses releasing latanoprost, the TRS allowed to compare for the first time drug release mechanisms in the presence of cells under dynamic and static conditions and highlighted the rate-limiting barrier created by the cells as well as the amount of drug that can be lost into the supernatant/"tear film".

6.2 Recommendations for Future Work

Recommendations relate to both the dynamic drug release model and the tear replenishment system.

The goal of designing the dynamic drug release model was to provide a cost efficient, open hardware/ open software instrument that can be adopted, modified and improved by other researchers for the purpose of dynamic drug release studies from drug eluting materials. While some may use commercially available contact lenses to study drug release, others might synthesize a novel hydrogel. To address the needs of different research groups, it is recommended that the design and the specifications of this system be published, ensuring open access to all researchers.

Furthermore, this design can be modified into a standardized high-throughput system, through which a large range of hydrogels and dye combinations can be screened rapidly to identify the best possible candidates for drug eluting materials.

While characterization of drug delivery devices can benefit from a more realistic ocular surface model, there is a much larger need to study the biocompatibility of ophthalmic materials such as new contact lenses, or multipurpose solutions and their interactions, or understanding the mechanism of corneal staining. It is recommended to employ the

tear replenishment system in investigating these possible interactions to gain a better understanding of the mechanisms involved in cell biocompatibility.

While the implemented experimental models took into consideration the microfluidic environment of the ocular surface and cells, these models do not reproduce the mechanical stimulation that is created from the movement of the eyelid through blinking. In an *in vitro* model of the ocular surface, this mechanical stimulation would be important as it allows some movement of the lens and thus promote tear exchange in the post-lens tear film and it may also affect drug release. While it may be difficult to add a moving component to simulate blinking in the TRS, it is recommended that efforts be undertaken to assess the current exchange of fluid between the lens and the curved corneal epithelial cell culture and further to improve the current design to promote fluid exchange at the back of the lens.

Most *in vitro* studies have taken place under static conditions. The TRS also offers new possibilities to investigate the effect of a dynamic environment in wound healing, inflammation and infection.

References

[1] B. Sjöquist, S. Basu, P. Byding, K. Bergh, and J. Stjernschantz. The pharmacokinetics of a new antiglaucoma drug, latanoprost, in the rabbit. *Drug Metabolism and Disposition*, 26(8):745–754, 1998.

[2] D. L. O. Wichterle. Hydrophilic gels for biological use. *Nature*, 185(4706):117–118, 1960.

[3] O. Wichterle and D. Lim. Cross-linked hydrophilic polymers and articles made therefrom. *US Patent RE27,401*, page 4, 1965.

[4] J. Sedlacek. Possibilities of application of ophthalmic drugs with the aid of gel-contact lenses. *Ceska a Slovenska Oftalmologie*, 21(6):509–512, 1965.

[5] C. J. White and M. E. Byrne. Molecularly imprinted therapeutic contact lenses. *Expert opinion on drug delivery*, 7(6):765–780, 2010.

[6] J. B. Ciolino, C. H. Dohlman, and D. S. Kohane. Contact lenses for drug delivery. *Seminars in Ophthalmology*, 24(3):156–60, 2009.

[7] C. C. Li and A. Chauhan. Modeling ophthalmic drug delivery by soaked contact lenses. *Industrial and Engineering Chemistry Research*, 45(10):3718–3734, 2006.

[8] D. M. Lehmann and M. E. Richardson. Impact of assay selection and study design on the outcome of cytotoxicity testing of medical devices: The case of multi-purpose vision care solutions. *Toxicology in Vitro*, 24(4):1306–1313, 2010.

[9] I. A. Maltseva, S. M. J. Fleiszig, D. J. Evans, S. Kerr, S. S. Sidhu, N. A. McNamara, and C. Basbaum. Exposure of human corneal epithelial cells to contact lenses *in vitro* suppresses the upregulation of human beta-defensin-2 in response to antigens of Pseudomonas aeruginosa. *Experimental Eye Research*, 85(1):142–153, 2007.

[10] M. Gorbet and C. Postnikoff. The impact of silicone hydrogel-solution combinations on corneal epithelial cells. *Eye & Contact Lens*, 39(1):42–47, 2013.

[11] R. Schoenwald. Ocular Pharmacokinetics. In *Textbook of Ocular Pharmacology*, pages 119–138. Springer Verlag, Berlin, m.l. sears edition, 1997.

[12] M. Araie and D. Maurice. The rate of diffusion of fluorophores through the corneal epithelium and stroma. *Experimental Eye Research*, 44(1):73–87, 1987.

[13] T. Saude. *Ocular Anatomy and Physiology*, volume 71 of *Basic Bookshelf for Eyecare Professionals Series*. Slack, 1994.

[14] J. Barar, A. R. Javadzadeh, and Y. Omidi. Ocular novel drug delivery: impacts of membranes and barriers. *Expert Opinion on Drug Delivery*, 5(5):567–581, 2008.

[15] K. B. Green-Church, I. Butovich, M. Willcox, D. Borchman, F. Paulsen, S. Barabino, and B. J. Glasgow. The international workshop on meibomian gland dysfunction: Report of the subcommittee on tear film lipids and lipid-protein interactions in health and disease. *Investigative Ophthalmology & Visual Science*, 52(4):1979–1993, 2011.

[16] M. Babizhayev. Ocular drug metabolism of the bioactivating antioxidant N-acetylcarnosine for vision in ophthalmic prodrug and codrug design and delivery. *Drug Development and Industrial Pharmacy*, 34(10):1071–1089, 2008.

[17] (World Health Organization). Global Initiative for the Elimination of Avoidable Blindness: action plan 2006-2011, 2007.

[18] (World Health Organization). Action Plan for the prevention of avoidable blindness and visual impairment, 2009.

[19] S. Kingman. Glaucoma is second leading cause of blindness globally. *Bulletin of the World Health Organization*, 82(11):887–888, 2004.

[20] U.S. Ophthalmologists' Assessment of Eye Care Medication Brands Brand Perceptions of Glaucoma, Age-Related Macular Degeneration, Allergic Conjunctivitis, and NSAID Medications. Technical report, Frost & Sullivan, 2010.

[21] C. B. Toris, B. T. Gabelt, and P. L. Kaufman. Update on the Mechanism of Action of Topical Prostaglandins for Intraocular Pressure Reduction. *Survey of Ophthalmology*, 53(6 SUPPL.):S107–20, 2008.

[22] D. Toscano, D. Ruppar, J. Brice, and G. Caressi. Analysis of the United States Glaucoma Pharmaceuticals Market Significant Opportunity Available to Address Unmet Needs. Technical report, Frost & Sullivan, 2012.

[23] M. Starr. Effects of Prostaglandin on Blood Flow in the Rabbit Eye. *Experimental Eye Research*, 11(2):161–169, 1971.

[24] G. Giuffrè. The effects of prostaglandin F2alpha in the human eye. *Graefe's Archive for Clinical and Experimental Ophthalmology*, 222(11):139–141, 1985.

[25] C. Xiang, M. Batugo, D. Gale, and T. Zhang. Characterization of human corneal epithelial cell model as a surrogate for corneal permeability assessment: metabolism and transport. *Drug Metabolism and Disposition*, 37(5):992–998, 2009.

[26] O. Camber, P. Edman, and L.-I. Olsson. Permeability of prostaglandin F2-alpha and prostaglandin across cornea *in vitro* F2-alpha esters. *International Journal of Pharmaceutics*, 29:259–266, 1986.

[27] B. Shah, V. Arora, M. Wadhwani, and S. K. Mishra. Prostaglandin analogs. *Journal of Current Glaucoma Practice*, 5(2):15–20, 2011.

[28] A. Russo, I. Riva, T. Pizzolante, F. Noto, and L. Quaranta. Latanoprost ophthalmic solution in the treatment of open angle glaucoma or raised intraocular pressure: a review. *Clinical Ophthalmology*, 2(4):897–905, 2008.

[29] T. Zhang, C. D. Xiang, D. Gale, S. Carreiro, E. Y. Wu, and Y. Eric. Drug Transporter and CYP P450 mRNA Expression in Human Ocular Barriers: Implications for Ocular Drug Disposition. *Drug Metabolism and Disposition*, 36(7):1300–1307, 2008.

[30] E. Mannermaa, K. S. Vellonen, and A. Urtti. Drug transport in corneal epithelium and blood-retina barrier: Emerging role of transporters in ocular pharmacokinetics. *Advanced Drug Delivery Reviews*, 58(11):1136–1163, 2006.

[31] S. Duvvuri, S. Majumdar, and A. K. Mitra. Role of metabolism in ocular drug delivery. *Current Drug Metabolism*, 5(6):507–15, 2004.

[32] M. E. Kraft, H. Glaeser, K. Mandery, J. König, D. Auge, M. F. Fromm, U. Schlötzer-Schrehardt, U. Welge-Lüssen, F. E. Kruse, and O. Zolk. The prostaglandin transporter OATP2A1 is expressed in human ocular tissues and transports the antiglaucoma prostanoid latanoprost. *Investigative Ophthalmology & Visual Science*, 51(5):2504–2511, 2010.

[33] K. M. Maxey, J. L. Johnson, and J. LaBrecque. The hydrolysis of bimatoprost in corneal tissue generates a potent prostanoid FP receptor agonist. *Survey of Ophthalmology*, 47(4 SUPPL. 1):S34–40, 2002.

[34] B. R. Conway. Recent patents on ocular drug delivery systems. *Recent Patents on Drug Delivery and Formulation*, 2(1):1–8, 2008.

[35] B. Booth, L. Vidal Denham, S. Bouhanik, J. T. Jacob, and J. M. Hill. Sustained-release ophthalmic drug delivery systems for treatment of macular disorders: present and future applications. *Drugs & Aging*, 24(7):581–602, 2007.

[36] K. Järvinen, T. Järvinen, and A. Urtti. Ocular absorption following topical delivery. *Advanced Drug Delivery Reviews*, (16):3–19, 1995.

[37] A. H. Wander. Long-term use of hydroxypropyl cellulose ophthalmic insert to relieve symptoms of dry eye in a contact lens wearer: case-based experience. *Eye & Contact Lens*, 37(1):39–44, 2011.

[38] Y. E. Choonara, V. Pillay, M. Danckwerts, T. R. Carmichael, and L. C. du Toit. A review of implantable intravitreal drug delivery technologies for the treatment of posterior segment eye diseases. *Journal of Pharmaceutical Sciences*, 99(5):2219–2239, 2010.

[39] E. Lavik, M. H. Kuehn, and Y. H. Kwon. Novel drug delivery systems for glaucoma. *Eye*, 25(5):578–586, 2011.

[40] J. Sangster. *Octanol-Water Partition Coefficients: Fundamentals and Physical Chemistry*. John Wiley & Sons, Incorporated, 1 edition, 1997.

[41] D. A. Porter, K. E. Easterling, and M. Sherif. *Phase Transformations in Metals and Alloys*. Chapman & Hall, 2nd edition, 2009.

[42] C. H. Powell, J. M. Lally, L. D. Hoong, and S. W. Huth. Lipophilic versus hydrodynamic modes of uptake and release by contact lenses of active entities used in multipurpose solutions. *Contact Lens and Anterior Eye*, 33(1):9–18, 2010.

[43] A. Mahomed and B. J. Tighe. The design of contact lens based ocular drug delivery systems for single-day use: Part (I) Structural factors, surrogate ophthalmic dyes and passive diffusion studies. *Journal of Biomaterials Applications*, 29(3):341–353, 2014.

[44] N. Tabuchi, T. Watanabe, M. Hattori, K. Sakai, H. Sakai, and M. Abe. Adsorption of actives in ophthalmological drugs for over-the-counter on soft contact lens surfaces. *Journal of Oleo Science*, 58(1):43–52, 2009.

[45] A. Mahomed, J. S. Wolffsohn, and B. J. Tighe. Structural design of contact lens-based drug delivery systems; *in vitro* and *in vivo* studies of ocular triggering mechanisms. *Contact Lens and Anterior Eye*, 39(2):97–105, 2016.

[46] A. Tieppo, K. M. Pate, and M. E. Byrne. *In vitro* controlled release of an anti-inflammatory from daily disposable therapeutic contact lenses under physiological ocular tear flow. *European Journal of Pharmaceutics and Biopharmaceutics*, 81(1):170–177, 2012.

[47] P. L. Ritger and N. A. Peppas. A simple equation for description of solute release I. Fickian and non-fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. *Journal of Controlled Release*, 5(1):23–36, 1987.

[48] A. Tieppo, A. C. Boggs, P. Pourjavad, and M. E. Byrne. Analysis of release kinetics of ocular therapeutics from drug releasing contact lenses: best methods and practices to advance the field. *Contact Lens and Anterior Eye*, 37(4):305–313, 2014.

[49] C. White, A. Tieppo, and M. Byrne. Controlled drug release from contact lenses: a comprehensive review from 1965-present. *Journal of Drug Delivery Science and Technology*, 21(5):369–384, 2011.

[50] C.-M. Phan, A. Hui, L. Subbaraman, and L. W. Jones. Insights to using contact lenses for drug delivery. *Clinical & Experimental Pharmacology*, 4(1):1–4, 2013.

[51] C. C. S. Karlgard, N. S. Wong, L. W. Jones, and C. Moresoli. *In vitro* uptake and release studies of ocular pharmaceutical agents by silicon-containing and p-HEMA hydrogel contact lens materials. *International Journal of Pharmaceutics*, 257(1-2):141–151, 2003.

[52] C. C. Peng and A. Chauhan. Extended cyclosporine delivery by silicone-hydrogel contact lenses. *Journal of Controlled Release*, 154(3):267–274, 2011.

[53] V. P. Costa, M. E. M. Braga, J. P. Guerra, A. R. C. Duarte, C. M. M. Duarte, E. O. B. Leite, M. H. Gil, and H. C. de Sousa. Development of therapeutic contact lenses using a supercritical solvent impregnation method. *Journal of Supercritical Fluids*, 52(3):306–316, 2010.

[54] J. Hillman, J. B. Marsters, and A. Broad. Pilocarpine delivery by hydrophilic lens in the management of acute glaucoma. *Transactions of the Ophthalmological Societies of the United Kingdom*, 95(1):79–84, 1975.

[55] A. Vaughan, J. Zhang, and M. Byrne. Enhancing Therapeutic Loading and Delaying Transport via Molecular Imprinting and Living/ Controlled Polymerization. *American Institute of Chemical Engineers*, 56(1):268–279, 2009.

[56] C. Alvarez-Lorenzo, F. Yañez, R. Barreiro-Iglesias, and A. Concheiro. Imprinted soft contact lenses as norfloxacin delivery systems. *Journal of Controlled Release*, 113(3):236–244, 2006.

[57] F. Yañez, L. Martikainen, M. E. M. Braga, C. Alvarez-Lorenzo, A. Concheiro, C. M. M. Duarte, M. H. Gil, and H. C. De Sousa. Supercritical fluid-assisted preparation of imprinted contact lenses for drug delivery. *Acta Biomaterialia*, 7(3):1019–1030, 2011.

[58] C. C. Peng, J. Kim, and A. Chauhan. Extended delivery of hydrophilic drugs from silicone-hydrogel contact lenses containing Vitamin E diffusion barriers. *Biomaterials*, 31(14):4032–4047, 2010.

[59] J. Kim, C. C. Peng, and A. Chauhan. Extended release of dexamethasone from silicone-hydrogel contact lenses containing vitamin E. *Journal of Controlled Release*, 148(1):110–116, 2010.

[60] M. J. Taylor, S. Tanna, and T. Sahota. *In vivo* study of a polymeric glucose-sensitive insulin delivery system using a rat model. *Journal of pharmaceutical sciences*, 99(10):4215–4227, 2010.

[61] M. Ali, S. Horikawa, S. Venkatesh, J. Saha, J. W. Hong, and M. E. Byrne. Zero-order therapeutic release from imprinted hydrogel contact lenses within *in vitro* physiological ocular tear flow. *Journal of Controlled Release*, 124(3):154–162, 2007.

[62] C.-M. Phan, M. Bajgrowicz, H. Gao, L. Subbaraman, and L. W. Jones. Release of fluconazole from contact lenses using a novel *in vitro* eye model. *Investigative Ophthalmology & Visual Science*, 93(4):387–394, 2016.

[63] M. Bajgrowicz, C.-M. Phan, L. N. Subbaraman, and L. Jones. Release of ciprofloxacin and moxifloxacin from daily disposable contact lenses from an *in vitro* eye model. *Investigative Ophthalmology & Visual Science*, 56(4):2234–2242, 2015.

[64] H. Lorentz, M. Heynen, L. M. M. Kay, C. Y. Dominici, W. Khan, W. W. S. Ng, and L. Jones. Contact lens physical properties and lipid deposition in a novel characterized artificial tear solution. *Molecular vision*, 17(9):3392–405, 2011.

[65] H. Lorentz and M. Heynen. Using an *in vitro* model of lipid deposition to assess the efficiency of hydrogen peroxide solutions to remove lipid from various contact lens materials. *Current Eye Research*, 37(9):777–786, 2012.

[66] S. Reichl, C. Kölln, M. Hahne, and J. Verstraelen. *In vitro* cell culture models to study the corneal drug absorption. *Expert Opinion on Drug Metabolism & Toxicology*, 7(5):559–578, 2011.

[67] H.-S. Huang, R. D. Schoenwald, J. L. Lach, H.-S. Huang, and R. D. Schoenwald. Corneal penetration behavior of β-blocking agents: II - assessment of barrier contributions. *Journal of Pharmaceutical Sciences*, 72(11):1272–1279, 1983.

[68] H.-S. Huang, R. D. Schoenwald, and J. L. Lach. Corneal Penetration Behaviour of beta-blocking agents III: *In Vitro-In Vivo* Correlations. *Journal of Pharmaceutical Sciences*, 72(11):1279–1281, 1983.

[69] R. Schoenwald and H. Huang. Corneal penetration behavior of beta-blocking agents I: Physicochemical factors. *Journal of Pharmaceutical Sciences*, 72(11):4–10, 1983.

[70] S. Hariharan, M. Minocha, G. P. Mishra, D. Pal, R. Krishna, and A. K. Mitra. Interaction of ocular hypotensive agents (PGF2 alpha analogs-bimatoprost, latanoprost, and travoprost) with MDR efflux pumps on the rabbit cornea. *Journal of Ocular Pharmacology and Therapeutics*, 25(6):487–98, 2009.

[71] H. Kidron, K. S. Vellonen, E. M. Del Amo, A. Tissari, and A. Urtti. Prediction of the corneal permeability of drug-like compounds. *Pharmaceutical Research*, 27(7):1398–1407, 2010.

[72] B. Sjöquist and J. Stjernschantz. Ocular and systemic pharmacokinetics of latanoprost in humans. *Survey of Ophthalmology*, 47 Suppl 1, 2002.

[73] M. York and W. Steiling. A critical review of the assessment of eye irritation potential using the Draize rabbit eye test. *Journal of Applied Toxicology*, 18(4):233–240, 1998.

[74] C. Debbasch, C. Ebenhahn, N. Dami, M. Pericoi, C. Van Den Berghe, M. Cottin, and G. J. Nohynek. Eye irritation of low-irritant cosmetic formulations: correlation of *in vitro* results with clinical data and product composition. *Food and Chemical Toxicology*, 43(1):155–165, 2005.

[75] F. Castro-Muñozledo. Corneal epithelial cell cultures as a tool for research, drug screening and testing. *Experimental Eye Research*, 86(3):459–469, 2008.

[76] H. Musther, A. Olivares-Morales, O. J. D. Hatley, B. Liu, and A. Rostami Hodjegan. Animal versus human oral drug bioavailability: do they correlate? *European Journal of Pharmaceutical Sciences*, 57(1):280–291, 2014.

[77] T. Moriyama, I. Asahina, M. Ishii, M. Oda, Y. Ishii, S. Enomoto, J. D. Zieske, V. S. Mason, M. E. Wasson, S. F. Meunier, C. J. Nolte, N. Fukai, B. R. Olsen, N. L. Parenteau, S. Wakitani, T. Goto, R. G. Young, J. M. Mansour, D. Ph, V. M. Goldberg, A. I. Caplan, E. J. Orwin, A. Hubel, D. Ph, H. A. Awad, D. Ph, D. L. Butler, D. Ph, P. Malaviya, D. Ph, B. Huibregtse, A. I. Caplan, and D. Ph. Basement membrane assembly and differentiation of cultured corneal cells: importance of culture environment and endothelial cell interaction. *Experimental Cell Research*, 4(4):415–427, 1994.

[78] C. K. Postnikoff, R. Pintwala, S. Williams, A. M. Wright, D. Hileeto, and M. B. Gorbet. Development of a curved, stratified, *in vitro* model to assess ocular biocompatibility. *PLoS ONE*, 9(5):e96448, 2014.

[79] M. Griffith, R. Osborne, R. Munger, X. Xiong, C. Doillon, N. Laycock, M. Hakim, Y. Song, and M. Watsky. Functional Human Corneal Equivalents Constructed from Cell Lines. *Science*, 286(5447):2169–2172, 1999.

[80] M. Hornof, E. Toropainen, and A. Urtti. Cell culture models of the ocular barriers. *European Journal of Pharmaceutics and Biopharmaceutics*, 60(2):207–225, 2005.

[81] S. Reichl, J. Bednarz, and C. C. Müller-Goymann. Human corneal equivalent as cell culture model for *in vitro* drug permeation studies. *The British journal of ophthalmology*, 88(4):560–5, 2004.

[82] N. L. Parenteau, V. S. Mason, and O. R. Bjorn. *In vitro* cornea equivalent model. *US Patent 5374515*, 1994.

[83] S. D. Klyce. Electrical profiles in the corneal epithelium. *The Journal of Physiology*, 226(2):407–29, 1972.

[84] A. K. Mitra. *Ophthalmic drug delivery systems*. Marcel Dekker, New York, 2nd edition, 2003.

[85] K. Araki-Sasaki, Y. Ohashi, T. Sasabe, K. Hayashi, H. Watanabe, Y. Tano, and H. Handa. An SV40-immortalized human corneal epithelial cell line and its characterization. *Investigative Ophthalmology & Visual Science*, 36(3):614–21, 1995.

[86] S. E. Wilson, J. Weng, S. Blair, Y. G. He, and S. Lloyd. Expression of E6/E7 or SV40 large T antigen-coding oncogenes in human corneal endothelial cells indicates regulated high-proliferative capacity. *Investigative Ophthalmology & Visual Science*, 36(1):32–40, 1995.

[87] M. Dutot, E. Reveneau, T. Pauloin, R. Fagon, C. Tanter, J.-M. Warnet, and P. Rat. Multipurpose solutions and contact lens: modulation of cytotoxicity and apoptosis on the ocular surface. *Cornea*, 29(5):541–549, 2010.

[88] A. M. Zicari, F. Occasi, A. Cesoni Marcelli, V. Lollobrigida, C. Celani, L. Indinnimeo, G. Tancredi, G. De Castro, and M. Duse. Habitual snoring in children with previous

allergic sensitization. *International Journal of Immunopathology and Pharmacology*, 26(2):565–570, 2013.

[89] A. Pauly, M. Meloni, F. Brignole-Baudouin, J. M. Warnet, and C. Baudouin. Multiple endpoint analysis of the 3D-Reconstituted corneal epithelium after treatment with benzalkonium chloride: Early detection of toxic damage. *Investigative Ophthalmology & Visual Science*, 50(4):1644–1652, 2009.

[90] J.-E. Chang, S. Basu, and V. Lee. Air-interface condition promotes the formation of tight corneal epithelial cell layers for drug transport studies. *Pharmaceutical Research*, 17(6):670–676, 2000.

[91] T. B. Sørensen and F. T. Jensen. Tear flow in normal human eyes. Determination by means of radioisotope and gamma camera. *Acta Ophthalmologica*, 57(4):564–581, 1979.

[92] S. Mishima, A. Gasset, S. D. Klyce, and J. L. Baum. Determination of tear volume and tear flow. *Investigative Ophthalmology & Visual Science*, 5(3):264–276, 1966.

[93] J. Baca, D. Finegold, and S. Asher. Tear glucose analysis for the noninvasive detection and monitoring of diabetes mellitus. *The Ocular Surface*, 5(4):280–293, 2007.

[94] L. O'Toole. Therapeutics in practice - Disorders of the tears and lacrimal system. *Optometry Today*, pages 47–52, 2005.

[95] N. Ferrell, R. R. Desai, A. J. Fleischman, S. Roy, H. D. Humes, and W. H. Fissell. A microfluidic bioreactor with integrated transepithelial electrical resistance (TEER) measurement electrodes for evaluation of renal epithelial cells. *Biotechnology and Bioengineering*, 107(4):707–716, 2010.

[96] S. Reichl and C. C. Müller-Goymann. The use of a porcine organotypic cornea construct for permeation studies from formulations containing befunolol hydrochloride. *International Journal of Pharmaceutics*, 250(1):191–201, 2003.

[97] I. Schneider, K. Maier-Reif, and T. Graeve. Constructing an *in vitro* cornea from cultures of the three specific corneal cell types. *In Vitro Cellular & Developmental Biology - Animal*, 35(9):515–26, 1999.

[98] U. Becker, C. Ehrhardt, M. Schneider, L. Muys, D. Gross, K. Eschmann, U. F. Schaefer, and C. M. Lehr. A comparative evaluation of corneal epithelial cell cultures for assessing ocular permeability. *Alternatives to Laboratory Animals*, 36(1):33–44, 2008.

[99] H. Gupta, T. Velpandian, and S. Jain. Ion- and pH-activated novel in-situ gel system for sustained ocular drug delivery. *Journal of Drug Targeting*, 18(7):499–505, 2010.

[100] S. Reichl, S. Döhring, J. Bednarz, and C. C. Müller-Goymann. Human cornea construct HCC - An alternative for *in vitro* permeation studies? A comparison with human donor corneas. *European Journal of Pharmaceutics and Biopharmaceutics*, 60(2):305–308, 2005.

[101] A. Attama, S. Reichl, and C. Müller-Goymann. Sustained release and permeation of timolol from surface-modified solid lipid nanoparticles through bioengineered human cornea. *Current Eye Research*, 34(8):698–705, 2009.

[102] K. S. Vellonen, M. Malinen, E. Mannermaa, A. Subrizi, E. Toropainen, Y. R. Lou, H. Kidron, M. Yliperttula, and A. Urtti. A critical assessment of *in vitro* tissue models for ADME and drug delivery. *Journal of Controlled Release*, 190:94–114, 2014.

[103] V. P. Ranta, M. Laavola, E. Toropainen, K. S. Vellonen, A. Talvitie, and A. Urtti. Ocular pharmacokinetic modeling using corneal absorption and desorption rates from *in vitro* permeation experiments with cultured corneal epithelial cells. *Pharmaceutical Research*, 20(9):1409–1416, 2003.

[104] M. Griffith, M. Watsky, C. J. Doillon, and Y. Song. Artificial Cornea. *US Patent 6645715*, 2003.

[105] Z. Friedman, R. C. Allen, and S. M. Raph. Topical acetazolamide and methazolamide delivered by contact lenses. *Archives of Ophthalmology*, 103(7):963–6, 1985.

[106] E. M. Hehl, R. Beck, K. Luthard, R. Guthoff, and B. Drewelow. Improved penetration of aminoglycosides and fluoroquinolones into the aqueous humour of patients by means of Acuvue contact lenses. *European Journal of Clinical Pharmacology*, 55(4):317–323, 1999.

[107] M. R. Jain. Drug delivery through soft contact lenses. *The British Journal of Ophthalmology*, 72(1):150–154, 1988.

[108] J. Xu, X. Li, and F. Sun. *In vitro* and *in vivo* evaluation of ketotifen fumarate-loaded silicone hydrogel contact lenses for ocular drug delivery. *Drug delivery*, 18:150–158, 2011.

[109] X. Tian, M. Iwatsu, and A. Kanai. Disposable 1-Day Acuvue (R) Contact Lenses for the Delivery of Lomefloxacin to Rabbits' Eyes. *Eye & Contact Lens*, 27(4):212–5, 2001.

[110] X. Tian, M. Iwatsu, K. Sado, and A. Kanai. Studies on the uptake and release of fluoroquinolones by disposable contact lenses. *Contact Lens Association of Ophthalmologists*, 27(4):216–20, 2001.

[111] Y. Shirasaki. Molecular Design for Enhancement of Ocular Penetration. *Journal of Pharmaceutical Sciences*, 97(7):2462–2496, 2008.

[112] M. Sachdev, U. Yadava, and N. Bamrolia. Pharmacokinetics of antiglaucoma medications. *Journal of Current Glaucoma Practice*, 5(2):21–26, 2011.

[113] S. Basu, B. Sjöquist, J. Stjernschantz, and B. Resul. Corneal permeability to and ocular metabolism of phenyl substituted prostaglandin esters *in vitro*. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 50(4):161–168, 1994.

[114] J. B. Ciolino, C. F. Stefanescu, A. E. Ross, B. Salvador-Culla, P. Cortez, E. M. Ford, K. A. Wymbs, S. L. Sprague, D. R. Mascoop, S. S. Rudina, S. A. Trauger, F. Cade, and D. S. Kohane. *In vivo* performance of a drug-eluting contact lens to treat glaucoma for a month. *Biomaterials*, 35(1):432–439, 2014.

[115] S. Mohammadi, L. Jones, and M. Gorbet. Extended latanoprost release from commercial contact lenses: *In vitro* studies using corneal models. *PLoS ONE*, 9(9):1–10, 2014.

[116] M. Gorbet, R. Peterson, D. McCanna, C. Woods, L. Jones, and D. Fonn. Human corneal epithelial cell shedding and fluorescein staining in response to silicone hydrogel lenses and contact lens disinfecting solutions. *Current Eye Research*, 39(3):245–56, 2014.

[117] K. L. Bandamwar, E. B. Papas, and Q. Garrett. Fluorescein staining and physiological state of corneal epithelial cells. *Contact Lens and Anterior Eye*, 37(3):213–223, 2014.

[118] A. Soluri, A. Hui, and L. Jones. Delivery of Ketotifen Fumarate by Commercial Contact Lens Materials. *Optometry and Vision Science*, 89:1140–1149, 2012.

[119] S. Mohammadi, C. Postnikoff, A. M. Wright, and M. Gorbet. Design and development of an *in vitro* tear replenishment system. *Annals of Biomedical Engineering*, 42(9):1923–1931, 2014.

[120] A. H. Lefebvre. *Atomization and Sprays.* CRC Press, NewYork, 1988.

[121] J. M. Tiffany. The viscosity of human tears. *International Ophthalmology*, 15(6):371–376, 1991.

[122] J. Tiffany, N. Winter, and G. Bliss. Tear film stability and tear surface tension. *Current Eye Research*, 8(5):507–515, 1989.

[123] C. Baudouin, A. Labbé, H. Liang, A. Pauly, and F. Brignole-Baudouin. Preservatives in eyedrops: The good, the bad and the ugly. *Progress in Retinal and Eye Research*, 29(4):312–334, 2010.

[124] J. T. Whitson and W. M. Petroll. Corneal epithelial cell viability following exposure to ophthalmic solutions containing preservatives and/or antihypertensive agents. *Advances in Therapy*, 29(10):874–888, 2012.

APPENDICES

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Appendix B: Fractional Solute Release Ratio Graphs

When studying drug release from polymeric devices under perfect sink condition, it is common to use fractional solute release ratio, M_t/M_∞. The perfect sink condition allows extraction of most of the solute from the polymer network. Therefore, normalizing the release over time to the maximum total release will enable an easier comparison among the lens materials.

In the small sink model (fixed volume model) used in this research, only a limited amount of drug is released from contact lens material over the course of experiments (48h). Thus, the release results were normalized to the uptake by each lens. In this appendix the fractional solute release ratio graphs are presented. It must be noted that these graphs do not replicate the release behavior of tested contact lens materials under perfect sink conditions.

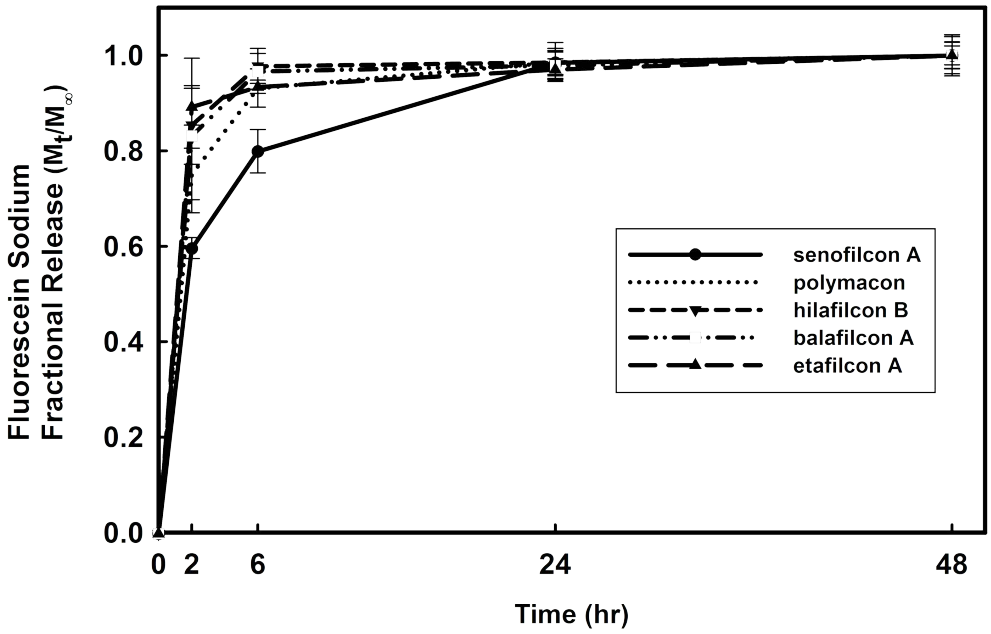

Figure B.1: Fractional solute release ratio of fluorescein sodium in fixed volume release model

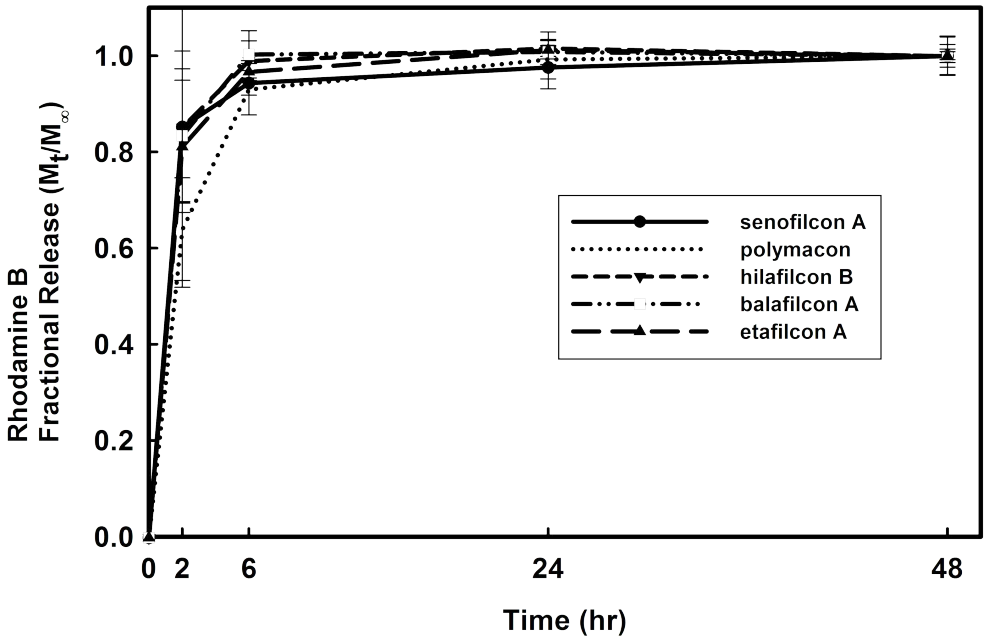

Figure B.2: Fractional solute release ratio of rhodamine B in fixed volume release model

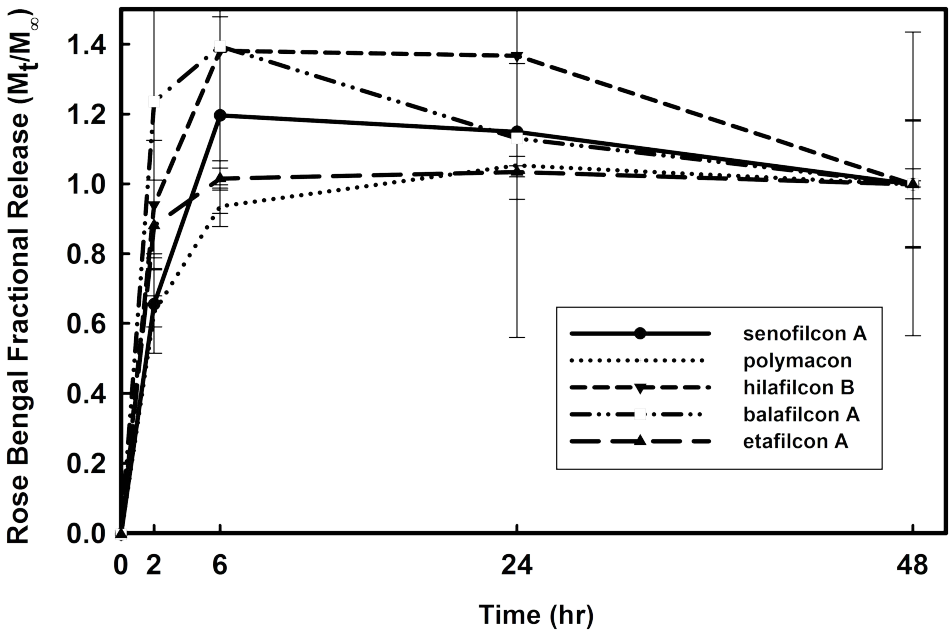

Figure B.3: Fractional solute release ratio of rose bengal in fixed volume release model

Appendix C: Release of Surrogate Drugs

In this appendix, the release and release to uptake ratio graphs for fixed volume model as well as the cell model for 48h are presented and compared to the 24h release in dynamic drug release model.

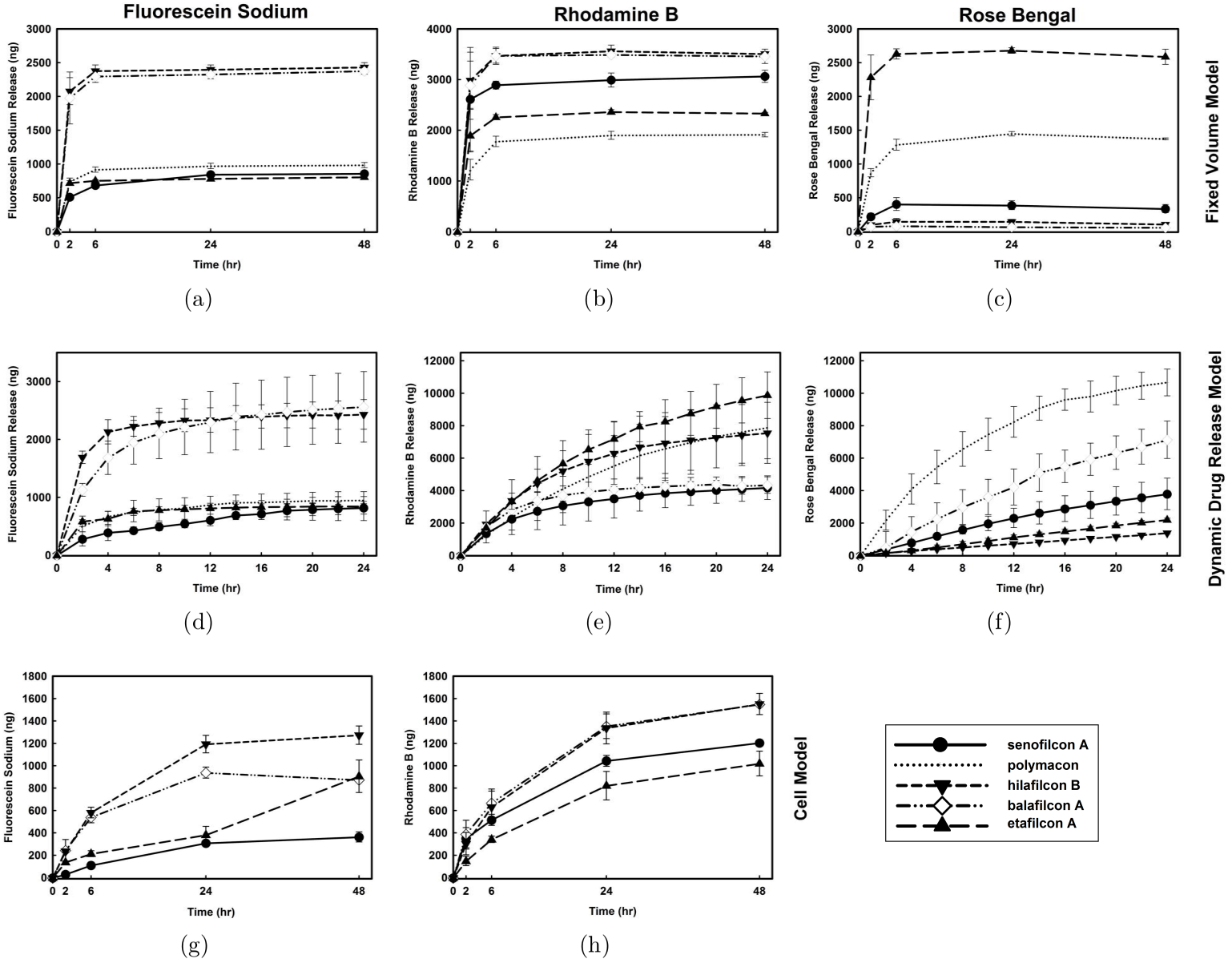

Figure C.1: Release results comparison among the contact lens materials, surrogate drugs, using various release models. (a), (b), and (c) Fixed Volume Model: release of surrogate drugs from contact lenses in the fixed volume model. (d), (e), and (f) DDRM: release of the drugs in dynamic drug release model. (g) and (h) Cell Model: release of fluorescein sodium and rhodamine B in *in vitro* cell model.

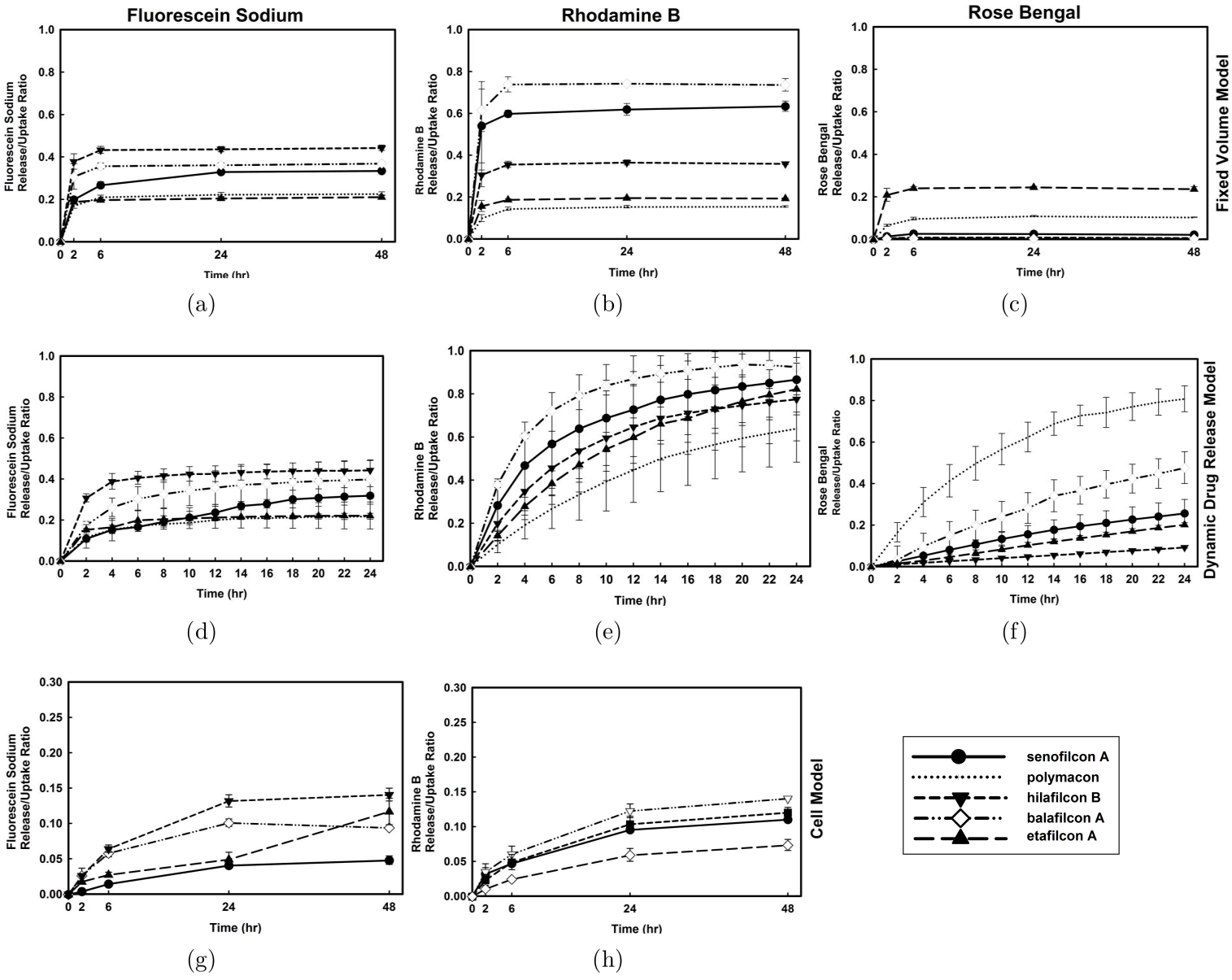

Figure C.2: Release to uptake ratio results. Comparison among the contact lens materials, surrogate drugs, in three release models. (a), (b), and (c) Fixed Volume Model: release of surrogate drugs from contact lenses in the fixed volume model. (d), (e), and (f) DDRM: release of the drugs in dynamic drug release model. (g) and (h) Cell Model: release of fluorescein sodium and rhodamine B in *in vitro* cell model.