Development of Micropatterned, Mucoadhesive, Ocular Films for the Treatment of Diabetic Keratopathy

by

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ABSTRACT

According to the CDC report in 2017, approximately 9.4% of the US population suffers from diabetes. The ocular manifestations of diabetes include diabetic retinopathy, glaucoma, cataract and ocular surface diseases. Research shows that around 47-64% of diabetic individuals suffer from dysfunctional corneal wound healing in the eye, known as diabetic keratopathy. The presence of free oxygen radicals, advanced glycation products and the absence of mucins in the eyes of diabetic individuals disrupts the homeostasis required for epithelial wound healing. Current treatment for corneal wound healing is mostly symptomatic and includes administration of antibiotic drops, bandage lenses, artificial tears and punctal plugs. However, these are symptom-based treatments and there remains a need for a cause-based approach. Rebamipide eye drops are used in dry eye disease in Japan because of its ability to improve mucin secretion and maintain tear film stability. Additionally, it has shown epithelial wound healing activity along with the ability to scavenge reactive oxygen species and reduce inflammation. In this study, we have fabricated micropatterned, mucoadhesive films containing rebamipide that have a sustained release of drug from the polymer matrix. We show that these thin films containing cellulose and Eudragit polymers form clear and distinct patterns of good resolution. Micropatterned films also show good tensile strength and flexibility. Cytotoxicity studies on corneal stem cells show that the 48%EPO+32%FS30D formulation has the least toxicity with a good sustained release of drug in 24 hours. We also demonstrate the higher mucoadhesive properties of micropatterned films as
compared to plain, unpatterned films on the ocular mucosa. Finally, we show the preliminary development of an ex-vivo lacrimation model of the retention of films on the sclera using enucleated porcine eyeballs.

**Keywords:** Diabetes, diabetic keratopathy, ocular surface diseases, micropatterns, mucins, Rebamipide, films
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1.0 Introduction

Diabetes is a chronic disease which affects the way the body utilizes and metabolizes blood sugar. As reported by the CDC, around 30 million adults in the United States have diabetes and it is the seventh leading cause of death. Diabetes is a serious illness due to the numerous co-morbidities associated with it such as nerve damage, kidney disease, heart disease, and ocular problems, among others. Although treatment can significantly alleviate or slow these manifestations and control blood sugar, there exists no cure for diabetes as of today. One among the numerous organs affected by chronically high blood glucose and its impaired metabolism is the eye.

The ocular complications associated with diabetes include glaucoma, diabetic retinopathy, cataract, diabetic papillopathy and surface abnormalities. Diabetic ocular surface diseases are a cluster of abnormalities occurring on the anterior ocular surface of the eye. Diabetic Keratopathy, first reported by Schultz et. al., is the term used to describe these ocular surface complications. It includes the occurrence of corneal surface erosions and delayed wound healing in the cornea. Around 47-64% of the diabetic patients were reported to have some kind of epithelial lesions on the cornea. The corneal epithelium is an essential barrier to the entry of external pathogens into the eye. A breach of this barrier renders a diabetic patient vulnerable to numerous ocular infections. Further, non-healing ulcerations cause the development of scar tissue and severely impair vision. Therefore, there is an urgent need for a simple and effective treatment method for the prevention of recurrent corneal erosions in diabetic patients.
1.1 Anatomy and Physiology of the Ocular Surface

![Figure 1 Anatomy of the eye (Adapted from Nigel et al.5)](image)

The anterior segment of the eye consists of the cornea, sclera, conjunctiva, iris, and the pupil. The cornea and the sclera converge at the limbus, which forms a ring around the cornea. The outermost transparent covering of the sclera is a thin layer of mucus-producing cells called the conjunctiva. This layer is continuous with the inside of the superior and inferior palpebrae (eyelids). Covering the cornea is a tri-layered tear film that comprises of a lipid layer, an aqueous layer and a mucin layer that protect the eye from pathogens, dust and the shear caused due to blinking.6

The sclera is the white, fibrous, and strong outer covering of the eye comprising of collagen and elastic fibers. The cornea is the transparent, convex, and avascular portion of the eye through which light is transmitted. The average diameter of the cornea is reported to be between 11 and
12mm with a central thickness of around 0.5mm.\textsuperscript{7, 8} The cornea consists of the epithelium, the Bowman layer, stroma, the Descemet membrane, and the endothelium. The epithelium consists of 4-6 layers of squamous corneal epithelial cells containing tight junctions that are replaced every 7-10 days.\textsuperscript{9} Epithelial inflammation and abrasions in diseases such as diabetic keratopathy cause the loss of tight junctions leading to the vulnerability of the eye towards pathogens. The basal layer of epithelial cells is attached to the Bowman membrane by hemidesmosomes. Hemidesmosomes are cellular junctions that enable cells to tightly adhere to the underlying basement membrane.\textsuperscript{10} In diabetic keratopathy, a decrease in the number of hemidesmosomes has been reported.\textsuperscript{11} As a result, there is decreased adhesion of the epithelium to the basal lamina. The corneal limbus consists of stem cells that migrate and differentiate to replace lost epithelial cells continually. They also serve as a barrier to prevent the migration of conjunctival cells across the cornea.\textsuperscript{12}
1.2 Corneal Wound Healing

An injury to the surface of the cornea initiates a cascade of events that repair and restore the cornea to its normal state. Corneal epithelial wound healing occurs with the help of corneal limbal epithelial cells that are stem cells found in the limbus. Corneal wound healing begins with cell death at the site of injury and is a series of migration, proliferation and differentiation of corneal epithelial cells. Soon after corneal injury, a latent phase ensues in which dead cells are

Figure 2 Corneal wound healing process (adapted from Yoon et. al. 13)
removed and the cells at the periphery of the wound begin to synthesize structural proteins in preparation for migration.14 15 This is followed by the migration of epithelial cells from the periphery to cover the wound area and ensure wound closure.16 The limbal epithelial cells divide and differentiate into corneal epithelial cells to restore cellular mass in the wounded region. Finally, the newly formed cellular layer forms permanent adherent junctions to the basement membrane called hemidesmosomes after the entire wound is closed.14, 15 Thoft et. al. have hypothesized corneal wound healing to be an X+Y=Z process in which proliferation of epithelial cells (X) and the centripetal migratory motion of peripheral cells (Y) serves to make up for the loss of epithelium (Z) due to injury.17

In diabetic keratopathy, the corneal wound healing process is highly impaired and results in chronic corneal inflammation and erosions. The cornea is one of the most innervated tissues in the body. The corneal nerves are important in maintaining the corneal epithelium and in homeostasis. Chronic diabetes results in the loss in sensitivity of corneal nerves, persistent inflammation, deposition of disruptive advanced glycation end-products (AGE) in the basement membrane and repeated corneal erosions and scarring.2, 18, 19, 20 AGE deposition is hypothesized to cause basement membrane thickening and improper adhesion of corneal cells to the membrane.21 Repeated inflammation and the resultant generation of oxidative stress primarily contributes to the non-healing epithelial erosions.22 Higher levels of reactive oxygen species (ROS) has been reported to decrease the number of tight junctions in the cornea, compromising its barrier function.23 This severely affects the corneal wound healing process due to a disruption in ocular homeostasis. Recurring erosions that do not heal may finally transition to opaque scar tissue formation, thus impairing vision.
Diabetic individuals also often experience tear film instability and low secretion of mucins on the ocular surface, which contributes to a dry eye-like state. Corneal mucins play an important role in maintaining the lubrication of the corneal surface and preventing cell to cell adhesion between the cornea and the eyelid. They have also been reported to have protective functions by acting as a penetrative barrier to pathogens and external molecules. Overall, the ocular environment in diabetic keratopathy is hostile and this in turn impairs proper healing of corneal erosions and injuries.

Figure 3 Causes of Diabetic Keratopathy
1.3 Topical Ocular Drug Delivery

Topical delivery on the ocular surface is the preferred method of administration of drugs for the treatment of ocular surface disorders and form about 90% of all marketed ocular formulations. 26 Drugs are instilled directly into the ocular cavity in the form of drops, gels, inserts, ointments, microneedles, suspensions and other lipid and polymeric systems. 27

Eyedrops remain the top choice for ocular drug delivery due to the ease of application and patient compliance. However, due to the high clearance rate from the ocular surface, 95% of the administered dose is washed away within a few minutes of instillation. 28 The residence time of an instilled drug on the eye is only about 5 minutes. 29 Current research is therefore focusing on improving the retention of topical dosage forms on the ocular surface (Table 1).

Ophthalmic ointments and gels are designed to improve the release and residence of drug on the ocular surface by increasing the viscosity of the formulation. Ocular ointments are developed with drug incorporated into semi-solid lipids such as lanolin and paraffin. The limitation of ointments include the disturbance of vision due to the lipoidal nature of the vehicle, thus reducing patient acceptability. Further strategies to improve the residence of drug at the target site involve the development of ocular gels. Ophthalmic gelling systems are solutions that utilize a stimulus such as temperature, enzymes or pH to convert to a viscous gel. 30 However, gelling systems also cause a blurring of vision due to their viscosity. Gels may also cause reflexive blinking, resulting in rapid clearance of the formulation from the eye. Colloidal ophthalmic dosage forms include liposomes, micelles, nanoemulsions, nanoparticles and microparticles. 31 Nanoparticles, emulsions and liposomes may be coated or functionalized with mucoadhesive polymers or groups to adhere to the ocular surface. Amniotic membrane (AM) grafts are also widely used as a post-operative healing treatment and for limbal stem cell deficiency (LSCD). 32
However, AM grafts are unreliable due to possible risks of disease transmission and incompatibility.\textsuperscript{33}

Ocular inserts are solid dosage forms that are usually inserted into the conjunctival cul-de-sac. They have high retention potential and the ability to deliver therapeutic doses effectively to the target site. They allow a larger residence time of drug in the ocular cavity as compared to conventional eye drops. One of the earliest sustained release ocular insert system that was widely accepted was the pilocarpine Ocusert® marketed by Alza Corporation. Other examples of successful ocular insert formulations include the Lacrisert® by Bosch and Lomb for the treatment of dry eye, and the ocular therapeutic system (Minidisc).\textsuperscript{34} Literature has shown the development of contact lens-based drug delivery.\textsuperscript{35, 36, 37} However, this did not translate to commercialization due to variabilities in material properties and poor processability.\textsuperscript{38}

Polymeric thin films are types of ocular inserts consisting of a thin, flexible, polymeric drug loaded matrix. A wide variety of film forming polymers can be used in ocular polymeric thin films such as cellulose, cellulose derivatives, polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), poly (lactic-co-glycolic acid) (PLGA), and chitosan, to name a few. The drug release from polymeric films can be modified by incorporating sustained release polymers such as Eudragit® and high molecular weight cellulose derivatives in the film matrix. Thin films are superior to conventional eyedrops and other ocular dosage forms due to their ability to provide high drug bioavailability at the target site.\textsuperscript{39} Mucoadhesive polymeric films are non-invasive and serve as an effective means for delivering and retaining drug for the treatment of ocular surface diseases. Due to their thin and flexible nature, they can be placed in the cul-de-sac with good tolerability. Since ocular films deliver the dose directly to the target site without much loss, this system can allow
reductions in total dose administered. Films also provide greater stability and shelf-life as compared to aqueous formulations.

Table 1 Methods for improving retention of ocular dosage forms

<table>
<thead>
<tr>
<th>Method</th>
<th>Dosage Forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhancing viscosity</td>
<td>Gels, ointments, In-situ gelling systems</td>
</tr>
<tr>
<td>Mucoadhesion</td>
<td>Coated nanoparticles, emulsions</td>
</tr>
<tr>
<td>Ocular inserts</td>
<td>Thin films, microneedle devices, Ocusert, Lacrisert</td>
</tr>
<tr>
<td>Colloidal systems</td>
<td>Liposomes, nanoparticles</td>
</tr>
</tbody>
</table>
1.4 Barriers To Ocular Drug Delivery

1.4.1 Tear Film And Tear Turnover

The first barrier to topical drug delivery in the eye is the tear film and its turnover. The tear film is composed of an outer lipid layer secreted by the Meibomian glands and the inner aqueous layer, which serves as a barrier to drug permeability. The volume of tears in the eye can vary from 7 to 10 µl. The tear turnover rate is about 16% per minute, which increases the wash-off of drug from the surface of the eye.

1.4.2 Tight Junctions

The tight junctions between corneal epithelial cells or the zona occludens (ZO) prevent the permeation of drugs through the paracellular route. The ZO has high paracellular resistance values of about 120 Ω.cm-2 (human corneal epithelial cells). High tight junction resistance values in other tissues such as the gastric mucosa (2000 Ω.cm-2), colon (300-400 Ω.cm-2), and human bronchia (~766 Ω.cm-2) have also been reported by studying in-vitro cell models. The tight epithelial junctions also serve as effective barriers to drug permeability by preventing the entry of polar molecules.

1.4.3 Nasolacrimal Drainage

The nasolacrimal duct is responsible for the drainage of pre-corneal fluids away from the ocular space. The maximum volume that can be retained in the eye is about 30µl, while the rest of
the instilled volume is drained away. Additionally, instillation of topical preparations cause reflexive blinking, which results in faster drainage of the formulation.

These anatomical and physiological barriers pose a challenge to conventional topical ocular drug delivery with respect to dose retention in the eye. Therefore, drug delivery systems with prolonged residence and sustained release in the eye are required to reduce the loss of drugs due to continued drainage from the ocular surface.
1.5 Mucoadhesive Drug Delivery

Mucoadhesive drug delivery systems take advantage of the interaction of the anionic mucin glycoprotein chains with various polymeric excipients to form temporary adhesive bonds to improve residence of dosage form. This mechanism is widely explained by the five theories of mucoadhesion:46, 47, 48

1. Wetting theory: It describes the spreading of the dosage form over the mucosal surface with a low angle of contact.

2. Diffusion theory: This theory describes the interpenetration of the long mucin chains with the polymer chains to form a strong adhesive bond depending on the depth of penetration.

3. Electronic theory: It involves the exchange of electrons between the anionic mucin glycoproteins and the mucoadhesive polymers.

4. Adsorption theory: It involves weak surface forces such as ionic and Van der Waal’s interactions.

5. Fracture theory: It describes the force involved in the separation of the two surfaces after mucoadhesion.

Mucoadhesive drug delivery systems are widely used in a variety of routes including oral, intra-nasal, intra-ocular, buccal, sublingual, vaginal and rectal sites. Mucoadhesive polymer excipients possess the ability to spread and bind to surface mucins, thereby forming strong, temporary adhesive bonds on the mucosal surface and increasing retention time of the dosage forms. Some of the most commonly used mucoadhesive polymers are given in table 2. A combination of wetting and swelling activates these polymers allows them to interact with the mucins, thereby allowing adhesion and retention of the dosage form.
<table>
<thead>
<tr>
<th>Source</th>
<th>Mucoadhesive Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>Chitosan, hyaluronic acid, sodium alginate, gelatin, agarose, gums</td>
</tr>
<tr>
<td>Semi-synthetic</td>
<td>Cellulose derivatives: Carboxymethyl cellulose (CMC), Hydroxypropyl methylcellulose (HPMC), Hydroxyethyl cellulose (HEC), Methylcellulose (MC), Sodium carboxymethyl cellulose (Na-CMC)</td>
</tr>
<tr>
<td>Synthetic</td>
<td>Poly (acrylic acid)-based polymers, PVA, PVP, Poly (ethylene oxide)</td>
</tr>
</tbody>
</table>

There are various factors governing the formation of mucoadhesive bonds between the dosage form and the mucosal surface. Some of these factors are given in table 3.
Table 3 Factors affecting mucoadhesion (John et. al. 46 and Shaikh et. al. 48)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect on Mucoadhesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>Higher molecular weight polymers interact by entanglement while lower molecular weight polymers interact by interpenetration of chains.</td>
</tr>
<tr>
<td>Spatial conformation of chains</td>
<td>Polymer chains with exposed active binding groups show greater mucoadhesion than polymers having coiled chains that shield the binding groups by virtue of their spatial conformation.</td>
</tr>
<tr>
<td>Polymer cross-linking</td>
<td>High cross linking prevents hydration of polymer chains and decreases mucoadhesion. Optimum cross-linking is desired to allow swelling and hydration for maximum mucoadhesion</td>
</tr>
<tr>
<td>Charge</td>
<td>Mucins have a negative surface charge and are able to interact well with oppositely charged polymers.</td>
</tr>
<tr>
<td>Polymer concentration</td>
<td>Increase in polymer concentration results in the increase in the number of chains available for interaction with surface mucins. However, beyond optimum concentration, the coiling of polymer chains results in poor mucoadhesion.</td>
</tr>
<tr>
<td>pH</td>
<td>In polymers that have ionizable groups, changes in pH will significantly affect mucoadhesion depending on the pKa of the polymer.</td>
</tr>
</tbody>
</table>
The current treatment for diabetic keratopathy is focused on a symptom-based approach as opposed to cause-based approaches. Artificial tears are majorly prescribed to make up for the damaged tear film and reduced lacrimation by increasing the viscosity of tears and improving tear break-up time. Bandage contact lenses are also used which are protective hydrogel lenses placed over the surface of the cornea to prevent further injury. Antibiotics are administered...
for prophylactic purposes to prevent further infection of the compromised cornea by external pathogens. In severe cases, tarsorrhaphy of the eyelids is performed to minimize the exposed surface of the cornea. This involves suturing a part or whole of the eyelids together temporarily or permanently to allow the cornea to heal. These treatments, although protective in nature, do not address the underlying cause or assist in wound healing through re-epithelialization of the abraded tissue. To address this problem, research has focused on developing therapeutic growth hormones such as epithelial growth factor (EGF), opioid growth factors, gene therapies, topical insulin, stem-cell treatments, and pharmacological molecules. However, none of these therapies have translated to clinical treatments due to their complexity and variability. Therefore, there is a need for specific treatment that restores the ocular homeostasis and promotes corneal wound healing.
1.7 Research Hypothesis

Diabetic keratopathy is a lesser known, potentially sight-threatening, corneal epithelial disease that affects about 47-64% of the diabetic population. Diabetic keratopathy can be associated with a wide array of complications such as increased inflammation, persistent epithelial erosions, non-healing ulcerations, decreased formation of tear fluid, increased generation of reactive oxygen species, and decreased corneal sensitivity. Additionally, diabetic keratopathy is characterized by the loss of both ocular surface and circulatory mucins. Mucins are an important part of the ocular surface. The ocular surface contains both membrane-associated and circulatory mucins. The conjunctiva of the eyelids contains goblet cells that produce the circulating mucins MUC5AC, MUC2 and MUC7.6 The cornea expresses membrane associated mucins, mainly MUC1, MUC4 and MUC16.25 Mucins maintain ocular homeostasis and allow normal functioning of ocular tissues. Therefore, the lack of mucins in diabetic keratopathy increasingly contributes to the poor healing of corneal wounds.

Currently employed therapies for the management of diabetic keratopathy are primarily focused on symptomatic treatment, largely involving improved ocular lubrication and prophylaxis against bacterial infections. However, none of the current treatments focus on targeting the cause of corneal inflammation and persistent erosions. Hence, the development of a simple, safe and effective treatment of the underlying cause of diabetic keratopathy is the need of the hour.
Rebamipide, a quinolinone drug (Figure 5) manufactured by Otsuka Pharmaceuticals in Japan, has proven to be efficacious in improving the secretion of mucins in both gastric and ocular mucosa.\textsuperscript{61, 62} Rebamipide was first introduced as a mucin secretagogue for the treatment of gastric ulcers in H.pylori infections and dyspepsia.\textsuperscript{63} Further studies on rebamipide’s secretagogue activity led to successful clinical trials and the establishment of 2\% rebamipide topical ocular suspension as a treatment option for patients with dry eye disease.\textsuperscript{61, 64} Rebamipide was shown to improve the stability of the tear film by increasing ocular surface mucin production.\textsuperscript{65, 66, 67} It was also proven to improve the secretion of soluble mucins secreted by the conjunctival goblet cells by increasing their numbers.\textsuperscript{68} Along with improving mucin secretion, rebamipide also decreased the expression of TNF-\( \alpha \) and consequently the expression of interleukin-6 and 8, which resulted in reduced inflammation.\textsuperscript{69, 70} Further, rebamipide was also shown to reduce ocular inflammation caused due
to the presence of reactive oxygen species. Two case studies reporting the efficacy of rebamipide eye drops on the resolution of persistent corneal epithelial erosions have been published, also demonstrating the efficacy of rebamipide in a diabetic individual with keratopathy. Therefore, rebamipide is a good candidate for the treatment of diabetic keratopathy that targets the underlying causes of corneal epithelial erosions.

The marketed 2% suspension of rebamipide currently has a recommended dosing of 4 drops a day in each eye for 4 weeks for the treatment of dry eye disease. In diabetic keratopathy, the newly formed epithelial tissue is extremely fragile and could possibly be disturbed by the frequent dosing requirements of rebamipide. Additionally, higher dosing frequencies reduce patient convenience and compliance. Therefore, sustained release dosage forms such as mucoadhesive polymeric thin film devices can reduce the dosing frequency of the drug by improving the retention of the drug in the eye. The flexible devices may be placed within the conjunctival cul-de-sac away from the inflamed corneal site to elute small quantities of drug throughout the day. However, as discussed above in diabetic keratopathy, the ocular surface has been reported to suffer from a lack of mucins, which may pose a challenge to the mucoadhesive retention of the thin film device. Therefore, conventional methods of improving mucoadhesion may not be effective.

An interesting study by Arzt et. al. describes the ability of numerous species of insects to stick to smooth surfaces by virtue of the microstructures on their limbs. Studies on insect and gecko adhesion mechanisms elucidated that the microscopic projections or setae on their limbs dictate their ability to adhere to vertical surfaces. Literature indicates that the microscopic setae and their surface roughness increases the surface area in contact with substrates and allows Van der Waals forces to result in strong surface adhesion. Gorb et. al describe a bioinspired tape with
microscopic features that possesses superior adhesive strength and lower contamination potential by virtue of its topography. Hence, the incorporation of micropatterns on polymeric films could potentially serve as an additional retentive mechanism to supplement mucoadhesion in the event of low surface mucin availability. Therefore, in this study, we hypothesize that micropatterned, mucoadhesive films will be retained for long periods on the ocular surface and provide a sustained release of rebamipide for the treatment of diabetic keratopathy.
2.0 Materials and Methods

2.1 Materials

Rebamipide was purchased from TCI America (Portland, OR). EUDRAGIT® FS 30 D and EUDRAGIT® EPO copolymers were kindly gifted by Evonik Industries (Piscataway, NJ). Methocel™ K4M (Hydroxypropylmethyl cellulose K4M) was purchased from Colorcon (West Point, PA) and Natrosol™ 250 (hydroxyethyl cellulose) was obtained from Ashland Global Chemical Company (Wilmington, DE). The plasticizer, polyethylene glycol 400 (PEG 400), was purchased from TCI America (Portland, OR). Polydimethylsiloxane (PDMS) was purchased as the Sylgard 184 Silicone Elastomer Kit from Dow Corning (Midland, MI). Keratinocyte serum free media (KSFM) and Dulbecco’s modified Eagle media: Nutrient mixture F-12 (DMEM/F-12) were purchased from Thermo Fisher Scientific (Waltham, MA). Newborn Calf Serum (NCS) was obtained from Sigma-Aldrich (St. Louis, MO). 70µm nylon mesh sterile cell strainers for cytotoxicity studies were purchased from Fisher Scientific (Hampton, NH). Other chemicals and reagents used in formulation preparation were of analytical grade.
2.2 Methods

2.2.1 Film Preparation

Figure 6 Preparation of micropatterned films from PDMS molds

(A) Soft-lithography preparation of PDMS mold from SU8 template and preparation of micropatterned film by solvent casting. (B) Macroscopic images of SU8 template, PDMS mold and micropatterned film

Photolithography was used to fabricate silicon wafer master templates having uniform micro-posts of SU-8 photoresist. Soft lithography techniques were then employed to transfer these master patterns on to Polydimethylsiloxane (PDMS) molds using the Sylgard elastomer kit. Micropatterned films with or without rebamipide were prepared by solvent casting of polymeric solutions on the PDMS molds. Briefly, PEG 400 (plasticizer) was weighed and mixed with water to form a uniform solution. Calculated quantities of rebamipide were accurately weighed and
transferred to the solution, forming a fine, milky dispersion. This was followed by sequential addition of polymers with continuous stirring. The polymer dispersion was allowed to stir for 4-5 hours to ensure uniform distribution of rebamipide and Eudragit, and hydration of cellulose. The dispersions were then poured into the PDMS molds and subjected to vacuum cycles to dispel the air incorporated during preparation. The polymeric dispersions were dried in an oven at 70°C for 14-15 hours. The films were gently peeled from the mold and packed in aluminum foil for storage.

2.2.2 Formulation Of Polymeric Micropatterned Films

The polymer solution consisted of a blend of polymers with sustained release properties. The bulk of the solid content of the films consisted of pH sensitive, sustained release poly(meth)acrylate polymers (Eudragits). The two Eudragit polymers chosen were Eudragit FS30D, a methacrylic copolymer and Eudragit EPO, an aminoalkyl methacrylate copolymer. Eudragit FS30D dissolved beyond pH 7 due to its anionic carboxylic acid groups while Eudragit EPO dissolved below pH 5 due to its cationic dimethylaminoethyl groups. Cellulose polymers Natrosol 250L and HPMC K4M were incorporated in smaller amounts as the mucoadhesive component of the films. The total solid content of the films was maintained at 10% w/w of the film solution of which 80% w/w consisted of Eudragits while 20% w/w consisted of cellulose polymers.

Four different formulations were prepared containing varying amounts of Eudragits (Table 5). Since EPO is obtained in powder form, two variants of the EPO-containing formulation were prepared: 80% EPO, which is a fine dispersion of EPO in neutral solution and 80% EPO-A which contains EPO powder dissolved in acidified water along with other components. Therefore, the 80%EPO formulation was a dispersion of EPO in film solution while the 80%EPO-A was a uniform solution of EPO in acidified film solution.
Table 4 Formulation nomenclature

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Sustained Release Polymer</th>
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<tr>
<td>80%FS30D</td>
<td>EUDRAGIT® FS 30 D</td>
</tr>
<tr>
<td>80%EPO</td>
<td>EUDRAGIT® EPO</td>
</tr>
<tr>
<td>48%EPO+32%FS30D</td>
<td>EUDRAGIT® EPO and FS 30 D</td>
</tr>
<tr>
<td>80%EPO-A</td>
<td>EUDRAGIT® EPO</td>
</tr>
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</table>

Table 5 Film compositions (% w/w of solution)

<table>
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<tr>
<th>Film Component</th>
<th>80%FS30D (% w/w)</th>
<th>80%EPO (% w/w)</th>
<th>48% EPO+32% FS30D (% w/w)</th>
<th>80% EPO-A (% w/w) (Water pH &lt;5)</th>
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<tr>
<td>EUDRAGIT® FS 30 D</td>
<td>26.65</td>
<td>-</td>
<td>10.67</td>
<td>-</td>
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<tr>
<td>EUDRAGIT® EPO</td>
<td>-</td>
<td>8</td>
<td>4.8</td>
<td>8</td>
</tr>
<tr>
<td>HPMC K4M</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HEC 250L</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PEG 400</td>
<td>4</td>
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<tr>
<td>Distilled Water</td>
<td>67.35</td>
<td>86</td>
<td>78.54</td>
<td>86</td>
</tr>
</tbody>
</table>

2.2.3 Physical Characterization Of Films

2.2.3.1 Appearance

The micropatterned films were imaged with a Carl Zeiss Primovert inverted microscope (Carl Zeiss Microscopy LLC, WhitePlains, NY) to visualize the patterns on the surface. Images of
different magnifications were captured from the top view and side view to observe pattern shape and height.

2.2.3.2 Weight and Thickness

The weight of each film formulation was recorded using a Mettler Toledo DualRange analytical balance (Mettler Toledo, Columbus, OH). Thickness of patterned and unpatterned films was recorded at several points on the film using the Mitutoyo Absolute thickness gauge (Mitutoyo, Japan), the mean and SD were calculated.

2.2.4 Tensile Strength

The tensile strength of the micropatterned films was measured using the ADMET MTestQuattro (ADMET, Inc., Norwood, MA) instrument with a 250lbf load cell. The film thickness was measured, and the film was cut into 10 x 20mm² rectangular pieces free from surface imperfections and bubbles. The piece was secured between the grips of the tensile tester positioned at a distance of 10mm from each other. The grips were slowly pulled apart at a rate of 10mm/min till the film fractured. The load required to fracture the film was noted as the breaking load. The ultimate tensile strength (UTS) and Young’s modulus of elongation was calculated using the following formulae:

\[ \text{Ultimate Tensile Strength (UTS)} = \frac{\text{Breaking Load}}{\text{Cross sectional area}} \]

\[ \text{Stress} = \frac{\text{Load}}{\text{Cross sectional area}} \]

\[ \text{Strain} = \frac{\text{Change in length of film}}{\text{Original length of film}} \]
Young's Modulus = \frac{Stress}{Strain}
2.2.5 Cytotoxicity Of Micropatterned Films On HCLE Cells

Immortalized Human Corneal Limbal Epithelial (HCLE) cells are stem cells in the cornea that differentiate to form the epithelial layer. Immortalized HCLE cells were generously provided to us by Dr. Robert Shanks (Eye & Ear Institute, UPMC, Pittsburgh, PA). The cells were cultured in keratinocyte serum free medium (KSFM) enriched with 25μg/ml bovine pituitary extract (BPE) and 0.2ng/ml epidermal growth factor (EGF). All cultures were maintained at 37°C with 5% CO2 in a humidified incubator.

Patterned (SQ100D100) films were cut into 12mm inserts containing a 0.25 mg, 0.5mg or 1 mg dose of Rebamipide each. Patterned placebo control films containing no drug were prepared and cut. The films were sewed on to the bottom of nylon mesh well inserts (Figure 7) and then sterilized under UV light for 30 minutes. Solutions of 0.25mg, 0.5mg and 1mg rebamipide were prepared in media as controls. HCLE cells were seeded into 6 well plates at a cell density of 0.75 million cells per well and allowed to adhere overnight. The media was aspirated, and the films were inserted into the well with the patterned side facing the cells. Pure drug in media was added as the reference control. Positive (only cells) and negative (no cells) controls containing no treatments were prepared simultaneously. 4ml of fresh media was added to all the wells and incubated at 37°C with 5% CO2. After 24 hours the treatment was removed, and the media was aspirated. 3ml media with 10% v/v resazurin was added to each well and incubated for 1-4 hours to allow metabolism of resazurin. Samples were collected from each well and analyzed for fluorescence with a SpectraMax M5e multi-mode microplate reader (Molecular Devices, San Jose, CA) with absorbance at 560nm and emission at 590nm. Fluorescence of the wells was calculated relative to the positive control to obtain cell viability.
Figure 7 Nylon mesh well inserts with films
2.2.6 In Vitro Release Of Rebampide From Films

2.2.6.1 Drug Content Of The Films

A stock solution of 1mg/ml of rebamipide was prepared in pH 7.4 phosphate buffer (Table 6). Serial dilutions were performed in buffer to obtain a concentration range of 5-50µg/ml. A full spectrum scan was performed to obtain the maximum absorption wavelength of Rebamipide. A standard curve was plotted using UV Spectrophotometry (NanoDrop OneC; ThermoFisher Scientific, PA) at 327nm.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity Given</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Phosphate monobasic monohydrate</td>
<td>20.214 g</td>
</tr>
<tr>
<td>Sodium phosphate dibasic heptahydrate</td>
<td>3.394 g</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>q.s 1000ml</td>
</tr>
</tbody>
</table>

Three drug-loaded films from each formulation were cut into 10mm or 12mm diameter inserts, weighed and transferred into conical tubes with 10mL of pH 7.4 phosphate buffer. The tubes were ultrasonicated in a Branson® bath sonicator (Thomas Scientific, Swedesboro, NJ) to completely dissolve the drug in the buffer. The tubes were centrifuged at 3000 rpm for 5 minutes and the supernatant was collected. The drug content was measured using UV Spectrophotometry (NanoDrop OneC; ThermoFisher Scientific, PA) at 327nm.
2.2.6.2 Release Of Rebamipide From Micropatterned Films

The in-vitro release of rebamipide-loaded films was performed in pH 7.4 phosphate buffer to mimic the pH of tear fluid. The media was placed in a 1.8ml glass vial to simulate the low volumes of the ocular cavity while maintaining good sink conditions. Films were cut into small circular inserts (10 or 12mm diameter) and placed in the vials in 1ml of buffer solution. The vials were then placed in an orbital incubator shaker (Excella E25; New Brunswick Scientific, Edison, NJ) at 37°C and 100 rpm. A 0.4ml of sample was withdrawn from each vial at predetermined intervals and replaced with equal volumes of buffer. A placebo film of each formulation was used as a blank control for measurement. The samples were centrifuged at 1000 rpm and the supernatant was collected. The amount of rebamipide released from the films was analyzed using UV spectrophotometry at 327 nm.
2.2.7 Ex-Vivo Mucoadhesion Of Films On Porcine Intestinal And Ocular Tissues

The mucoadhesive properties of the film formulations were tested using a TA.XT Plus C (Stable Microsystems, UK) texture analyzer. The formulations were first screened for their mucoadhesive behavior on the abundantly available porcine intestinal mucosa, followed by porcine ocular tissue sourced from a local slaughterhouse (Thoma Meat Market, Saxonburg, PA). The tissues were transported to the laboratory in a temperature-controlled ice box.

The porcine intestinal tissue was cleaned and cut into pieces of 2cm, placed individually in resealable pouches and stored at -80°C. Prior to the experiment, the intestinal tissue was thawed, cleaned and rinsed with PBS and blotted to remove excess mucins. The tissue was then clamped on to a flat tissue holder at the base of the instrument. The load cell was first calibrated with a weight of 2kg. The film was firmly attached to an 8mm diameter probe with double-sided adhesive tape, which was then affixed to the load cell. The probe was lowered on to the tissue at a speed of 0.5 mm/sec with a force of 10g. A contact time of 60 seconds was maintained to allow the probe to equilibrate on the tissue. The probe was then lifted, and the force required to detach the probe from the mucosal surface was recorded as the peak detachment force (PDF). The work done to detach the two surfaces was recorded as the work of adhesion (WOA).

Enucleated porcine eyes were immersed in PBS containing 1% L-Glutamine, 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin (preservation media) prior to transportation. The eyes were rinsed thoroughly with PBS. The connective tissues were removed from the globes, and the cornea, sclera and eyelids were carefully cut and stored separately in preservation media at 4°C. Within 3 hours of dissection, the ocular tissues were removed from the preservation medium and rinsed with PBS. The experimental procedure for mucoadhesion on ocular tissues remained
the same as that for intestinal tissue. The peak detachment force and work of adhesion was recorded for the films using the sclera, cornea and eyelid tissues.

Figure 8 Texture analyzer setup for mucoadhesion
2.2.8 Ex-Vivo Ocular Retention Of Micropatterned Films

The ability of the film to remain adhered when placed on the ocular surface was studied using a newly developed ex-vivo porcine eyeball model. Enucleated porcine eyeballs were cleaned of surrounding connective tissue and placed in preservation media at 4°C. A test setup was constructed as shown in Figure 9 A. A syringe containing PBS maintained at 34°C with the help of a heating jacket was placed in a microfluidic syringe pump. The syringe was connected to a 20G needle by means of tubing. The tube and needle assembly were maintained in place by a clamp within an incubator set at 34°C, the temperature of the ocular surface. The eyeball was rinsed in PBS and held in place inside a beaker containing PBS with the help of a glass vial (Figure 9 B). Two 5mm circular pieces of each SQ100D100 and unpatterned 48%EPO+32%FS30D films were gently placed on the frontal sclera. A pink dye was incorporated into the formulation for visualization. The beaker was covered in parafilm to prevent over-drying of the eyeball and placed under the needle. The pump was turned on and set to deliver 6 drops/min of PBS to the surface of the eye to simulate the flow of tears over the eyeball (Figure 9C). The needle was adjusted such that the drops delivered to the eye were able to evenly spread over the surface. The entire system was maintained undisturbed for a period of 5 hours. The films were observed every 30 minutes for displacement or detachment from the surface. Images of the experimental set up and procedure were recorded with the help of a smartphone camera and documented.
Figure 9 Ex-vivo retention time of ocular film
3.0 Results and Discussion

3.1.1 Film Formulation

Drug bioavailability and clearance are the greatest challenges faced by periocular drug delivery systems. Ophthalmic films are attractive platforms for increasing the residence of drugs on the ocular surface and to sustain drug release. They offer the flexibility for the incorporation of various novel materials such as mucoadhesives and sustained release polymers. Polymeric thin films are prepared by the addition a drug to a film-forming polymeric matrix. The polymers and plasticizers used to fabricate the films in this study are categorized as Generally Recognized as Safe (GRAS) by the FDA. The two major classes of polymers used in the formulation include mucoadhesive cellulose polymers and sustained release Eudragit polymers. Hydroxypropyl methylcellulose (HPMC) K4M is a viscous cellulose polymer used to increase the viscosity of artificial tears and as an excipient in sustained release preparations. It has a viscosity of 4000 MPa.s in a 2% w/v solution in water. Hydroxyethyl cellulose 250L (Natrosol 250L) is a lower viscosity polymer of 75-150 MPa.s in a 5% w/v solution. Cellulose polymers interact with mucin by forming hydrogen bonds between their carboxylic groups and the mucin glycoproteins. Eudragits are methacrylate copolymers that are commonly used sustained release coating polymers for oral drug delivery. Eudragit FS30D is an anionic, carboxylate containing poly(meth)acrylate copolymer dispersion while Eudragit EPO is a cationic, amine containing poly(meth)acrylate polymer powder. These polymers are capable of forming a sustained release matrix that retards the rate of release of drug from the film. Therefore, together the cellulose and eudragit polymers allow sustained release of drug and lend mucoadhesive properties to the film.
3.1.2 Polymeric Films Form Good Three-Dimensional Patterns of High Fidelity

On macroscopic visual inspection, the micropatterned films appeared smooth, uniform, flexible and translucent. Light microscopy images of the micropatterned films clearly show the formation of distinct, 3 dimensional patterns. Figure 10A, 10B and 10C show the formation of the uniform, cuboidal micropatterns at different magnifications. Images of the transverse section of these films confirm the 100-micron height of the 3D pattern projections (Figure 10D and 10E). The patterns were named according to their shape, pattern size and distance between two patterns. As an example, square micropatterns of 100 microns with an inter-pattern distance of 100 microns were named SQ100D100 (Figure 10F). The average weight and thickness of these films is given in Table 7. The thickness of micropatterned films was observed to be around 0.3mm, which is comparable to commercially available formulations such as the Ocusert™ (0.25-0.4mm) and the Lacrisert™ (1.7mm diameter). It was observed that placebo 80%FS30D films had good flexibility and visual appeal while its drug loaded counterpart showed considerable opacity. All the other formulations remained translucent upon loading with rebamipide.
Figure 10 Light microscopy images of micropatterned films
Surface view of SQ100D100 patterns at (A) 10X, (B) 20X and (C) 40X magnifications. Transverse section of the film at (D) 10X and (E) 20X showing the height of the patterns. (F) Schematic depicting the pattern dimensions and nomenclature.

Table 7 Physical characterization of films (Mean±SD, N=3)

<table>
<thead>
<tr>
<th>FORMULATION</th>
<th>WEIGHT OF WHOLE FILM (g)</th>
<th>THICKNESS (mm)</th>
</tr>
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<tbody>
<tr>
<td>80%FS30D</td>
<td>2.604±0.249</td>
<td>0.305±0.008</td>
</tr>
<tr>
<td>80%EPO</td>
<td>2.815±0.148</td>
<td>0.327±0.014</td>
</tr>
<tr>
<td>48%EPO+32%FS30D</td>
<td>2.961±0.042</td>
<td>0.317±0.015</td>
</tr>
<tr>
<td>80%EPO-A</td>
<td>2.898±0.133</td>
<td>0.315±0.008</td>
</tr>
</tbody>
</table>
3.1.3 Micropatterned Polymeric Films Show Good Mechanical Properties

The ultimate tensile strength (UTS) of a film measures the maximum load that the film can withstand before it breaks. The Young’s modulus of the film measures its stiffness or ability to withstand stretching due to the application of external load. Higher values of UTS and lower values of Young’s modulus indicate that the film can withstand high loads and is an elastic solid. Ocular inserts must be able to withstand the shear stress in the ocular cavity due to blinking. The films must also be easy to handle during the manufacturing process and must be able to withstand peeling from the mold and cutting.

As observed in Figure 11, the 80% FS30D formulation seems to have the lowest Young’s modulus values as compared to the other formulations, while the 80% EPO and 80% EPO-A formulations have the largest values. The composite formulation 48%EPO+32%FS30D has intermediate values of Young’s modulus, which correlates well with the two extreme formulations. According to literature, polymers with lower values of Young’s modulus are classified as elastomeric polymers and possess high flexibility and elasticity. It can be inferred that Eudragit FS30D shows more elastomeric properties as compared to Eudragit EPO. Additionally, the values for Young’s modulus of films (~0.02 – 0.3 MPa) are comparable to those obtained from commercially and historically available hydrogel contact lenses (0.1 – 0.8 MPa), as described in Table 8.

UTS follows a reverse trend as compared to Young’s modulus, with 80%FS30D having the highest values. As expected, the UTS values of the composite 48%EPO+32%FS30D formulation lie between the two extremes. Typically, the UTS of polymers greatly depends on their molecular weight. Higher molecular weight polymers possess structural entanglements which significantly improves their strength as compared to loose-chained, light polymers. Indeed, it
can be observed that the molecular weight of Eudragit FS30D (~280,000 g/mol) is much greater than that of Eudragit EPO (~47,000 g/mol), which correlates well with this theory.

It can also be observed from the figure that unpatterned films have significantly higher Young’s modulus and UTS values as compared to their corresponding patterned films. This is reflected in the observation that in the microscopic fracture characteristics of the film, patterned films indeed fracture in the plain area between the patterns (Figure 11B and 11C) suggesting that the area between patterns is more prone to fracture due to decreased thickness. It may thus be inferred that the unpatterned films have greater potential of withstanding higher loads of extension as compared to patterned films.
Figure 11 Tensile testing of film formulations

A schematic of the mechanical testing setup; (B) and (C) Microscopic images of fracture site in unpatterned and micropatterned films; (D) Measurement of Young’s modulus and ultimate tensile strength of unpatterned films, SQ100D100 films and SQ100D200 films by the ADMET MTest Quattro. Data presented as mean ± standard deviation for 9 replicates in each group. * p<0.5, One-way ANOVA with Tukey’s post hoc test
### Table 8 Tensile (Young’s) modulus of commercially available contact lenses

(adapted from Bhamra et. al.)

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Manufacturer</th>
<th>Young’s Modulus (MPa)</th>
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<tr>
<td>Cibasoft</td>
<td>Ciba Vision</td>
<td>0.8</td>
</tr>
<tr>
<td>SeeQuence</td>
<td>Bausch &amp; Lomb</td>
<td>0.6</td>
</tr>
<tr>
<td>Surevue</td>
<td>J&amp;J Healthcare</td>
<td>0.3</td>
</tr>
<tr>
<td>B &amp; L Softlens</td>
<td>Bausch &amp; Lomb</td>
<td>0.2</td>
</tr>
<tr>
<td>Medalist 66</td>
<td>Bausch &amp; Lomb</td>
<td>0.1</td>
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</table>
3.1.4 Eudragit Containing Micropatterned Films Are Non-Toxic To The Regenerating Corneal Limbal Epithelial Cells

Human corneal limbal epithelial cells (HCLE) are stem cells of the corneal epithelium that reside at the junction between the cornea and the conjunctiva, known as the corneoscleral limbus. HCLE cells play a major role in epithelial regeneration after corneal injury and thus are the most important players of corneal wound healing. In the event of corneal injury and abrasion, HCLE cells from the limbal niche migrate towards the wound and differentiate to form renewed corneal epithelium. In diabetic keratopathy, the persistent inflammation, recurrent erosions and poor anchorage of basal epithelial cells to the cell membrane results in the loss of equilibrium in the X+Y=Z process of wound healing. Rebamipide, being a scavenger of inflammatory reactive oxygen species and a mucin secretagogue, has been known to significantly improve ocular homeostasis. The main objective of this study was to determine the effect of film formulations and rebamipide on the viability of HCLE cells using the AlamarBlue cell viability assay.

AlamarBlue or resazurin is a redox dye that allows the indirect quantification of cell viability through cellular proliferation and metabolism. The dye is non-toxic, cell-permeable and water soluble. On reduction of resazurin by cellular metabolism, the dye changes to a fluorescent red molecule, resorufin (Figure 12). Fluorescence quantification can be performed at excitation and emission wavelengths of 560nm and 590nm respectively to indirectly obtain viable cell numbers through metabolic activity.
Exposure of HCLE cells to placebo films of 80%FS30D for 24 hours resulted in around 39% viability. FS30D is soluble above pH 7 and thus results in the dissolution of almost the entire film on incubation in media. Interestingly, 80%FS30D also led to loss in inter-cellular tight junctions of the cells. Exposure to the 80%EPO and 80%EPO-A resulted in even lower viability values of about 4-8%. On microscopic evaluation, a large volume of particulate matter can be observed in the well with a loss in cell junctions. EPO being insoluble below pH 5, precipitates out as particles from the surface of the film due to its high concentration in the formulation. On the other hand, exposure of cells to placebo films of 48%EPO+32%FS30D resulted in a very high viability of about 95% as compared to untreated control (Figure 13 A).

Rebamipide is marketed by Otsuka Pharmaceuticals for the treatment of dry eye as a 2% suspension and has a recommended dose of 4 drops a day. A drug loading of 4mg corresponds to the daily dose of rebamipide in a single eye. However, only about 5% instilled formulations are retained on the target site in the eye. It can thus be crudely inferred that the total bioavailable dose of rebamipide in a day to the eye is only around 0.2mg. Research shows that rebamipide
produces a significant increase in membrane associated mucin secretion in concentrations as low as 10µM in human corneal epithelial cells in vitro. Therefore, the least toxic 48%EPO+32%FS30D composition was formulated into films containing 0.25mg, 0.5mg and 1mg rebamipide. Further testing with these drug-loaded 48%EPO+32%FS30D films resulted in the gradual increase of cytotoxicity with increasing drug loading (Figure 13 B). However, the viability values were comparable to those of HCLE cells when exposed to the same concentrations of pure rebamipide in media. Therefore, it can be inferred that 48%EPO+32%FS30D films containing rebamipide are non-toxic to HCLE cells.

Figure 13 Cell viability

(A) % Viability of HCLE cells after exposure to placebo films of different compositions

(B) % Viability of HCLE cells when exposed to pure drug and drug-loaded films of 48%EPO+32%FS30D when compared to placebo. Data presented as mean ± standard deviation for n= 3 to 4 in each group.
3.1.5 Micropatterned Polymeric Films Show A Sustained Release Of Rebamipide Over 24 Hours

Eudragit EPO and Eudragit FS30D serve as release rate modifiers in the micropatterned film formulations. Eudragit EPO remains unionized in the release buffer of pH 7.4 and does not dissolve, thus serving as a sustained release polymer. Eudragit FS30D swells and forms a permeable matrix at pH 7.4 that dissolves with time. In-vitro drug release for rebamipide from the films was performed to determine the sustained release property of the films. Firstly, the standard curve of rebamipide was prepared within the range of 5µg/ml to 50µg/ml and was found to be consistently reproducible (Figure 14). Further, to optimize the protocol and assess the release profiles of the different compositions, 10mm circular samples theoretically containing 2.8mg drug were cut from the films and placed in 1ml of pH 7.4 phosphate buffer. The release profile for rebamipide from the four different formulations is shown in Figure 15. Films containing 80% FS30D dissolved within 3 hours and provided a burst release of drug due to the property of FS30D
to dissolve above pH 7. The 48%EPO+32%FS30D formulation resulted in insoluble films that released approximately 90% of rebamipide in 24 hours. The formulations containing 80% EPO showed a good sustained release of drug with about 60% release in 24 hours. Eudragit EPO does not dissolve at ocular pH (7.4), allowing the slower release of drug from the matrix. Thus, increasing the percentage of EPO polymer in the films improves the release profile of rebamipide. However, according to the cytotoxicity studies, the 80% FS30D, 80%EPO and the 80%EPO-A formulations show high levels of cell death. Therefore, the 48%EPO+32%FS30D formulation is the optimum composition for a non-toxic film with a good release profile.

Further, films of 12mm diameter (approximately 1sq. cm) containing 0.25mg, 0.5mg and 1mg doses of rebamipide in the 48%EPO+32%FS30D were analyzed for drug release (Figure 16). It was observed that the formulations containing 0.25mg and 0.5mg drug released about 80% of drug within the first 3 hours. However, the films containing 1mg of Rebamipide released upto 80% of the drug only after 12 hours. This profile was similar to that observed with higher drug loading of 2.8mg/insert. Current research indicates that the daily dose of rebamipide suspension for the treatment of dry eyes is 1 drop in each eye, 4 times a day.6 This ophthalmic film formulation of 1mg of Rebamipde per insert allows a sustained release of drug from the matrix, enabling the reduction of dosing frequency and may help improve patient compliance.
Figure 15 Release of rebamipide from different formulations (2.8mg/film)

Figure 16 Release of rebamipide from 48% EPO+32% FS30D formulations containing different loadings of rebamipide.
3.1.6 Micropatterned Films Possess Higher Mucoadhesion To Ocular Tissues As Compared To Unpatterned Films

Mucoadhesion is the adhesive force between a material and a mucosal membrane. Mucoadhesive drug delivery systems take advantage of the adhesive interactions between biomembranes and polymer excipients to increase the retention time of the dosage form. Retention on the ocular surface is paramount for the sustained release of the drug from the film. Peak detachment force is a measure of the force required to detach a material from a mucosal surface while work of adhesion measures the work done to separate the two surfaces. Literature indicates that mucoadhesion is a means to prevent the movement of the dosage form in the eye, thereby preventing further inflammation.

As seen in Figure 18 for porcine intestinal tissue, only 80%FS30D showed a significant difference between work of adhesion for unpatterned and patterned films of SQ100D100 and SQ100D200 patterns. The peak detachment force for the same formulation, although not significant, showed a similar trend. However, the porcine intestinal mucosal tissue contains an abundance of mucins as compared to the ocular surface. This could result in the masking of the effect of patterns on tissue adhesion. Additionally, the folds present on the surface of intestinal mucosa resulted in high tissue-to-tissue variability. In an attempt to obtain more relevant results, the films were then tested for mucoadhesion on porcine ocular tissue. Owing to difficulty in processing and short stability of porcine ocular tissues, only formulations with promising release profiles (80%EPO-A) and cytotoxicity values (48%EPO+32%FS30D) were tested.

The 48%EPO+32%FS30D formulation showed a significant difference in the work of adhesion on the cornea with the SQ100D200 patterns (Figure 19). There were no significant differences between patterned and unpatterned films on the sclera and eyelid. However, similar
trends were observed between 80%EPO-A and 48%EPO+32%FS30D on the sclera, with the SQ100D100 and SQ100D200 patterns. A simple explanation for this behavior could be that the more crowded 100D100 patterns are able to hold on tighter to the surface mucins as compared to the more spaced-out 100D200 patterns. Additionally, the 100D100 patterns have a higher surface area for greater contact with the tissue as compared to both the 100D200 and the unpatterned films. Therefore, we can infer that micropatterns enhance the mucoadhesion and retention of the ophthalmic films on the ocular surface.

In diabetic keratopathy, placement of the films on the sensitive cornea would result in further injury, inflammation and impairment of vision. Patterned films show a good mucoadhesion on the sclera as seen in Figure 19. The ocular inserts therefore may be placed on the sclera within the conjunctival cul-de-sac, akin to other marketed ocular inserts such as pilocarpine Ocuserts® and cellulose-based lubricant inserts or Lacrisert®. This prevents the interaction of the device with the injured cornea. The improved mucoadhesive properties of micropatterned films will enable these films to adhere to the ocular surface after placement and prevent it from dislodging.
Figure 17 Ex-vivo mucoadhesion on porcine intestinal and ocular tissue

(A) Porcine intestinal tissue; (B) Porcine cornea, eyelid and sclera (left to right); (C) Removal of excess connective tissue from the surface of porcine eye; (D) Tissue clamp; (E) Texture analyzer mucoadhesion set-up
Figure 18 Mucoadhesion on porcine intestinal tissue

Data presented as mean ± standard deviation for 18 replicates in each group. * p<0.5, One-way ANOVA with Tukey’s post hoc test.
**Figure 19** Mucoadhesion of 48% EPO+32% FS30D on porcine ocular tissues

Data presented as mean ± standard deviation for 6 to 9 replicates in each group. * p<0.5, One-way ANOVA with Tukey’s post hoc test
3.1.7 48% EPO+32% FS30D Films Are Retained On The Sclera For More Than 5 Hours In The Ex-Vivo Eyeball Model

The ocular surface has a tear volume of about 7-10µl with a tear turnover rate of 1.2 µl per minute. In an effort to understand the behavior of ocular inserts in the presence of lacrimation, an ex-vivo model was developed using porcine eyeballs. The inserts were placed on the anterior sclera that possessed the conjunctival membrane (Figure 20). It was observed that both the patterned and unpatterned 48% EPO+32% FS30D films were retained on the anterior sclera for more than 5 hours. There was no observed dislocation due to simulated lacrimation at 6 drops/minute. It was also observed that the inserts turned visibly opaque on hydration. The pink dye incorporated in the formulation slowly leached out of the film, indicating the constant flow of PBS over the insert. An interesting observation in this study was that the ocular films do not lose their integrity on hydration. On manual removal of films from the surface of the eyeball after completion of the test, it was observed that the films detached without breaking or losing structure. Therefore, this implies that the ocular films can be removed intact after usage. However, this model is still preliminary and requires optimization. Further studies involving acceleration of the drop rate and reducing the distance between the two inserts on the eye may provide better insights on the difference in adhesion between patterned and unpatterned films.
Figure 20 Ex-vivo retention study of ocular films on the surface of porcine eye
4.0 Conclusions And Future Directions

The current study has successfully demonstrated the formulation of mucoadhesive, micropatterned polymeric films containing rebamipide as a candidate for the treatment of diabetic keratopathy. The utilization of release modifying Eudragit polymers combined with the soluble, viscous matrix-building cellulose polymers HPMC K4M and HEC 250L resulted in a non-bioerodable ocular insert with sustained release properties. Further, the study shows that solvent casting of films on PDMS molds yield robust micropatterns with good fidelity.

Micropatterned films had varying tensile properties depending on the formulation. The 80% FS30D formulation showed very elastic and flexible behavior while the 80% EPO and 80% EPO-A formulations were tough and brittle. The 48%EPO+32%FS30D formulation showed intermediate values of elasticity and toughness due to blended properties of both Eudragits. Patterned films seemed to be less tough as compared to unpatterned films and fractured along the space between the patterns. The load cell used for the tensile studies was 250lbf, which may not be sensitive enough to capture smaller changes in load with time. Therefore, future studies with a more sensitive load cell are required to confirm the tensile behavior of these films more closely.

Cellular viability of HCLE cells when exposed to placebo films of all formulations was found to be greatest in the combination formulation of 48%EPO+32%FS30D. Viability also seemed to decrease with an increase in drug content from 0.25mg to 1mg drug per insert. The formulations containing 80% FS30D and 80%EPO were extremely cytotoxic with almost negligible cell viability due to polymer dissolution and precipitation respectively. Additional cytotoxicity studies with stratified corneal epithelial cells and conjunctival epithelial cells may be
performed in the future to obtain a complete picture of the cellular effect of the thin film formulation on the eye.

We have successfully achieved a sustained release of up to 87% rebamipide in 24 hours, thus reducing dosing frequency of rebamipide. Additionally, we have demonstrated the incorporation of rebamipide, a poorly water-soluble drug, into the polymeric thin film formulation. However, the drug loading efficiency for 0.25mg, 0.5mg and 1mg rebamipide loaded inserts was only about 80% of added drug. Therefore, further studies must focus on dose optimization, improvement in loading efficiency.

Further, we show the differences in mucoadhesive properties of films between different parts of the ocular surface including the cornea, sclera and eyelids. Since the film formulation was designed to be placed on the anterior sclera within the ocular cul-de-sac, it is interesting to observe that SQ100D100 micropatterns seem to have better mucoadhesion as compared to the unpatterned counterpart. In diabetic keratopathy, there is a decrease in goblet cells, circulating mucins and membrane associated mucins on the ocular surface. Here, the micropatterned films having greater mucoadhesion due to their Velcro-like effect could be advantageous in providing additional retention of the dosage form. Additionally, the preliminary ex-vivo ocular study showed that the 48%EPO+32%FS30D films remain attached to the sclera for over 5 hours even under the continuous flow of PBS over the inserts. Future studies in this direction can be focused on the effects of ocular shear force on the ocular inserts caused due to the blinking motion of the eye. Detachment studies supplemented with shear adhesion studies can be used to more completely predict the behavior of these films when placed in the eye.

In conclusion, this research study demonstrates the potential of micropatterned, mucoadhesive films in sustained drug delivery to the ocular surface and reduce the dosing
frequency of rebamipide in diabetic keratopathy. Through the use of rebamipide, we have developed a cause-based therapeutic approach for the treatment of diabetic keratopathy. Additionally, we have developed a novel method of enhancing the mucoadhesion of ocular films by the fabrication of micropatterned surfaces. Further studies are required to assay the functional effect of rebamipide-loaded on the secretion of ocular mucins and to decrease the insert size to one that is closer to commercially accepted products. Finally, due to the hostile nature of the ocular environment in diabetic keratopathy, an additional parameter that remains to be studied is the effect of micropatterned films on ocular inflammation.
Bibliography


