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Research paper

Delivery of ionic molecules to anterior chamber by iontophoretic contact lenses

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ABSTRACT

Ophthalmic delivery via iontophoresis is currently achieved by placing an electrode on the cornea with a counter electrode on the ear or forehead. Here we test the feasibility of placing both electrodes diametrically opposite on a contact lens to create field gradients to transport ionic drugs into the cornea.

Commercial lenses loaded with nile blue and fluorescein as hydrophobic and hydrophilic drug analogs, respectively were placed on cadaver rabbit eyes. Electric field gradients were created by placing cathode and anode on the lens diametrically opposite to each other. The incorporation of an electric field (0.125 m–0.250 mA), showed an increased uptake of nile blue and the quantity was a function of the duration of the electric field and the amount of applied current. Similar increases in flux were observed for fluorescein. Confocal fluorescence imaging also shows increased penetration of the dyes in presence of the field. An equivalent circuit model suggests that the field gradients are much stronger in the direction perpendicular to the cornea, which results is minimal short circuiting of current through the tear film. The incorporation of an electric field into a lens could be a less invasive and more effective approach to achieve ophthalmic drug delivery via iontophoresis.

1. Introduction

Ophthalmic drugs are most commonly delivered via eye drops in spite of significant drawbacks. Although nearly 90% of the current market for non-invasive ocular drugs are in eye drop form [1], these offer a very low bioavailability. The inefficient drug uptake requires multiple applications of the eye drops a day and still cannot be used to treat retinal diseases [2]. Although topical drugs are sufficient for a majority people afflicted by ocular diseases such as glaucoma, inflammation, bacterial or fungal infections, some require injections to treat their conditions such as corneal neovascularization which can be treated with anti-VEGF agents [3]. However, these injections can lead to irritation and infection, or in more serious cases, endophthalmitis [4,5]. The deficiencies of administering an intrastromal injection has led to the exploration of alternative approaches to the delivery of larger molecules including less invasive injection sites, intra-ocular inserts, sclera implants, and iontophoresis. Although they sound promising, each come with a new set of complications such as difficulty of device insertion, low efficacy, and patient discomfort [6–9]. There is a clear need and opportunity for novel approaches to address these shortcomings. The problem arises from the limitations of the passive diffusion through the endothelial and epithelial cell layers in the eye that have low permeabilities and offer the greatest resistance in transporting drugs efficiently to the aqueous and the vitreous humor. However, low drug permeability through cellular networks has been overcome in the past through iontophoresis, a process that incorporates an electric field that drives the migration of charged species to facilitate delivery of molecules to both aqueous and vitreous humor [10].

Recent technology that incorporates iontophoresis for targeted drug delivery to the eye utilizes large reservoirs with an embedded electrode [11]. The counter electrode is then placed on the forehead or ear, resulting in a complex current pathway potentially resulting in large variability and a need for large voltage. Another shortcoming of the technology is that a bulky device could cause the user discomfort stemming from the need to keep their eye open for the duration of the procedure, preventing the replenishment of the tear film [12–14]. The approach is effective and necessary for the non-invasive delivery of much larger molecules such as RNAi or large growth factors [15]. Thus, there is a clear need to design a more compact system for ocular iontophoresis.

Abbreviations: HEMA, hydroxyethylmethacrylate; PBS, phosphate buffered saline; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); RGB, red, green, blue; B/W, black and white; ROI, region of interest

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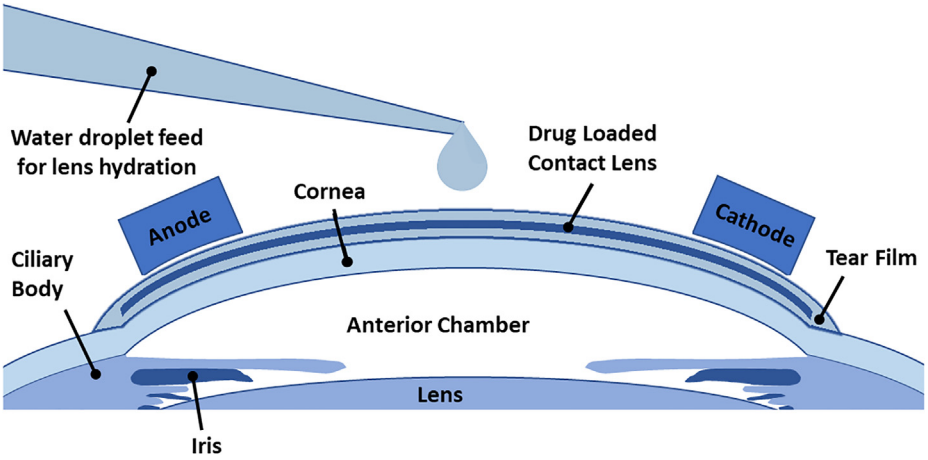

Fig. 1. Ex vivo arrangement of drug loaded lens and electrodes on a cadaver rabbit eye. The electrodes were connected to a constant current power source with variable control. The electrodes rested on top of the lens but were not incorporated directly into the contact lens itself. The water droplet feed allows the continued hydration of the drug loaded contact lens.

Iontophoresis has been used more commonly for transdermal drug delivery. The device for transdermal iontophoresis (skin patch) [10,16–18] includes both a cathode and an anode on the same patch. When a voltage is applied, current flows from anode, into the skin and then back out into the cathode to complete the circuit. Cationic drugs are loaded near the anode while anionic drugs are loaded near the cathode so that the field created by the applied voltage drives drugs into the skin. Here we consider the possibility of building the entire iontophoresis assembly on a contact lens, similar to how both cathode and anode are placed on the same skin patch. In our proposed design, the contact lens contains two electrodes that are spatially separated and connected to an external power supply. Recent research on microfabrication can allow building the electrodes and the battery into the lens, allowing a non-invasive approach to deliver ophthalmic drugs via iontophoresis [19–21]. Here though we use an external supply and use ex vivo rabbit eyes and electrochemical circuit modeling to assess the viability of such an approach.

The anatomy and physiology of the human cornea is significantly different from that of the skin. While skin surface is relatively dry, the human eye contains a fluid tear film that could create a short circuit between the cathode and the anode, minimizing any electric field penetration into the inner layers of the cornea. So a key question for the viability of this proposed design is whether the bypass current through the tear film eliminate the possibility of any significant drug transport into the cornea. Another critical issue is whether the rate of gas production due to breakdown of water will lead to an unacceptable rate bubble formation. Additionally, cornea is a more sensitive tissue compared to skin so it is critical to determine whether the electrochemical processes lead to significant damage to the cornea. We investigate these key questions here in this exploratory study.

2. Methods

2.1. Materials

Mature cadaver rabbit eyes were purchased frozen from Pel-Freez Biologicals (Rogers, AR). The nile blue A dye (hydrophobic, 75% dye content, MW: 354 g/mol, net charge: +1) and fluorescein (hydrophilic, MW: 332 g/mol, net charge: −1) were purchased from Sigma-Aldrich (St. Louis, MO). Electrode materials were either made from generic lead-free, silver bearing solder or was 3D printed with conductive PLA filament (Proto Pasta, Vancouver, WA) [22]. Cadaver rabbit eyes were fixed in Margo solution containing paraformaldehyde and glutaraldehyde purchased from Fisher Scientific (Hampton, NH). Soaking agents for lenses included phosphate buffered saline (PBS), 1 M HEPES, a HEPES buffer containing sodium chloride, calcium chloride, magnesium chloride, and potassium phosphate were all purchased from Fisher Scientific (Hampton, NH). Commercial contact lenses Acuvue Oasys (14 mm diameter, 8.4 mm base curve, 0.0 power) were used in the study.

2.2. Lens preparation

For studies with nile blue, Acuvue Oasys contact lenses, with a partition coefficient of roughly 30, were loaded by soaking in a 1 mg/mL solution in DI water for two days, resulting in roughly 400 μg of nile blue after loading. To investigate the effect of buffer, lenses were soaked in either pure HEPES (1 M) or HEPES buffer (NaCl 115 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.2 mM, K_2HPO_4 2.4 mM, HEPES 1 M) for 10 min prior to placement on the cadaver eyes. The molecular weight of HEPES is 238 allowing rapid uptake by the contact lenses.

For studies with fluorescein, Acuvue Oasys lenses, with a partition coefficient of 4, were soaked in a 100 mM HEPES solution at 0.1 mg/mL overnight, which was determined to be significantly longer than the time for equilibration and resulted in roughly 26 μg of fluorescein in the lens.

2.3. Application of electric field

The field was applied through two circular electrodes placed over the lens. The electrodes were attached to a power source set to the desired current (Fig. 1). A syringe pump was configured to gently place a drop of PBS about 30 μL in volume every 30 s on the center of the lens to form a thin film on the surface. The thin film prevents drying of the lens and the cornea and also served as an approximate mimic of the in vivo tear film. It was also critical to ensure that the lens was submerged in a liquid film so that bubbling of gases, if any, was easily visible. The field was applied for the desired duration and then the power source was shut down and lenses were removed.

2.4. Effect of parameters

Experiments with nile blue were conducted for three different choice of solutions (DI water, 1 M HEPES, 1 M HEPES buffer), and each of these cases was explored for three different combinations of current and time. The strengths of the electric fields were 0.25 mA (high current) and 0.125 mA (low current). Time durations were either 30 min (short time) or 60 min (long time) for DI water only. Then, for samples soaked in HEPES and HEPES buffer solutions were ran for either 60 min (short time) or 120 min (long time). The effects of buffers were chosen to be studied with nile blue because it doubles as a pH indicator. When the pH is either acidic or neutral, nile blue has a blue hue. However, when the solution becomes basic (pH ≈ 10) the color gradually changes to pink. The ability to visualize the diffusion of the drug and observe the

progression of the ionic species generated at the electrodes is ideal for the quantification of drug delivery and monitor the severity of the electrochemical reactions. Control studies were included in which eyes were not exposed to any dye, and another set that had dye loaded lenses were placed on the eyes, but field was not applied. Additionally, in another set, the eyes were exposed directly to 1 mg/mL nile blue solution in DI water for 60 min without application of field. Since the effect of buffers was already explored using nile blue, the experiments for fluorescein transport were conducted using only 100 mM HEPES, which was considered to be optimal to minimize the potential for damage to the cornea. Fluorescein transport was explored as a function of time of application and magnitude of current.

2.5. Measuring nile blue transport

After the field was removed, the cadaver eyes were imaged in triplicate by a Nikon DSLR camera and the images were processed through ImageJ to determine whether nile blue was delivered to the eye. After direct imaging, each set of cadaver eyes were fixed in Margo formulation [23]. The fixative solution consists of 1% paraformaldehyde and 1.25% glutaraldehyde in PBS and the eyes were submerged for 40hr to ensure complete fixation. After fixation, the eyes were cross-section in hemispherical form to include both regions of the cornea that were adjacent to the electrodes and put in a glass bottom dish filled with PBS. The dishes were mounted on a Nikon A1 laser scanning confocal microscope to observe the distribution of nile blue throughout the cornea. All images were captured and processed with identical imaging properties.

2.6. Measuring fluorescein transport

Fluorescein, a fluorescent molecule, was loaded into lenses in a 100 mM HEPES solution at 0.1 mg/mL. Delivery of fluorescein into the cornea was measured in triplicate for each variation of time and current and for the control which was purely driven by diffusion. After applying field for the desired duration, the eyes immediately had the aqueous and vitreous harvested. Each sample was centrifuged at x5000 g for 10 min in Qor Labs mini centrifuge to remove any residual tissue. Relative fluorescent intensity of each sample was found using a Quantech fluorometer and the corresponding concentrations of fluorescein was found through a calibration curve.

2.7. In vitro release of fluorescein

The release was carried out by loading the lens with 0.1 mg/mL fluorescein in an Acuvue Oasys lens overnight for uptake. After the uptake of fluorescein, the lens was removed and lightly blotted to remove residual fluorescein from the surface. The release was carried out under sink condition by soaking the lens in 5 mL of PBS. It can be assumed that 100% of the drug release occurred after reaching equilibrium.

2.8. Model

A simplified COMSOL model of the electric field distribution through the cornea was constructed to probe the feasibility of having two electrodes on a single contact lens (Fig. 2). The geometry of the cornea and contact lens with embedded electrodes were approximated as a flat surface for simplicity. The geometry incorporated all layers of the corneal tissue, the tear film, and contact lens with properties from Table 1. The electric field was generated using the 'AC/DC' module with a fixed current density from one electrode at a value translating to an overall current of 0.125 mA which was a value used in ex vivo experiments.

3. Results

3.1. Transport of nile blue

3.1.1. Imaging by camera

The nile blue was loaded into the lenses of three separate solutions. The first was in deionized water (DI) because the absence of any other salts or buffers results in nearly all of the current through the lens to be carried by the charged drug, therein maximizing the transport. The second was a concentrated HEPES solution which is a zwitterionic buffer that is commonly used in cell biology. The zwitterionic HEPES buffer is ideal for maintaining the pH near the electrodes in spite of the electrodic reaction. The last solution was a concentrated HEPES buffer with common physiological salts to mitigate the changes in stroma. Previous studies have shown that the loss of negatively charged ions lead to a structural change in corneal stroma fibrils which cause a decrease in the transparency of the cornea [26–28].

In order to quantify drug delivery, diffusion efficacy was measured by simultaneous comparison of four separate eyes, each corresponding to a different condition. Three of the four eyes had a drug loaded lens and an electric field applied to the system while the fourth served as a control. The eyes used to observe drug transport had lenses that were soaked in the same drug solution to ensure equal loading concentration. The applied electric fields were chosen to achieve 0.25 mA (high current) and 0.125 mA (low current). Samples for lenses soaked in DI water only were ran for either 30 min (short time) or 60 min (long time). Then samples soaked in HEPES and HEPES buffer solutions were ran for either 60 min (short time) or 120 min (long time). Upon inclusion of HEPES and HEPES buffer, the drug loaded into the lenses was no longer the dominant carrier of current, therefore requiring a longer time interval for drug delivery. Images after drug delivery were processed through ImageJ and were separated into RGB, red, and B/W channels. (Fig. 3C) The amount of nile blue delivered can be seen by the darker ROI that arises from the blue hue produced by the dye. Therefore, as presence of nile blue increases, the lower the ROI gray value.

3.1.2. Confocal fluorescent imaging

In order to observe the transport of a charged species and the distribution throughout the cornea, confocal microscopy of cadaver rabbit eyes was conducted for each lens loading solution used in previous experiments. After the completion of the diffusion experiments, the whole eyes were fixed in a paraformaldehyde and glutaraldehyde solution, preserving the eyes and the nile blue. After fixation, the eyes were cross-sectioned to include the areas that were adjacent to the electrodes and imaged on a confocal microscope. The FITC channel shows the fibrous structure of the stroma and the regions of high cellular density due to the autofluorescent property of rabbit tissue while the far-red images are of nile blue (Fig. 4). Contrasting the rate of delivery was done by first establishing the intensity and distribution of nile blue administered to a cadaver rabbit eye by free solution diffusion and from a drug loaded contact lens for 1hr (Fig. 4A). Then, the three situations incorporating the electric field at high current for a short time duration with lenses loaded with DI water, HEPES, and HEPES buffer respectively were considered (Fig. 4B–D).

The results indicate that at high current, electrochemically generate species produce a caustic solution that damages the cornea at the cathode. However, upon incorporation of HEPES, the effects were neutralized. Conflictingly, the HEPES produced an osmotic effect at the anode that reduced the thickness of the cornea which disrupted the native organization of the collagen matrix in the stroma and resulted in a loss in transparency even though there was some small degree of recovery when the cornea was submerged in PBS for a several minutes. As a result, the most ideal lens would be loaded with HEPES in the area near the cathode to prevent damage from electrochemically generated species and the area near the cathode would be loaded with DI water to prevent any osmotic side effects.

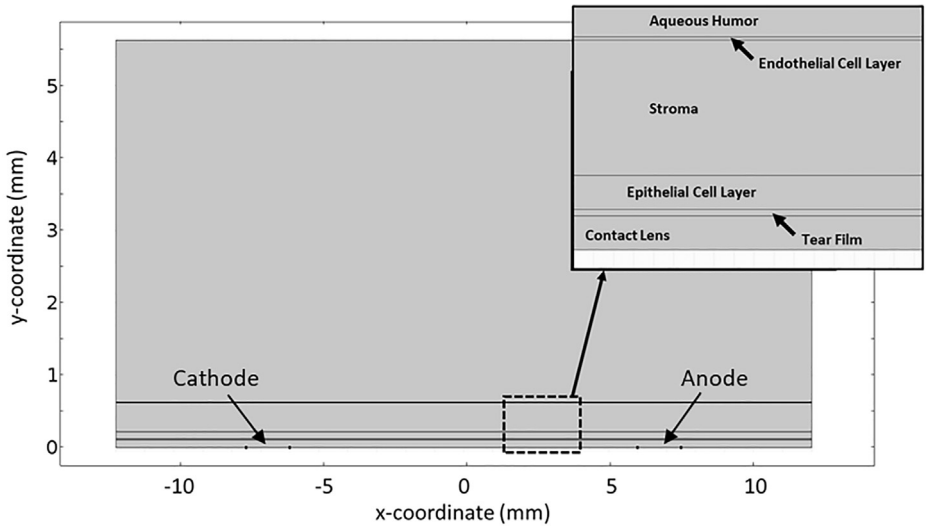

Fig. 2. COMSOL representation of the corneal assembly. The geometry of the simplified layers of the cornea and the electrodes that provide an electric field. The enlarged section depicts the layers of the cornea, the tear film, and contact lens.

Table 1
Values used to in the dimensional analysis and scaling comparison to approximate the bioavailability of nile blue in the cornea.

Parameter	Symbol	Value	Units
Distance between electrodes	ℓ	10^{-2}	[m]
Thickness of lens	h_L	10^{-4}	[m]
Thickness of tear film	h_T	10^{-5}	[m]
Thickness of epithelial layer	h_{EP}	10^{-4}	[m]
Thickness of stroma	h_S	$4 \cdot 10^{-4}$	[m]
Thickness of endothelial layer	h_{END}	$3 \cdot 10^{-4}$	[m]
Thickness of aqueous humor	h_{AQ}	10^{-3}	[m]
Resistivity of contact lens	ρ_L	$4 \cdot 10^{-3}$	[S/m]
Resistivity of tear film	ρ_T	1.5	[S/m]
Resistivity of epithelial layer [24]	ρ_{EP}	$4 \cdot 10^{-4}$	[S/m]
Resistivity of stroma [24]	ρ_S	0.2	[S/m]
Resistivity of endothelial layer [24]	ρ_{END}	$6.6 \cdot 10^{-3}$	[S/m]
Resistivity of aqueous humor	ρ_{AQ}	1.5	[S/m]
Diffusivity in tear film	D_T	10^{-9}	[m^2/s]
Diffusivity in epithelial cell layer [25]	D_{EP}	$5 \cdot 10^{-12}$	[m^2/s]

A lens was cut in half and one half was soaked in HEPES while the other was soaked in a nile blue solution with DI water. Then an electric field was applied at high current for a short time duration, the case with no damage at the anode with DI water lenses. After completion (Fig. 5A), the cornea had a small deposition of nile blue localize at the area adjacent to the anode and no damage in the area near the cathode. Similarly, a situation with a hybrid lens was constructed but the lens loaded with nile blue was soaked in PBS because it is more physiologically acceptable for a contact lens rather than DI water. The results of the PBS hybrid setup, nearly identical to that of the DI water lens which is expected due to the low salt concentrations in PBS. In order to confirm that the cornea was unchanged in thickness during the process, confocal images were taken of the cornea at regions adjacent to the anode and cathode (Fig. 5B). The image shows that the structure of the stroma and cellular layers are like that of a healthy corneal cross section. Furthermore, the nile blue is well distributed in the region near the anode, similar to previous experiments.

3.1.3. COMSOL simulation for the distribution of the electric field in the cornea

The current density from one electrode was fixed at a value translating to an overall current of 0.125 mA which was a value used in ex vivo experiments. The distribution of the electric potential shows that a great deal of the voltage drop is through the contact lens but also the epithelial cell layer. (Fig. 6) The streamlines, depicting the flow of current, indicate that a majoring of the current penetrates through the epithelial and endothelial cell layers and into the aqueous humor. The low voltage drop across all layers past the epithelium demonstrates the drugs of interest would be under less influence from the electric field once in the stroma. This means that the drug is more likely to freely diffuse to the interior of the eye rather than fully following the flow of current to the counter electrode. The model supports the claim about the distribution of the electric field through the cornea and although the model is a 2D simplification, it demonstrates that a physiologically relevant current density ($> 5\,A/m^2$) can provide substantial current through the front of the eye without short circuiting through the contact lens or the tear film.

3.1.4. Electrophoretic velocity of nile blue through the epithelial cell layer

The distribution of nile blue in the cornea with an electric field at various currents and time durations can be further explained by a simple analysis of the electrophoretic velocity (v_{ep}) (Eq. (1)). The electrophoretic velocity is mainly a function of the charge of the species (z), the within the epithelium, overall voltage, and the distance between the electrodes.

$$v_{ep} = \frac{zeDV}{kT\ell} \tag{1}$$

Due to the hydrophobic nature of nile blue, the rate of diffusion is quite small in comparison to hydrophilic drugs when in the epithelium. Using values from Table 1 and assuming a V_{ep} of 2 V on a single half of the eye, after 30, 60, and 120 min the total distance travelled in the epithelium is only 69, 139, and 278 μm respectively. In the case of the shortest time duration, this is roughly only 2/3 of the epithelium. This explains why there is a localization of nile blue when the system was applied for a short time duration with a low current for DI water lenses in Fig. 4. The nile blue had not yet penetrated the epithelial layer and dispersed within the cornea in regions with higher overall diffusion rates. Additionally, this is why there is no overall aggregation of nile blue in the region adjacent to the counter electrode for any of the time durations used in the experiments. Then, for the HEPES and HEPES buffer after 60 min, the nile blue was able to traverse the entire epithelium and permeate the stroma. However, the distance travelled may not be reliable as the stromal collagen matrix has reorganized, resulting in a variation of the inherent properties.

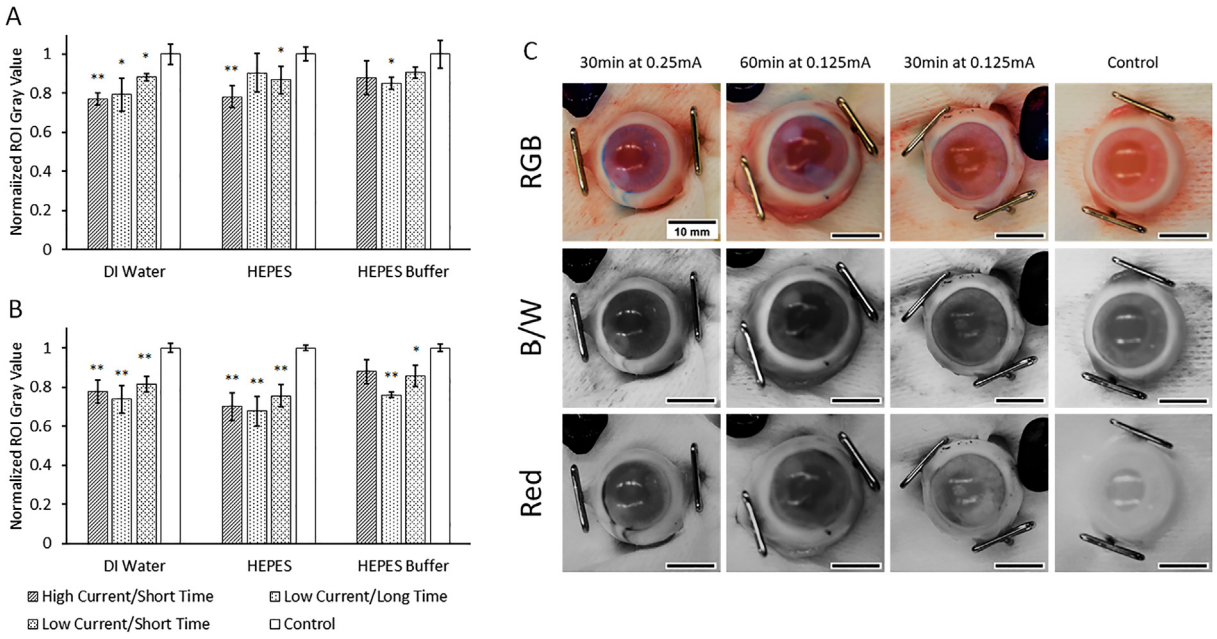

Fig. 3. ROI analysis of cadaver rabbit eyes after delivery of nile blue from drug loaded lenses. Graphs show the normalized RBG (A) and red (B) ROI values for the cadaver rabbit eyes for all three lens conditions with the three different current and durations and the control eye. (C) One set of cadaver rabbit eyes after conclusion of nile blue delivery from DI water lenses. (∗∗: p-value less than 0.05, ∗: p-value less than 0.1, n = 3) (scale bar: 10 mm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.1.5. Transport of fluorescein

Fluorescein, a hydrophilic and fluorescent, was loaded into lenses in a 100 mM HEPES solution at 0.1 mg/mL. In order to quantify the delivery of the hydrophilic drug to the interior of the eye, the experiments were conducted at three different combinations of time and current and a control without current. However, since fluorescein has a much higher permeability than nile blue, the application times were reduced to 15 min (short time) and 30 min (long time) but still used the same current values because they were chosen to border the limit of physiologically safe current density. After the duration of the experiments, the interior of the eye was harvested and fluorescence was quantified using a fluorometer. The results indicated that the final concentration

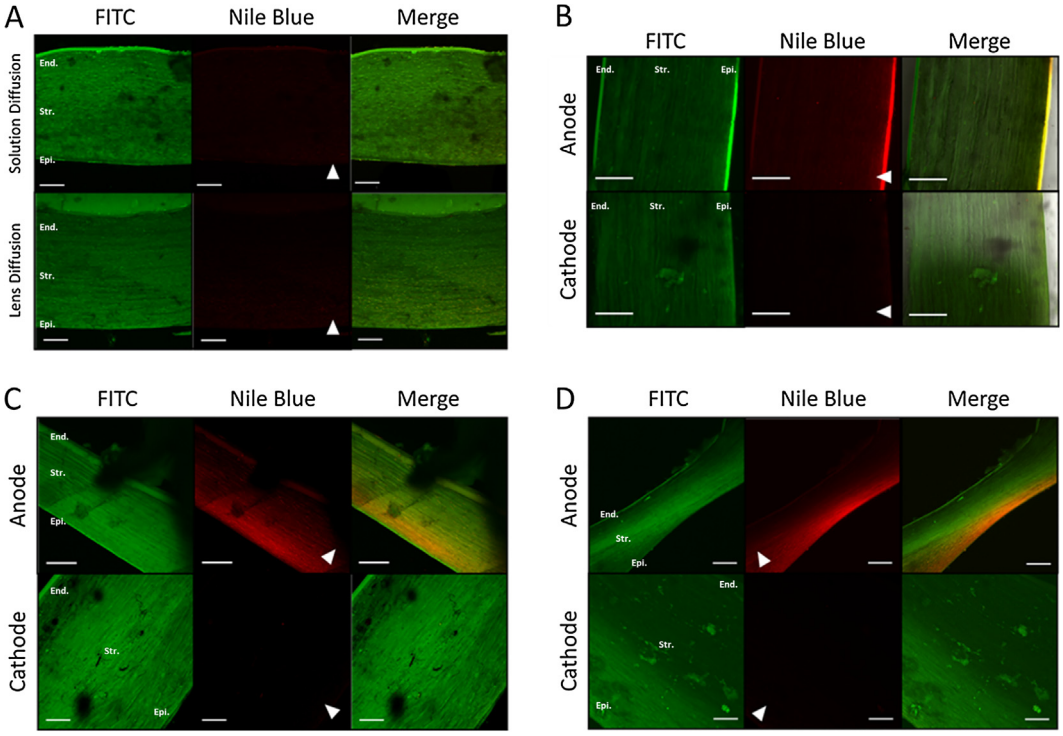

Fig. 4. Confocal images of cross-sectioned cadaver rabbit eyes after delivery of nile blue. (A) The diffusion of nile blue through the cornea delivered via free standing solution and another with a drug loaded lens for 60 min. (B–D) Corneas of regions near anode and cathode after delivery at 0.25 mA for 30 min from drug loaded lens soaked in DI water (B), for 60 min and pre-soaked in HEPES (C), for 60 min and pre-soaked in a HEPES buffer solution (D). Arrows indicated the direction of the diffusion profile from the front of the cornea to the interior of the eye. (scale bar: 200 μm).

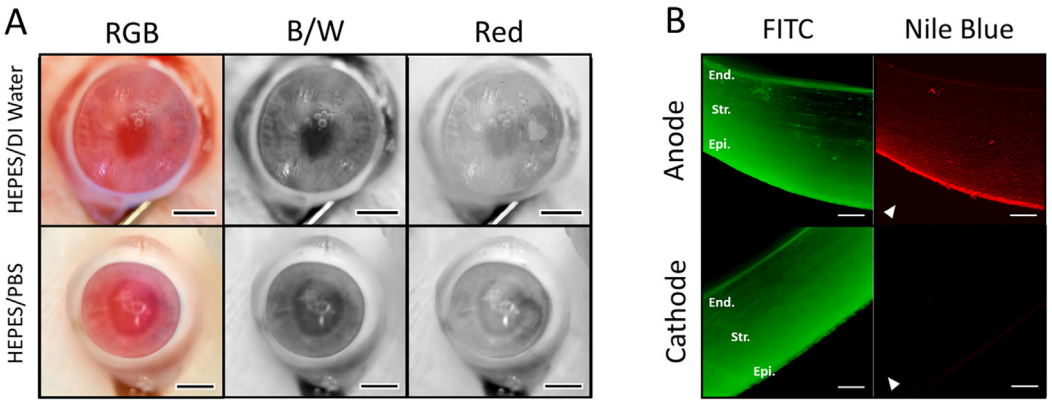

Fig. 5. Results of hybridized lens. (A) Cadaver rabbit eye after delivery of nile blue at high current for 30 min with hybridized lens configurations. Anodic region (right half) had nile blue loaded lens soaked in DI water or PBS and cathodic region (left half) had HEPES soaked lens. (scale bar: 5 mm) (B) Confocal images of hybridized lens configuration with PBS and nile blue at the anode and the HEPES lens at the cathode at high current for 30 min. FITC shows intact stroma and red channel illustrates nile blue distribution after delivery from the drug loaded lens. (scale bar: 200 μm).

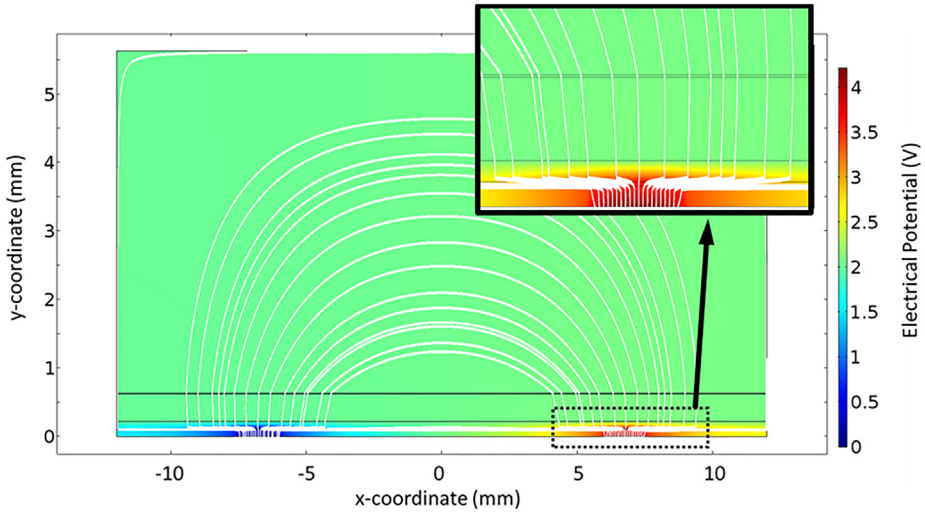

Fig. 6. Electric potential distribution through the anterior region of the eye. Simplified corneal assembly with electrodes on opposite sides of a contact lens. The voltage drop can be seen to take place across the contact lens and the epithelial cell layer while the streamlines depict the current path between the electrodes.

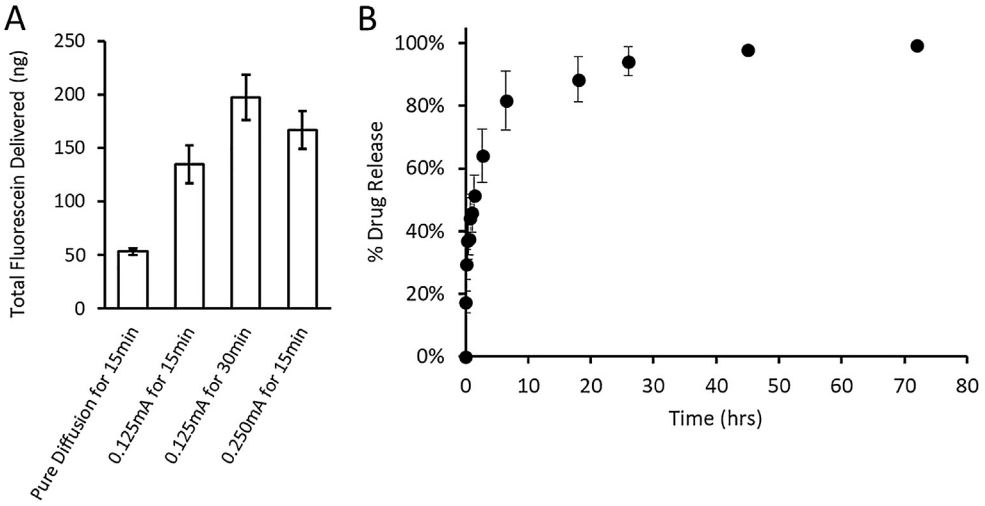

Fig. 7. Quantification of fluorescein delivered to the interior of the eye. (A) The concentration of fluorescein within the vitreous and aqueous humor after the application of the electric fields and the control experiment which was driven by diffusion only. (n = 3) (B) The release profile of fluorescein from a contact lens which was used to find the diffusion coefficient from the lens. (n = 5).

in the interior of the eye (vitreous and anterior chambers) was lowest for the case without electric field and was highest for the low current and long time application (Fig. 7A).

3.1.6. Circuit equivalent model for the electrically driven transport of a drug through the cornea

An equivalent circuit model of the cornea was constructed to describe the concentration profiles and determine the relative effect of

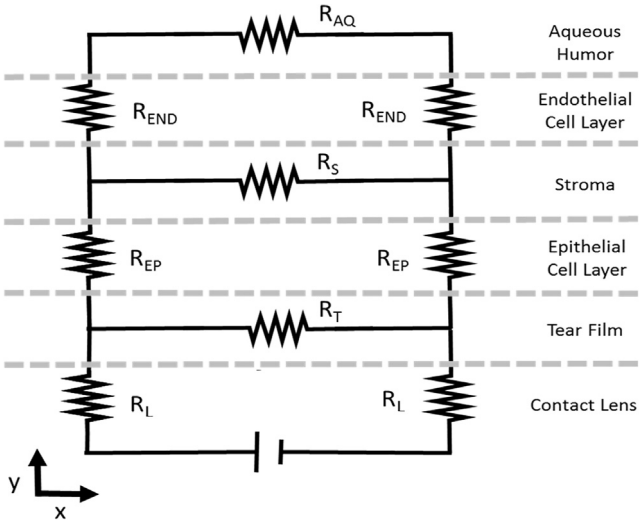

Fig. 8. Circuit diagram of the cornea. Representative circuit diagram for the resistive layers of the cornea with a voltage source in the contact lens while the resistances for each layer of the cornea are oriented in the direction of the dominant potential gradient.

short circuiting through the tear film. From the model, a fundamental dimensional analysis could estimate the efficacy of drug transport through the cornea and the bioavailability. The model assumes that the cornea can be visualized as a simple circuit diagram with each layer having its own resistance (Fig. 8).

The first step in understanding the drug transport is to determine the current distribution between the epithelium and the tear film which can be estimated by the ratio of resistances through each layer. The layers with lower resistivity (tear film (T), stroma (S), and aqueous humor (AQ)) will have a predominant current in the x-direction while the layers with higher resistivity (epithelium (EP), endothelium (END), and contact lens(L)) will exhibit current in the y-direction.

$$R_j = \rho_j \ell / h_j \qquad (AQ, S, T) \tag{2}$$

$$R_i = \rho_i h_i / \ell \qquad (END, EP, L) \tag{3}$$

Each layer of the cornea has a known resistance as a function of resistivity (ρ), the height of the respective layer (h), and the distance between electrodes (ℓ)(Eqs. (2) and (3). Taking the ratio of the voltage drop through the tear film and epithelium will determine if the length scale of the tear film is high enough to overcome the resistivity of the epithelial cell layer. The voltage drops (ΔV_T, ΔV_{EP}), were found and approximated using properties from Table 1 as a function of the overall potential (V) applied to the system (Eqs. (4) and (5). The ratio of voltage drops was found to be roughly 1 which means that the total resistance through the tear film is on the order as the resistance through the epithelial cell layer and a significant fraction of current passes through the epithelium. If the ratio of voltage drops had been much less than 1, it would mean that the tear film acted as a short-circuit and much of the current would not be able to penetrate the epithelium.

$$\Delta V_T = V / \left(1 + \frac{4h_T}{\rho_T L^2}[\rho_L h_L + \rho_{EP} h_{EP}]\right) \tag{4}$$

$$\Delta V_{EP} = V / (2 + 2[\rho_L h_L / \rho_{EP} h_{EP}]) \tag{5}$$

The next step in determining the bioavailability was to determine the relative time scales for drug transport across the tear film (τ_T) and the epithelium (τ_{EP}). In the case of the time scale for the tear film, the concentration gradient was only be in the x-direction and attributed to the electrophoretic flux as a function of diffusivity (D_T), the charge of the drug (e), and the electric potential (φ_T) (Eq. (6)). After scaling, substitution, and assuming the potential gradient in the tear film was a constant value ($\varphi_T\ \Delta V_T$), the time scale for the drug transport across the tear film was characterized as the amount of drug in the tear film over the rate of electrophoretic transport through the tear film (Eq. (7)).

$$\frac{\partial C}{\partial t} = \frac{D_T e}{kT}\frac{\partial}{\partial x}\left(C\frac{\partial \varphi_T}{\partial x}\right) \tag{6}$$

$$\tau_T = \frac{kTL^2}{eD_T \Delta V_T} \tag{7}$$

In the case of concentration gradient in the epithelium, there was assumed to be no concentration gradient in the x-direction and that the drug transport was purely in the y-direction as a function of the electrophoretic flux driven by the potential gradient (Eq. (8)). However, since the potential through the epithelium is not constant, the $\partial\varphi_{EP}/\partial y$ term was assumed to be the voltage drop across the epithelium (ΔV_{EP}) over the height of the epithelium (h_{EP}). Therefore, the time scale for drug transport across the epithelial cell layer was defined as the amount of drug in the tear film over the rate of electrokinetic transport through the epithelium (Eq. (9)).

$$\frac{\partial C}{\partial t} = \frac{D_{EP} e}{kT}\frac{\partial}{\partial y}\left(C\frac{\partial \varphi_{EP}}{\partial y}\right) \tag{8}$$

$$\tau_{EP} = \frac{kTh_{EP}h_T}{eD_{EP}\Delta V_{EP}} \tag{9}$$

$$\tau_T/\tau_{EP} = z\left(\frac{D_{EP}}{D_T}\right)\left(\frac{\rho_{EP}}{2h_T\rho_T}\right)\left(\frac{\ell^2\rho_T + 4h_T[\rho_L h_L + \rho_{EP}h_{EP}]}{[\rho_L h_L + \rho_{EP}h_{EP}]}\right) \tag{10}$$

$$Bioavailability\ \frac{\tau_T/\tau_{EP}}{1 + \tau_{T/\tau_{EP}}} \tag{11}$$

The time scales for the drug transport across the epithelium (τ_{EP}) and through the tear film (τ_T) characterize the time for the drug in the tear film to either go through the epithelium or to pass through the tear film and aggregate on the counter electrode. Taking the ratio of τ_{EP} to that of τ_T characterizes the expected ratio of drug delivered through the epithelium compared to that through the tear film (Eq. (10)). The relation approximates the bioavailability of the drug, or simply the amount of drug that remains in the tear film compared to the amount of drug that is delivered across the epithelial cell layer and into the stroma (Eq. (11)). A bioavailability less than 0.5 indicates that most of the drug remains in the tear film due to the shorter time scale in the tear film. However, if the bioavailability is greater than 0.5, then most of the drug is delivered across the epithelium due to the longer time scale in the tear film. Using drug properties for a lipophilic drug (approximated properties of nile blue in the cornea) [29] and the electrode configuration used in the experiments, the bioavailability is approximated to be nearly 99%. Meaning that almost all the drug that leaves the lens via electrokinetic transport follows the electric field through the epithelium instead of short circuiting through the tear film. As previously shown in the confocal images of the cross-sectioned cadaver rabbit eyes (Fig. 4), a large portion of the nile blue was transported into the epithelium instead of aggregating near the surface of the counter electrode, supporting the model prediction of almost 100% bioavailability into the cornea. If short circuiting through the tear film was significant, there would be significant nile blue in the cornea near the cathode, which was clearly not the case based on both modeling and ex vivo experiments. Therefore, the design with two electrodes on a single drug loaded contact lens shows potential in regards of transport of a charged species into the cornea.

4. Discussion

4.1. Transport of nile blue

A drug can passively diffuse through the endothelial and epithelial

cell layers and into the aqueous humor but the time scale for drug diffusion can be longer than the residence time of the drug in tears, in which case a majority of the drug is lost through the conjunctiva or tear drainage. An electric field can drive a charged drug or drug solution through diffusive barriers faster and additionally loading the drug in the contact lens can increase the residence time in tears. In ex vivo experiments on cadaver rabbit eyes, a drug loaded lens was placed over the eye and two electrodes, each 3 mm in diameter, were placed near the periphery at opposite sides of the lens. (Fig. 1) A power source, held at constant current, was connected to the electrodes providing the electric field to the system. Nile blue was chosen as the drug analog due to the positive charge, size comparable to ophthalmic drugs, ease of detection, and the ability to act as a pH indicator. Over the course of the experiments with DI water lenses, the area adjacent to the cathode did demonstrate a color change to a pinkish hue, indicating that the pH had become quite basic and can potentially inflict damage to the ocular surface. However, when buffers were added to the lens, this change was much less noticeable and mitigated the harmful effects from the electrochemically generated species.

4.1.1. Effect of buffer on drug delivery

The typical dominant electromotive force that drives drug delivery in iontophoresis is through charge repulsion. However, when additional salts are added to the system, magnitude of the net repulsive force on the drug itself is reduced and the buffer itself becomes an important carrier of the current, resulting in less drug being delivered to the target region. This can be seen in the experiments with nile blue with ROI analysis.

The nile blue delivery shown in Fig. 3C demonstrates that the application of high current for short time and half the current but for double the time have similar drug delivery to the cornea. The nile blue is well distributed throughout the cornea near the anode except for the experiment with low current for a short time. In this case, the nile blue is localized to the area where the anode was placed. Since nile blue is a positively charged dye, this is the area the drug would be expected to penetrate the epithelial cell layer, confirming that the drug delivery is noticeably aided by the presence of an electric field and that contributions from passive diffusion were very minor.

In the case of the lenses loaded in DI water, all conditions showed significant quantities of nile blue. The cases with high current for a short time and low current for a longer time interval showed very similar values, indicating that the time and magnitude of the current applied has a proportional relationship to the amount of drug delivered. The case with the least amount of nile blue delivered compared to the control was the low current for a short time which is expected if the current and duration are proportional to drug delivery. These results clearly indicate that passive diffusion from the lens is insignificant compared to the electrical flux.

The next set of drug loaded lenses were soaked in a concentrated HEPES solution. The trials were conducted at the same currents but for double the amount of time to compensate for the percentage of current now carried by a species other than nile blue. The results are similar to those of the DI water lenses in that the case with high current for a short time had the highest amount of drug transport. The general trend also indicates that passive diffusion has negligible contributions for the delivery of nile blue. The final set of lenses were soaked in a HEPES buffer that included common salts to balance the transport of ionic species. However, the salts became the notable carrier of current through the system and the only case that consistently saw a significant presence of nile blue was with low current for a long time duration while the other cases showed a smaller and similar degree of transport in contrast to each other. This is indicative of passive diffusion becoming a substantial means of transport, although there were still noticeable contributions from the electric field.

4.1.2. Confocal fluorescence imaging

It has been established that the incorporation of an electric field can significantly increase the rate of the transport of a charged species into the cornea. To characterize the slow rate solely diffusion driven transport, two situations were constructed. The delivery of nile blue from a contact lens and the delivery from a standing solution. After 1 hr of administration, very minor amounts of nile blue were present throughout the cornea in both cases. The solution diffusion case demonstrated a very uniform distribution of nile blue but with very small concentration. The diffusion from the contact lens illustrated a faint gradient in the distribution of nile blue with the highest concentration regime at the front of the cornea. This shows that the pure diffusion driven transport has a difficult time penetrating the dense epithelial cellular layer. Once more, there were no indications that a significant amount of nile blue had been delivered through the cornea.

Upon incorporating an electric field and the application through the drug loaded lens soaked in DI water (Fig. 4B), the distribution of nile blue near the anode illustrates a high concentration gradient with the peak concentration at the front of the cornea while the concentration decreases as it progresses towards the interior of the eye. In the case of the cathodic region and adjacent area with only pure diffusion, there is only a small degree of nile blue localizing near the outer surface with a similar distribution to the anodic region but to a much lesser extent. However, unlike experiments conducted with HEPES in the contact lens (Fig. 4C and D), there was no significant change in the thickness of the cornea at either electrode. This is most likely due to the lack of buffering ions in the contact lens that would lead to osmotic differences across the cornea. Therefore, the case with DI water lenses without any buffering agent had the greatest preservation of the stromal structure and had a minimal change in transparency at the anode.

When the drug loaded lenses were pre-soaked in a concentrated HEPES solution (Fig. 4C) with high current for a short time duration, there was no obvious cellular damage to the cornea from the production of sodium hydroxide at the cathode. Similarly, the highest concentration of nile blue was at the front of the cornea in the region directly adjacent to the anode. However, the distribution of nile blue demonstrated a much more linear gradient throughout the cornea except for the endothelium which was more concentrated than the preceding region of the stroma. This can be attributed to the higher diffusive resistance in the endothelium, leading to greater accumulation of nile blue than in the stroma. Then near the cathode, there was no nile blue visible which is expected for delivery on a short time scale. Even if the electric field is applied for much longer durations, after the nile blue penetrates the endothelium, the drug rapidly diffuses into the aqueous humor. Furthermore, the regions with the highest voltage drop, or the regions through which a majority of current passes are the tear film and epithelial cell layer. This results in an almost negligible amount of the electric field to act on any other area of the cornea. Then in the case of the drug loaded lenses pre-soaked in the HEPES buffer (Fig. 4D) with high current for a short time duration, the results were almost identical to that of the HEPES soaked lenses apart from there being less nile blue to penetrate the cornea and there being less accumulation of nile blue in the endothelium. Another observation for both the HEPES and HEPES buffer lenses was the variability in the thickness of the cornea near the electrodes. The region near the anode demonstrated a noticeable decrease in thickness. This can most likely be attributed to the change in osmolarity near the electrodes as the electrochemically generated species permeate the cornea and possibly influence of the electric field on the cells themselves. The change in thickness can also explain the loss in transparency after the completion of the experiments due to the variation in the stromal structure.

The confocal images show that the electric field drives the nile blue into the epithelium and potentially into the stroma. After the drug has been deposited through the most significant diffusion barrier, the epithelium, it can passively diffuse through the remaining cornea and into the aqueous humor. Due to the high diffusive resistance through the

epithelium compared the remaining cornea, if the drug only penetrates half of the epithelium, the drug has a high likelihood of passing into the interior of the eye rather than diffusing back out through front of the eye. However, it is very apparent that ionic transport and electrochemical reactions have a harmful impact on the viability of the cornea after the drug delivery process.

4.1.3. Potential for damage

There are two potential mechanisms that can cause damage to the cornea. At the cathode, the generation of sodium hydroxide would result in a caustic solution that could cause damage. At the anode, the electric field driven ion flux could cause osmotic swelling or shrinking to reduce the transparency of the cornea. The damage due to swelling or shrinking could be reversible. Detailed analysis of damage to the cells would involve concentration measurements in the tears to determine whether specific chemicals were released from the cells. Also, fluorescence staining to measure barrier function and histology to directly visualize the cells would be important. Since this is a preliminary study, we used transparency of the cornea as the first indication for damage.

The damage done to the cornea when nile blue was delivered varied with current and time duration. For contact lenses soaked in DI water, the cornea remained transparent suggesting no significant damage. At high current and short time, the cathode region remained transparent but there was damage at the anodic region which is a result of the electrochemically generated species. The low current and long-time interval had damage at both the anodic and cathodic regions. This is most likely due to the combination of electrochemically generated species and the change in the stromal fibrils that results in a loss of transparency. This pattern reveals that without any buffer to moderate the electrochemically generated species at the cathode combined with a sufficiently high enough current and time duration, there will be irreversible damage done to the surface of the cornea. Similarly, with a long enough time duration, the orientation of the corneal stroma fibrils undergoes a structural change and a loss in transparency. Once the eye was re-suspended in PBS for a short time (5–10 min) the damage at the anode would occasionally regress and regain transparency while the damage at the cathode was typically seen to be unrecoverable. These patterns were noticeably similar to the cases that incorporated HEPES and HEPES buffer into the lenses but there was no visible damage to the cornea in the region near the cathode. This confirms that a buffering agent can protect the cornea from sodium hydroxide generated at the electrode. Additionally, in these experiments we did not observe any bubbling which suggests that the rate of gas generation was sufficiently small. However, the rearrangement in to the stromal fibrils was persistent in both cases when the electric field was applied for the longer time durations and at high current.

Based on the studies with nile blue, it was deduced that a lower concentration of HEPES loaded into the lens would minimize the potential for damage and so that solution was used in studies with fluorescein. There was no damage to the cornea when fluorescein was delivered by soaking the lenses in 100 mM HEPES buffer.

4.1.4. Lens design to reduce adverse effects from electric field

The results of the nile blue ROI analysis (Fig. 3) and initial confocal imaging (Fig. 4) indicate that at high current, there is an increase in pH due to electrochemically generated species at the cathode. A HEPES solution and HEPES buffer were incorporated into the lenses and inhibited the damage at the cathode. However, the addition of these solutions caused an osmotic effect at the anode that reduced the thickness of the cornea and greatly reduced transparency. Therefore, a hybrid lens system was explored to prevent damage at the cathode while preserving the thickness of the thickness of the cornea. The hybrid lens incorporated HEPES at the cathode in both arrangements and then either DI water or PBS at the anode along with nile blue. The initial test with DI water was to simply explore the effectiveness of the system to deliver nile blue and the incorporation of PBS was done to make the system more physiologically relevant. After supplying a high current for a short time duration, the results of both arrangements were nearly identical, revealing no apparent damage at the cathode while demonstrating a small deposit of nile blue at the site of the anode. Confocal imaging revealed that the cornea was indeed of typical thickness and that the nile blue is well dispersed throughout the cornea, confirming that the hybrid lens design can achieve drug delivery while minimizing the damaging components of the previous trials.

A hybrid lens would be difficult to design so an alternative approach was tested for minimizing damage while exploring fluorescein transport. This approach was based on the observation that the 1 M HEPES buffer minimized the damage at cathode but its ionic strength was too high resulting in damage at the anode. It was hypothesized that a 100 mM HEPES buffer may also prevent damage at the cathode while reducing the potential for damage at the anode due to the osmotic effects. This hypothesis was tested while exploring transport of fluorescein and results suggest that the 100 mM HEPES is ideal for minimizing the potential for damage at either electrode.

4.2. Fluorescein transport

In the case of fluorescein, the amount of drug delivered directly correlate with the duration and strength of the electric field. The results show that the addition of an electric field, across all combination of time and field strength, increases the delivery of the drug in comparison to the control. Furthermore, the high current had more fluorescein delivered than the low current case when applied for the same duration. Then when the time duration was doubled and had a low current value applied, it demonstrated the most fluorescein delivered to the interior of the eye. The reason the concentration did not more than double when time was also doubled was due to the release profile from the contact lens. When a lens begins a drug release, it initiates with a burst release (Fig. 7B). Therefore, a significantly higher fraction of the drug is released in the first 15 min than in any following 15 min segment. The decrease in release from the lens with time implies leads to a sub-linear increase in the transport into the cornea. However, it is clear that application of field significantly increases the mass of fluorescein that reaches across the cornea. Increasing duration of field application beyond the release duration from the lens will not lead to any further increase in mass delivered. If longer durations with smaller currents are preferred, the diffusivity of the hydrophilic molecules can be reduced by vitamin E loading, which is done by soaking the lens in an ethanol and vitamin E solution [30–33].

5. Conclusions

The outcomes of the ex vivo experiments and equivalent circuit analysis suggest that a contact lens containing both the cathode and the anode could be useful for iontophoretic based drug delivery to the eye. There are possible benefits of a more compact device that could be easy to use and there may be benefits of higher bioavailability and better predictability because the electrical field distribution can be accurately predicted. Additionally, the compact device would be much more convenient for patients due to the smaller and more localized electric field than other devices and could be worn like a normal contact lens without severely obstructing the field of vision. The longer wear time enables the use of lower currents compared to other devices and reduces the potential for damage to the ocular surface. However, the device itself would of course show the greatest benefit for patients that require the delivery of larger therapeutic compounds due to their lower permeabilities.

However, there are many challenging issues that must be addressed. First, there is certainly a potential for toxicity due to the electrodic reaction that causes a caustic environment at the cathode. The results of the nile blue experiments demonstrated that the generation of

electrochemical species can raise the pH to unsafe levels without a buffer. Furthermore, this issue is clearly a key problem that needs to be addressed as other medical devices and recent patents for condensed electrical system on a lens have buffering reservoirs or sacrificial electrodes to control the pH [15,34]. This issue can potentially be addressed by using a suitable buffer, such as HEPES, that is loaded in the contact lens. A high strength buffer is suitable for cathode but it could cause osmotic swelling at the anode, which is undesirable even though it is likely reversible. Adjusting the ionic strength could potentially manage this issue as well. Integrating both electrodes in a contact lens along with a battery source is challenging but rapid technological advances have already led to contact lenses with electrodes and antennas to generate power wirelessly so it may be feasible to design a compact system that can be placed on the eye while the patient can continue to blink. While these results are promising, significant issues remain unexplored including detailed toxicity analysis and design of a compact lens containing the power source. Furthermore, a detailed study on relevant macromolecules, materials for electrodes, optimization of electrode placement to minimize entrance into the field of vision, and the costs for operation and manufacturing compared to current devices needs to be explored for the clinical future of the proposed device. Considering the promising results shown here, these other issues will hopefully be explored by researchers interested in drug delivery both to the front and to the back of the eye as well.

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