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ORIGINAL ARTICLE

Controlled delivery of pirfenidone through vitamin E-loaded contact lens ameliorates corneal inflammation

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Abstract

Chemical injury by alkali burn is a major cause of corneal blindness in the clinical setting. Current management advocates multiple therapies aimed to prevent inflammation, initiate quick re-epithelialization, arrest the fibrosis, and avoid dry eye and pain by using bandage contact lenses. We hypothesized sustained delivery of the anti-inflammatory, antifibrotic drug pirfenidone through vitamin E-loaded contact lenses as a logical single approach to counter the pathology involved. Vitamin E particles were created in situ in commercial silicon hydrogel contact lenses by soaking the lenses in a vitamin E-ethanol solution. The vitamin E-laden lenses were then placed into pirfenidone-saline solution to load the drug into the lens. The contact lenses were evaluated by both in vitro and in vivo means. For in vitro, lenses were placed into 3 mL of saline solution. The concentration of pirfenidone released was measured by UV-vis spectrophotometry. The contact lenses were implanted in rabbit eyes following the alkali burn; the drug availability in the aqueous humor was evaluated by HPLC at various time points 10 min, 30 min, 2 h, and 3 h; and gene expression of inflammatory cytokines IL-1β, TNF-α, and TGF-β1 was evaluated in the cornea at the end of the study period. In another group of rabbits inflicted with alkali injury, the corneas were graded after 7 days of contact lens implantation with and without pirfenidone. A mathematical model was developed for delivery of the drug to the cornea and aqueous humor after a contact lens is inserted in the eye. The model was validated with experimental data and used to determine the bioavailability both for contact lenses and eye drops. In vitro release of unmodified commercial contact lenses saw a release time of approximately 20 min, with a partition coefficient of 2.68 ± 0.06. The release of pirfenidone from 20% vitamin E-loaded lenses saw a release time of approximately 80 min, with a partition coefficient of 4.20 ± 0.04. In vivo, the drug was available in the aqueous humor for up to 3 h. Gene expression of inflammatory cytokine IL-β1 and profibrotic growth factor TGF-β1 was significantly suppressed in corneas treated with pirfenidone contact lenses. A week after the alkali burn, the eyes with pirfenidone contact lenses showed significant improvement in corneal haze in comparison to the control eyes. About 50% of the drug loaded in the lens reached the aqueous humor compared to 1.3% with eye drops. Vitamin E-loaded contact lenses serve as a suitable platform for delivery of pirfenidone following alkali burn in rabbit eyes; positive pre-clinical outcome identifies it as promising therapy for addressing corneal inflammation and fibrosis. The bioavailability is about 40-fold higher for contact lenses compared to that for eye drops.

Keywords Vitamin E-loaded contact lens · Pirfenidone · Alkali burn injury

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Introduction

Chemical injuries, a potentially blinding condition, constitute 11.5–22.1% of ocular traumas [1]. Alkali materials are found more commonly in building materials and cleaning agents, and injuries from these materials occur more frequently than acid injuries. Inflammation followed by scarring is the major sequel of an alkali burn, leading to vision impairment [2]. Pirfenidone is an antifibrotic agent used to treat idiopathic pulmonary fibrosis [3], (https://www.esbriet.com/). The antifibrotic and anti-inflammatory properties of pirfenidone have made it of interest in ocular surgery as a post-operation antiscarring agent [4–6].

Oral medication has poor effectiveness owing to low availability in the anterior segment of the eye, and so eye drops make up a large majority of current delivery methods for most ocular drugs to the anterior of the eye [7]. Despite their widespread use, eye drops suffer from a low bioavailability, due to a rapid drainage of the applied drop through the canaculi. This drainage occurs over the course of several minutes, leading to a bioavailability of < 5% [8].

In recent years, contact lenses have been suggested as an alternative method of targeted delivery to the eye [9]. Contact lenses do not suffer from the drainage issue found in eye drops, leading to a much higher bioavailability to the cornea [10]. However, unmodified contact lenses tend to release loaded drugs too quickly, risking toxicity to the ocular tissue [11]. This quick release is especially prevalent in small molecules like pirfenidone (molecular weight = 185.22 g/mol). Previous work by Chauhan et al. has found that integration of vitamin E diffusion barriers into silicone hydrogel contact lenses extended the release for both hydrophobic and hydrophilic drugs [12–14] including small drugs such as cysteamine (m.w. = 77.15 g/mol) [15].

In acute clinical inflammatory conditions of the cornea like accidental alkali burns, an immediate viable approach may be to provide an anti-inflammatory, antifibrotic drug through contact lenses, with the additional advantage of prevention of dry eye and protection of pain incited to the highly sensitive and inflamed corneal surface due to blinking movements.

This paper examines for the first time the efficacy of pirfenidone-loaded contact lenses in an animal model of chemical injury with the objective of exploring the possibility of future clinical use as bandage contact lens in chemical burns and other inflammatory conditions of the cornea. As a secondary goal, this paper examines the pharmacokinetics and bioavailability of pirfenidone from contact lenses. This paper is the first to demonstrate a bioavailability of approaching 50% for corneal drug delivery by contact lens.

Materials and methods

Materials

Pirfenidone (> 98%) was purchased from Carbosynth (Compton, UK). ACUVUE OASYS® contact lenses (power = − 3.5, BC = 8.8) were purchased from Johnson & Johnson (Jacksonville, FL). Phosphate-buffered saline 1× without calcium and magnesium (PBS) was purchased from Mediatech, Inc. (Manassas, VA). Ethanol (200 proof) and vitamin E (DL-alpha tocopherol, > 96%) were purchased from Sigma-Aldrich. All chemicals were used as received without further purification.

Vitamin E loading

Commercial contact lenses were removed from blister packs and soaked in de-ionized (DI) water for 15 min. Vitamin E solutions were prepared by dissolving vitamin E in ethanol. The lenses were removed from the DI water, placed into the vitamin E/ethanol solution, and soaked for 24 h. The lenses were then removed from the vitamin E/ethanol solution and placed in approximately 100 mL of DI water for 1 min. This step removes all ethanol remaining in the lens, while the vitamin E is retained, resulting in phase separation and formation of vitamin E aggregates that act as diffusion barriers. The lenses were then removed and dipped into 200-proof ethanol for 5 s to remove any remaining surface deposits of vitamin E. The lenses were immediately placed into 100 mL of fresh DI water, where they soaked for 1 h, to again remove any remaining ethanol. The lenses were removed and placed into fresh 100 mL of DI water, where they soaked for an additional 24 h. After this, the lenses were placed into 3 mL of PBS until ready for drug loading and release.

In vitro drug loading and release

Pirfenidone solution (1 mg/mL) was prepared by dissolving pirfenidone into PBS. Vitamin E lenses were removed from the storage solution and placed into the 0.1% pirfenidone/PBS solution. Control lenses were taken from blister packs and placed into DI water for 15 min before being placed into 3 mL of drug solution. The lenses remained in the drug solution for 72 h. For the uptake, both $t = 0$ and 72-h measurements were obtained with a UV-vis spectrophotometer (ThermospectronicGenesys 10S UV). The spectra were measured from 190 to 500 nm. Two milliliters of solution was withdrawn, measured, and returned to the loading vial.

After the loading period, the lenses were removed from the drug solution, dabbed gently with a Kimwipe to remove excess solution on the surface, and then placed into 3 mL of PBS. The spectra (190–500 nm) of the solution were measured periodically using a UV-vis spectrophotometer

(ThermospectronicGenesys 10S UV). Two milliliters of solution was withdrawn, measured, and returned to the release vial. A $t = 0$ measurement was obtained as the control spectra prior to immersion of the lens. The measured spectra were compared to a known calibration curve to determine the concentration of the measurement. Measurements were stopped after the concentration remained unchanged over 24 h.

In vitro modeling of release

The in vitro release data was evaluated to determine the partition coefficient and diffusivity in the lens. The partition coefficient, K_g, can be determined by the ratio of equilibrium concentrations between the lens and surrounding PBS. Due to the large volume ratio of solution to lens and hydrophilicity of pirfenidone, the loading and release can be assumed to be under perfect source and sink conditions, respectively. The partition coefficient can thus be calculated by the following equation:

$$K_g = \frac{C_{g,f}}{C_{l,f}} = \frac{V_r(C_{r,f})}{V_g C_l} \tag{1}$$

where V_g is the volume of the lens, C_l is the concentration of the loading solution, and V_r and $C_{r,f}$ are the volume and final concentration of the release medium, respectively. The diffusivity D can be obtained by fitting the data to the diffusion control model.

While transport in the lens occurs in both the radial and thickness directions, the thickness, on the order of 80 μm, is significantly smaller than the diameter, on the order of 1.5 cm. This difference in length scale means that diffusion out of the lens in the direction of thickness will occur much faster. The transport of drug in the lens can be approximated by a one-dimensional diffusion equation:

$$\frac{\partial C_g}{\partial t} = D\frac{\partial^2 C_g}{\partial y^2} \tag{2}$$

where C_g is concentration, t is time, D is diffusivity, and y is distance. For the system, $y = 0$ is taken to be the center of the lens, with h being half the thickness of the lens and the location of the boundary. The boundary conditions are as follows:

$$\frac{\partial C_g}{\partial y}(y = 0) = 0 \tag{3}$$

$$C(y = h) = K_g C_r \approx 0 \tag{4}$$

where C_{water} is the concentration of pirfenidone in the surrounding medium. Under sink conditions, the concentration in the surrounding medium is much lower than the concentration of the lens and can be treated as zero. With these boundary conditions, the equation can be solved analytically using separation of variables. The concentration profile of the release medium is given by the following equation:

$$C_g = \sum_{n=0}^{\infty} \frac{(-1)^n 4 C_{g,i}}{(2n+1)\pi} \cos\left(\frac{(2n+1)\pi}{2h} y\right) e^{-\frac{(2n+1)^2\pi^2}{4h^2} Dt} \tag{5}$$

where $C_{g,\,i}$ is the initial concentration in the lens, which is taken to be $K_g{*}C_{l,\,f}$. This equation can be converted to describe the release medium using a mass balance:

$$\Delta \text{pirfenidone}_{water} = \text{pirfenidone}_{in} \tag{6}$$

where the only source of pirfenidone is the lens. This release can be described as the flux (described by Fick's 1st Law as the concentration gradient times diffusivity) multiplied by surface area. This gives the following equation:

$$V_r \frac{dC_r}{dt} = -DA_s \frac{\partial C_g}{\partial y}\bigg|_{y=h} \tag{7}$$

where A_s is the total surface area of the lens and V_r is the volume of the release medium. The equation can then be normalized (divided by the total mass released) to give the fraction or percentage of drug released:

$$\%\text{pirfenidone released} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(-\frac{(2n+1)^2\pi^2}{4h^2} Dt\right) \tag{8}$$

The measured data for release is fitted to Eq. 8 by minimizing the sum of squared errors over the entire duration of the experiment.

Animal studies

All animal studies were conducted with prior permission of the Institutional Animal Ethics committee and as per the recommendation of the Association for Research in Vision and Ophthalmology (ARVO). The study was conducted in adult New Zealand White rabbits of either sex weighing between 2 and 2.5 kg; the animals were examined to rule out pre-existing ophthalmic disorders. Anesthesia was induced with a combination of xylazine HCl at 5 mg/kg wt I/M and ketamine HCl at 35 mg/kg wt I/M; topical anesthesia was induced with proparacain drops.

An alkali burn was induced in one eye of a New Zealand White rabbit (8 Nos) with topical application of 1 N NaOH for 30 s and hereafter rinsed with sterile normal saline. The treatment group received contact lenses loaded with 20% vit E + levofloxacin and 0.05% pirfenidone (4 Nos), and the control eye was treated with one drop of sterile PBS (4 Nos).

Levofloxacin was included in the contacts to avoid infection during the study. Release of levofloxacin from vitamin E-loaded contact lenses has been explored previously [16].

Based on in vitro release kinetics, 0.1 mL aqueous humor was collected from treated and corresponding control eyes under topical anesthesia at various time points, i.e., collection was performed at 10 min, 30 min, 2 h, and 3 h post-implantation. The aqueous humor was preserved in − 80° for detection of the drug in the aqueous humor by HPLC.

At the end of the study period, the animals were euthanized and the corneas of both treated and control eyes were harvested and preserved in trizol for evaluation of gene expression of inflammatory cytokines IL-1β, TNF-α, and TGF-β1.

In another batch of eight rabbits, corneal inflammation and injury were induced with alkali burn as above. In four eyes, a contact lens with 20% vit E + levofloxacin and 0.05% pirfenidone was implanted while the other four eyes received a contact lens with 20% vit E + levofloxacin without pirfenidone. Temporary tarsoraphy was performed to prevent the contact lens from displacing; antibiotic drops were instilled in both groups for 7 days through the openings in the lid suture. The eyes were regularly monitored for signs of infection. On the 7th day, the eyes were examined clinically by flourescein dye test under a slit lamp biomicroscope and the opacity of the cornea in both the groups was graded blindly by a standard scoring method by Fantes et al. [17]: grade 0, complete clear cornea without any trace of haze; grade 0.5, a faint haze detectable only by oblique illumination; grade 1, mild haze but not interfering with visibility of iris details; grade 2, more prominent haze with mild obscuration of iris details; grade 3, opacity of moderate density easily detectable under direct illumination with partial obscuration of iris details; and grade 4, complete opacity, with no visibility of structures in the anterior chamber.

HPLC for detection of pirfenidone in aqueous humor following delivery through contact lenses

HPLC was performed using a Shimadzu (Japan) HPLC system. All reagents used in the procedure were of HPLC grade. The mobile phase consisted of acetonitrile and water (23:77, *v*/*v*) containing 0.2% acetic acid. The mobile phase was filtered through a 0.45-μm Millipore filter before use, and a flow rate of 1 mL/min was applied in the system. The detection wavelength for pirfenidone was set at 310 nm.

Pirfenidone was dissolved in methanol and diluted to obtain standard solutions in the concentration range of 100 to 250 μg per ml methanol. The samples were run, and a standard curve was obtained. Control and treated aqueous humor was added to 1 mL methanol and centrifuged at 12,000 rpm for 10 min. The supernatant was collected in fresh centrifuge tubes and was evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 200 μL methanol and run in the system for detection of pirfenidone. The standard curve was linear, and the equation relating to the area of peaks (Y) against the concentration of pirfenidone (X) was $Y = 2505X - 72{,}490$, and $R^2 = 0.994$.

Gene expression of inflammatory cytokines IL-1β, TNF-α, and profibrotic growth factor TGF-β1 in corneal tissues treated either with PBS- or with pirfenidone-loaded contact lenses following alkali burn

Total RNA was extracted from corneas of normal, control, and pirfenidone contact lens-treated eyes using TRIZOL® reagent (Cat No. 15596-018, Invitrogen, Life Technologies, USA) following standard protocol. The concentration of RNA was determined, and cDNA was transcribed according to the kit protocol (Revert Aid™ First Strand cDNA Synthesis Kit, #K1622, Fermantas). Real-time PCR was performed with IL-beta-1 (F: TGGCTCAACAGTCACCTAAAC, R: GGGTGGTCAAAGTTCCATCATA), TNF-α (F: CCTTCCTCTCCTCAGATGTTTC, R: ACGGGTCA GTCACCAAATC), TGF-β (F: ATAGTCTTCCTGCC GGTCCT, R: TGGGGAGCTTTATGTGCCAG), and GAPDH (F: TCAACAGCAACTCCCACTCTTCCA, R: ACCCTGTTGCTGTAGCCGTATTCA) primers using SYBR^R Premix Ex Taq™ (Perfect Real Time), TaKaRa #PR041A in a real-time PCR cycler (ABI Prism 7500 Sequence Detection System; Applied Biosystems, Foster City, CA, USA). Relative mRNA levels in each sample were calculated after normalization to GAPDH mRNA expression using the DD CT method.

Animal modeling

The collected data was modeled using a mass balance of drug in the aqueous humor. The general equation can be described as

$$\Delta \text{pirfenidone}_{\text{aq.humor}} = \text{pirfenidone}_{\text{in}} - \text{pirfenidone}_{\text{out}} \quad (9)$$

The source of pirfenidone is the contact lens. However, not all of the drug released from the contact lens will reach the aqueous humor, as portions will be lost to the tear film. The aqueous humor also has an outflow of liquid which will remove pirfenidone from the system. The time scale of pirfenidone transporting through the cornea can be modeled as the thickness (hc) squared divided by the diffusivity of the drug through the cornea. The cornea has a thickness of approximately 400 μm [18] and a diffusivity of $4–10 \times 10^{-6}$ cm^2/s [19]. This gives a diffusion time of

$$t_d, \text{cornea} \sim \frac{hc^2}{D} = 2.4 \text{ to } 6.7 \text{ min} \quad (10)$$

The time scale for aqueous humor drainage can be estimated as the ratio of the aqueous humor (~ 300 μL) and drainage rate (~ 3 μL/min). This gives an overturn time of

$$t_{q,aq} \sim \frac{V_{aq}}{q_{aq}} = 100 \text{ min} \quad (11)$$

Since the time scale for the concentration equilibration in the cornea is much larger than the aqueous humor turnover time ($t_{d,\ cornea} < t_q$), we can assume that the cornea concentration is in equilibrium with that in the aqueous humor. This allows us to simplify the influx of pirfenidone into the cornea/aqueous, yielding the following mass balance:

$$V_{aq}\frac{dC_{aq}}{dt} = j_a - q_{aq}C_{aq} \quad (12)$$

where V_{aq} is the volume of the aqueous and cornea, C_{aq} is the concentration in the aqueous humor and cornea, t is time, j_a is the flux of drug into the cornea, and q_{aq} is the volumetric drainage rate from the aqueous humor. The mass transfer resistance between the cornea and the aqueous humor is negligible compared to the time scale of the experiment, so both the cornea and aqueous humor can be lumped together into a single compartment of volume 390 μL and drainage rate of 2.68 μL/min, which are both based on reported values for rabbit ocular tissue [14, 17, 20].

Determining the flux of drug from the post lens tear film into the cornea will require developing a mass transfer model for the post lens tear film and integrating that with a non-sink model for drug release from the contact lens [11]. Below, we justify a simpler model that treats the post lens tear film as a sink environment allowing the use of sink release from the lens.

The diffusion time in the tear film can be approximated as the thickness of the tear film (h_{POLTF}) divided by the permeability of the cornea (K_{cornea}).

$$t_{d,tear} \sim \frac{h_{POLTF}}{K_{cornea}} = 100 \text{ s} \quad (13)$$

As the transport across the tear film is much faster than the release from the lens, particularly for the vitamin E lenses, which take upwards of 100 min, we can assume the release from the lens will follow close to perfect sink conditions. This allows the flux term into the cornea to be modeled as

$$j_a = f\sum_{n=0}^{\infty}\frac{2C_{g,i}D}{h}\exp\left(-\frac{(2n+1)^2}{4h^2}Dt\right) \quad (14)$$

where h is half the thickness of the lens, D is diffusivity, and f represents the fraction of the released drug from the lens that reaches the cornea.

As shown from the time scale analysis, the above model implicitly neglects any mass transfer resistance within the cornea and also assumes that the drug transport from the lens to the tears can be described as sink release. It is noted that all parameters in the model are obtained either from literature or from fitting in vitro data so the model for the in vivo pharmacokinetics does not have a fitting parameter.

Scanning electron microscopy imaging

The microstructure of the vitamin E barriers in ACUVUE® OASYS™ lenses was explored by SEM imaging on a FEI Nova NanoSEM 430. Samples were prepared by drying the hydrogel at ambient conditions for 1 day. Dried samples were then cut with a precision blade to expose the cross-sectional area and placed onto carbon tape. The samples were then coated with a single pass of gold-palladium and imaged.

Results

In vitro releases

Figure 1 shows the releases of pirfenidone from ACUVUE® OASYS® contact lenses along with the solid lines that are best fits based on the sink model as shown per Eq. 8. The diffusivity and partition coefficients calculated are shown in Table 1, and the ratio of the diffusivity in the vitamin E-loaded lenses and the control lens is plotted in Fig. 2 as a function of the vitamin E loading. An unmodified contact lens reached a 90% release in approximately 20 min. Loading a contact lens with 20% vitamin E increased this duration to approximately 80 min, and 40% loading increased the time to approximately 260 min. This trend of increase in release time vs vitamin E loading can be seen in Fig. 2.

Animal studies

Pirfenidone was available in aqueous humor for up to 3 h following delivery through contact lens

The concentrations in the aqueous humor (average of four eyes at each time point) of 0.05% pirfenidone in aqueous humor when delivered through 20% vitamin E-loaded contact lenses following alkali burns in rabbit eyes at various time points were 10 min, 62.38 ± 13.77 μg/mL; 30 min, 51.87 ± 7.03 μg/mL; 2 h, 36.15 ± 2.77 μg/mL; and 3 h, 16.04 ± 18.54 μg/mL (Fig. 3).

Use of pirfenidone-loaded contact lenses ameliorated inflammation following alkali burns in rabbit eyes

The clinical observation as well as gene expression of inflammatory cytokines demonstrated improved response in eyes

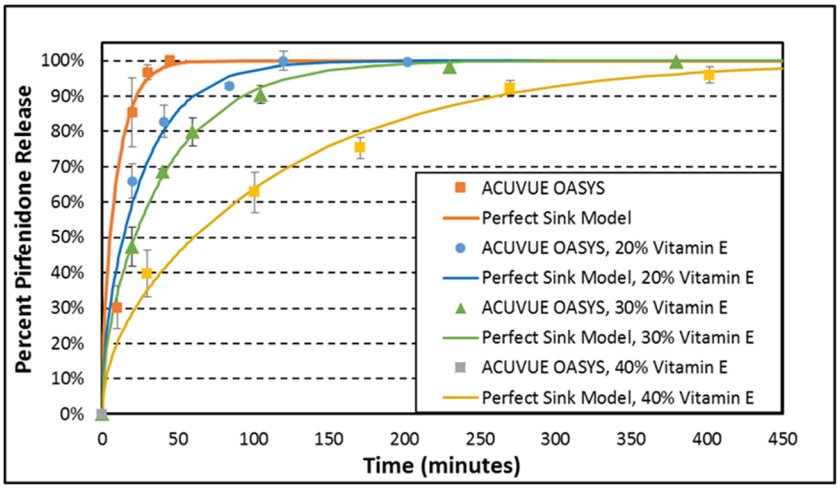

Fig. 1 In vitro release of pirfenidone from ACUVUE® OASYS® contact lens with increasing vitamin E loading and fit using perfect sink model (± Stdev. $n = 3$)

with pirfenidone contact lenses.The clinical observation at the end of 3 h following alkali burns shows reduced pigmentation and congestion in the eyes treated with pirfenidone contact lenses in comparison to untreated control eyes (Fig. 4).

There was a significant increase in the gene expression of inflammatory cytokines IL-1β ($P = 0.00216$), TNF-α ($P = 0.03669$), and TGF-β1 ($P = 3.5494E6$) in alkali burn eyes in comparison to normal eyes, whereas expression of these genes was significantly reduced for IL-1β ($P = 0.00358$), TNF-α ($P = 0.03936$), and TGF-β1 ($P = 0.00251$) in pirfenidone contact lens-treated eyes in comparison to alkali burn corneas (Fig. 5).

To evaluate the influence of a single time treatment with pirfenidone contact lenses on corneal fibrosis, we evaluated the eyes at a later time point. On the 7th day, the pirfenidone contact lens-treated eyes scored significantly ($P = 0.02401$) less haze in comparison to control eyes (Fig. 6); two out of four eyes with pirfenidone contact lenses had complete re-epithelialization compared to untreated controls where all the eyes had flourescein retention indicating incomplete healing (Fig. 6).

Discussion

Effect of vitamin E on in vitro release

As seen in Table 1, the partition coefficient of pirfenidone increases with higher vitamin E loading. While pirfenidone is hydrophilic, it does have an aromatic ring which most likely surface adsorbs onto the vitamin E phase in the lens (Fig. 7). Evidence for surface adsorption comes from the lower increase in diffusion time with vitamin E loading compared to other molecules, such as timolol [12], which saw ~50× increase. The 3× increase at 20% vitamin E loading is similar to hydrophobic drugs, which again suggests a similar hydrophobic interaction between the pirfenidone and vitamin E [13].

Table 1 Effect of vitamin E loading on the partition coefficient and diffusivity on ACUVUE® OASYS® (± Stdev., $n = 3$)

Vitamin E loading (%)	Partition coefficient	Calculated diffusivity (mm^2/min)
0	2.68 ± 0.06	5.987E−5 ± 8.31E−6
20	4.20 ± 0.04	2.236E−5 ± 3.83E−6
30	4.37 ± 0.26	1.603E−5 ± 1.21E−6
40	4.69 ± 0.33	8.188E−6 ± 1.93E−7

As seen in Fig. 2, the increase of diffusivity exhibits a quadratic behavior. As shown in ref. [9], the path length scales as $h(1 + \alpha(\varphi - \varphi^*))$, where h is the half thickness of the lens, α is a term unique to each lens and drug combination that depends on microstructure and particle size, φ is the volume ratio of vitamin E in the dry gel, and $(\varphi - \varphi^*)$ is the fraction that is present as vitamin E diffusion barriers (inset in Fig. 2). Using the time scale for diffusion of l^2/D, where l is the path length and D is the diffusion coefficient, and modeling the increase in thickness as $h = h_0(1 + \varphi/3)$, where h_0 is half the thickness of a pure lens, the following relationship can be modeled:

$$\tau \sim \frac{h_0^2}{D}\left(1 + \frac{\varphi}{3}\right)^2\left(1 + \alpha\left(\varphi - \varphi^*\right)\right)^2 \tag{15}$$

The term $\left(1 + \frac{\varphi}{3}\right)^2$ does not make a contribution greater than 2 and, for the range of vitamin E loadings analyzed, only approaches 1.2. This term can be neglected, and the ratio of τ to τ_0, or the time scale of the vitamin E-loaded lens to the pure lens, can be modeled as

$$\frac{\tau}{\tau_0} \sim \left(1 - \alpha\varphi^*\right)^2 + 2\varphi\left(\alpha - \alpha^2\varphi^*\right) + \alpha^2\varphi^2 \tag{16}$$

The values can then be determined using a least squares, quadratic fit to the data. This gives an α value of 45.0 and a φ^* value of 18.11. The value of φ^* represents the vitamin E that is adsorbed into the lens and so is not contributing to the barriers, and α is a measure of the aspect ratio of the barriers. Similar behavior has been observed in the transport of other

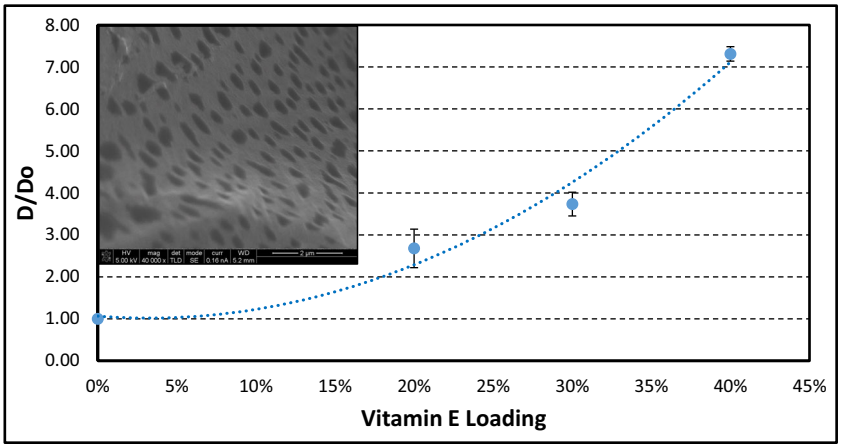

Fig. 2 Ratio of diffusivity, D, and control diffusivity, D_o, as a function of vitamin E loading, φ. (± Stdev. $n = 3$). The solid line is the quadratic fit using Eq. 8.(Insert shows image of vitamin E-loaded particles in ACUVU18 OASYS taken by FEI Nova NanoSEM 430)

ophthalmic drugs in vitamin E-loaded contact lenses [12, 13, 17]. As seen in Table 1, the partition coefficient increased with increasing vitamin E loading. The overall trend is linear when compared to vitamin E loading, with an R^2 value of 0.9261, suggesting an interaction between vitamin E and the drug, possibly due to adsorption of the drug on the surface of the vitamin E barriers. Alternatively, the fraction of the loaded vitamin E that is adsorbed on the gel network could contribute to an increase in the partition coefficient.

Corneal scarring/fibrosis is the major sequel to several forms of insults to the cornea including alkali burns. Loss of corneal transparency is the second highest cause of vision impairment [21]; therefore, appropriate management to prevent corneal fibrosis is imperative. There is yet no specific therapy in clinical practice to address corneal fibrosis; we have previously identified pirfenidone nanoformulation drops as promising therapy to prevent corneal scarring [22] and have also reported on the anti-inflammatory and antifibrotic effects of pirfenidone in preventing fibrosis leading to proliferative vitreoretinopathy [4]. Since contact lenses are proving excellent modality for sustained drug delivery to the ocular surface and bandage contact lenses offer potential advantages, i.e., assist in the retention of drug on the cornea and prevent drying of the cornea and pain incited on the corneal surface by the blinking movement, we chose to study the efficacy of vitamin E-loaded contact lenses for delivery of pirfenidone in cornea injured by alkali burns.

Vitamin E loading significantly enhances drug loading and sustained delivery to the aqueous humor. Also, the predictions of the model for drug concentration in the aqueous humor are in good agreement with the data, suggesting validity of the model and also suggesting that the assumption of 50% bioavailability is valid. Our study shows the availability of pirfenidone in the aqueous humor for up to 3 h. While a previous study also has reported improved availability of pirfenidone delivered through contact lenses [23], our study was conducted in a disease model of an alkali burn to evaluate drug delivery through contact lenses in an inflamed cornea. Additionally, the previous study used only control contact lenses with a rapid release rate and did not quantify bioavailability in the aqueous humor [23]. If the drug release is rapid, the contact lenses need much higher initial drug loadings to sustain a measurable drug concentration at a specific target duration, such as at 3 h. This is reflected in the very high drug concentration in the aqueous at initial times in the previous study [23] compared to our study. Consistent with drug availability, the gene expression of inflammatory cytokines IL-1β, TNF-α, and TGF-β1 was significantly reduced in cornea

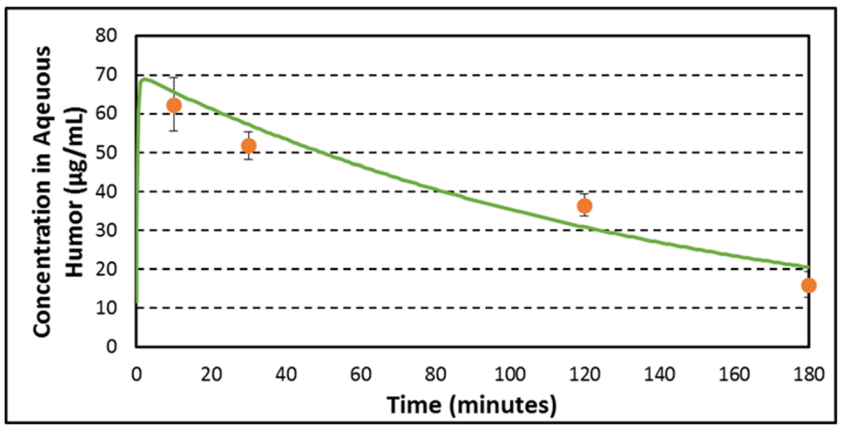

Fig. 3 In vivo data collected from aqueous humor and fitted with model assuming 50% bioavailability, 2.68 μL/min aqueous humor drainage rate, and 390 μL combined volume of cornea and aqueous humor [16]

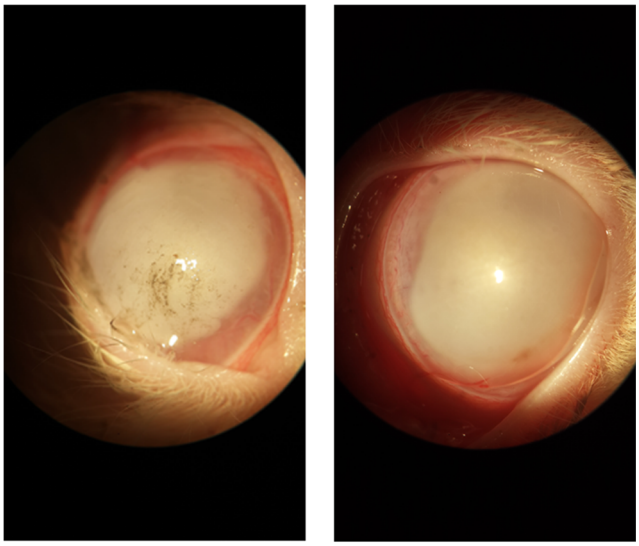

Fig. 4 Pirfenidone-loaded contact lens reduced corneal inflammation and pigmentation. Slit-lamp image of the cornea from alkali-burnt eyes showed huge inflammation and pigmentation (**a**) whereas the image of the cornea treated with pirfenidone-loaded contact lens showed reduced inflammation and pigmentation (**b**)

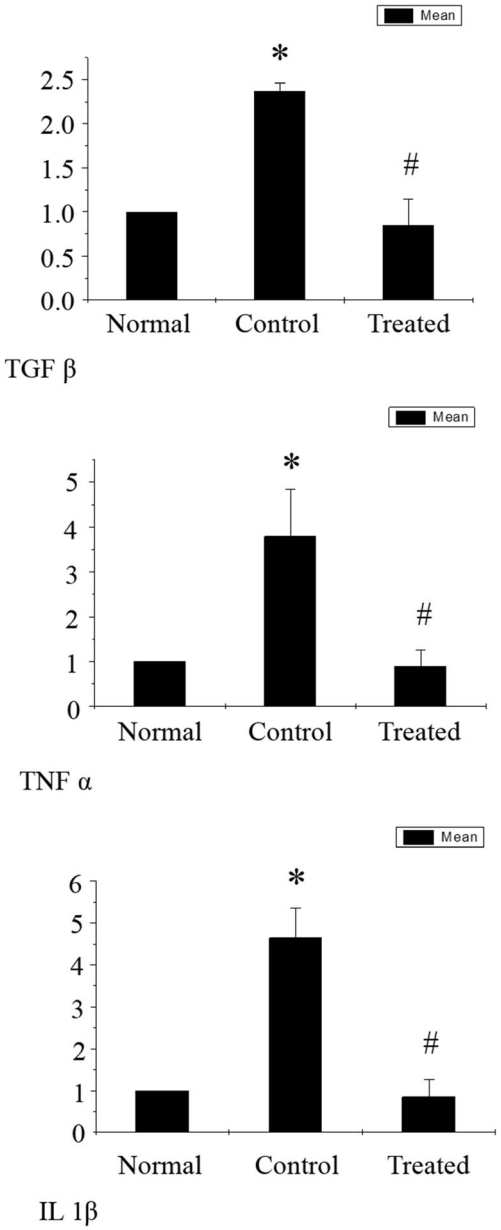

Fig. 5 Treatment with pirfenidone-loaded contact lens prevented increased expression of cytokines following alkali burn. The expression of TGF-β1, TNF-α, and IL-1β was significantly ($p < 0.05$) increased in alkali burn corneas in comparison to normal cornea and their expression in pirfenidone-loaded contact lens-treated corneas was significantly ($P < 0.05$) reduced in comparison to untreated controls. ($N = 4$); asterisk, $P < 0.05$ vs normal; number sign, $P < 0.05$ vs control

treated with pirfenidone-loaded lenses in comparison to untreated control eyes at 3 h. Clinical appearance also seemed improved in pirfenidone-treated eyes. The upregulation of IL-1β has been reported to be high during the initial inflammatory phase following the alkali burn [24, 25], and TGF-β1 contributes most significantly towards epithelial mesenchymal transition of corneal keratocytes to myofibroblasts, leading to fibrotic changes in the transparent cornea [26]. Therefore, we sought to investigate the influence of pirfenidone on these cytokines. Expression of cytokine TNF-α in the eye has been reported to significantly increase following an alkali burn, and use of an anti-TNF-α agent reduces the pathologies involved [25]; consistent with this report, we have reported a significant increase in gene expression of TNF-α in the control and its reduction in pirfenidone-treated eyes in respect to control levels. This is the first study showing reduced expression of inflammatory cytokine and growth factor with pirfenidone treatment following an alkali burn in the cornea. The anti-inflammatory effect of pirfenidone has been reported in several other disease conditions as well [27, 28].

To evaluate the influence of extended single-time wear of pirfenidone-loaded contact lenses on corneal healing and fibrosis, we evaluated the eyes 1 week following the alkali burn; the corneal haze score was significantly less in pirfenidone-treated eyes, and improved healing was also observed in these eyes. Although the drug was available for up to 3 h, suppressing the initial acute inflammatory response, i.e., cytokine IL-1β and profibrotic growth factor TGF-β1, may have contributed to improved outcome after a week in comparison to untreated controls; therefore, it is expected that daily use of fresh pirfenidone-loaded contact lenses will improve corneal opacity, reduce discomfort and pain, and enhance corneal healing [26]. Although sustained-release topical formulations are effective and beneficial for ophthalmic drug delivery, use of contact lenses for drug delivery to the cornea may add additional advantages like protection of the ocular surface while it

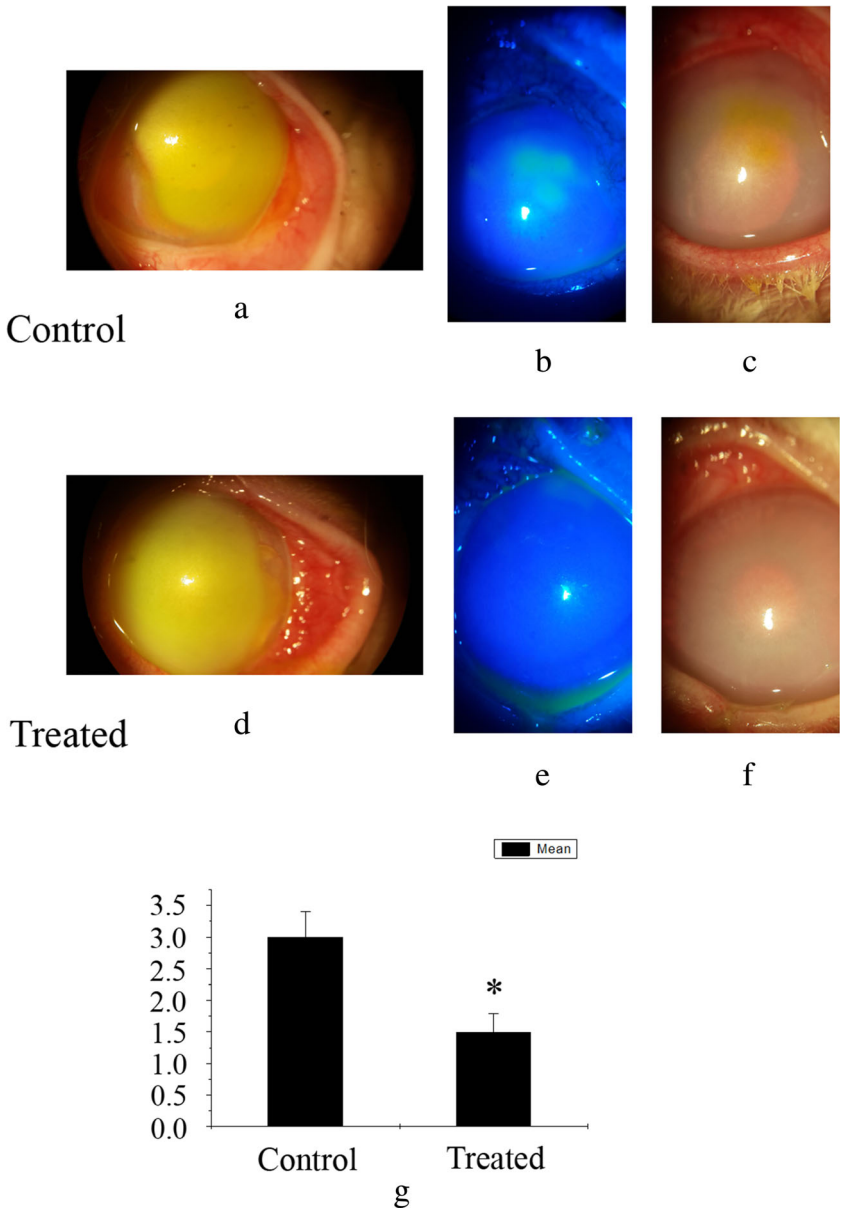

Fig. 6 Treatment of alkali burn corneas with pirfenidone-loaded contact lens induced early re-epithelialization and reduced corneal haze. Control corneas responded with pigmentation on day of injury (**a**), incomplete re-epithelialization (**b**), and pronounced central haze on day 7 (**c**); the pirfenidone contact lens-treated eyes responded with reduced pigmentation (**d**), complete re-epithelialization (**e**), and reduced haze in central cornea, the pupil is well demarcated behind the mildly hazy cornea (**f**). Average haze score of control and treated corneas is presented as mean ± se, $N = 4$; asterisk, P 0.05 vs control

heals, prevention of dry eye, and also the minimizing of local irritation on the cornea inflicted during blinking.

Expansion and validation of model

In order to examine the predictive effectiveness of the model described by Eqs. 6 and 7, the in vivo data collected by Yang et al. was fitted [23]. This paper used various contact lenses and eye drops to deliver pirfenidone to an in vivo rabbit model and measured drug concentration in the tear film, cornea, aqueous humor, conjunctiva, and sclera. The concentrations in the tear film and aqueous humor are modeled as described below.

The contact lens data for the aqueous humor can be calculated as above, except that the value of diffusivity and initial loading are obtained based on the data reported by Yang et al. [23]. The predicted concentration in the aqueous humor (Fig. 8) is in very good agreement with the measurements which supports the validity of the model and further supports the assumption of 50% bioavailability for drug delivered via contact lenses.

To determine the concentration in the tear film after placing a contact lens, a mass balance is needed on the tear film [10].

$$V_{\text{tear}}\frac{dC_{\text{tear}}}{dt} = j_{\text{lens}}A_{\text{s}} - K_{\text{cornea}}A_{\text{s}}C_{\text{tear}} - K_{\text{conjuctiva}}A_{\text{conjuctiva}}C_{\text{tear}} - q_{\text{tear}}C_{\text{tear}} \tag{17}$$

where j_{lens} is given by Eq. 14 using $f = 0.5$, which assumes that the drug released from the anterior surface is released into the

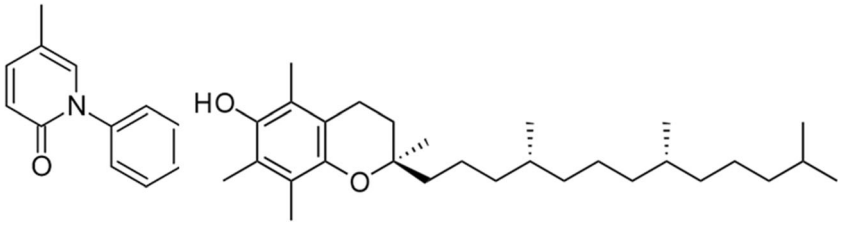

Fig. 7 While pirfenidone (left) is hydrophilic, its aromatic group most likely interacts with vitamin E (right), leading to an increase in partitioning

tears. In the above equation, V_{tear} is the total volume of the tear film; q_{tear} is the drainage rate to the canaliculi; K_{cornea} and $K_{conjunctiva}$ are the permeability of the cornea and conjunctiva to pirfenidone, respectively; and A_{cornea} and $A_{conjunctiva}$ are the areas of the cornea and conjunctiva, respectively. The values of these parameters in this model were obtained from literature and are listed in Table 2.

The permeability of the cornea is based on the predictive model from Edwards et al. [34]. The permeability of the conjunctiva is not available, but is most likely in the order of magnitude of 1×10^{-5} cm/s [35]. As detailed in [11], Eq. 18 can be simplified by using asymptotic techniques and assuming that the time upscale for drug uptake is smaller than axial equilibration. This assumption eliminates the conjunctiva term, giving

$$V_{tear}\frac{dC_{tear}}{dt} = j_{lens}A_{lens} - K_{cornea}A_{cornea}C_{tear} - qC_{tear} \tag{18}$$

This assumption of time scale loss to the conjunctiva is supported by conjunctiva concentration measurements from [23], which reach a peak concentration of less than 5 μg/mg at 60 min, a concentration much lower and much later than the peak concentration of the cornea, suggesting a higher partitioning into the cornea compared to the conjunctiva. The predicted concentration in the tears is in good agreement with the measured values (Fig. 8), again suggesting that the model for predicting the in vivo concentrations is accurate and that conjunctival uptake of pirfenidone is much smaller than corneal uptake.

For eye drops, the delivery of the drug can be assumed to occur at $t = 0$, followed by loss of the drug to the cornea, conjunctiva, and drainage. For modeling tear concentration after instilling eye drops, the mass balance is modified to the following:

$$\frac{dV_{tear}C_{tear}}{dt} = -K_{cornea}A_{lens}C_{tear} - K_{conjuctiva}A_{conjuctiva}C_{tear} - qC_{tear} \tag{19}$$

where V_{tear} is the dynamic tear volume and the eye drop instillation results in an initial concentration = $M_0/(V_{tear} + V_{drop})$, where M_0 and V_{drop} are the mass of the drug and volume of the eye drop, respectively. The dynamic tear volume increases significantly after eye drop instillation and then decreases gradually to the original value because the tear instillation results in an increase in the drainage rate, with the tear film reaching its equilibrium volume in around 5 min [10]. A detailed model that incorporates the changes in drainage rate due to eye drop instillation has been derived, but here, for simplicity, we assume that the tear volume returns to baseline rapidly at a constant rate of 6 μL/min (or a decrease of $V_{drop} + V_{tear}$ to V_{tear} in 5 min) for the first 5 min. Under this assumed drainage rates and assuming a pseudo-steady state in the eye, i.e., the time scale for change of volume is slower compared to that for the concentration decline, Eq. 19 can be solved to yield

$$C_{tear} = C_0 e^{-\left(\frac{q + K_{cornea}A_{cornea} + K_{conjuctiva}A_{conjuctiva}}{V_{tear}(t)}\right)t} \tag{20}$$

$$V_{tear}(t < 5\ \text{min}) = 32.5\ \mu\text{L} - 6\ \mu\text{L/min} \times \text{time and } V(t > 5\,\text{min}) = 2.3\ \mu\text{L} \tag{21}$$

The permeability of the conjunctiva to pirfenidone can be calculated by fitting an exponential curve to the measured data, yielding a calculated value of 1.2×10^{-5} cm/s.

Table 2 Parameters used in predictive models

Term	Value	Source
h (half thickness of contact lens)	40 μm	[29]
A_{lens}	230 mm^2	–
V_{drop}	30 μL	–
V_{tear}	2.3 μL	–
Tear film drainage rate (q_{tear})	0.1 μL/min	[30]
Increased tear film drainage rate (eye drop)	6 μL/min	[10]
K_{cornea}	1.3E−5 cm/s	[31]
A_{cornea}	104 mm^2	[32]
$A_{conjuctiva}$	17.68 cm^2	[33]
Volume of aqueous humor + cornea	390 μL	[16]
Aqueous humor drainage rate (q_{aq})	2.68 μL/min	[16]

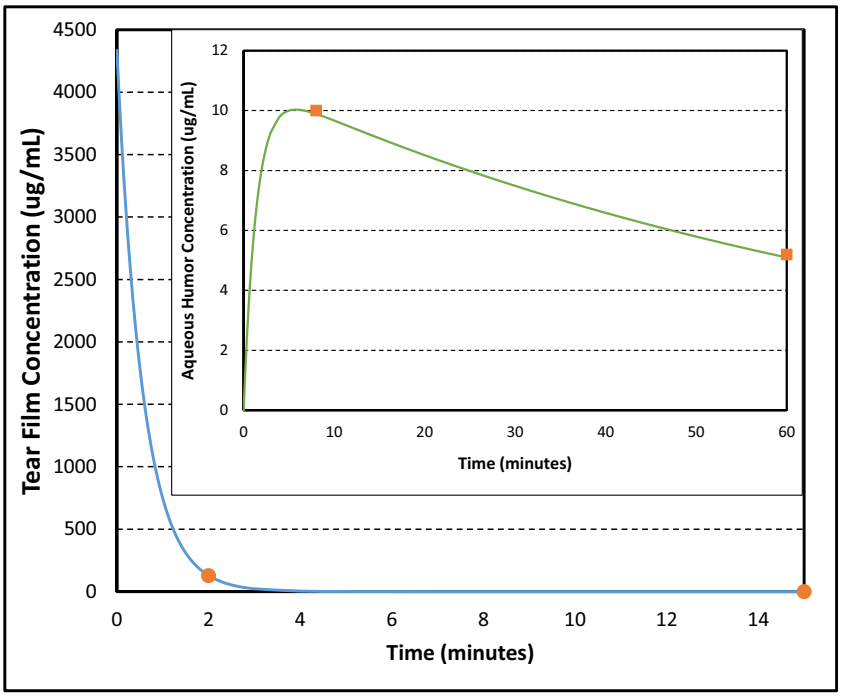

Fig. 8 In vivo data from Yang et al. [19] for contact lens delivery of pirfenidone along with fits based on our model (solid lines). Top: aqueous humor assuming 50% bioavailability. Bottom: tear film concentration

A disadvantage to fitting the eye drop data is that by the time of the second measurement at 15 min, the tear film had reached a negligible drug concentration. This leaves only one nonzero measured value for the model to predict. Much more frequent and rapid measurements would be needed to further validate the model in the tear film for eye drops. However, this issue does highlight the large disadvantage of eye drops of having a very quick residency time of the drug in the tear film.

The concentration in the aqueous humor can be then calculated by a mass balance similar to Eq. 12:

$$V_{\mathrm{aq}}\frac{dC_{\mathrm{aq}}}{dt} = K_{\mathrm{cornea}}A_{\mathrm{cornea}}C_{\mathrm{tear}} - q_{\mathrm{aq}}C_{\mathrm{aq}} \tag{22}$$

where C_{tear} is determined from Eq. 20. The concentration of the drug in tears and aqueous humor predicted from this model is in reasonable agreement with the measured values reported by Yang et al. [21] (Fig. 9). It is interesting to note

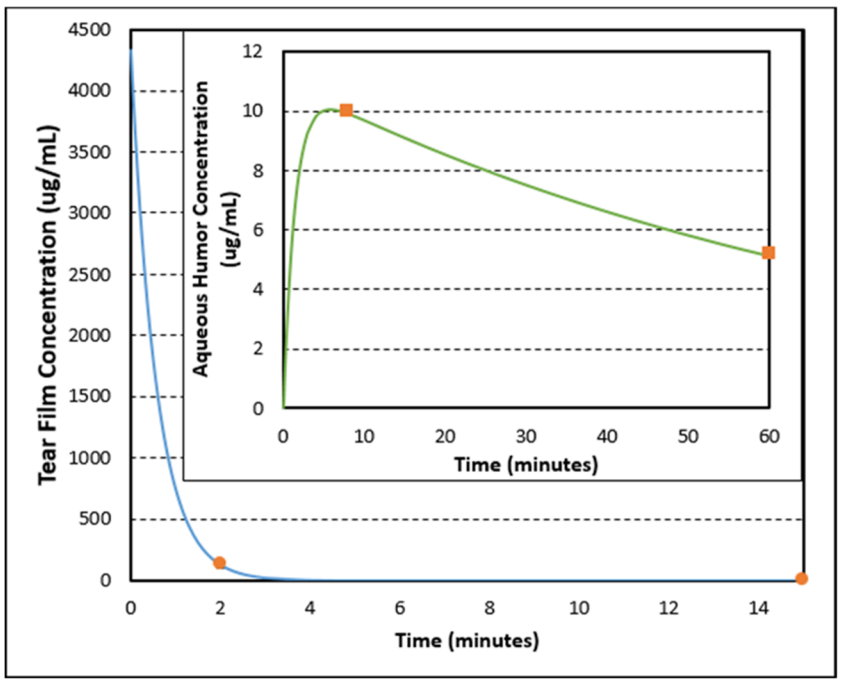

Fig. 9 In vivo data from Yang et al. [19] for eye drop delivery of pirfenidone along with fits based on our model (solid lines)

that while the residence time of the drug in tears is only a few minutes, the residence time in aqueous humor is about an hour. The residence time in aqueous is governed by the time for aqueous turnover which is about 100 min, while the mass of the drug that reaches the aqueous is determined by the permeability of the cornea and the residence time in tears. The cornea acts as a low-permeability barrier that leads to very low bioavailability, but on the other hand traps the small mass of drug that enters the cornea-aqueous humor region.

The bioavailability for the drug delivered via eye drops can then be calculated ($f_{\text{eye drops}}$) as

$$f_{\text{eye drops}} = K_{\text{cornea}} A_{\text{cornea}} \int_0^{\infty} \frac{C_{\text{tear}}}{Mo} dt \tag{23}$$

By using the tear concentration based on Eq. 20, we calculated the value of $f_{\text{eye drops}}$ to be 1.3%, which is about 40-fold lower compared to that for contact lenses. This order of magnitude difference shows the effectiveness of contact lenses in these pirfenidone studies while also supporting previous studies of other ocular drugs and their increased efficacy through the application of both modified and unmodified soft contact lenses.

Conclusion

In conclusion, the release of pirfenidone was extended from 15 to 20 min to 80 min by vitamin E loading of 20% in ACUVUE® OASYS® contact lenses. This increase in release time follows a similar quadratic trend similar to other molecules tested with vitamin E-loaded lenses. The mechanistic pharmacokinetic modeling shows that 50% of the drug loaded in the lens reaches the cornea which is about 50-fold higher compared to eye drop formulations. The mathematical model accurately predicts the concentrations in the tear film and the aqueous humor. Gene expression of inflammatory cytokine IL-1β and profibrotic growth factor TGF-β1 was significantly suppressed in corneas treated with pirfenidone contact lenses. A week after the alkali burn, the eyes with pirfenidone contact lenses showed significant improvement in corneal haze in comparison to the control eyes. This makes contact lenses a prime candidate for future use with pirfenidone.

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Nomenclature α, aspect ratio of barrier; φ, vitamin E loading in lens; φ^*, absorbed vitamin E in lens; τ, time scales of pirfenidone release of vitamin E loaded; τ_0, time scales of pirfenidone release of control lens; $A_{\text{conjunctiva}}$, area of conjunctiva; A_s, surface area of contact lens; C_{aq}, concentration of pirfenidone in aqueous humor/cornea system; C_g, concentration of pirfenidone in contact lens; $C_{g,\,f}$, concentration of pirfenidone in contact lens after loading; $C_{g,\,i}$, concentration of pirfenidone in contact lens after loading; $C_{l,\,f}$ concentration of pirfenidone in loading solution; C_0, concentration at time zero for tear film after the elution of the eye drop; C_r, concentration of pirfenidone in release medium; $C_{r,\,f}$, final concentration of release medium; C_{tear}, concentration of pirfenidone in tear film; D, diffusion coefficient of gel; f, fraction of drug released from the lens that reaches the cornea; $f_{\text{eye drops}}$, bioavailability of eye drops; h, half thickness of contact lens; h_c, thickness of cornea; h_{POLTF}, thickness of tear film; j_a, flux of pirfenidone into cornea; j_{lens}, flux of pirfenidone from lens; K_g, partition coefficient of pirfenidone in contact lens; $K_{\text{conjunctiva}}$, permeability of pirfenidone in conjunctiva; K_{cornea}, permeability of pirfenidone in cornea; M_o, total mass of pirfenidone in an eye drop; q_{aq}, volumetric drainage rate from aqueous humor/cornea system; q_{tear}, drainage rate of tear film; t, time; t_d, diffusion time of pirfenidone in contact lens; $t_{d,\,\text{aq}}$, diffusion time of pirfenidone in aqueous humor; $t_{d,\,\text{tear}}$, diffusion time of pirfenidone in tear film; $t_{q,\,\text{aq}}$, time scale of drainage from aqueous humor; $t_{q,\,\text{tear}}$, time scale of drainage from tear film; V_{aq}, volume of aqueous humor and cornea; V_g, volume of contact lens; V_r, volume of release medium; V_{tear}, volume of tear film; y, axis of thickness

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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