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Dual drug delivery from vitamin E loaded contact lenses for glaucoma therapy

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ABSTRACT

Glaucoma patients frequently instill eye drops multiple times each day, which is a cause for reduced compliance. Additionally, eye drops suffer from other limitations including low bioavailability, which can lead to side effects. We propose to develop drug-eluting contact lenses for managing glaucoma with increased bioavailability and improved compliance.

Contact lenses are developed for extended simultaneous release of timolol and dorzolamide, both of which are commonly prescribed hydrophilic drugs. The extended release is achieved by loading lenses with vitamin E barriers. *In vitro* release studies are performed with control and vitamin E loaded lenses for both drugs loaded separately and then together in the same lens. The safety and efficacy of combination therapy by contacts are demonstrated in a Beagle model of glaucoma.

Simultaneous loading of timolol and dorzolamide increases the release duration of both drugs. Also vitamin E incorporation is highly effective in increasing the release durations of both drugs to about 2-days. The lenses loaded with both drugs exhibited superior IOP reduction compared to eye drops with about 6-fold lower drug loading. More importantly, combination therapy by continuous wear of vitamin E loaded contact for 2-days, followed by a new set of contacts for another two days, reduced IOP during the 4 days of wear time and for another 8 days after removal of the contacts.

Vitamin E loading is very effective for providing combination therapy by contact lenses due to the increase in release durations of several drugs. The contact lens based therapy reduces IOP with lower drug dose compared to eye drops and may significantly improve the compliance as the effect of the therapy lasts significantly longer than the wear-duration.

1. Introduction

Glaucoma affects about 60.5 million people, leaving 8.4 million with bilateral blindness [1,2]. The World Health Organization estimates that by 2020 the number of cases for blindness due to glaucoma will increase to 12 million [2]. In the U.S., approximately 120,000 are blind from glaucoma, accounting for 9–12% of all cases of blindness. The financial impact of glaucoma on the US economy is in excess of $1.5 billion annually [3]. Currently, most of the antiglaucoma medications are applied topically through eye drops which are not very efficient. Due to the short residence time of drug and physiological and anatomical barriers in the eye, less than 5% of active ingredients can reach to the target tissue, with the remaining drug reaching other organs through the systemic circulation resulting in unwanted side effects [4,5]. To compensate for the low bioavailability, eye drops are often prescribed with high-frequency dosing regimens which exacerbate the side effects and additionally, reduce patient compliance. The poor patient compliance is a major problem for treating chronic diseases such as glaucoma because patients feel no instant benefit from treatment, which is accompanied by unpleasant side effects [6–8].

Contact lenses have been proposed as a potential candidate for ophthalmic drug delivery for improving bioavailability and patient compliance. The drug released from the contact lenses into the thin tear film in between the lens and the cornea has a residence time of up to 30 min, which leads to an estimated bioavailability as large as 50% [9,10]. The higher bioavailability allows reduction in the mass of drug instilled, thereby reducing the systemic uptake and the associated undesired side effects. Unfortunately, the release durations of most ophthalmic drugs from commercial contact lenses are a few hours, which is a limiting factor in drug delivery via contact lenses [11,12]. Recently, studies have focused on

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developing novel methods for increasing the drug release durations, as summarized in several good reviews [13–16]. Chauhan and coworkers have developed extended drug release contact lens modified by vitamin E diffusion barriers which significantly increase the drug release duration while retaining transparency and other critical lens properties [17,18]. The safety and efficacy of the vitamin E loaded contact lenses were also proven in *in vivo* studies in a Beagle dog model of glaucoma [19,20]. These studies showed that vitamin E loaded contact lenses could be safely worn for extended duration of four days, with a continuous release of timolol resulting in IOP reduction comparable to eye drops, but with a reduced dose of 20% compared to the drops.

Vitamin E loaded lenses are particularly suitable for glaucoma therapy because most patients require multiple medications to control the IOP. As demonstrated by Peng et al. [17], the presence of vitamin E diffusion barriers in the contact lens reduces the drug transport rate by increasing the diffusion path length in the lens matrix, and thus the approach is effective for a large number of drugs [18,21–24]. In this study, we report *in vitro* and *in vivo* studies on the feasibility of simultaneously delivering glaucoma drugs timolol maleate and dorzolamide hydrochloride from vitamin E loaded contact lenses. These drugs were chosen because both of these drugs are commonly prescribed and the mixture of timolol and dorzolamide is also commercially available (Cosopt®). Timolol, a β-adrenergic antagonist and dorzolamide, a carbonic anhydrase inhibitor decrease IOP by inhibiting the production of aqueous humor, but through different mechanisms [25]. Previous studies have confirmed a better efficacy in lowering IOP by combination therapy than monotherapy [26–28] and fixed combination showed comparable clinical effect as concomitant therapy [29–32]. To our knowledge this is the first study proving the efficacy of simultaneous release of glaucoma drugs from contact lenses.

2. Materials and methods

2.1. Materials

Timolol maleate (⩾98%) and (±)-α-Tocopherol (synthesized vitamin E, ⩾96%) were purchased from Sigma–Aldrich Chemicals (St. Louis, MO, USA). Dorzolamide hydrochloride was purchased from Taizhou Crene Biotechnology Co., Ltd. (Taizhou, Zhejiang, China). Ethanol (200 proof) was purchased from Decon Laboratories Inc. (King of Prussia, PA, USA). Dulbecco's phosphate buffered saline (PBS) was purchased from Mediatech, Inc. (Manassas, VA, USA). All chemicals were used as received without further treatment. The contact lenses used in this study are senofilcon A (ACUVUE® OASYS™, Vistakon, Fl, USA) with diopter −3.50, based curve 8.4 mm and diameter 14.0 mm of which detailed composition is proprietary information. No further modification was done to the lenses for *in vivo* studies due to the similarity in cornea size and shape between the Beagle dogs and human.

2.2. Preparation of vitamin E loaded contact lenses

Higher vitamin E loading concentration results in quadratic increase in drug release duration, but also impairs critical contact lens properties such as oxygen and ion permeability. As shown in the previous study [17], senofilcon A loaded with a vitamin E concentration of 20% (weight of loaded vitamin E in the lens/weight of dried lens) retains all critical properties as an extended wearable contact lens. The 20% vitamin E loaded lens is transparent in appearance with only 3% increment in lens diameter which is likely to have minimum interference with the ability of the lenses to be worn successfully and to correct refractive error.

Commercial contact lenses were removed from the blister packs, rinsed with deionized (DI) water several times and then soaked in 3 ml of 40 mg/ml vitamin E-ethanol solution for 24 h. The contact lenses swelled significantly in ethanol solution which facilitates the vitamin E to diffuse into the lens matrix. Ethanol has been extensively used in contact lens manufacturing for triggering esterification of carboxyl-containing polymer [33], detaching the lens from the mold [34], extracting unreacted components in the gel [35] or coating phosphorylcholine for colored-contact lens [36] and should have negligible impact on lens' optical properties. After reaching equilibrium, the lenses were gently blotted with Kimwipes and then soaked in 350 ml of DI water to extract ethanol for an hour. The same extraction step was repeated twice until the ethanol concentration in the DI water bath was under the detection limit of UV–Vis spectrophotometry. During the extraction process, vitamin E was mostly retained in the lens because of the very poor solubility in water. The extraction of ethanol led to oversaturation of vitamin E in the lens that caused phase separation into the diffusion barriers. After extraction steps, the lens was dipped in pure ethanol for few seconds and submerged in DI water for another 1 min to wash off the vitamin E aggregates depositing on the lens surface. The vitamin E loaded lenses were then kept in 5 ml of fresh PBS solution for later use. Three contact lenses were dried in air before and after loading step to confirm the amount of vitamin E loaded into the lenses by measuring the difference in dried weights. After the dry weight measurements, these lenses were discarded and not used in the following experiments.

2.3. Determination of releasing profiles by in vitro experiments

2.3.1. Measurement of individual drug releasing profiles

Drug loaded contact lenses were prepared by soaking the lens into 3.5 ml of 0.8 mg/ml timolol maleate-PBS solution or 3.5 ml of 0.75 mg/ml dorzolamide hydrochloride solution. The control contact lenses were soaked in drug solution for 24 h and increased to 4 days for vitamin E modified ones. Next, the lenses were removed and gently blotted to remove residual drug solution on the surface. The *in vitro* drug release experiment was carried out under sink condition by soaking the drug loaded lens into 2 ml of fresh PBS and it can be assumed 100% drug release after reaching equilibrium. The concentration of drug released to PBS was measured at predetermined time points by using UV–Vis spectrophotometry (Thermospectronic Genesys 10 UV, Rochester, NY, USA) in the range of 228–315 nm.

2.3.2. Measurement of simultaneous drug releasing profiles

To load with two different drugs, the control lenses were soaked in 3.5 ml of PBS containing timolol and dorzolamide at concentrations of 2.8 mg/ml and 2.5 mg/ml, respectively; while the vitamin E modified lenses were soaked in 3.5 ml of PBS containing timolol and dorzolamide at concentrations of 12.75 mg/ml and 20 mg/ml, respectively. The time of soaking in the solution was 24 h and 4 days for control and vitamin E modified contact lenses, respectively. Next, the lens was taken out from the solution, gently blotted and the drug release experiments were carried out by soaking the lens in 2 ml of fresh PBS and measured the drug concentration in PBS periodically. The measured UV spectrum was a linear combination from the two individual drugs, namely timolol and dorzolamide. The individual drug concentration can be determined by applying the least square fit method as described in Ref. [37]. Briefly, the measured UV spectrum can be expressed as:

$$Abs_\lambda = \alpha \times Timolol_\lambda + \beta \times Dorzolamide_\lambda \quad (1)$$

Table 1
Summary of the design for *in vivo* animal studies.

Therapy	Study design	Dose[a]	Time to release 90% of loaded drug[a]
Commercial Cosopt® eye drops	Drugs were given twice a day for 4 days. The IOP and heart rate measurement continues for one more day.	D[b]: ~670 μg/drop T[b]: ~205 μg/drop	
Pure senofilcon A	Contact lens was replaced daily for 5 days.	D: 217.8 ± 3.3 μg/lens	D: ~3 h
		T: 60.6 ± 1.2 μg/lens	T: ~1.2 h
20% VE loaded senofilcon A	Replaced once after 48 h of wearing. Lens was taken off at 96 h and no lens was on the eye after that. IOP was kept measuring until the 12th day.	D: 676.7 ± 2.0 μg/lens	D: ~48 h
		T: 191.4 ± 0.7 μg/lens	T: ~48 h

[a] Dose and release duration were determined by *in vitro* experiments.
[b] D represents dorzolamide and T represents timolol.

where Abs_λ is the UV absorbance measured at wavelength λ, and $Timolol_\lambda$ and $Dorzolamide_\lambda$ are the reference UV absorbance of timolol and dorzolamide at wavelength λ, respectively. By substituting the UV absorbance measured from 240 to 315 nm into Eq. (1), we can obtain a series of algebraic equations with only two unknowns, constant α and β. A least square fit method is then applied to calculate the optimum values of α and β. Finally, the concentration of timolol in the sample solution can be calculated by α times the concentration of the reference timolol solution; and the same for dorzolamide. This method was validated by measuring standard solutions of timolol and dorzolamide mixtures and the determined concentration showed only about 1% of error.

2.3.3. Determine effective diffusivity of drug in contact lens

The effective diffusivities of the drugs were determined by fitting the short-time release data to the Higuchi equation, i.e.,

$$\frac{M_t}{M_\infty}(release\%) = \frac{2}{\sqrt{\pi}}\sqrt{\frac{Dt}{h^2}} \quad (2)$$

where D is the effective diffusivity of the drug; h is the half thickness of the contact lens, which is assumed to be uniform over the lens and equals to 40 μm; M_t is the accumulated mass of drug released at time t; and M_∞ is the accumulated mass of drug release when t approaches infinity.

2.4. Evaluation of therapy efficacy and safety by in vivo animal studies

Ten Beagle dogs affected by various stages of primary open-angle glaucoma were enrolled in the animal studies. The animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at University of Florida (UF). The entire experiment procedures and the animal care and housing were performed in compliance with the statement and guidelines of Association for Research in Vision and Ophthalmology (ARVO) and the IACUC at UF.

The IOP was measured and used as an indication of the efficiency of the therapy. In addition, timolol has been known to cause cardiovascular side effects such as arrhythmia and bradycardia [38,39] which reflects on dogs' heart rate soon after the drug administration. Heart rate measurement was thus used as an indication of the severity of the side effects. Baseline measurement of IOP and heart rate of each individual dog was established prior to receiving any treatment. Three different treatment therapies were investigated including using eye drops, control contact lenses and vitamin E loaded contact lenses. Table 1 summarized the experiment design of the three therapy methods and the details of the animal study are as follows:

2.4.1. Washout period

Studies showed that the halftime of timolol in plasma was about 2.5–5.5 h in human and 1 h in dogs with oral dosing [40–42]. Similar pharmacokinetic studies showed that topical eye drops of dorzolamide had a halftime around 2–3 h in human ocular tissue [43]. To prevent the cross interaction, a one week of washout period was included prior to each of the therapy method including baseline measurement.

2.4.2. Baseline measurement

Before having any treatment, IOP of both eyes and heart rate were measured 4 times daily at 9:00, 10:00, 11:00 and 16:00 and continue for 5 days to establish the baseline for each individual dog. The IOP readings were measured by using applanation tonometry (Tono-Pen-XL, Mentor O and O, Norwell, MA, USA) after applying one drop of topical anesthetic agent (proparacaine hydrochloride, 0.5%). The heart rates were measured by directly feeling the dog's sphygmus.

2.4.3. Eye drop study

Commercial Cosopt® eye drops were used in the study. Each drop (30 μl) of Cosopt® contains roughly 205 μg of timolol and 670 μg of dorzolamide. The therapy consisted of instilling eye drops b.i.d. (9:00 and 16:00) in a randomly chosen eye with the other eye as control. The treatment continued for four days and IOP and heart rate were measured at 9:00, 10:00, 11:00 and 16:00 for each day of the four days treatment and one day more after the treatment stopped.

2.4.4. Control contact lens

The lens preparation procedure for the animal studies was exactly the same as the lens used in *in vitro* studies. As in the eye drop study, a randomly chosen eye was treated with contact lenses with the other used as control. The lenses were designed to load roughly 60 μg of timolol and 220 μg of dorzolamide which was roughly one-third of the dosing of one drop of Cosopt® (Table 1). Each lens was worn for 24 h and replaced daily with a fresh one at 9:00 after the IOP and heart rate were measured. Except for the first measurement at the beginning of the treatment,

the IOP measurements were done with the lens on the eye, which has been shown to not impact the IOP measurements. IOP and heart rate were measured during the 5 days of treatment period.

2.4.5. Vitamin E loaded contact lens

20% vitamin E loaded senofilcon A was designed to release about 200 μg of timolol and 680 μg of dorzolamide which was roughly equal to one drop of Cosopt® formulation. The lens was placed on the eye for a consecutive of 48 h and then replaced with a fresh vitamin E loaded lens and wore for another 48 h. The lens was placed in only one eye and the other eye was remained untreated as control. Measurement of IOP and heart rate continued for the four days during which the contact lenses were in the eyes and 8 more days following the lens removal.

2.4.6. Safety assessment

Eyes were observed during the treatment periods and any eye discomfort, redness, discharge, etc. were recorded and quantified based on the McDonald and Shadduck scoring system as described in Dermatotoxicology [44].

2.5. Statistical analysis

The average of IOP and heart rate for each day was compared with subsequent measurements by utilizing Tukey's HSD and ANOVA tests for repeated measurements to determine whether there was a significant difference ($p < 0.05$) between the different drug delivery methods. The measured parameters for the drug-treated eyes were also compared to both baseline and untreated eyes. The same method was used to analyze the effective diffusivities of timolol and dorzolamide calculated from *in vitro* experiments. The analysis was performed in statistical software R (version R-2.15.2 for Window®, R Development Core Team).

3. Results

3.1. In vitro drug release experiment

3.1.1. Drugs loaded individually

The release profiles of timolol and dorzolamide from 0% or 20% vitamin E loaded senofilcon A normalized to 100% drug release for better comparison of release duration are shown in Fig. 1. The sink conditions ensured that the entire mass of the loaded drug was released as evident from negligible drug remaining in the lenses after the release. The mass of drug released was used to calculate

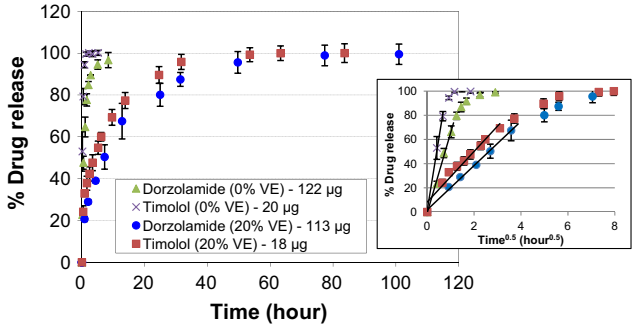

Fig. 1. Release profiles of timolol and dorzolamide released from different lenses with or without vitamin E loaded. The same release profiles expressed as % Drug release vs. square root of time are also shown. The solid lines are the best fit straight lines. The effective diffusivities can be calculated by equating the slopes of the fitted lines to Eq. (2). "% Drug release" is calculated as the ratio of cumulative release at any time and that after equilibrium is achieved. The cumulative release at equilibrium is indicated in the legends. Data are shown as mean ± SD with $n = 3$.

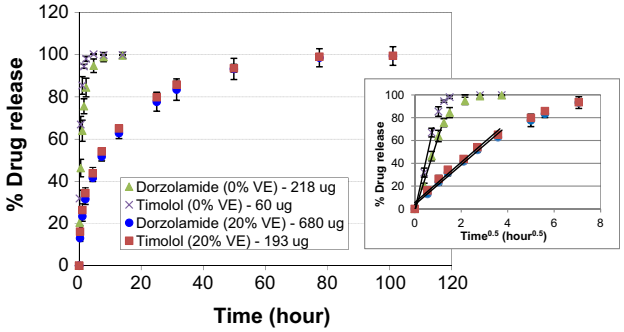

Fig. 2. Release profiles of timolol and dorzolamide released simultaneously from the same lens with or without vitamin E loaded. The same release profiles expressed as % Drug release vs. square root of time is also shown in the figure. The solid lines are the best fit straight lines. The effective diffusivities can be calculated by equating the slopes of the fitted lines to Eq. (2). "% Drug release" is calculated as the ratio of cumulative release at any time and that after equilibrium is achieved. The cumulative release at equilibrium is indicated in the legends. Data are shown as mean ± SD with $n = 3$.

the loading efficiency. For the 20% vitamin E loaded senofilcon A, the drug loading amounts in the lens is 191.4 μg of timolol and 676.7 μg of dorzolamide after soaking in 3.5 ml of 12.75 mg/ml timolol and 20 mg/ml dorzolamide mixture solutions. After loading, only 1.5% of timolol and 3.4% of dorzolamide were loaded into the lens matrix. The results are comparable with the control lenses. The loading efficiency can be increased by reducing the fluid volume and increasing the drug concentration. In this study we chose to load in larger fluid volumes to maintain sink conditions so that the loaded concentration was in equilibrium with the initial concentration. In fact the optimal strategy would be to add the drug to the blister-pack solution which is a fraction of an mL in some cases (0.2 ml for "Miru") and load the drug during the lens storage.

The release durations of timolol and dorzolamide, defined as the duration for 90% release, are 0.7 and 2.5 h, respectively from the senofilcon A lenses. The release durations significantly increase to 24.6 h for timolol and 36.0 h for dorzolamide after the incorporation of 20% of vitamin E. Fig. 1 shows the same release profile of % drug release as a function of square root of time with the solid lines as the best fits to the short-time release data. The fits are good with the value of R^2 larger than 0.95, which proves the transport of both drugs in the contact lens with or without vitamin E modification is diffusion-controlled.

3.1.2. Drugs loaded together

The release profiles from studies in which both drugs were loaded into the same lens are shown in Fig. 2. The release durations of timolol and dorzolamide are 1.2 and 3.0 h, respectively, and significantly increase to 42.2 and 42.3 h after the incorporation of vitamin E into the lens. The plots of % drug release as a function of square root of time in Fig. 2 are linear with $R^2 > 0.96$, which indicates the transport of both drugs in the lens is still diffusion-controlled despite the two drugs are diffusing simultaneously in the same lens.

The release durations and the calculated effective diffusivities of timolol and dorzolamide releasing separately or simultaneously from control or vitamin E loaded lens are all summarized in Table 2. Compared to separate loading and release, the average release durations increase for both drugs if they are loaded into the same lens. The increase in release duration from control contact lens is significant for timolol ($p = 0.0459$) but not for dorzolamide ($p = 0.1767$). The average release durations of both drugs also increased if they are releasing simultaneously rather than separately from vitamin E modified lenses, but still, the difference is

Table 2
Summary of release durations[a] and effective diffusivities[b] from *in vitro* experiments.

	Release duration (h) (released separately)	Release duration (h) (released simultaneously)	Diffusivity (10^{-5} mm^2/h) (released separately)	Diffusivity (10^{-5} mm^2/h) (released simultaneously)
Control lens Timolol	0.7	1.2	207.90 ± 61.36	105.40 ± 9.53
Dorzolamide	2.5	3.0	66.84 ± 14.10	51.50 ± 7.91
20% vitamin E loaded lens Timolol	24.6	42.2	6.17 ± 0.05	3.91 ± 0.10
Dorzolamide	36.0	42.3	4.34 ± 1.24	3.80 ± 0.06

[a] Release duration is defined as the time to reach 90% of the release amount at equilibrium.
[b] Data are presented as mean ± SD with $n = 3$.

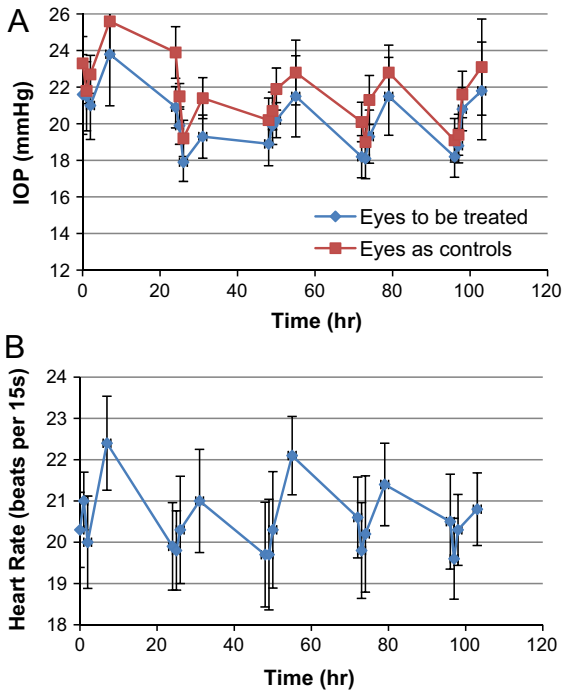

Fig. 3. Baseline (A) IOP and (B) heart rate measurement of 10 Beagle dogs. For each dog, only one randomly chosen eye (5 in left eyes and 5 in right eyes) was receiving treatment and the other eye was untreated as a control. Data are presented as mean ± SEM.

only significant for timolol ($p < 10^{-6}$ for timolol and $p = 0.4921$ for dorzolamide).

3.2. Animal studies

3.2.1. Baseline measurement

The baseline measurement of IOP and heart rate over 5 days is shown in Fig. 3. Both IOP and heart rate showed a circadian rhythm each day [45]. The average IOP of "eyes to be treated" and "eyes as control" over all 5 days was 20.16 ± 0.36 mmHg and 21.57 ± 0.31 (mean ± SEM), respectively. The IOP difference between the two sets was significant ($p = 0.0034$), and so the efficacy of any treatment was gauged by comparisons of the IOP after treatment with the baseline for the same set of eyes. The average of heart rate over all 5 days was 20.49 ± 0.24 beats per 15 s. There was no significant difference in day-to-day variation of heart rates ($p = 0.9150$) and IOP of "eyes to be treated" ($p = 0.1360$) and "eyes as control" ($p = 0.0627$). Table 3 summarized the average IOP and heart rate from baseline and from treatments of using eye drops, control contact lenses and contact lenses loaded with vitamin E.

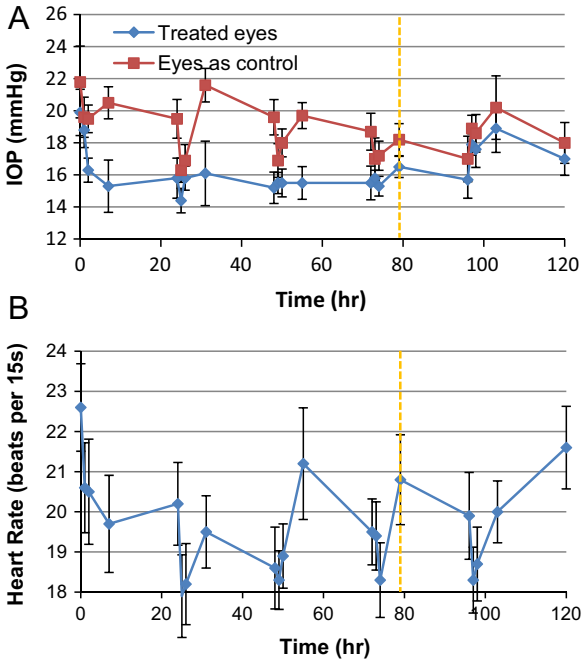

Fig. 4. (A) IOP and (B) heart rate measurement of 10 Beagle dogs with Cosopt® eye drops treatment. For each dog, only one randomly chosen eye (5 in left eyes and 5 in right eyes) was receiving treatment and the other eye was untreated as a control. The dash line at 79 h indicates the time the last eye drop was given. Data are presented as mean ± SEM.

Table 3
Summary of IOP and heart rate (mean ± SEM) of the baseline measurement and after receiving the three different drug delivery methods.

Method	Study period (days)	IOP of treated eyes (mmHg)[a]	IOP of untreated eyes (mmHg)[a]	Heart rate (beats per 15 s)[a]
Baseline	5	20.16 ± 0.36	21.57 ± 0.31	20.49 ± 0.24
Eye drops	5	*16.37 ± 0.28	*18.79 ± 0.26	*19.56 ± 0.23
Control contact lenses	5	*15.49 ± 0.29	20.66 ± 0.23	*21.99 ± 0.23
Vitamin E loaded contact lenses	12	*15.81 ± 0.18	21.03 ± 0.23	21.19 ± 0.21 (first 5 days) *22.81 ± 0.26 (last 5 days)[b]

[a] The symbol * indicates significant difference from baseline ($p < 0.05$).
[b] The reported heart rate is separated into two groups, which are the average over the first 5 days (with the contact lenses on the eyes) and the last 5 days (after the removal of the contact lenses).

3.2.2. Eye drops treatment

The variations of IOP and heart rate with Cosopt® eye drop treatment are shown in Fig. 4. The last eye drop treatment was given at 79 h which is indicated as the dash line in the figure, but the IOP and heart rate measurement continued for one more day. As shown in Fig. 4, the average IOP in the treated eyes decreased 2.05 ± 0.58 mmHg ($p = 0.1177$) from day 1 to day 2 and increased 1.75 ± 0.57 mmHg ($p = 0.2441$) from day 4 to day 5, although the differences were not significant. The comparison between treated eyes and untreated eyes showed significant differences from day 1 to day 3 which were 2.78 ± 0.81 ($p = 0.0288$), 3.05 ± 0.78 ($p = 0.0094$) and 3.13 ± 0.51 mmHg ($p = 0.0067$), respectively, but became insignificant in day 4 and day 5 with a difference of 2.00 ± 0.51 ($p = 0.3148$) and 1.15 ± 0.45 mmHg ($p = 0.9288$). The average IOP difference between treated eyes and untreated eyes over the five days' study was 2.42 ± 0.28 mmHg ($p < 10^{-6}$). When compared to baseline, the IOP of treated eyes was significantly lower with a difference of 2.58 ± 0.75 ($p = 0.0136$), 4.63 ± 0.50 ($p < 10^{-6}$), 4.73 ± 0.46 ($p < 10^{-6}$), 4.38 ± 0.53 ($p < 10^{-6}$) and 2.63 ± 0.70 mmHg ($p = 0.0110$) from day 1 to day 5 and the average difference over the five days' study was 3.79 ± 0.28 mmHg ($p < 10^{-6}$). Note that the IOP average of untreated eyes was also significantly lower than baseline for day 2 (3.00 ± 0.71 mmHg, $p = 0.0003$), day 3 (3.02 ± 0.47 mmHg, $p = 0.0003$), day 4 (3.80 ± 0.37 mmHg, $p < 10^{-6}$) and day 5 (2.90 ± 0.58 mmHg, $p = 0.0006$) with the only exception in day 1 (1.22 ± 0.63 mmHg, $p = 0.5030$).

The average heart rate variation during eye drops treatment in each day was not significantly different from each other with the largest difference being observed in between day 1 and day 2 which was 1.9 beats per 15 s ($p = 0.0684$). When compared to baseline, the average heart rate over the 5 days' study was significantly lower by 0.93 ± 0.23 beats per 15 s ($p = 0.0056$).

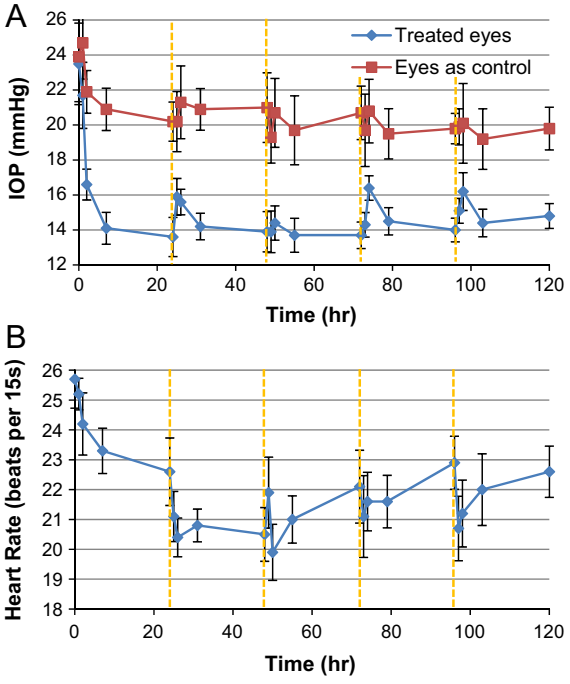

Fig. 5. (A) IOP and (B) heart rate measurement of 10 Beagle dogs with treatment of wearing timolol and dorzolamide loaded contact lenses. For each dog, only one randomly chosen eye (5 in left eyes and 5 in right eyes) was receiving treatment and the other eye was untreated as a control. The dash lines indicate the time the contact lenses were replaced with fresh ones. Data are presented as mean ± SEM.

3.2.3. Treatment with control contact lenses

The variations of IOP and heart rate treated with timolol and dorzolamide-loaded control contact lenses are shown in Fig. 5. Each lens was worn for 24 h and then replaced with a fresh one at the times indicated by the dash lines in the figure. The average IOP in the treated eyes decreased significantly with a difference of 4.15 mmHg ($p < 10^{-4}$) from day 1 to day 2, but was not significantly different among day 2 to day 5. The comparison between treated eyes and untreated eyes showed significant differences from day 1 to day 5 which were 3.83 ± 0.91 ($p = 0.0101$), 5.53 ± 0.90 ($p < 10^{-5}$), 6.15 ± 0.94 ($p < 10^{-6}$), 5.38 ± 0.77 ($p < 10^{-4}$) and 4.98 ± 0.69 mmHg ($p = 0.0001$), respectively, and the average difference over the five days' study was 5.17 ± 0.38 mmHg ($p < 10^{-6}$). When compared to baseline, the treated eyes showed significantly lower IOP with a difference of 5.33 ± 0.47 ($p < 10^{-6}$), 6.18 ± 0.51 ($p < 10^{-6}$), 5.43 ± 0.39 ($p < 10^{-6}$) and 5.23 ± 0.42 ($p < 10^{-6}$) from day 2 to day 5, but not significant in day 1 with a difference of 1.18 ± 0.99 mmHg ($p = 0.6580$). The overall five days' IOP of treated eyes and untreated eyes was lower than baseline by 4.67 ± 0.29 mmHg ($p < 10^{-6}$) and 0.92 ± 0.38 mmHg ($p = 0.0663$), respectively.

As shown in Fig. 5B, the average heart rate in day 1 was significantly higher than that of day 2 and baseline measurement by 3.40 ($p < 10^{-4}$) and 4.12 ($p < 10^{-6}$) beats per 15 s, respectively. After day 1, the average heart rate variation during control contact lens therapy showed no significant difference from day 2 to day 5 ($p = 0.7058$). The average heart rate over the five days' study was significantly higher than baseline by 1.50 ± 0.23 beats per 15 s ($p < 10^{-4}$).

3.2.4. Treatment with 20% vitamin E loaded contact lenses

The variations of IOP and heart rate during the therapy of using 20% vitamin E loaded contact lenses are shown in Fig. 6. Each vitamin E modified contact lens was designed to be worn for 48 h. The dash line at 48 h in Fig. 6 indicates the time the lenses were replaced by fresh one. The other dash line at 96 h indicates the time the lenses were removed. The IOP and heart rate measurement continued from 96 to 288 h without any lens placed on the eyes. This result suggested that the IOP reduction effect was able to sustain for another whole week after a total of 96 h of vitamin E modified contact lens treatment. Note that no data were collected during 120–168 h (day 6–7) and were not included in the subsequent analysis.

The average IOP in treated eyes in day 1 was significantly higher than the rest of the days from day 2 to day 11 ($p = 0.0179$), but not day 12 ($p = 0.4607$). The comparison between treated eyes and untreated eyes was not significantly different in day 1 ($p = 0.0833$) but was significantly different from day 2 to day 12 with the largest difference in day 8 by 7.15 ± 0.93 mmHg ($p < 10^{-6}$) and the smallest difference in day 12 by 4.13 ± 0.61 mmHg ($p = 0.0011$). The average difference between treated eyes and untreated eyes over the 12 day's study was 5.22 ± 0.21 mmHg ($p < 10^{-6}$). Except day 1, the average IOP from day 2 to day 12 was all significantly lower than baseline with the largest difference in day 8 by 5.48 ± 0.57 mmHg ($p < 10^{-6}$), the smallest difference in day 12 by 3.43 ± 0.88 mmHg ($p = 0.0001$) and the average difference over the 12 days' study was 4.35 ± 0.18 mmHg ($p < 10^{-6}$). The average IOP of untreated eyes in vitamin E loaded contact lenses treatment was insignificantly lower than baseline by 0.54 ± 0.23 mmHg ($p = 0.1686$).

As shown in Fig. 6B, the heart rate increased gradually after the removal of contact lenses at 96 h. A comparison between the average heart rate over the first 5 days (with contact lenses on the eyes) and the last 5 days' measurement (after the removal of the lenses) showed a significant difference of 1.62 beats per 15 s ($p < 10^{-5}$). In addition, there was no significant difference in day-to-day

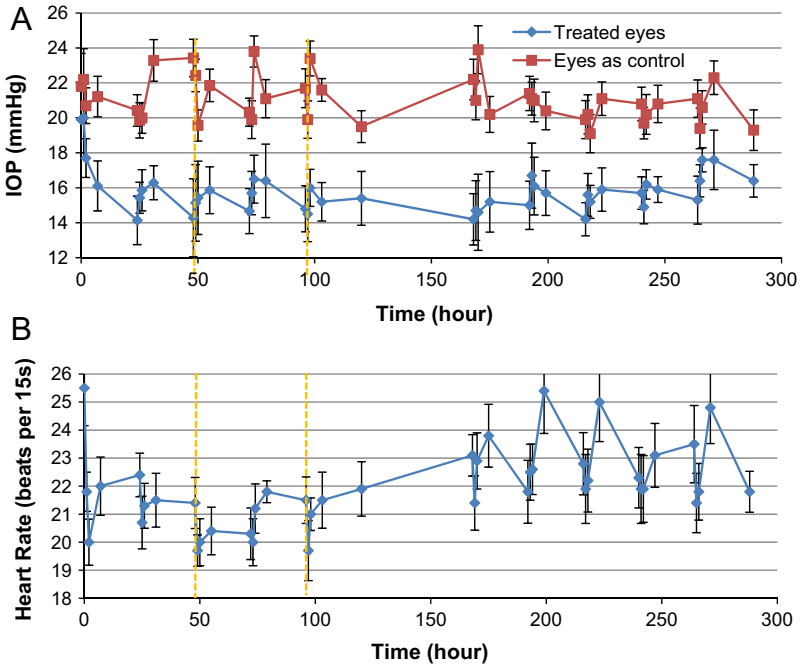

Fig. 6. (A) IOP and (B) heart rate measurement of 10 Beagle dogs with treatment of wearing 20% vitamin E modified contact lenses loaded with timolol and dorzolamide. For each dog, only one randomly chosen eye (5 in left eyes and 5 in right eyes) was receiving treatment and the other eye was untreated as a control. The dash line at 48 h indicates the time the contact lenses were replaced with a fresh one. The other dash line at 96 h indicates the time the contact lenses were removed. No lens was on the eye during 96–288 h. No data were collected during 120 to 168 h. Data are presented as mean ± SEM.

variation in the first 5 and the last 5 days' measurement, except for the difference between day 1 and day 3 by 1.95 beats per 15 s ($p = 0.0226$). The comparison to the baseline showed that the average of heart rate over the first 5 days was insignificantly higher by 0.70 ± 0.19 beats per 15 s ($p = 0.0909$), but was significantly higher by 2.32 ± 0.27 mmHg ($p < 10^{-6}$) over the last 5 days.

4. Discussion

4.1. Extended multiple drug delivery

In our previous studies, we have demonstrated that the drug release duration from contact lenses can be effectively increased by incorporating vitamin E as diffusion barriers. Hydrophilic drugs such as timolol and dorzolamide have negligible solubility in the vitamin E aggregates, and the drug molecules are forced to diffuse along a tortuous, longer pathway in the lens. Our results here show that about 20% vitamin E incorporation increased the release duration 35 and 14-fold for timolol and dorzolamide, respectively, when drugs were loaded individually. When the drugs were loaded into the same lens, the release durations of both drugs again increased 35 and 14-fold for timolol and dorzolamide, respectively. Interestingly, compared to release separately, the release durations of timolol and dorzolamide increased roughly 1.7 and 1.2-fold, respectively, as the two drugs were releasing simultaneously from control contact lens. After the lens was loaded with vitamin E, the release durations of timolol and dorzolamide also increased around 1.7 and 1.2-fold if the two drugs were releasing simultaneously compared to release separately. The mechanism of this phenomenon is not clear but could be attributed to interaction of the drugs in the lens potentially through hydrogen bonding. This effect makes the release durations of timolol and dorzolamide almost identical in the senofilcon A lens loaded with 20% of vitamin E (Fig. 2), which is a highly desirable attribute in a combination release device.

4.2. Therapeutic efficacy and safety evaluation

The therapeutic efficacy of using vitamin E loaded contact lens for extended multiple drugs delivery was investigated in the animal study and compared to eye drops and contact lens without modification. All three ophthalmic drug delivery methods decreased the Beagle dogs' IOP significantly from baseline as the results shown in Table 3. The control contact lens treatment decreased the IOP more significantly than eye drops by 0.88 ± 0.28 mmHg ($p = 0.0294$), despite the sixfold lower drug dosing compared to eye drops (Table 1). The result was consistent with other *in vivo* [19,20,46–49] and modeling studies [9] that showed the drug delivery by contact lens could provide a higher bioavailability. Similarly, the therapy from vitamin E modified contact lens lowered IOP during lens wear by comparable magnitude as eye drops but with 4-fold lower drug loading. Additionally, the IOP reduction was maintained even after the removal of the contacts, and on factoring that into the analysis, the vitamin E loaded contacts achieved comparable IOP reduction with 11-fold lower drug dose compared to eye drops. In our animal studies, except occasional mild redness, no ocular toxicity was observed during the administration of the three different types of therapies and in the follow-up of one-week examination after the treatments were stopped. To explore whether contact lens use can mitigate the systemic side effects, the heart rate changes were measured during the treatment. Studies on both Beagle dogs and human showed a statistically significant decrease of heart rate to as large as 10% after receiving acute or chronic dosing of timolol eye drops [26,50–52]. In our animal study, the average heart rate significantly decreased 4.5% from baseline with the eye drops treatment. However, the average heart rate significantly increased 7.3% with the treatment using control contact lenses. Since neither of the drugs or use of contacts should increase the heart rate, we conjecture higher heart rate with contacts is an artifact of baseline shifting. In fact, the heart rate measured right before the treatment (at time = 0 h in both Figs. 5 and 6) was much higher than the baseline,

which suggests a shift in baseline. This unexpected increase in heart rate made it more complicated to conclude that using contact lenses to deliver the drug was able to reduce the systemic side effects. However, the significant difference in heart rate ($p < 10^{-5}$) between the first 5 days (with the lenses on the eyes) and the last 5 days (after the lenses were removed) in the treatment of using vitamin E modified contact lenses suggested that the systemic side effects was significantly reduced after the removal of contact lenses, while the IOP was still effectively controlled. On the other hand, the average IOP of the untreated eyes was significantly decreased only in eye drops treatment while not in the contact lenses therapies (Table 3) which could be a firmly support that contact lenses therapies were able to mitigate the systemic side effects. The contralateral IOP reduction is commonly attributed to the systemic adsorption of drugs from conjunctiva and nasolacrimal sac where the drugs are then transported to the untreated eye by systemic circulation [26,53–55].

While improved bioavailability is clearly beneficial, the prolonged IOP reduction is perhaps the biggest benefit of the contact lens based therapy. As shown in Fig. 6, the IOP reduction effect sustained for another whole week after the lenses were removed at 96 h. The prolonged IOP reduction effect most likely resulted from the creation of drug depots in the ocular tissues. Cornea is a tri-laminate structure consisting of epithelium, stroma and endothelium with a thickness of roughly 50, 350 and 13 μm, respectively. Epithelium and endothelium are high lipid cellular layers which form a significant resistance to the transport of hydrophilic molecules. Stroma, lying in between the two lipid layers, is a thick hydrophilic layer contains roughly 80% of water which becomes a natural reservoir for hydrophilic drugs. The detailed transport and accumulation of timolol and dorzolamide through cornea has not been explored but the relevant mechanisms could be discerned at least partially from a study by Gupta et al. [56], in which a custom-built confocal scanning fluorescence microscope was employed to determine the depth-resolved trans-corneal penetration of sodium fluorescein through the rabbit cornea as it diffused from the endothelium to the epithelium side. The transient trans-corneal fluorescence profiles showed that the fluorescein accumulated in stroma and reached equilibrium concentrations in about six hours, which is significantly shorter than the release duration from the vitamin E loaded contacts. This suggests that during the lens wear the drug concentration in the stroma will be in equilibrium with that in the tears and thus considerable drug would accumulate. However the drug accumulation will stop when the contacts are removed on the 4th day of the study and following that in about six hours, a majority of the drug in the stroma would diffuse across the endothelium into the aqueous humor, followed by clearance through the drainage. Essentially the rate of drug transport across the endothelium and through the stroma is far too rapid to account for the long IOP reduction effect observed in our experiments. The study by Gupta et al. [56] also showed that the concentration of fluorescein in the epithelium increased very gradually with a total time for equilibration estimated to be about 4–7 days. The long time for equilibration is due to the slow transport of the hydrophilic molecules across the lipid-bilayers of the cells in the epithelium. It is noted that during the time for equilibration, fluorescein could transport across the epithelium by diffusing in between the cells but could not accumulate inside the cells. Thus, the transport of hydrophilic molecules can occur through the paracellular pathway or through the slow transcellular pathway. When ophthalmic drugs are delivered via eye drops, the residence time is far too short for drugs to partition inside the epithelium cells, and so the only transport pathway is through the tight junctions in between the cells. In the presence of vitamin E loaded contact lenses, the continuous release for four days allows significant drug accumulation inside the epithelium cells. After the lenses are removed, the accumulated drug can then diffuse out and that process would also take a long time of 4–7 days, which could lead to the prolonger IOP reduction. While this hypothesis appears to be plausible, further studies are needed to prove its validity. There are alternative mechanisms that could lead to prolonger IOP reduction. Ocular tissues could also get saturated with drug during the 4-days of release with contacts, and then slowly release the drug after the contacts are removed. Ahmed and Patton [57] showed timolol accumulation in posterior segment when the drug was delivered as eye drops to anesthetized rabbits with blocked tear drainage ducts. Also the accumulation after 4-h was 4 and 6-fold higher in cornea and iris-ciliary body in the group with blocked drainage compared to control. Timolol was also reported to accumulate in Tenon capsule with long-term topical administration [58]. Another study investigated the distribution of dorzolamide in pigmented rabbit's ocular tissues after acute or repeated instillation of 2% dorzolamide eye drops [59]. Dorzolamide was found to have a strong binding to iris-ciliary body, retina and choroid with a much slower elimination rate than in cornea. Compared to acute instillation, repeated instillation (b.i.d., for 10 days) significantly increased the concentration of dorzolamide in these ocular tissues (e.g. 10 times higher in retina). These studies along with the fluorescein transport study suggest that both drugs could accumulate in various tissues during the 4 days of continuous therapy, and then serve as the depots for the next few days to maintain the IOP reduction. Compared to the treatment of using vitamin E modified contact lens, the residence time of eye drops on the cornea is too short for the drug to accumulate and form depots in the eye and thus the IOP starts to increase back up to baseline after the eye drop treatment stops (Fig. 4). It is, however, not clear how long the IOP reduction effect can be sustained with the treatment with control contact lenses without vitamin E, since the IOP was not measured after the lens' removal. Peng et al. [20] reported a similar prolonged IOP reduction effect after ceasing the timolol delivery from contact lens with or without vitamin E modification, but the IOP was only measured one day more after the lens removal.

5. Conclusions

Glaucoma therapy by eye drops suffers from several deficiencies including poor compliance and low bioavailability. Several researchers have proposed an alternative approach for glaucoma therapy based on drug eluting contact lenses. Previous *in vitro* and *in vivo* results have shown that contact lenses can manage IOP with significantly lower drug payload compared to eye drops. Here we further show the benefits of glaucoma therapy by contact lenses. In particular we focus on a combination therapy because a majority of the glaucoma patients need to instill multiple drugs to effectively manage the IOP. The combination of timolol and dorzolamide is commercially available as eye drops and here we show the feasibility of combination therapy by contact lenses by doing *in vitro* release studies and *in vivo* IOP reduction studies with lenses loaded with both drugs. The co-loading of timolol and dorzolamide increases the release duration of the both drugs suggesting drug-drug interaction possibly due to hydrogen bonding. Even with co-loading the release durations are not sufficiently long for extended drug delivery and so vitamin E loading is explored to increase the release durations. The vitamin E loading increases release duration of both drugs resulting in a lens that can provide extended drug delivery for about 2-days. The contact lenses loaded with both drugs show superior IOP reduction compared to eye drops with 3–4-fold lower drug payload compared to eye drops. However, a more important benefit of the combination therapy

by the vitamin E loaded lenses was that the IOP reduction was maintained for about a week after removal of the contact lenses. This extended IOP reduction is likely due to the formation of depots during the lens-wear phase, followed by a slow release after the lens is removed. The depot effect could potentially be due to slow accumulation of the drugs inside the corneal epithelium cells, or the posterior segment, or possibly due to binding to high affinity targets such as iris-ciliary body and tenon capsule. The continuous exposure of the epithelium to the drugs did not result in any toxicity. Based on the entire study duration, the vitamin E loaded lenses achieved superior IOP reduction than drops at about 11-fold lower drug dose. The extended IOP reduction after the removal of contact lens opens the exciting possibility of achieving long term reduction in IOP with lens wear for a short time, such as a week-long therapy with one-day wear. Such a treatment approach would allow more patients to use the contacts and may significantly improve the compliance. Thus, vitamin E loaded contact lenses could be very useful for managing glaucoma with significantly higher bioavailability and lower side effects, and improved patient compliance. While these results are encouraging, there are several factors that may limit the feasibility of using contacts for glaucoma therapy. Patients that normally do not wear contacts may be hesitant to use this modality. Future studies are needed to explore the viability and efficacy of this approach in humans.

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