Published in final edited form as:

J Control Release. 2016 April 28; 228: 48–57. doi:10.1016/j.jconrel.2016.02.041.

Sustained reduction of intraocular pressure by supraciliary delivery of brimonidine-loaded poly(lactic acid) microspheres for the treatment of glaucoma

B. Chiang^{a,1}, Y.C. Kim^{b,1}, A.C. Doty^{c,1,2}, H.E. Grossniklaus^d, S.P. Schwendeman^c, and M.R. Prausnitz^{a,b,*}

^aWallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta GA 30332, USA

^bSchool of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta GA 30332, USA

^cDepartment of Pharmaceutical Sciences, The Biointerfaces Institute, University of Michigan, North Campus Research Complex, 2800 Plymouth Road, Ann Arbor MI 48109, USA

^dDepartment of Ophthalmology, Emory University School of Medicine, Atlanta GA 30322, USA

Abstract

Although effective drugs that lower intraocular pressure (IOP) in the management of glaucoma exist, their efficacy is limited by poor patient adherence to the prescribed eye drop regimen. To replace the need for eye drops, in this study we tested the hypothesis that IOP can be reduced for one month after a single targeted injection using a microneedle for administration of a glaucoma medication (i.e., brimonidine) formulated for sustained release in the supraciliary space of the eye adjacent to the drug's site of action at the ciliary body. To test this hypothesis, brimonidine-loaded microspheres were formulated using poly(lactic acid) (PLA) to release brimonidine at a constant rate for 35 days and microneedles were designed to penetrate through the sclera, without penetrating into the choroid/retina, in order to target injection into the supraciliary space. A single administration of these microspheres using a hollow microneedle was performed in the eye of New Zealand White rabbits and was found to reduce IOP initially by 6 mm Hg and then by progressively smaller amounts for more than one month. All administrations were well tolerated without significant adverse events, although histological examination showed a foreign-body reaction to the microspheres. This study demonstrates, for the first time, that the highly-targeted

*Corresponding author at: School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta GA 30332, USA. prausnitz@gatech.edu (M.R. Prausnitz).

¹These authors contributed equally to this work.

²Current address: Discovery Pharmaceutical Sciences, Pharmaceutical Sciences & Clinical Supply, Merck Sharp and Dohme Corp., 33 Ave Louis Pasteur, Boston MA 02460, USA.

Author contributions

BC and YCK performed experiments. ACD fabricated microspheres and performed *in vitro* characterization experiments. HEG prepared and evaluated histological slides. BC, YCK, ACD, SPS, and MRP designed the study and wrote and edited the manuscript.

Competing interests

YCK and MRP are inventors on patents that have been licensed, and MRP has a significant financial interest in a company that is developing microneedle-based products for ocular delivery (Clearside Biomedical). This potential conflict of interest has been disclosed and is overseen by Georgia Tech and/or Emory University.

delivery of brimonidine-loaded microspheres into the supraciliary space using a microneedle is able to reduce IOP for one month as an alternative to daily eye drops.

Keywords

Suprachoroidal space; Glaucoma; Brimonidine; Microneedles; New Zealand White rabbit

1. Introduction

Primary open-angle glaucoma is a leading cause of blindness in the United States, affecting nearly 2 million individuals with an annual cost of \$2.9 billion [1,2]. Glaucoma is the most common form of optic neuropathy, where loss of retinal ganglion cell axons permanently disrupts transmission of visual information from the retina to the brain [1,3,4]. Over decades, patients experience a painless and gradual loss of vision starting from the periphery and eventually claiming central vision [3,5]. Intraocular pressure (IOP) is the only modifiable risk factor [6, 7] and reducing IOP prevents the progression of glaucoma-related vision loss [5,8].

IOP is mediated by the balance of aqueous humor production and aqueous humor removal [9]. Aqueous humor is a clear liquid that is secreted by the ciliary body. Clearance of aqueous humor occurs through either the trabecular meshwork into the episcleral veins or the uveoscleral outflow pathway into the suprachoroidal space [9,10]. Medical and surgical therapy for glaucoma seeks to control IOP by reducing production of aqueous humor and/or increasing clearance of aqueous humor [5,11]. Topical eye drops, such as timolol, latanoprost, and brimonidine, are commonly-used FDA-approved medical therapies for glaucoma patients. Brimonidine is a α_2 -adrenergic agonist that both decreases aqueous humor secretion by the ciliary body and increases aqueous humor clearance [12]. Because topical eye drops can have low bioavailability through the cornea (<5%), some regimens call for multiple eye drops per day to ensure sufficient drug dosing (e.g., brimonidine eye drops are prescribed three times per day) [11,13].

1.1. The need for improved patient adherence with administration of IOP-lowering drugs

Patient adherence to topical eye drops is low, estimated to be only 41% to 76% [11,14–18]. Due to the chronic nature of glaucoma and the rigorous administration schedule, it can be difficult for patients to administer their eye drops on a regular basis. Since any loss of vision is permanent, increasing patient adherence to the regimen will preserve functional vision and decrease progression to blindness [11].

Patient adherence to the eye drop regimen can be increased through methods, such as memory tools that remind patients, and improved formulations that do not require refrigeration or require simpler administration regimens [11]. But perhaps the most attractive method to improve eye drop adherence is through the use of controlled-release drug delivery systems that obviate the need for the patient to take eye drops at all. While brimonidine-loaded drug delivery systems for the management of glaucoma have been studied before [19–22], we seek to determine the efficacy of a targeted controlled-release system delivered using a microneedle adjacent to brimonidine's site of action in the ciliary body.

1.2. Injections targeting the supraciliary spacing using microneedles

The supraciliary space is the anterior-most region of the suprachoroidal space. The suprachoroidal space is a potential space in the eye found between the sclera (the fibrous collagenous layer that contains the eye) and the choroid (the rich vascular network that supplies nutrients to the outer retina). The suprachoroidal space has been explored as a site for ophthalmic drug delivery in preclinical and recent clinical studies (e.g., NCT01789320 and NCT02255032) [23–38], motivated by higher bioavailability compared with topical eye drops [28,32] and the ability to target drug delivery to the choroid that lines the suprachoroidal space, the adjacent retina or, most recently, the ciliary body that forms its most anterior boundary.

Injections are targeted to the suprachoroidal (and supraciliary) space using individual hollow microneedles with a length matched to the thickness of the sclera and conjunctiva that enable access to the suprachoroidal space with a procedure comparable to an intravitreal injection, which is a method of ophthalmic drug delivery regularly performed in the outpatient clinic setting [28,32,39]. Microneedle injections in the suprachoroidal space were originally designed as a treatment for posterior-segment diseases. This study seeks to treat glaucoma, which is an anterior-segment disease, by targeting drug delivery to the supraciliary space [37].

In our initial study, a bolus microneedle injection of glaucoma drugs (including brimonidine) into the supraciliary space was able to reduce IOP with significant dose sparing compared with topical eye drops [37]. Furthermore, fewer ocular side effects are expected since the drug is compartmentalized in the suprachoroidal space away from other non-target tissues (e.g., lens, cornea).

In this study, we hypothesize that IOP can be reduced for one month after a single microneedle injection of brimonidine formulated for sustained release using PLA into the supraciliary space of the eye. Brimonidine was chosen because it is an FDA-approved IOP-lowering agent currently prescribed to glaucoma patients [12,13,40] and is pharmacologically active in the rabbit [19–22,41]. Due to increased bioavailability, a microneedle injection into the supraciliary space should reduce the dose needed, compared with topical eye drops, thereby allowing a relatively small injection to contain sufficient drug for extended therapy. The successful implementation of this technique could enable a sustained-release treatment for glaucoma patients without the need to administer topical eye drops.

2. Materials and methods

2.1. Materials

Brimonidine tartrate, poly-lactic acid (PLA) with an inherent viscosity (i.v.) of 0.20 dL/g (free acid terminated, RESOMER® 202H), and polyvinyl alcohol (PVA, 80% hydrolyzed, MW ~9000–10,000) were purchased from Sigma-Aldrich (St. Louis, MO). PLGA (75:25, i.v. = 1.13 dL/g, ester terminated) was purchased from Durect (Cupertino, CA). All solvents used were HPLC grade and were purchased from Fisher Scientific (Waltham, MA), and unless otherwise noted, all other chemicals were purchased from Sigma-Aldrich.

2.2. Removal of low molecular weight acids from PLA

PLA (~5 g) was dissolved in 10 mL of CH₂Cl₂ at room temperature and then added to a stirring ddH₂O bath maintained at 60 °C. After evaporating CH₂Cl₂ for 3 h the aqueous phase containing water-soluble, low molecular weight acids was removed while the water-insoluble, higher MW polymer remained as a solid in the vessel as a result of the organic solvent evaporation. The resulting higher MW polymer was dried under a vacuum and stored at -20 °C until use [42].

2.3. Microsphere preparation

Microspheres were prepared using oil-in-water (o/w) emulsion solvent-evaporation methods. First, brimonidine and the selected polymer(s) (Table 1) were dissolved in 1 mL CH₂Cl₂. Two mL 5.0% (w/v) PVA was added and vortexed to create the o/w emulsion, which was then poured into a stirring bath of 0.5% (w/v) PVA to allow for CH₂Cl₂ evaporation and microsphere hardening. After 3 h, the hardened microspheres were screened to 20–45 µm using sieves, washed with ddH₂O, then lyophilized and stored at -20 °C for future use.

2.4. Scanning electron microscopy

Prior to imaging, lyophilized microspheres were mounted using double-sided carbon tape and coated with a thin layer of gold under a vacuum. Scanning electron microscopy (SEM) images were then taken using a Hitachi S3200N scanning electron microscope (Hitachi, Japan). Images were obtained using EDAX software.

2.5. Determination of brimonidine loading and encapsulation efficiency

Prepared microspheres (~5 mg) were dissolved in 1 mL acetonitrile. The resulting solution was filtered and analyzed for brimonidine content by ultra-performance liquid chromatography (UPLC), as described below. Percentage loading and encapsulation efficiency were calculated using Eqs. (1) and (2), respectively.

$$\%w/w \text{ loading}(L_A) = (\text{mass of brimonidine} / \text{total mass of microspheres}) \times 100 \quad (1)$$

$$\% \text{ encapsulation efficiency}(EE) = (\text{actual loading} / \text{theoretical loading}) \times 100. \quad (2)$$

2.6. In vitro release kinetics of brimonidine

Microspheres (~5 mg) were suspended in 1 mL phosphate-buffered saline + 0.02% Tween 80 (PBST, pH 7.4) at 37 °C under mild agitation. As brimonidine tartrate is highly water soluble [43], 1 mL of release media was sufficient to ensure sink conditions for the duration of release. Microspheres were separated from media at each time point by centrifugation at 8000 rpm for 5 min. Then, release media was completely removed and replaced at 1, 3, 5 and 7 days and weekly thereafter for 7 weeks. Release media was assayed for brimonidine content by UPLC, as described below.

2.7. Brimonidine quantification

Brimonidine content in loading solutions and release media was determined using a UPLC system (Waters, Milford, MA). The mobile phase was composed of 40:60 (acetonitrile:ddH₂O) and the flow rate was set to 0.5 mL/min. Samples and standards prepared in either acetonitrile or PBST were injected (8 μ L) onto a C18 (Acquity BEH C18, 1.7 μ m, 2.1 $\times$ 100 mm) column maintained at 30 $^{\circ}$ C. Brimonidine was detected at 254 nm.

2.8. Microneedle fabrication

A 27-gauge (OD = 0.41 mm; ID = 0.21 mm) hypodermic needle (Becton Dickinson, Franklin Lakes, NJ) was used as the starting material. The needle was first pre-shortened to $\sim$ 1.5 mm in length by cutting the needle using a cordless rotary tool (Dremel 800, Robert Bosch Tool Corporation, Mount Prospect, IL). The needle was further shortened and the bevel was produced by grinding the shortened needle at a 60 $^{\circ}$ angle against the sanding band attachment of the rotary tool. A constant stream of water was flowed through the needle bore to prevent heat buildup and the metal from melting. This was done under a stereoscope to ensure that the needle length was 750 ± 50 μ m. If the needle was too long, the process was repeated; if the needle was too short, the plastic hub was carefully removed with a razor. Each needle was inspected for needle length, absence of metal shavings, and sharpness. If needed, the needle was filed with sandpaper and/or electropolished (ESMA E399, South Holland, IL), as previously described [28].

2.9. In vivo experimental treatment groups

All in vivo experiments were carried out in albino New Zealand white rabbits (Charles River, Wilmington, MA) and were approved by the Georgia Institute of Technology Institutional Animal Care and Use Committee. Practices complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Although bioavailability and distribution of brimonidine is affected by the presence of pigment in the eye, albino rabbits were used to facilitate visualization of microspheres and since an albino rabbit animal model was used in a previous study [41,44]. The treatment groups are listed in the Table 2.

Before the injection procedure, animals were anesthetized with a subcutaneous injection of ketamine and xylazine. An eye drop of proparacaine (Akorn, Lake Forest, IL) was given 5 min prior to the injection. The eye was proptosed to facilitate injection. A hollow microneedle with a length of 750 μ m (see Fig. 2) and a 250 μ L glass chromatography syringe (National Scientific, Rockwood, TN) were used to make injections 3 mm posterior to the limbus.

To ensure delivery near the ciliary body, the total injection volume was divided into multiple injections of 10–20 μ L each. Injections were spaced approximately equally around the limbus while avoiding the extraocular muscles. To inject a total volume of 50 μ L, three injections of 16.7 μ L each were made supranasal, supratemporal, and infranasal. To inject 100 μ L, five injections of 20 μ L each were made; the same 3 injection sites, as in the 50 μ L dose, were used with an additional 2 injections infratemporal and temporal. The fluid carrier was 5% (w/v) carboxymethyl cellulose (CMC; average M_w 700 kDa; Sigma-Aldrich) in

Hank's buffered salt solution (HBSS; Mediatech, Manassas, VA). For all groups, only one eye per rabbit received treatment; the contralateral eye did not receive any treatment during the study.

For the supraciliary delivery of HBSS group (SC-HBSS), a total of 100 μ L of HBSS was injected into the supraciliary space. For the supraciliary delivery of CMC group (SC-CMC), 50 μ L of 5% (w/v) CMC in HBSS was injected. For the supraciliary delivery of blank microspheres group (SC-blank), 50 μ L of 30% blank microspheres (w/v) and 5% (w/v) CMC in HBSS was injected. For the low-dose delivery of brimonidine-loaded microspheres group (SC-low dose), 50 μ L of 30% brimonidine-loaded microspheres (w/v) and 5% (w/v) CMC in HBSS was injected into the supraciliary space. For the high-dose delivery of brimonidine-loaded microspheres group (SC-high dose), 100 μ L of 30% brimonidine-loaded microspheres (w/v) and 5% (w/v) CMC in HBSS was injected into the supraciliary space. The total injected volume was increased from the SC-low dose to SC high-dose because particle concentrations higher than 30% (w/v) particle in 5% CMC clogged the 27 gauge microneedle.

After the injection, animals were monitored until mobile. If there was significant redness, inflammation, or irritation, a steroid/antibiotic ointment was applied to the eye. If the animal looked agitated or in pain after the procedure, 200 μ L of buprenorphine was given subcutaneously. Animals were euthanized with an injection of pentobarbital through the marginal ear vein at the end of the experiment. The eyes were enucleated and some were processed histologically.

For the topical delivery group (TOP), brimonidine eye drops (0.15% Alphagan, Allergan, Irvine, CA) were administered 3 times per day at 8:30 am, 2:30 pm, and 8:30 pm unilaterally to the upper conjunctival sac without anesthesia.

2.10. Intraocular pressure monitoring

For the animals that received a microneedle injection, IOP was measured in both eyes with a TonoVet (iCare, Vantaa, Finland) rebound tonometer between 10 am and 2 pm. To measure IOP, the animal was removed from the cage and restrained in a towel for at least 5 min. IOP was measured at least 3 times in both eyes. If there was a significant variation between recordings, additional time was given to acclimate the animal to its surroundings before attempting to measure IOP again. The baseline IOP was established by measuring IOP daily for a week prior to the injection. IOP was not taken on the day of injection because the ketamine cocktail used had a significant impact on IOP [37]. IOP was monitored on 1, 3, 5, 7, 10, 14, 17, 20, 24, 27, 30, 33, and 43 days post-injection. Animals that received topical eye drops had their IOP measured at 4 pm daily for a week using the same methods.

2.11. Histology

After the animals were euthanized, some eyes were enucleated and immediately fixed in 10% formalin. The eyes were serially sectioned, and sections were stained with hematoxylin–eosin (H&E) or periodic acid Schiff (PAS). Light microscopic examination was performed on all histologic sections for any signs of anatomical changes and foreign

body reaction. Histological sections were photographed at 20× magnification (DP 71; Olympus, Tokyo, Japan).

2.12. Statistical analysis

All values are mean $\pm$ standard error of the mean (SE), unless otherwise specified. Time traces of IOP were calculated in two ways: by finding the difference from the average IOP pre-treatment and by finding the difference between the treated and contralateral eyes. Repeated-measures ANOVA was used to compare the time course data. The dose–response curve was generated by calculating the area under the curve (AUC) using the trapezoid rule. Fisher's Least Square Difference test ($\alpha = 0.05$) was performed to determine statistical significance for individual time points.

3. Results

3.1. Characterization of brimonidine-loaded microspheres

Our first goal was to encapsulate brimonidine into microspheres for sustained release for up to one month (Fig. 3). We initially encapsulated brimonidine in free-acid terminated PLA (800PLA), which resulted in low efficiency and high initial burst (24-hour release; Fig. 1). This is probably because brimonidine is highly water-soluble, which caused leaching of brimonidine into the aqueous phase during microsphere formulation, leading to poor loading [45]. The highly water-soluble nature of brimonidine also probably contributed to high initial burst due to rapid dissolution of poorly encapsulated, surface-associated drug. Initial burst is generally an undesirable property of controlled-release formulations, as it may cause exposure to toxic drug levels.

To improve loading efficiency and decrease initial burst from microspheres, we employed three alternative formulation strategies for encapsulation of brimonidine: (i) increasing PLA concentration to 1000 mg/mL (abbreviated 1000PLA), (ii) blending PLA with PLGA (PLA/PLGA), and (iii) treating the PLA to remove low molecular weight acids (800PLA-T). The first two strategies increased brimonidine loading and decreased initial burst, believed to be due to a denser polymer matrix, which is more efficient in trapping the molecule during microsphere formulation [46]. Although these formulations improved loading and burst, the long-term release from these microspheres in vitro was incomplete through five weeks and was thus deemed unfavorable (Fig. 1).

To further optimize the brimonidine release kinetics, we removed acidic monomers and oligomers to improve polymer stability during storage and effectively increase molecular weight of the bulk PLA. It has been shown that biodegradable polymers may contain 10–20 μ mol of acid per 100 mg of polymer following synthesis. Furthermore, the lactic acid monomers and oligomers formed during polymerization may catalyze the degradation of the polymer during storage, leading to a decline in PLA molecular weight and a buildup of additional acidic byproduct [42]. Higher molecular weight polymers create a denser matrix during microsphere manufacturing, leading to more efficient encapsulation [42].

This final formulation was prepared with the same free-acid terminated PLA 202H as used in our initial formulation. However, prior to microsphere manufacturing, we stirred the

dissolved polymer in a hot water bath to remove low molecular weight, water-soluble acids; a strategy that has been used successfully in the past to encapsulate the peptide leuprolide with high efficiency and resulting in low initial burst [42]. Using these methods, we were able to greatly improve brimonidine loading in PLA to achieve $85 \pm 0.4\%$ efficiency (Table 3) These microspheres also exhibited reduced initial burst in vitro ($8.0 \pm 1.3\%$) as compared to the initial 800PLA microspheres ($16.5 \pm 0.2\%$). Although this formulation did not achieve complete drug release at the end of the experiment, it was more than 75%.

3.2. Targeted injection localized to the supraciliary space

Our next objective was to target injection of the brimonidine-loaded microspheres to the supraciliary space adjacent to the ciliary body. Microneedles ($750 \pm 50 \mu\text{m}$ in length) were fabricated for this study (Fig. 2). We and others have seen that injection of larger volumes leads to spread over a larger area of the suprachoroidal space [41, 47–49]. To localize our formulation at the site of injection at the supraciliary space, we wanted to minimize the injection volume and therefore needed to give multiple injections in order to deliver the target dose. Histological images were acquired for all supraciliary microsphere injection groups (SC-blank, SC-low dose, and SC-high dose) to assess the degree of localization of the particles at the injection site. These images showed that the injected particles were localized at the anterior suprachoroidal space, which is bordered by the ciliary body (Fig. 3), which we call the supraciliary space.

To further image supraciliary injection, we added 1% (w/v) red-fluorescent microspheres ($1 \mu\text{m}$ diameter) to blank microspheres injected into the supraciliary space at multiple locations around the limbus. Sixty-nine days after injection, fluorescence imaging of eyes cut radially from the posterior pole to the limbus and then splayed open similarly show localization of the injected microspheres at the sites of injection in the anterior suprachoroidal space near the ciliary body (Fig. 4). This result is consistent with previous reports that a low volume injection of CMC was able to be localized in the anterior suprachoroidal space [37].

3.3. Effect of supraciliary delivery of brimonidine-loaded microspheres on intraocular pressure

Next, our goal was to inject brimonidine-loaded microspheres into the supraciliary space of rabbit eyes to reduce IOP for one month. The rabbits had a baseline IOP of $12.2 \pm 2.1 \text{ mm Hg}$ (mean $\pm$ SD). As a positive control, brimonidine was given topically (TOP group) in the form of the clinical product Alphagan®, which was administered topically three times per day for five days to mimic chronic use. This dosing regimen corresponds to approximately $75 \mu\text{g}$ of brimonidine administered per eye drop and $1125 \mu\text{g}$ of brimonidine administered over the course of five days.

In the TOP group, we saw a consistent IOP drop of 2–4 mm Hg in the treated eye (one-way ANOVA, $p = 0.003$, Fig. 5A) with a magnitude similar to those reported previously for the length of the treatment [50]. Five days after stopping eye-drop administration, the IOP of the treated eye had returned to baseline (Fisher's LSD test, $p = 0.86$). The IOP of the contralateral eye in the TOP group also showed a significant reduction (one-way ANOVA, $p = 0.03$, Fig. 5A) with a magnitude similar to the treated eye (two-way ANOVA, $p = 0.26$,

Fig. 5B). Because the body weight of the rabbits (2–4 kg) was more than an order of magnitude less than a human, it is likely that the brimonidine that cleared from the treated eye (that was administered a human dose of drug) was sufficient to cause an effect on the contralateral effect, either through a systemic cardiovascular effect or a contralateral therapeutic effect [51].

Guided by this positive control, we next injected brimonidine-loaded microspheres into the supraciliary space. To improve localization of delivery to the ciliary body by avoiding flow of the injectate away from the injection site, the microspheres were formulated with CMC to increase viscosity, and the total material injected was divided into multiple small injections (10–20 μ L) at multiple locations around the eye, as was done previously to localize to the supraciliary space [41]. We delivered 15 mg (SC-low dose, containing 0.45 mg of brimonidine, Fig. 5C–D) and 30 mg (SC-high dose, containing 0.9 mg of brimonidine, Fig. 5E–F) of microspheres into the supraciliary space. To control for the effect of the injection of microspheres, we also injected 15 mg of blank microspheres (SC-blank) as a negative control (Fig. 5G–H).

Both groups that received brimonidine-loaded microspheres (i.e., SC-low dose and SC-high dose) experienced a decline in IOP of the treated eye of as much as 6 mm Hg when compared to the contralateral eye (repeated-measures ANOVA, $p = 0.025$ and 0.002 , respectively). It is notable that the contralateral eye showed no significant change in IOP (repeated-measures ANOVA, $p = 0.92$ and 0.73 , respectively), which is consistent with localization of the drug to the site of injection. The SC-low dose group showed a reduction in the IOP of the treated eye for 14 days after the injection (Fisher's LSD test, $p = 0.005$). The SC-high dose group showed a reduction in the IOP of the treated eye for 33 days (Fisher's LSD test, $p = 0.03$). These data are consistent with our central hypothesis that IOP can be reduced for one month after a single injection of brimonidine formulated for sustained release in the supraciliary space.

In the negative control (SC-blank), the “treated” eye had an IOP reduction of 3 mm Hg compared to the contralateral eye 3 days after injection (one-way ANOVA, $p = 0.02$), which returned to baseline within 5 days (Fisher's LSD test, $p = 0.35$). This effect seen in the negative control might be due to an inflammatory response caused by the injection, reduced aqueous humor production due to disruption of the ciliary body, or increased uveoscleral outflow due to stretching of the ciliary muscles [52,53].

3.4. Effect of fluid carrier on intraocular pressure

To further elucidate the cause of IOP reduction in the SC-blank group, we examined the effect of the injection technique and fluid carrier on IOP. We injected HBSS (SC-HBSS, Fig. 5I–J) or 5% (w/v) CMC in HBSS (SC-CMC, Fig. 5K–L) into the supraciliary space to monitor their impact on IOP. There was a change in IOP from baseline for both SC-HBSS and SC-CMC groups that was evident immediately after injection (Fisher's LSD test, $p = 0.01$), but was no longer evident at the next measurement 2 days later (Fisher's LSD test, $p = 0.84$). Possible explanations of this brief IOP reduction include: increased uveoscleral outflow due to ciliary muscle stretching, a short inflammatory reaction causing blood-

aqueous-barrier breakdown and decreased aqueous humor production due to ciliary body distention. Further studies are needed to explore this phenomenon.

There was no difference between treated eyes of SC-CMC (Fig. 5C–D) and SC-blank groups (Fig. 5G–H) (repeated-measures ANOVA, $p = 0.22$). This result shows that the blank microspheres had little intrinsic effect on IOP. Therefore, the IOP reduction in the treated eye seen in the SC-low dose and SC-high dose groups is believed to be due primarily to the effect of brimonidine release from the microspheres.

3.5. Integrated pharmacodynamic response of treatment groups

As a characterization of the integrated pharmacodynamic responses, we determined the area under the curve (AUC) for all treatment groups by integrating the difference in IOP between the treated and contralateral eyes over time (Fig. 6). The expected response of the brimonidine eye drop (TOP) regimen extrapolated over 30 days was 70 mm Hg-days (Fig. 6, dotted line). Unlike the other values presented in Fig. 6, the response was calculated by finding the IOP reduction of the treated eye minus the IOP of the baseline (i.e., before eye drop application) over the course of one day of eye drop application, and extrapolating the effect by 30 days. There was no statistical difference between the SC-blank group and the SC-HBSS group (Fisher's LSD test, $p = 0.99$) or the SC-CMC group (Fisher's LSD test, $p = 0.93$). The SC-low dose group also did not reach statistical significance compared with the SC-blank group (Fisher's LSD test, $p = 0.38$) because there was considerable variation in the response among the five animals in the SC-low group. There was a significant response with the SC-high dose of brimonidine-loaded microspheres compared with the SC-blank group (Fisher's LSD test, $p = 0.017$).

The high-dose brimonidine-loaded microspheres were necessary to ensure a consistent sustained effect for one month. All animals in the SC-high dose group had a sustained reduction in IOP for at least three weeks. On the other hand, there was appreciable variation among the responses of the animals in the SC-low dose group. Out of the six rabbits in the SC-low dose group, one rabbit experienced no IOP drop in the treated eye and another had the strongest response out of all the rabbits tested. This variation in IOP response is possibly due to inter-animal differences in sensitivity to brimonidine or the microspheres releasing at the lower limit needed for a pharmacologic response. Since an increased dose resulted in a prolongation of the effective duration, the release rate from the microspheres might have decreased after 14 days, suggesting non-linear drug release rate.

3.6. Initial safety assessment

During the experiment, rabbits were inspected on a daily basis by veterinary staff and/or study investigators. The microneedle injection site was barely visible immediately post-injection (Fig. 7A) and was not visible 1 day post-injection (Fig. 7B). Some rabbits had mild redness 1 day after injection, and were subsequently treated with an antibiotic/steroid ointment. Three days after injection, all eyes were quiet and there were no signs of redness or irritation, or apparent distress in the rabbits. No serious adverse events were noted, and the rabbits at least maintained their weight from pre-injection. Assessment of visual acuity

and visual fields were not performed on the rabbits though we would be unlikely to detect any deficit since the injection was localized to the far periphery of the suprachoroidal space.

To further assess the safety of brimonidine-loaded microspheres in the supraciliary space, we performed histology on the enucleated eyes. The slides were read by a board-certified ocular pathologist. All eyes that received a supraciliary injection demonstrated separation of the suprachoroidal space at the anterior region (Fig. 8). However, further studies are required to determine if this might be an artifact from the histology process.

Due to the histologic/staining process, the PLA microspheres were dissolved leaving behind voids where the microspheres were located. There was a significant foreign body response among the microspheres in the supraciliary and subconjunctival spaces (Fig. 8). We hypothesize that this response may be due to the acidic byproducts of the PLA microsphere degradation or impurities in the microsphere from the fabrication process, because previous injections of polystyrene microspheres did not cause inflammation [28,32]. The foreign body response was present in both blank (Fig. 8C) and brimonidine-loaded microspheres (Fig. 8B), which further suggested that the accumulation of macrophages was due to the presence of microspheres (i.e., and not due to the drug itself). The presence of the PLA microspheres and associated foreign-body response also appeared at the injection site (Fig. 8D, arrow) [54]. Nevertheless, it is important to note that the eye did not look inflamed and the animals did not show signs of pain, irritation or distress. Further experiments are needed to investigate this.

We were not able to observe the time frame of complete microsphere degradation. The PLA microspheres were still present 46 and 69 days after the injection (Fig. 10B–D). It is important to note that all brimonidine was released by these times (as seen with the in vitro drug release). As the degradation rates of this polymer are well known [55,56] and certainly much slower than the release rate of the drug, especially in vivo, and intact microspheres remained at the end of the release experiment, we did not explore the erosion and degradation behavior of the microspheres further. Now that we have initial proof-of-principle data on the success of in vivo delivery, further formulation work on PLGA microspheres for brimonidine and related drug molecules is warranted.

The inability of the injection site to heal was concerning. A delayed healing response may be due to the release of byproducts from the particles that delayed healing, or the mass effect of the particles. This hole through the sclera may be a risk for infection, although it is expected the conjunctiva healed over the wound limiting access to the wound.

4. Discussion

Current glaucoma medical therapies are dominated by topical administration. Due to the low bioavailability of eye drops, typically less than 5% of the drug penetrates into the eye and reaches the target site (i.e., ciliary body) [11]. The remaining 95% of the drug enters systemic circulation and can cause side effects throughout the body [11]. For example, brimonidine use causes dry mouth in 33% of patients [57]. Because of the low bioavailability of drug at the ciliary body, patients are often required to administer multiple eye drops daily. Patient adherence suffers due to the complexity and rigor of the treatment

required to control glaucoma [14–18,58,59]. It is estimated that adherence with use of topical eye drops is as low as 41% [11,59].

Glaucoma therapy delivered in a highly-targeted manner to the ciliary body could reduce side effects by lowering the required dose. Supraciliary delivery allows the highly targeted delivery of glaucoma drugs to the ciliary body with significant dose sparing [37]. This dose sparing translates into a lower daily dose as well as fewer side effects. In this study, we delivered 0.9 mg of brimonidine loaded into slow-releasing polymeric microspheres as a single injection and demonstrated an IOP reduction for more than one month in the treated eye. The amount of brimonidine delivered via commercial eye drops three times per day for an equivalent time would result in a 7.5-times greater dose. Consistent with the lower administered dose, IOP reductions in the contralateral eye were not seen with the brimonidine-loaded microspheres groups compared with the topical eye drop administration. This increase in bioavailability during controlled release of brimonidine is lower than previously reported for bolus delivery of brimonidine in the suprachoroidal space [41]. We hypothesize that this may be due to the strong binding affinity of brimonidine to α_2 adrenergic receptors [40,50] and the fast clearance from the SCS [29,31]. With a bolus injection, brimonidine is expected to bind all available receptors and causes a biological response for up to 12 h [41]. However, with a controlled release system, brimonidine is continually released in small quantities, and this must overcome the clearance of brimonidine from the SCS. Because of its strong binding affinity, not all the brimonidine release will be able to bind receptors since the receptors are already occupied by previously released brimonidine. This brimonidine is essentially ‘wasted’, which may at least partially explain the lower than expected dose sparing. To our knowledge, this is the first time that controlled-release drug delivery systems for the management of glaucoma have been injected into the supraciliary space.

In this study, we have shown reduced IOP for one month, however further optimization of microsphere formulation and injection procedure could increase the duration of effect and further reduce the required dosage. Since glaucoma patients typically make regular visits to the eye clinic every 3–6 months, we envision an outpatient procedure performed by an ophthalmologist consisting of a single supraciliary microneedle injection of glaucoma drugs encapsulated within microspheres to adequately control IOP for the next 3–6 months. This would obviate the need for the patient to administer eye drops multiple times per day, and thus drastically raise patient adherence. Future studies will address the development of microparticles that release glaucoma drugs for 3–6 months and their use in the eye.

Histology sections showed foreign body response due to the presence of microspheres in the supraciliary space. This may be due to impurities in the microsphere fabrication process, or particle degradation byproducts, though further studies are needed to determine the cause. Nevertheless, the animals did not show signs of distress or redness in the eye. Furthermore, the microspheres persisted in the supraciliary space despite complete drug release. Further studies are required to improve the biocompatibility of materials injected into the supraciliary space.

The success of our approach is based on the combination of two advances: the development of sustained-release brimonidine microspheres and their placement adjacent to the drug's site of action in the ciliary body using microneedles. Roughly zero-order release of brimonidine with minimal burst effect was achieved by formulating the drug into PLA microspheres using PLA which was first treated to remove acidic monomers and oligomers to improve polymer stability during storage and effectively increasing molecular weight of the bulk PLA used for formulation. The resulting PLA microspheres had higher molecular weight and a denser matrix, which enabled efficient encapsulation, slow and continuous release with minimal burst in the first day. By increasing the PLA molecular weight or using an ester end-capped PLA in future studies, the release rate could be extended further, perhaps to achieve the target of 3–6 months of sustained release. Using different biodegradable polymers, such as poly(trimethylene carbonate), could reduce the inflammatory response to the microspheres because this polymer does not produce acidic byproducts during its degradation by surface erosion [60,61]. Other polymers might also biodegrade in a time frame matched to the drug release.

Placement of the sustained-release microspheres in the supraciliary space served a number of functions. First, forming a drug depot adjacent to the ciliary body enabled dose sparing, which is not only of interest to reduce side effects, but also enables a smaller injection to contain enough drug for a longer duration of effect. Second, the supraciliary space is not in the visual axis and therefore should not affect vision the way eye drops or intravitreal injections can [62,63].

Third, injection into the supraciliary space is expected to be safe and straightforward to perform on an outpatient basis. This is because the microneedle used for injection inherently targets the supraciliary space due to its length and the injection procedure is almost identical to how current intravitreal injections are routinely performed, except for using a shorter needle. Studies in animals and humans have shown that microneedle injections into the suprachoroidal space have been well tolerated [26,27,35,38,48].

Finally, the supraciliary space is believed to be a safe place to deposit drug formulations. The sclera and choroid are not tightly attached and thereby form a potential space that can be temporarily expanded without apparent long-term adverse effects [31,38,48,63–68]. Sustained-release formulations placed into the suprachoroidal space of animals and humans have been well tolerated [26,27,36,38].

Acknowledgments

Funding

This work was supported by the National Eye Institute grants EY007092 (BC), EY025154 (BC), EY017045 (BC, YCK, MRP), EY022097 (BC, YCK, MRP), and EY06360 (HEG), and an unrestricted departmental grant from the Research to Prevent Blindness (HEG).

We thank Donna Bondy for administrative support.

References

1. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006; 90:262–267. [PubMed: 16488940]
2. Quigley HA, Cassard SD, Gower EW, Ramulu PY, Jampel HD, Friedman DS. The cost of glaucoma care provided to Medicare beneficiaries from 2002 to 2009. *Ophthalmology*. 2013; 120:2249–2257. [PubMed: 23769330]
3. Tasman, W.; Jaeger, E. *Duane's Ophthalmology*. 15. Lippincott Williams & Wilkins; Philadelphia, Pa: 2009.
4. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet*. 2004; 363:1711–1720. [PubMed: 15158634]
5. Gooch N, Molokhia SA, Condie R, Burr RM, Archer B, Ambati BK, Wirosko B. Ocular drug delivery for glaucoma management. *Pharmaceutics*. 2012; 4:197–211. [PubMed: 24300188]
6. Gordon MO, Beiser JA, Brandt JD, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller JP, Parrish RK 2nd, Wilson MR, Kass MA. The ocular hypertension treatment study: baseline factors that predict the onset of primary open-angle glaucoma. *Arch Ophthalmol*. 2002; 120:714–720. discussion 829-730. [PubMed: 12049575]
7. Sommer A. Relationship between intraocular pressure and primary open angle glaucoma among white and black Americans. *Arch Ophthalmol*. 1991; 109:1090. [PubMed: 1867550]
8. Lavik E, Kuehn MH, Kwon YH. Novel drug delivery systems for glaucoma. *Eye (Lond)*. 2011; 25:578–586. [PubMed: 21475311]
9. Ethier CR, Johnson M, Ruberti J. Ocular biomechanics and biotransport. *Annu Rev Biomed Eng*. 2004; 6:249–273. [PubMed: 15255770]
10. Alm A, Nilsson SF. Uveoscleral outflow—a review. *Exp Eye Res*. 2009; 88:760–768. [PubMed: 19150349]
11. Ghate D, Edelhauser HF. Barriers to glaucoma drug delivery. *J Glaucoma*. 2008; 17:147–156. [PubMed: 18344762]
12. Toris CB, Gleason ML, Camras CB, Yablonski ME. Effects of brimonidine on aqueous humor dynamics in human eyes. *Arch Ophthalmol*. 1995; 113:1514–1517. [PubMed: 7487618]
13. Konstas AG, Stewart WC, Topouzis F, Tersis I, Holmes KT, Stangos NT. Brimonidine 0.2% given two or three times daily versus timolol maleate 0.5% in primary open-angle glaucoma. *Am J Ophthalmol*. 2001; 131:729–733. [PubMed: 11384568]
14. Robin AL, Novack GD, Covert DW, Crockett RS, Marcic TS. Adherence in glaucoma: objective measurements of once-daily and adjunctive medication use. *Am J Ophthalmol*. 2007; 144:533–540. [PubMed: 17686450]
15. Tsai JC, McClure CA, Ramos SE, Schlundt DG, Pichert JW. Compliance barriers in glaucoma: a systematic classification. *J Glaucoma*. 2003; 12:393–398. [PubMed: 14520147]
16. Rotchford AP, Murphy KM. Compliance with timolol treatment in glaucoma. *Eye (Lond)*. 1998; 12(Pt 2):234–236. [PubMed: 9683946]
17. Patel SC, Spaeth GL. Compliance in patients prescribed eyedrops for glaucoma. *Ophthalmic Surg*. 1995; 26:233–236. [PubMed: 7651690]
18. Gurwitz JH, Glynn RJ, Monane M, Everitt DE, Gilden D, Smith N, Avorn J. Treatment for glaucoma: adherence by the elderly. *Am J Public Health*. 1993; 83:711–716. [PubMed: 8484454]
19. Ibrahim MM, Abd-Elgawad AH, Soliman OA, Jablonski MM. Natural bioadhesive biodegradable nanoparticle-based topical ophthalmic formulations for management of glaucoma. *Transl Vis Sci Technol*. 2015; 4:12. [PubMed: 26175958]
20. Fedorchak MV, Conner IP, Medina CA, Wingard JB, Schuman JS, Little SR. 28-Day intraocular pressure reduction with a single dose of brimonidine tartrate-loaded microspheres. *Exp Eye Res*. 2014; 125:210–216. [PubMed: 24983144]
21. Deokule SP, Baffi JZ, Guo H, Nazzaro M, Kaneko H. Evaluation of extended release brimonidine intravitreal device in normotensive rabbit eyes. *Acta Ophthalmol (Copenh)*. 2012; 90:e344–e348.

22. De TK, Rodman DJ, Holm BA, Prasad PN, Bergey EJ. Brimonidine formulation in polyacrylic acid nanoparticles for ophthalmic delivery. *J Microencapsul.* 2003; 20:361–374. [PubMed: 12881116]
23. Kim YC, Edelhauser HF, Prausnitz MR. Particle-stabilized emulsion droplets for gravity-mediated targeting in the posterior segment of the eye. *Adv Healthc Mater.* 2014; 3:1272–1282. [PubMed: 24652782]
24. Abarca EM, Salmon JH, Gilger BC. Effect of choroidal perfusion on ocular tissue distribution after intravitreal or suprachoroidal injection in an arterially perfused ex vivo pig eye model. *J Ocul Pharmacol Ther.* 2013; 29:715–722. [PubMed: 23822159]
25. Kadam RS, Williams J, Tyagi P, Edelhauser HF, Kompella UB. Suprachoroidal delivery in a rabbit ex vivo eye model: influence of drug properties, regional differences in delivery, and comparison with intravitreal and intracameral routes. *Mol Vis.* 2013; 19:1198–1210. [PubMed: 23734089]
26. Gilger BC, Abarca EM, Salmon JH, Patel S. Treatment of acute posterior uveitis in a porcine model by injection of triamcinolone acetonide into the suprachoroidal space using microneedles. *Invest Ophthalmol Vis Sci.* 2013; 54:2483–2492. [PubMed: 23532526]
27. Tyagi P, Barros M, Stansbury JW, Kompella UB. Light-activated, in situ forming gel for sustained suprachoroidal delivery of bevacizumab. *Mol Pharm.* 2013; 10:2858–2867. [PubMed: 23734705]
28. Patel SR, Berezovsky DE, McCarey BE, Zarnitsyn V, Edelhauser HF, Prausnitz MR. Targeted administration into the suprachoroidal space using a microneedle for drug delivery to the posterior segment of the eye. *Invest Ophthalmol Vis Sci.* 2012; 53:4433–4441. [PubMed: 22669719]
29. Wang M, Liu W, Lu Q, Zeng H, Liu S, Yue Y, Cheng H, Liu Y, Xue M. Pharmacokinetic comparison of ketorolac after intracameral, intravitreal, and suprachoroidal administration in rabbits. *Retina.* 2012; 32:2158–2164. [PubMed: 23099451]
30. Seiler GS, Salmon JH, Mantuo R, Feingold S, Dayton PA, Gilger BC. Effect and distribution of contrast medium after injection into the anterior suprachoroidal space in ex vivo eyes. *Invest Ophthalmol Vis Sci.* 2011; 52:5730–5736. [PubMed: 21685338]
31. Olsen TW, Feng X, Wabner K, Csaky K, Pambuccian S, Cameron JD. Pharmacokinetics of pars plana intravitreal injections versus microcannula suprachoroidal injections of bevacizumab in a porcine model. *Invest Ophthalmol Vis Sci.* 2011; 52:4749–4756. [PubMed: 21447680]
32. Patel SR, Lin AS, Edelhauser HF, Prausnitz MR. Suprachoroidal drug delivery to the back of the eye using hollow microneedles. *Pharm Res.* 2011; 28:166–176. [PubMed: 20857178]
33. Peden MC, Min J, Meyers C, Lukowski Z, Li Q, Boye SL, Levine M, Hauswirth WW, Ratnakaram R, Dawson W, Smith WC, Sherwood MB. Ab-externo AAV-mediated gene delivery to the suprachoroidal space using a 250 micron flexible microcatheter. *PLoS One.* 2011; 6:e17140. [PubMed: 21347253]
34. Kim SH, Galban CJ, Lutz RJ, Dedrick RL, Csaky KG, Lizak MJ, Wang NS, Tansey G, Robinson MR. Assessment of subconjunctival and intrascleral drug delivery to the posterior segment using dynamic contrast-enhanced magnetic resonance imaging. *Invest Ophthalmol Vis Sci.* 2007; 48:808–814. [PubMed: 17251481]
35. Olsen TW, Feng X, Wabner K, Conston SR, Sierra DH, Folden DV, Smith ME, Cameron JD. Cannulation of the suprachoroidal space: a novel drug delivery methodology to the posterior segment. *Am J Ophthalmol.* 2006; 142:777–787. [PubMed: 16989764]
36. Einmahl S, Savoldelli M, D'Hermies F, Tabatabay C, Gurny R, Behar-Cohen F. Evaluation of a novel biomaterial in the suprachoroidal space of the rabbit eye. *Invest Ophthalmol Vis Sci.* 2002; 43:1533–1539. [PubMed: 11980871]
37. Kim YC, Edelhauser HF, Prausnitz MR. Targeted delivery of anti-glaucoma to the supraciliary space using microneedles. *Invest Ophthalmol Vis Sci.* 2014
38. Goldstein DA, Srivastava SK, Nguyen Q, Noronha G, Widmann M. A phase 1/2 open-label, safety and tolerability study of triamcinolone acetonide administered to the suprachoroidal space in patients with non-infectious uveitis. *Invest Ophthalmol Vis Sci.* 2015; 56:3557. ARVO E-Abstract.
39. Rowe-Rendleman CL, Durazo SA, Kompella UB, Rittenhouse KD, Di Polo A, Weiner AL, Grossniklaus HE, Naash MI, Lewin AS, Horsager A, Edelhauser HF. Drug and gene delivery to the back of the eye: from bench to bedside. *Invest Ophthalmol Vis Sci.* 2014; 55:2714–2730. [PubMed: 24777644]

40. Walters TR. Development and use of brimonidine in treating acute and chronic elevations of intraocular pressure: a review of safety, efficacy, dose response, and dosing studies. *Surv Ophthalmol*. 1996; 41:S19–S26. [PubMed: 8970246]
41. Kim YC, Edelhauser HF, Prausnitz MR. Targeted delivery of antiglaucoma drugs to the supraciliary space using microneedles. *Invest Ophthalmol Vis Sci*. 2014; 55:7387–7397. [PubMed: 25212782]
42. Yamamoto, M.; Okada, H.; Ogawa, Y.; Miyagawa, T. Polymer, production and use thereof. European Patent. 86303417. Oct 21. 1987
43. Burke, JA.; Hughes, PM.; Zhang, K-M.; Lin, T.; Huang, G.; Jackson, BA.; Wheeler, LA.; Donn, RS. Intraocular implants and methods for improving vision. US Patent. US8293741. 2012.
44. Matsuo T, Cynader MS. Localization of alpha-2 adrenergic receptors in the human eye. *Ophthalmic Res*. 1992; 24:213–219. [PubMed: 1359487]
45. Chang, J.; Hughes, P. Oil-in-water method for making alpha-2 agonist polymeric drug delivery systems. US Patent. 7589057. Sep 15. 2009
46. Huang X, Brazel CS. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. *J Control Release*. 2001; 73:121–136. [PubMed: 11516493]
47. Kim YC, Oh KH, Edelhauser HF, Prausnitz MR. Formulation to target delivery to the ciliary body and choroid via the suprachoroidal space of the eye using microneedles. *Eur J Pharm Biopharm*. 2015; 95:398–406. [PubMed: 26036448]
48. Chen M, Li X, Liu J, Han Y, Cheng L. Safety and pharmacodynamics of suprachoroidal injection of triamcinolone acetate as a controlled ocular drug release model. *J Control Release*. 2015; 203:109–117. [PubMed: 25700623]
49. Gu B, Liu J, Li X, Ma Q, Shen M, Cheng L. Real-time monitoring of suprachoroidal space (SCS) following SCS injection using ultra-high resolution optical coherence tomography in guinea pig eyes. *Invest Ophthalmol Vis Sci*. 2015; 56:3623–3634. [PubMed: 26047049]
50. Burke J, Kharlamb A, Shan T, Runde E, Padillo E, Manlapaz C, Wheeler L. Adrenergic and imidazoline receptor-mediated responses to UK-14304-18 (brimonidine) in rabbits and monkeys. A species difference. *Ann N Y Acad Sci*. 1995; 763:78–95. [PubMed: 7677389]
51. Khan ZP, Ferguson CN, Jones RM. Alpha-2 and imidazoline receptor agonists: their pharmacology and therapeutic role. *Anaesthesia*. 1999; 54:146–165. [PubMed: 10215710]
52. Brubaker RF. Targeting outflow facility in glaucoma management. *Surv Ophthalmol*. 2003; 48(Suppl 1):S17–S20. [PubMed: 12852430]
53. Kaufman PL, Barany EH. Loss of acute pilocarpine effect on outflow facility following surgical disinsertion and retrodisplacement of the ciliary muscle from the scleral spur in the cynomolgus monkey. *Investig Ophthalmol*. 1976; 15:793–807. [PubMed: 824222]
54. Hikichi T, Yoshida A, Hasegawa T, Ohnishi M, Sato T, Muraoka S. Wound healing of scleral self-sealing incision: a comparison of ultrasound biomicroscopy and histology findings. *Graefes Arch Clin Exp Ophthalmol*. 1998; 236:775–778. [PubMed: 9801893]
55. Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev*. 1997; 28:5–24. [PubMed: 10837562]
56. Lin SY, Chen KS, Teng HH, Li MJ. In vitro degradation and dissolution behaviours of microspheres prepared by three low molecular weight polyesters. *J Microencapsul*. 2000; 17:577–586. [PubMed: 11038117]
57. Camras CB, Toris CB, Tamesis RR. Efficacy and adverse effects of medications used in the treatment of glaucoma. *Drugs Aging*. 1999; 15:377–388. [PubMed: 10600045]
58. Lee DA, Higginbotham EJ. Glaucoma and its treatment: a review. *Am J Health Syst Pharm*. 2005; 62:691–699. [PubMed: 15790795]
59. Reardon G, Kotak S, Schwartz GF. Objective assessment of compliance and persistence among patients treated for glaucoma and ocular hypertension: a systematic review. *Patient Prefer Adherence*. 2011; 5:441–463. [PubMed: 22003282]
60. Jansen J, Koopmans SA, Los LI, van der Worp RJ, Podt JG, Hooymans JMM, Feijen J, Grijpma DW. Intraocular degradation behavior of crosslinked and linear poly(trimethylene carbonate) and poly(D,L-lactic acid). *Biomaterials*. 2011; 32:4994–5002. [PubMed: 21507481]

61. Pêgo AP, Van Luyn MJA, Brouwer LA, van Wachem PB, Poot AA, Grijpma DW, Feijen J. In vivo behavior of poly(1,3-trimethylene carbonate) and copolymers of 1,3-trimethylene carbonate with D,L-lactide or ϵ -caprolactone: degradation and tissue response. *J Biomed Mater Res A*. 2003; 67A:1044–1054. [PubMed: 14613255]
62. Kim YC, Chiang B, Wu X, Prausnitz MR. Ocular delivery of macromolecules. *J Control Release*. 2014; 190:172–181. [PubMed: 24998941]
63. Rai Udo J, Young SA, Thrimawithana TR, Abdelkader H, Alani AW, Pierscionek B, Alany RG. The suprachoroidal pathway: a new drug delivery route to the back of drugs the eye. *Drug Discov Today*. 2015; 20:491–495. [PubMed: 25448755]
64. Mandelcorn ED, Kitchens JW, Fijalkowski N, Moshfeghi DM. Active aspiration of suprachoroidal hemorrhage using a guarded needle. *Ophthalmic Surg Lasers Imaging Retina*. 2014; 45:150–152. [PubMed: 24635157]
65. Patrianakos TD. Anatomic and physiologic rationale to be applied in accessing the suprachoroidal space for management of glaucoma. *J Cataract Refract Surg*. 2014; 40:1285–1290. [PubMed: 24943905]
66. Ianchulev, T. *Suprachoroidal Space as a Therapeutic Target*. Springer; New York: 2014.
67. Hoeh H, Ahmed II, Grisanti S, Grisanti S, Grabner G, Nguyen QH, Rau M, Yoo S, Ianchulev T. Early postoperative safety and surgical outcomes after implantation of a suprachoroidal micro-stent for the treatment of open-angle glaucoma concomitant with cataract surgery. *J Cataract Refract Surg*. 2013; 39:431–437. [PubMed: 23506920]
68. Danylkova N, Gupta N, Jhaveri CD, Gill MK. Suprachoroidal silicone oil migration following retinal detachment repair. *Ophthalmic Surg Lasers Imaging Retina*. 2013; 44:284–286. [PubMed: 23676233]

Fig. 1.

Representative SEM images of four microsphere formulations: (A) 800PLA, (B) 800PLA-T, (C) 1000PLA, and (D) PLA/PLGA. Scale bars represent 10 μm. (E) Brimonidine release from four polymer microsphere formulations in vitro. The media was PBST (pH 7.4, 37 °C). Data are expressed as mean ± SE (n = 3 replicates per group).

Fig. 2. Hollow microneedle measuring 750 μm in length is shown opposite a 50 μL drop from a conventional eye dropper. Scale bars represent 1 mm.

Fig. 3. Representative histological image of a section from the rabbit eye showing brimonidine-loaded microspheres suspended in 5% (w/v) CMC/HBSS 46 days after injection into the supraciliary space. Scale bar indicates 500 μm .

Fig. 4. Representative fluorescence image showing localization of red-fluorescent microspheres to the supraciliary space. Eyes were enucleated and imaged 69 days after injection of 1% (w/v) fluorescent polystyrene microspheres (1 μm diameter; ex: 580 nm, em: 605 nm; FluoSpheres, ThermoFisher Scientific, Waltham, MA) and 5% (w/v) blank microspheres suspended in 5% (w/v) CMC in HBSS. Bright-field image (A) displays the interior surface of rabbit eye. The center of the sample is the anterior segment, and the distal ends of the “petals” form the posterior pole. Each petal contains, from superficial to deep, the sclera (white), choroid, and retina. Fluorescence image (B) of same eye shows microspheres localized to the anterior suprachoroidal space.

Fig. 5.

Effect of brimonidine delivery on IOP in the rabbit eye. Change in IOP from baseline over time for topical brimonidine eye drops three times per day (A), brimonidine microspheres (low dose) (C), brimonidine microspheres (high dose) (E), blank microspheres (G), HBSS only (I), and 5% (w/w) CMC (K). Difference in IOP between treated and contralateral eye over time for topical brimonidine eye drops 3 per day (B), brimonidine microspheres (low dose) (D), brimonidine microspheres (high dose) (F), blank microspheres (H), HBSS only (J), and 5% (w/w) CMC (L). Data are expressed as mean $\pm$ SE ($n = 3-6$).

Fig. 6. Integrated pharmacodynamic response of treatment groups. Values were calculated by determining the area under the curve for differences in IOP between the treated and contralateral eyes (data from Fig. 5) for each animal. Bars represent mean values. Dotted line indicates the expected response achievable with brimonidine eye drops.

Fig. 7.
Representative images of the rabbit eye 5 min (A) and 1 day (B) after microneedle injection.
Microneedle injection site marked with arrow.

Fig. 8.

Representative histological images after microneedle injection of HBSS only (SC-HBSS, A) 88 days post-injection; blank microspheres (SC-blank, B) 46 days post-injection; and brimonidine-loaded microspheres (SC-low dose, C) 69 days post-injection. No inflammatory cells were noted in the SC-HBSS group (A inset), whereas accumulation of macrophages was seen in eyes that received SC-blank (B inset) and SC-low dose (C inset), as indicated by the darkly-stained nuclei (arrows) among the voids of where the microspheres were. The microspheres and associated foreign-body response could also be seen in the site of microneedle puncture across the sclera, indicated by an arrow, and in the subconjunctival space 69 days after injection (D). Scale bars indicate 500 μm .

Table 1

Microsphere formulation parameters.

Formulation name	Polymer	Polymer concentration (mg/mL-CH ₂ Cl ₂)	Theoretical w/w loading (L _T)
800PLA	PLA	800	6.00%
1000PLA	PLA	1000	5.00%
800PLA-T	PLA hot-water treated	800	6.25%
PLA/PLGA	50:50 Blend PLGA:PLA	500	10.00%

Table 2

Summary of in vivo experimental treatment groups.

Treatment group	Fluid carrier	Description
SC-HBSS	50 μ L of HBSS	Supraciliary delivery of saline
SC-CMC	50 μ L of 5% (w/v) CMC in HBSS	Supraciliary delivery of CMC
SC-blank	50 μ L of 5% (w/v) CMC in HBSS	Supraciliary delivery of blank microspheres
SC-low dose	50 μ L of 5% (w/v) CMC in HBSS	Supraciliary delivery of brimonidine-loaded microspheres (20–45 μ m) [low dose]
SC-high dose	100 μ L of 5% (w/v) CMC in HBSS	Supraciliary delivery of brimonidine-loaded microspheres (20–45 μ m) [high dose]
Top	N/A	Topical delivery of brimonidine

Table 3

Characterization of brimonidine encapsulation in microspheres. Data expressed as mean $\pm$ SE (n = 3).

Formulation name	Loading (w/w %)	Encapsulation efficiency (%)	Initial burst (%)
800PLA	2.3 $\pm$ 0.1	38 $\pm$ 1	16.5 $\pm$ 0.2
800PLA-T	5.3 $\pm$ 1.3	85 $\pm$ 0.4	8.0 $\pm$ 1.3
1000PLA	3.3 $\pm$ 0.3	67 $\pm$ 6	1.8 $\pm$ 0.3
PLA/PLGA	5.3 $\pm$ 0.7	53 $\pm$ 8	1.2 $\pm$ 0.1