Translational

Optimization of Subconjunctival Biodegradable Microfilms for Sustained Drug Delivery to the Anterior Segment in a Small Animal Model

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Purpose. We evaluated a biodegradable, sustained-release, prednisolone acetate (PA)-loaded poly[d,l-lactide-co-ε-caprolactone] (PLC) drug delivery system on its biocompatibility, feasibility and release characteristics in vitro and in vivo.

Methods. Blank and 40% PA-loaded PLC microfilms with a diameter of 2 mm were fabricated, and the degradation and drug release profiles of the microfilms were characterized in vitro and in vivo. The microfilms were implanted into the subconjunctival space of Lewis rats ($n = 48$). All eyes were assessed clinically using slit-lamp biomicroscopy, and graded with Hackett-McDonald ocular scoring system and anterior segment optical coherence tomography. Histologic and immunohistochemical analyses were performed comparing blank and PA-loaded microfilm groups. PA concentrations in the aqueous humor were determined by HPLC.

Results. Subconjunctivally-implanted PLC microfilms were able to deliver PA in a sustained manner over 3 months, with a steady rate of 0.002 mg/d in vivo. Eyes with either blank or PA-loaded implanted microfilms showed a very minimal inflammatory response at the insertion sites and mild degree of collagen encapsulation around the microfilms, with significantly less CD11c cells at 2 and 4 weeks ($P = 0.001$ and $P = 0.002$), and collagen formation at 2 weeks ($P = 0.001$) in the PA-loaded microfilm group. Anterior chamber PA levels were achieved, with concentrations at 76.7 ± 5.9, 70.3 ± 2.3, and 42.7 ± 4.1 ng/mL at 2, 4, and 12 weeks, respectively.

Conclusions. PA-loaded PLC microfilms display good biocompatibility, feasibility, and desirable sustained drug release profiles, and have the potential to exhibit antifibrotic and anti-inflammatory effects. This device is applicable to use in small animal models of anterior segment inflammation.

Keywords: poly[d,l-lactide-co-ε-caprolactone] (PLC), drug delivery system, anterior segment

Drug delivery to the anterior segment of the eye always has been a challenging issue. Topical eye drops, the most common route of administration of treatment for ocular anterior segment disease, has a low level bioavailability of 1% to 10%; hence, therapeutic concentrations may not be reached.[1] In addition, topical eye drops often have a short duration of action so that frequent application of drops is required. This may result in patients' discomfort due to ocular surface toxicity and the correct dosing regimen is highly dependent on patients' compliance. Therefore, different drug delivery systems have been investigated to address these limitations related to eye drops. Among them, the use of biodegradable polymeric implants to deliver a sustained drug level in the eye recently has become a topic of interest.[2–6]

A biodegradable implant is a matrix containing a biodegradable polymer, typically constructed from a synthetic aliphatic polyester of the poly-α-hydroxy acid family, such as polylactic acid (PLA), poly[d,l-lactide-co-glycolide] (PLGA) or poly[d,l-lactide-co-ε-caprolactone] (PLC).[4] All are proven vehicles for sustained drug release.[2,3] They have many advantages because they can be fabricated with various sizes, shapes, and thickness to modulate the amount and duration of drug release, tailoring to different ocular diseases and severities, since polyhydroxyesters are fabricated easily with predictable biodegradation kinetics.[7] They can provide a stable drug concentration over weeks to months,[3,8] and this sustained release eliminates the dependency of patients' compliance. Moreover, there is no need to remove the implant after the depletion of the drug, because the implant undergoes hydrolysis to the original monomers. For example, PLGA copolymers metabolize into lactic acid and glycolic acid, and PLC copolymers metabolize into lactic acid and caproic acid. These monomers are nontoxic, and eliminated safely via the Krebs cycle by conversion to carbon dioxide and water without causing any foreign body reactions.[2] Among different biodegradable polymers for ocular drug delivery, PLGA copolymers have been

used more frequently as vehicles.[9,10] PLC copolymers are relatively new, and very few ocular applications are reported in the literature.

Corticosteroids are used in a broad spectrum of ocular conditions and are among the most frequently prescribed drugs in ophthalmology.[11] They have a major role in the treatments of a wide range of ocular anterior segment diseases, for example, viral/allergic conjunctivitis, episcleritis, scleritis, keratitis, and uveitis, and in the managements of postoperative conditions, such as inflammation after cataract, glaucoma filtration, and corneal transplant surgery.[11] To circumvent the limitations of intensive application of topical therapy, alternative routes of drug administration often are used, for example, subconjunctival, sub-Tenon's, intracameral, or retrobulbar injection. However, the duration of action of injection-based administration is limited, and it results in the need for frequent reinjections to maintain therapeutic drug concentration. For example, it has been reported that the half-life of prednisolone after subconjunctival injection is only 38 minutes.[12]

Surodex (Oculex Pharmaceuticals, Inc., Sunnyvale, CA), a dexamethasone anterior segment drug delivery system, has been proven to be effective to treat inflammation after cataract surgery.[13] Even though satisfactory anti-inflammatory results were achieved, adverse side effects, such as implant migration in anterior chamber or peripheral anterior synechiae, have been reported.[14] Implantation of a subconjunctival sustained-release drug delivery system is a simpler and less invasive procedure, and would avoid anterior segment complications. This would be useful especially for patients who require long-term use of corticosteroids, for example, patients with chronic uveitis or corneal transplantation.

In our study, we evaluated the surgical feasibility, biocompatibility and drug release profile of a biodegradable prednisolone acetate (PA)-loaded PLC microfilm implanted subconjunctivally by in vitro and in vivo experiments. Using a small animal model, we aimed to develop a PLC-based drug delivery device providing a prolonged corticosteroid release to the ocular anterior segment.

Methods

Microfilm Fabrication

Copolymer PLC (d,l-lactide–to–ε-caprolactone molar ratio 70:30, with intrinsic viscosity of 1.6 dL/g; Purac Far East, Singapore), HPLC-grade dichloromethane, acetonitrile (Tedia, Fairfield, OH), PBS tablets (Sigma-Aldrich, Singapore), and prednisolone 21-acetate (≥97%; Sigma-Aldrich) were used in the study.

PA-loaded microfilms were prepared using a solution casting method.[15] PA and PLC with a predetermined drug loading percentage (40 wt %) were dissolved in dichloromethane to form a polymer solution. The drug and polymer mixture was cast on a glass plate using an automatic film applicator that can move the casting knife at a preset speed of 50 mm/s. The microfilm thickness (100 μm) was controlled by adjusting the height of the casting knife. Subsequently, the films were dried under a fume hood for one day, followed by drying in a vacuum oven at 37°C until the solvent level was less than 1% of the total weight, as measured using a thermo-gravimetric analyzer (TGA, Q500; TA Instruments, New Castle, DE). After drying, the microfilms were trephined into circular discs of 2 mm diameter.

In Vitro Studies

In Vitro Microfilm Degradation Study. Microfilms were immersed in closed vials containing 5 mL PBS. At 1, 2, 4, 8, and 12 weeks, samples ($n = 3$ for each time point) were taken out, rinsed with deionized water and dried in a 37°C vacuum oven for 7 days. Dried samples were dissolved in chloroform (1–2 mg/mL) and filtered through 0.22 μm regenerated cellulose syringe driven filters before testing. Weight average molar mass (M_w) and number average molar mass (M_n) of the samples were determined by gel permeation chromatography (GPC, Agilent 1100; Agilent Technologies, Santa Clara, CA) at 35°C, using Agilent PL gel 5 μm mixed-C column, under a flow rate of 1 mL chloroform per minute, using a Refractive Index Detector (RID). The microfilm degradation rate constant (λ) was the slope of the fitted line of natural log of M_n versus time, and a higher value of λ means a faster rate of degradation.

In Vitro Drug Release Study. Each PA-loaded microfilm was weighed before testing, and then immersed in an individual amber vial containing 5 mL PBS. Subsequently, at 3 days, and 1, 2, 4, and 12 weeks after insertion, the microfilms ($n = 3$ for each time point) were taken out, rinsed with deionized water, then dried in a vacuum oven at 37°C over a week. After drying, each microfilm was dissolved fully in 10 mL of acetonitrile, and then the amount of residual drug in each microfilm was determined by HPLC. The amount of drug released was quantified by subtracting the residual drug amount from the initial loading amount.

In Vivo Studies

Animals and Surgical Insertion of Microfilms. We used 48 eyes of 24 female Lewis rats (Rtl-l^{vl}) aged 8 to 10 weeks. All animals were treated in accordance with the tenets of the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research, and the protocol was approved by the Institutional Animal Care and Use Committee of SingHealth. After the animals had been anesthetized with intraperitoneal injection of ketamine hydrochlodride (50–75 mg/kg) and xylazil (5–8 mg/kg), the eyes were divided into two groups: blank microfilm (9 eyes) and PA-loaded microfilm (39 eyes) groups. Of the 39 PA-loaded microfilms-implanted eyes, 30 were used for the microfilm degradation and drug release studies in vivo, and the remaining nine eyes were used for histologic and immunohistochemical studies. For the surgical insertion of the microfilm, a subconjunctival pocket via blunt dissection just above the limbus was created with a 2.0 to 2.2-mm incision at the superotemporal aspect of the rat's eye, and the microfilm then was inserted into the subconjunctival pocket. Closure with two 10-0 nylon sutures was done to ensure secure implantation of the microfilm. The surgical procedures were performed by the same surgeon (YCL) who was masked to the types of the microfilms inserted. After microfilm insertions, topical tobramycin ointment was given 4 times daily for 3 days.

In Vivo Microfilm Degradation and Drug Release Studies. For the in vivo microfilm degradation study, the microfilms were retrieved from the subconjunctival space at 1, 2, 4, 8, and 12 weeks after insertion ($n = 3$ for each time point), and were tested by GPC as described in the in vitro studies to obtain M_w and M_n. For the in vivo drug release testing, the microfilms were retrieved at 3 days, and 1, 2, 4, and 12 weeks. The amount of the residual drug was obtained by using HPLC as described in the in vitro studies to derive the daily and cumulative drug release. In addition, 15 μL of aqueous humor from each eye ($n = 6$ at 2, 4, and 12 weeks, respectively) were aspirated using a 30-gauge needle. The aqueous humor was pooled to 30 μL for the analysis. The PA concentrations in the aqueous humor were analyzed by using HPLC.

Clinical Evaluation. Slit-lamp biomicroscopy was used to evaluate the anterior segments of the eyes, and the Hackett-McDonald ocular scoring system was used to evaluate the

conjunctival congestion (0–3), swelling (0–4), and discharge (0–3) around the microfilm insertion sites.[16] Anterior segment optical coherence tomography (ASOCT, RTVue; Optovue, Inc., Fremont, CA) scanning was used to assess the anatomic location of the microfilms. The clinical examinations were performed at 1 day after microfilm insertions and twice weekly thereafter.

Histology Analysis, Picrosirius Red, and Immunohistochemistry Staining. At 2, 4, and 12 weeks after insertions, three rats (6 eyes) for each time point were euthanized with overdose intraperitoneal pentobarbitone (60–150 mg/kg) followed by enucleation of eyes. The eyeballs (3 eyes for blank and PA-loaded microfilm group each) were embedded in OCT cryo-compound (Leica Microsystems, Nussloch, Germany), and then were cut into 7 μm slices using a cryostat (Microm HM 550; Microm, Walldorf, Germany).

The sections were stained with hematoxylin for 3 minutes and eosin for 2.5 minutes, and then were washed in tap water, semidried and mounted with paramount. The sections were viewed using a light microscope (Nikon Eclipse Ti-S; Nikon, Dusseldorf, Germany) in bright field mode. Six sections of each eye ($n = 3$ for blank and PA-loaded microfilm group each) were selected randomly. The thickness of the collagen capsule and microfilm, and the ratio of the length of microfilm surface lined by collagen compared to the entire length of microfilm surface were measured by using microscope scale bar. For the measurement of the thickness of the collagen capsule, six points on the microfilm margin with relatively anatomically minimal interference of subconjunctival Tenon tissue were selected randomly in each section. The sections also were stained with Picrosirius red (Sigma Sirius Red; Sigma, Steinheim, Germany) to determine the amount and extent of collagen encapsulation around the microfilms. When viewed with a polarized light, mature type I collagen fibers appear red or bright yellow. Six sections of each eye ($n = 3$ for blank and PA-loaded microfilm group each) were selected randomly. The area of the microfilm (F) and the area stained red or yellow adjacent to the microfilm (S) were outlined by using ImageJ software (available in the public domain at http://rsb.info.nih.gov/ij/). Semiqualification of the amount of collagen I was done based on the ratio of S/F: grade 1 (25% or less), grade 2 (25%–50%), grade 3 (50%–75%), and grade 4 (75%–100%).[17] All the histologicl measurements and grading of Picrosirius red staining were done by two masked independent observers (YCL and YP) in a ×100 microscopic field.

For immunohistochemistry analysis, sections were fixed with 4% paraformaldehyde for 10 minutes, washed with 1× PBS, blocked with 1% BSA in 1× PBS for 30 minutes, and incubated with mouse anti-rat CD11c (MCA1441; AbD Serotec, Oxford, UK) and CD45 (MCA43R; AbD Serotec) monoclonal primary antibodies for 1.5 hours at room temperature (M0851; Dako, Carpinteria, CA). After washing with 1× PBS, the sections were incubated with goat antimouse Alexa Fluor 488-conjugated secondary antibody (A11001; Invitrogen, Carlsbad, CA) at room temperature for 1 hour. Slides then were mounted with medium containing DAPI (UltraCruz Mounting Medium; Santa Cruz Biotechnology, Santa Cruz, CA). Sections were viewed and imaged with a fluorescence microscope (Zeiss Axioplan 2; Carl Zeiss, Oberkochen, Germany). For quantification of CD11c and CD45 stained cells, 5 nonoverlapping sections of each eye were chosen randomly for cell counting. The cells were counted in a ×400 microscopic field by a single masked observer (YCL).

Statistical Analysis

All data were expressed as mean ± SD. Statistical comparisons between blank and PA-loaded microfilm groups were performed using the Student's *t*-test. All data analyses were done with SPSS software package (SPSS, Inc., Chicago, IL).

Results

In Vitro and In Vivo Microfilm Degradation Studies

The M_w decreased gradually over the study period of 12 weeks, indicating the gradual degradation of the microfilms. The initial M_w was 155 to 170 kDa, and then it degraded by 85% of M_w approximately at the end of the study (Fig. 1A). The continuous decrease in the M_n also demonstrated the gradual degradation of the microfilms; this sort of degradation is referred to as "bulk" or homogeneous degradation. The degradation rate constant (λ) was 0.031 (day^{-1}) in vitro and 0.024 (day^{-1}) in vivo, indicating the microfilms degraded faster in vitro than in vivo (Fig. 1B).

In Vitro and In Vivo Drug Release Studies

There was an initial burst of 0.017 mg/d at day 3 in vitro. Subsequently, the amount of drug released reduced and became constant from the second week onwards, with a daily release amount of 0.004 mg/d. Comparatively, microfilms in vivo showed a steady daily release of approximately 0.002 mg/d without noticeable burst of release (Fig. 2A). The cumulative drug release profile revealed that PA-loaded microfilms achieved a steady, sustained release of drug in vitro and in vivo, although the in vivo drug release showed a slower release profile than that in vitro. The drug-loaded microfilms were almost exhausted of drug with 98.4% of release at 90 days in vitro, whereas there was 20.0% of loaded drug remaining in the microfilms after 90 days in vivo (Fig. 2B).

After insertions of PA-loaded microfilms subconjunctivally, PA concentrations were detected in the aqueous humor, with the levels of 76.7 ± 5.9, 70.3 ± 2.3, and 42.7 ± 4.1 ng/mL at 2, 4, and 12 weeks, respectively.

Clinical Evaluation

Slit-lamp examination revealed a mild degree of conjunctival vessels congestion around the margin of the microfilms at day 1 after insertion in either blank or PA-loaded microfilm group, but it resolved thereafter. The microfilms in both groups were visible at 12 weeks after insertions, without evidence of protrusion or dislocation (Figs. 3A–H). The mean total Hackett-McDonald ocular scores (0–10) assessing conjunctival congestion, swelling, and discharge were very low with the score of 0.63 ± 0.09, 0.07 ± 0.05, 0.05 ± 0.05, and 0 in the blank microfilm group, and 0.18 ± 0.08, 0.03 ± 0.03, 0, and 0 in the PA-loaded microfilm group at 1 day, and 2, 4, and 12 weeks, indicating the microfilms elicited minimal inflammation at the insertion sites. The PA-loaded microfilm group had a significantly less total score at day 1 ($P < 0.001$, Fig. 3I), and this resulted from the significantly less conjunctival vessels congestion. There was no sign of infection, neovascularization, bleeding, or scarring at the insertion sites.

The ASOCT images showed that all the microfilms in blank and PA-loaded microfilm group were placed in good anatomic positions subconjunctivally without any evidence of dislocation or protrusion throughout the three months (Fig. 4).

Histologic and Immunohistochemical Studies

Histologic sections with hematoxylin and eosin (H&E) staining revealed very minimal inflammatory cells within the subconjunctival space through the three months. There was a mild degree of fibrotic capsule formation around the microfilm seen

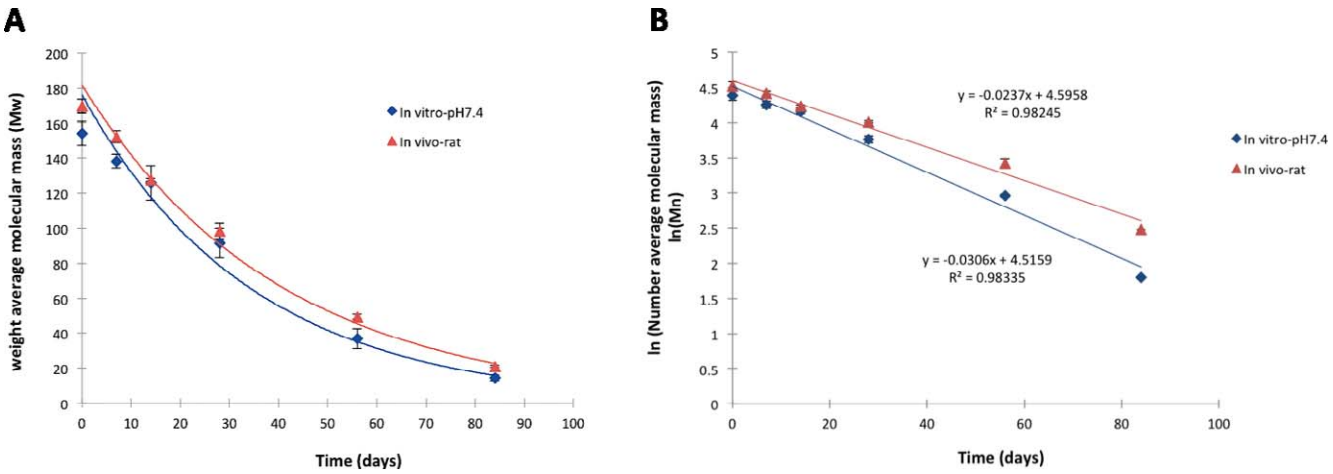

Figure 1. Graphs showing the degradation profiles of the microfilms. **(A)** Monitoring of M_w for the microfilms in vitro and in vivo. **(B)** Monitoring of M_n for the microfilms in vitro and in vivo.

from 2 weeks onwards after insertion (Fig. 5). The mean thickness of the collagen capsule in blank and PA-loaded microfilm groups was 5.29 ± 0.05 and 3.68 ± 0.05 μm at 2 weeks ($P = 0.80$), 7.12 ± 0.06 and 6.57 ± 0.05 μm at 4 weeks ($P = 0.56$), and 6.49 ± 0.05 and 6.37 ± 0.05 μm at 12 weeks ($P = 0.83$). The ratio of the length of microfilm surface lined by collagen to the entire length of microfilm surface for blank and PA-loaded microfilm groups was 81.33 ± 0.83% and 60.68 ± 0.63% at 2 weeks ($P = 0.001$), and 88.07 ± 0.27% and 85.55 ± 0.47% at 4 weeks ($P = 0.12$). The entire length of microfilm surface could not be determined at 12 weeks after insertions because the microfilms had degraded to fragments (Figs. 5C, 5F). The mean thickness of the microfilms in blank and PA-loaded microfilm groups was 98.8 ± 4.8 and 100.2 ± 4.2 μm at 2 weeks, 98.3 ± 4.0 and 98.4 ± 3.6 μm at 4 weeks, and 44.5 ± 2.6 and 42.8 ± 2.7 μm at 12 weeks. Picrosirius red staining for collagen further confirmed the amount and extent of collagen encapsulation of the microfilms. When viewed with a polarized light, a thin layer of mature type I collagen fibers was observed around the microfilm from 2 weeks onwards after insertion. The mean grading for the quantity of collagen in the blank microfilm group was 1.0 ± 0.0, 1.3 ± 0.3, and 1.6 ± 0.3 at 2, 4, and 12 weeks, respectively, and the mean grading for the PA-loaded microfilm group was 1.0 ± 0.0, 1.0 ± 0.0, and 1.3 ± 0.3 at 2, 4, and 12 weeks, respectively, indicating there was no excessive foreign body reaction and implant encapsulation in the subconjunctival space in groups (Fig. 6). Immunohistochemistry staining for CD11c cells and CD45 T cells showed minimal CD11c cells or CD45 T cells infiltrated around the microfilms in the subconjunctival space in either the blank or PA-loaded microfilm group, indicating there was only minimal inflammation after microfilm insertions (Figs. 7A–F, Supplementary Figs. S1A–S1F). There was a significant decrease in CD11c cells in the PA-loaded microfilm group compared to the blank microfilm group at 2 and 4 weeks after insertions ($P = 0.001$ and $P = 0.002$, Fig. 7G). There was no significant difference in the numbers of CD45 cells at 2, 4, and 12 weeks between the 2 groups ($P = 0.75$, $P = 0.46$, and $P = 0.76$; Supplementary Fig. S1G).

Discussion

In our study, we have developed a drug delivery system that has shown good biocompatibility/surgical efficacy, and released prednisolone acetate in a sustained and controlled manner over a period of 90 days for use in small animal models of disease.

Several other sustained ocular drug delivery systems composed of either nonbiodegradable or biodegradable polymers have been reported in the literature.[18] Polymeric systems have been used widely as implantable devices for

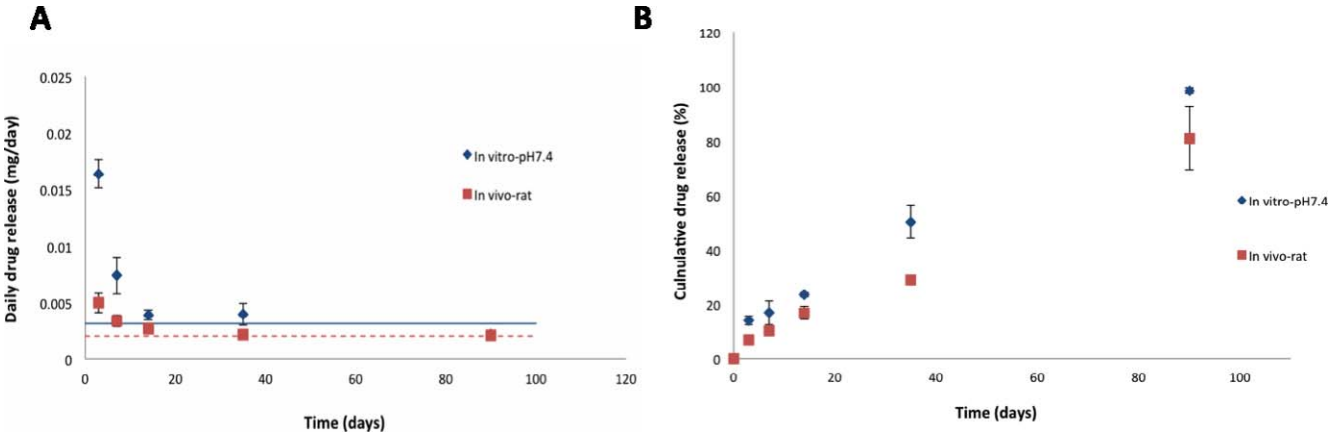

Figure 2. Graphs showing the drug release profiles of the microfilms. **(A)** The daily drug release from the 40% PA-loaded microfilms in vitro and in vivo. **(B)** The cumulative drug release from the 40% PA-loaded microfilms in vitro and in vivo.

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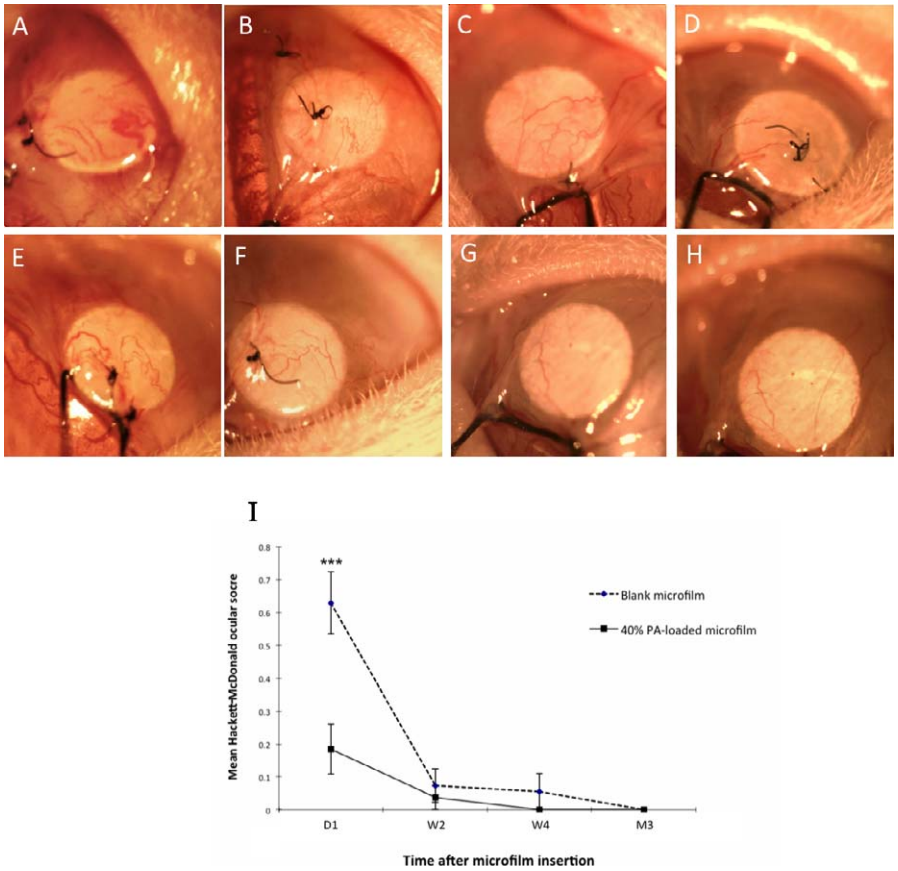

Figure 3. Slit-lamp photographs (**A**–**H**), and the changes in Hackett-McDonald ocular scores (**I**) of blank (**A**–**D**) and PA-loaded (**E**–**H**) microfilms at 1 day (**A**, **E**), and 2 (**B**, **F**), 4 (**C**, **G**), and 12 (**D**, **H**) weeks after insertions. Mild conjunctival vessels congestion around the margin of the microfilms was noted at 1 day after insertion, but resolved thereafter. There was no evidence of other clinically abnormal findings.

controlled release of drugs in various organs.[19,20] In ophthalmic use, PLGA copolymers are used more commonly, for example, incorporating timolol for intraocular pressure control,[21] all-trans retinoic acid to reduce muscle adhesion in strabismus surgery,[22] cyclosporin for treatment of uveitis,[23] or 5-fluorouracil for antifibrotic effects.[24] In our study, we used PLC copolymers instead. PLC is a relatively new copolymer that is made of poly(L-lactide) and poly(caprolactone), each of which has been approved by the Food and Drug Administration (FDA) in implantable products. Even though to our knowledge it has not been used previously in ophthalmology, its use has been reported in neurologic, orthopedic, and cardiovascular research.[25–27] In comparison with PLGA, PLC is quite hydrophobic as the caprolactone ester bonds of the copolymer are not easily hydrolyzed. Because of this slower hydrolysis rate, PLC microfilms degrade more slowly and, therefore, achieve a longer release.[28,29] Polymeric structural difference in crystallinity also affects degradation rates. PLGA is an amorphous copolymer and is easier to be degraded than a PLC copolymer, which has semicrystalline structure. Our previous studies have confirmed that PLC microfilms degrade slower than PLGA microfilms in vitro and in vivo,[30] which enables PLC to be a better candidate for a sustained ocular drug delivery system. Furthermore, PLGA copolymers have a higher glass transition temperature than PLC copolymers, which makes PLGA copolymers physically hard, while PLC copoly-

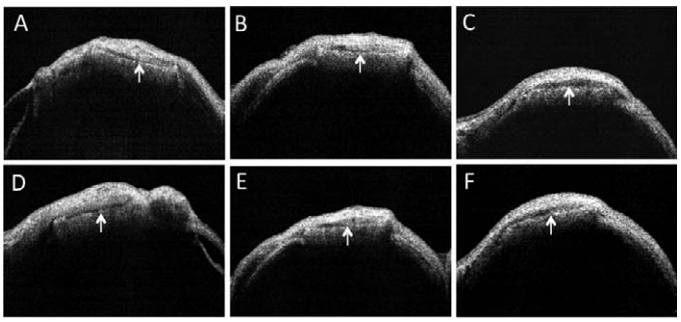

Figure 4. ASOCT scans of blank (**A**–**C**) and PA-loaded (**D**–**F**) microfilms at 2 (**A**, **D**), 4 (**B**, **E**), and 12 (**C**, **F**) weeks after insertions. It demonstrated the good anatomic placement of all microfilms implanted subconjunctivally (*arrow*) without any evidence of dislocation or protrusion throughout the three months.

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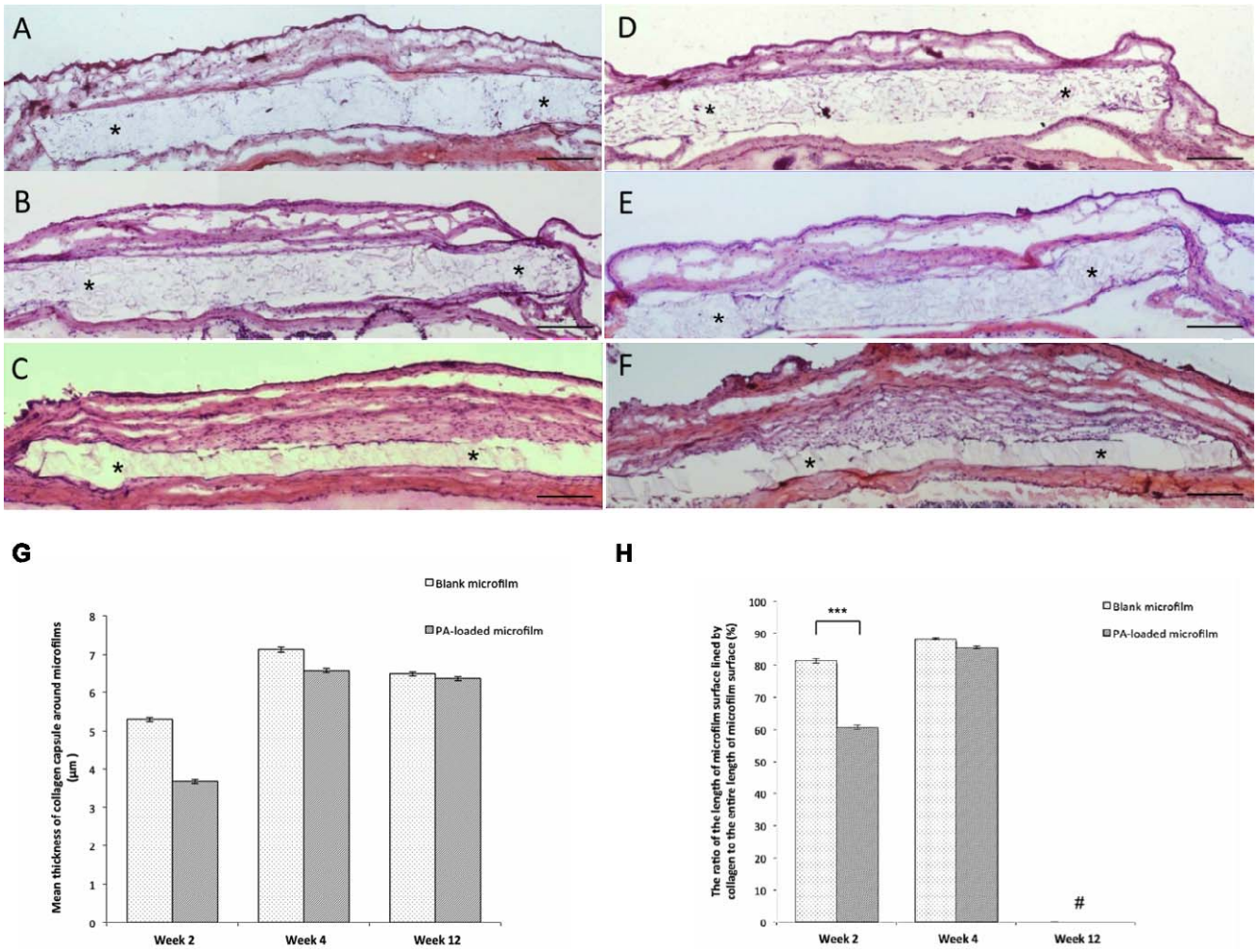

FIGURE 5. Histologic sections with H&E staining of blank (**A**–**C**) and PA-loaded (**D**–**F**) microfilms at 2 (**A**, **D**), 4 (**B**, **E**), and 12 (**C**, **F**) weeks after insertions. The mean thickness of the collagen capsule around the microfilm was measured (**G**). There was a significant reduction of the ratio of the length of microfilm surface lined by collagen to the entire length of microfilm surface in the PA-loaded microfilm group at 2 weeks ([**H**], $^{***}P = 0.001$. #The ratio could not be determined as the entire length of microfilm surface could not be determined at 12 weeks because the microfilms had degraded to fragments). *Asterisks* indicated the implanted microfilm. Original magnification: ×100. *Scale bar*: 100 μm.

mers appear to be soft and elastic.[31,32] Hence, we chose the softer material for the microfilm fabrication as it minimizes the possibility of surgical trauma during the implantation procedure as well as of extrusion after the implantation.

Among the clinically available corticosteroid preparations, PA has the highest aqueous concentration within 1 to 2 hours after application and maintains high levels for 24 hours due to its high rate of ocular penetration.[33–36] Moreover, the acetate analogue of prednisolone is more hydrophobic; hence, it has a higher tendency to stay attached to the PLC microfilms rather than be released within a short period of time. Therefore, we chose PA as the loading drug in our study. We detected PA concentrations in the aqueous humor with a steady level at 76.7 and 70.3 ng/mL at 2 and 4 weeks, which decreased to 42.7 ng/mL at 3 months. The trend of changes in PA concentrations in the aqueous humor corresponded to the drug release in vitro and in vivo. McGhee et al. reported that a mean peak concentration of 669.9 ng/mL was attained within 2 hours after applying 1 drop 1% PA eye drops in patients undergoing routine cataract extraction, and the drug levels decreased gradually to 99.5 ng/mL at 12 hours and 28.4ng/mL at 24 hours.[34] With regard to the drug delivery system, the amount of loaded drug is proportional to the size of the microfilms, and the size of the microfilms in our study was limited to the small eyeball of the rats. This accounts for our lower PA levels in the aqueous humor compared to that reported by McGhee et al.[34] We believe that future development of larger microfilms with higher PA loading concentration will equate to higher aqueous humor concentrations similar to those seen in patients following topical eye drops, since the smaller 2 mm diameter microfilms used in small animal models already have provided a PA concentration at 70 to 77 ng/mL in aqueous.

In our study, we demonstrated that PLC blank microfilms have good biocompatibility with minimal inflammatory and fibrotic reaction, confirmed by clinical observation, and histologic and immunohistochemical studies, which were consistent with the results in our previous study using a rabbit model.[30] We also found that PA-loaded microfilm group had significantly less collagen encapsulation at 2 weeks, and less CD11c infiltration around the microfilms at 2 and 4 weeks in comparison with the blank microfilm group. It is well known that corticosteroids possess antifibrotic and anti-inflammatory actions.[11,37,38] Our results indicated that our PA-loaded microfilms may have its applications to the clinical scenarios in which inflammation or fibrosis needs to be avoided.

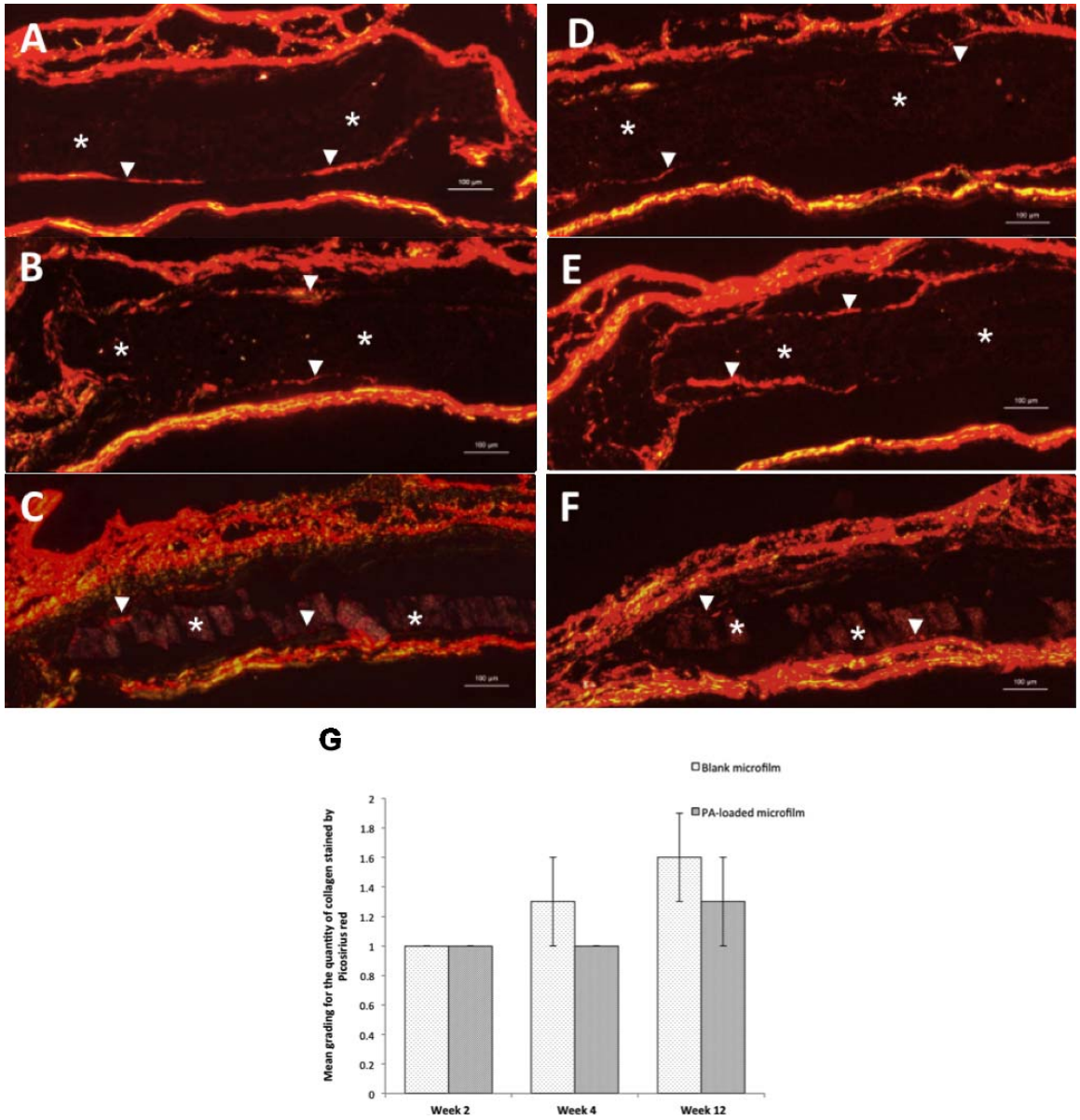

FIGURE 6. Picrosirius red staining for collagen of blank (**A**–**C**) and PA-loaded (**D**–**F**) microfilms at 2 (**A**, **D**), 4 (**B**, **E**), and 12 (**C**, **F**) weeks after insertions. Mild degree of mature collagen I formation (stained as *red* or *yellow*) was noted around the microfilms (*arrowhead*). The quantity of collagen was graded (**G**). *Asterisks* indicated the implanted microfilm. Original magnification: ×100. *Scale bar*: 100 μm.

The microfilm degradation rate and the drug release rate in vivo were slightly slower than those in vitro. This may be due mainly to the relative confined subconjunctival space in the rat's eye, such that the fluid surrounding the implanted microfilms was comparatively less than that in the in vitro setting, where the microfilms were bathed in PBS. In contrast, there was minimal "bathing" environment for the microfilms in the rats' subconjunctival space, that is, the released drug is not cleared fast, leading to some drug accumulation in vivo in the space surrounding the microfilms, and this may retard further release of PA from the microfilms. In the in vitro situation, there is a large excess of water present, which avoids this drug accumulation around the microfilms. In the terminology used in drug release studies, the "sink" condition is not maintained in vivo compared to in vitro, and hence there is slower release of drug in vivo.

Polyhydroxyesters, including PLC copolymers, may have two different modes of degradation: bulk degradation and surface erosion. In the bulk degradation mode, also called the homogeneous degradation mode, the polymers degrade slowly without appreciable volume or size loss until the product becomes water soluble and leaches out of the matrix, when volume or size change becomes detectable. In the surface erosion mode, also called heterogeneous degradation mode, there is a continuous decrease in volume or size as polymers degrade at the surface first, followed by dissolution of the surface layer.[30] In our study, the microfilms exhibited bulk degradation because the M_w and M_n decreased gradually throughout the study period, but no changes in the microfilm dimension or thickness (as seen in the histologic study) were observed during the initial 4 weeks. Furthermore, we also have examined the effect of PA loading onto microfilms, and we noted that blank and PA-loaded microfilms demonstrated quite similar degradation profiles (Supplementary Fig. S2), indicating PA loading did not affect the degradation results of microfilms.

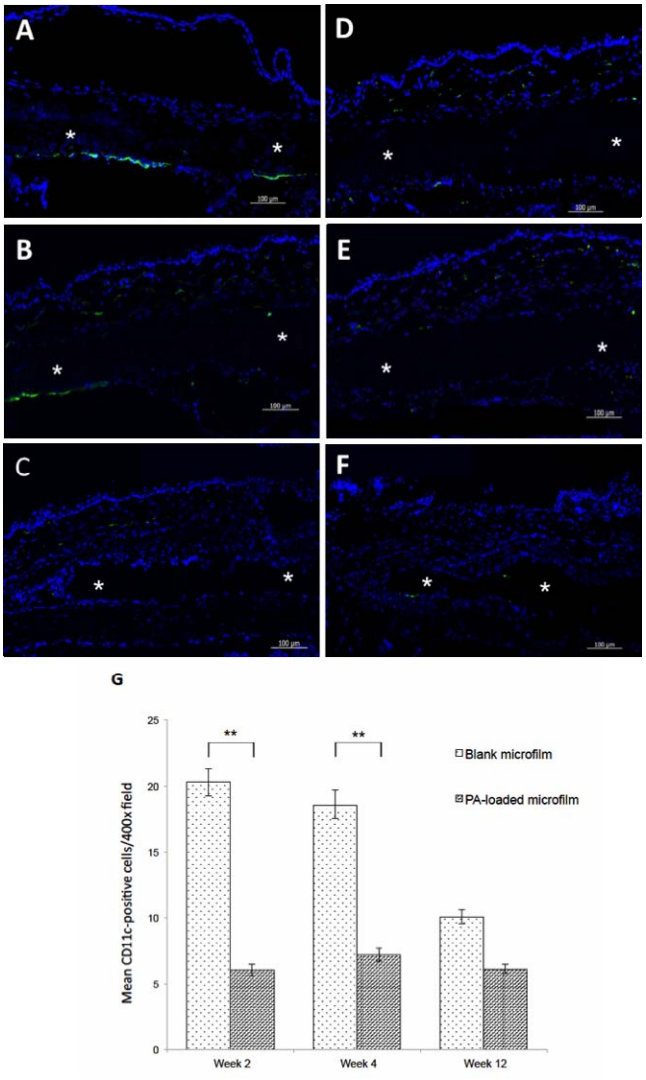

FIGURE 7. Immunohistochemistry staining for CD11c cells of blank (**A**–**C**) and PA-loaded (**D**–**F**) microfilms at 2 (**A**, **D**), 4 (**B**, **E**), and 12 (**C**, **F**) weeks after insertions. There was a significant reduction of CD11c cells in PA-loaded microfilm group at 2 and 4 weeks ([**G**], $^{**}P < 0.01$). Cell nuclei were stained *blue* with DAPI, and CD11c-positive cells were stained *green*. *Asterisks* indicated the implanted microfilm. Original magnification: ×100. *Scale bar*: 100 μm.

The dilemma in using corticosteroids in a drug delivery system is the ability to reverse unwanted steroid related side effects, for example, elevation of intraocular pressure, exacerbation of bacterial and viral infection, and posterior subcapsular cataract formation. In terms of reversing the corticosteroid effects due to its adverse reaction, it would be a simpler procedure to remove the microfilm from the subconjunctival space, compared to removing implanted devices (biodegradable or not) from the anterior chamber or vitreous cavity, or with the use of suspensions of nanoparticles as a drug delivery system for corticosteroids.

In conclusion, we have demonstrated the biocompatibility, feasibility, and desirable drug release profiles of the prednisolone acetate-loaded PLC microfilms in vitro and in vivo for use in small animal models of disease. It delivers a sustained and sufficient drug level, which meets clinical needs more efficiently, and it can be implanted subconjunctivally through a simple procedure. As corticosteroids have been used in a broad spectrum of ophthalmic inflammatory conditions, the PA microfilm provides a promising alternative to conventional eye drops.

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