Biomaterials 35 (2014) 432–439

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

In vivo performance of a drug-eluting contact lens to treat glaucoma for a month

Joseph B. Ciolino [a, b, c], Cristina F. Stefanescu [a, b, c], Amy E. Ross [a, b, c], Borja Salvador-Culla [a, b, c], Priscila Cortez [b, c], Eden M. Ford [b, c], Kate A. Wymbs [b, c], Sarah L. Sprague [b, c], Daniel R. Mascoop [b, c], Shireen S. Rudina [b, c], Sunia A. Trauger [d], Fabiano Cade [a], Daniel S. Kohane [b, c, *]

[a] *Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Boston, MA 02114, USA*
[b] *David H. Koch Institute for Integrative Cancer Research, 77 Massachusetts Ave, Building 76-66, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*
[c] *Laboratory for Biomaterials and Drug Delivery, Department of Anesthesiology, Division of Critical Care Medicine, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA*
[d] *Small Molecule Mass Spectrometry, Center for Systems Biology, Harvard University, Cambridge, MA 02138, USA*

ARTICLE INFO

Article history:
Received 15 August 2013
Accepted 10 September 2013
Available online 4 October 2013

Keywords:
Drug delivery
Contact lens
Sustained release
Latanoprost
Glaucoma
Ocular release

ABSTRACT

For nearly half a century, contact lenses have been proposed as a means of ocular drug delivery, but achieving controlled drug release has been a significant challenge. We have developed a drug-eluting contact lens designed for prolonged delivery of latanoprost for the treatment of glaucoma, the leading cause of irreversible blindness worldwide. Latanoprost-eluting contact lenses were created by encapsulating latanoprost–poly(lactic-co-glycolic acid) films in methafilcon by ultraviolet light polymerization. *In vitro* and *in vivo* studies showed an early burst of drug release followed by sustained release for one month. Contact lenses containing thicker drug–polymer films demonstrated released a greater amount of drug after the initial burst. *In vivo*, single contact lenses were able to achieve, for at least one month, latanoprost concentrations in the aqueous humor that were comparable to those achieved with topical latanoprost solution, the current first-line treatment for glaucoma. The lenses appeared safe in cell culture and animal studies. This contact lens design can potentially be used as a treatment for glaucoma and as a platform for other ocular drug delivery applications.

1. Introduction

Glaucoma is the leading cause of irreversible blindness worldwide [1]. The mainstay of preventive therapy is topical medications (drops) that reduce intraocular pressure (IOP). Unfortunately, only 1–7% of the medication in eye drops is absorbed, and the duration of effect is not sustained [2]. The excess medication typically washes down the nasolacrimal duct or spills onto the cheek, where it can potentially lead to side effects such as allergic blepharitis and contact dermatitis. Eye drops also can be difficult to administer and they can sting, burn, or cause a transient blurring of vision. All of these factors are believed to contribute to the notoriously poor patient adherence with glaucoma therapy, with an estimated adherence rate of less than 50% [3]. Poor adherence contributes to irreversible vision loss and is one of the biggest challenges facing eye care providers [4].

A noninvasive method of sustained ocular drug delivery could help improve adherence to glaucoma therapy by decreasing the frequency of drug administration. Controlling drug release from a contact lens has historically proven to be difficult [5]. Commercially available contact lenses can absorb and release drugs, but the duration of release tends to be limited to only several hours [5]. Recent research has focused on extending the duration of drug release through modification of the contact lens design [5,6]. However, a contact lens has yet to demonstrate the ability to elute a drug for weeks at a time in an animal model.

Here, we describe the formulation, characterization, and performance of a contact lens designed to elute a glaucoma medication for at least one month. The contact lens, composed of methafilcon, a high water content (55%) co-polymer of poly(hydroxyethylmethacrylate) and methacrylic acid [7,8], contains a drug–polymer film composed of poly(lactic-co-glycolic) acid (PLGA). PLGA is well-known for its

* Corresponding author. Department of Anesthesiology, 300 Longwood Ave, Children's Hospital Boston, Boston, MA 02115, USA. Tel.: +1 617 919 2364, +1 617 355 7327; fax: +1 617 730 0453.
E-mail addresses: daniel.kohane@childrens.harvard.edu, dskohane@mit.edu (D.S. Kohane).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.09.032

biocompatibility in many settings, biodegradability, and ability to control drug release kinetics, and it is approved by the Federal Drug Administration (FDA) in both ocular and systemic drug delivery devices [9]. Latanoprost is a first-line treatment for glaucoma, it is the most commonly prescribed anti-glaucoma drug in the United States [10], and it demonstrates minimal systemic or local toxicity at therapeutic doses [11].

2. Materials and methods

2.1. Materials

High molecular weight (118 kDa) 65:35 PLGA (65 glycolide:35 L-lactide) and 85:15 PLGA (85 glycolide:15 L-lactide) were obtained from Surmodics (Birmingham, AL). Irgacure 2959 was purchased from Ciba Specialty Chemicals Corporation (Tarrytown, NY). Latanoprost for incorporation into lenses ("commercial latanoprost") was obtained in an aqueous solution (50 μg/mL, Bausch and Lomb, Tampa, FL) and latanoprost standards were obtained in methyl acetate (50 mg/mL, Cayman Chemical, Ann Arbor, MI). Unpolymerized methafilcon was purchased in liquid form from Kontur Kontact Lens Company (Hercules, CA). Glucose, ethyl acetate, and all the other reagents were purchased from Sigma Aldrich (St. Louis, MO). Phosphate buffered saline (PBS, pH 7.4) was obtained from Invitrogen (Carlsbad, CA). Biopsy punches (3 mm) were obtained from Sklar Instruments (West Chester, PA).

2.2. Contact lens fabrication

PLGA (60 mg, 65:35 or 85:15) and 80 μL of latanoprost solution (50 mg/mL) were added to 920 μL ethyl acetate. 50 μL of the combined solution was then pipetted onto a concavity lathed into a cylinder of dry polymerized methafilcon (Kontur Kontact Lens Company). After 6 min of rotation on a spin coater (Model P6700, Speedline Technologies, Franklin, MA), the liquid ethyl acetate evaporated, leaving a drug–polymer film. Thinner 65:35 PLGA films were created by using a diluted solution that contained half the amount of polymer and latanoprost.

A 3 mm central aperture in the drug–polymer film was incised with a 3 mm biopsy punch. The hydrogel blanks containing the films were placed on a desiccator under vacuum for 3 days and then lyophilized for a day. The side of the films that was not yet in contact with methafilcon was then encapsulated in methafilcon by ultraviolet (UV) photopolymerization using a 400 W metal halide bulb (Loctite Zeta 7401, Loctite Corporation, Rocky Hill, CT). The methafilcon block was then lathed into a contact lens that consisted of the drug–PLGA film fully encapsulated in methafilcon (Table 1).

2.3. Scanning electron microscopy

The morphologies of the drug polymer films and the latanoprost-eluting contact lenses (CLs) were examined under scanning electron microscopy (SEM). Dry CLs and pre-encapsulated films were cross-sectioned and sputter coated with a gold–plutonium alloy under vacuum (Hummer 6.2, Anatech Limited, Union City, CA). Images were acquired with a JEOL 590 scanning electron microscope (JEOL JSM 5600 LV, USA Inc., Peabody, MA). The average thickness for each set of latanoprost–PLGA films was calculated by loading the respective SEM images, which contain a scale bar, into ImageJ and measuring the thickness of the film cross-section at the center and at each end.

2.4. In vitro drug release

Three sets of CLs were submerged in 5 mL of PBS solution that was pre-heated to 37 °C. The lenses and PBS were placed on a rotational shaker at 64 rpm. Each day, aliquots of the release media were sampled and the contact lenses were placed in fresh PBS. The aliquots of release media were stored at 4 °C, and latanoprost concentrations were quantified using an enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI).

2.5. Latanoprost molecular analysis

During lens construction and characterization, latanoprost was exposed to various environmental factors that could potentially result in drug degradation. To determine if latanoprost was altered, we analyzed release media (day 1) from $CL_{85:15,\ 45}$ ($n = 4$) by high-performance liquid chromatography in combination with high-resolution mass spectrometry (LC-MS). We monitored the presence of latanoprost and its most abundant degradation product, latanoprost free acid. We also analyzed commercial latanoprost solution (0.005%, Bausch and Lomb, Tampa, FL), latanoprost standard solution and latanoprost acid standard solution (both 50 mg/mL in methyl acetate, ≥98% purity, Cayman Chemical) by LC-MS. Latanoprost and latanoprost acid standards were used to form standard curves, which were used to quantify the concentrations of latanoprost and latanoprost acid in experimental samples.

Data were acquired on a Maxis Impact q-TOF mass spectrometer (Bruker Corporation, Billerica, MA) in combination with an Agilent 1200 HPLC, using LC-MS. An isocratic elution of water:acetonitrile:formic acid (45:55:0.05% *v*/*v*/*v*) was used at 200 μL/min flow rate. A Gemini Phenomenex 2.0 mm (5 μm particle size, 80 E pore size, 10 cm length; Phenomenex Inc., Torrance, CA) in combination with a 5 cm pre-column was used for the high-performance liquid chromatography (HPLC) separation. 0.1% formic acid was added to the sample solution to facilitate chromatographic elution, resulting in an acidic mobile phase (pH 4.0). Samples were run in positive ion mode to detect latanoprost and the same HPLC method was run in negative ion mode to monitor for latanoprost free acid [12]. The extracted ion currents with a threshold of ±0.005 Da for expected ions were used to monitor for the presence of the expected ions of unmodified latanoprost and latanoprost free acid.

2.6. Residual solvent analysis

When an organic solvent is used in the production product of a drug product, the FDA recommends weight loss analysis for initially measuring the mass of the residual solvent [13]. We sought to determine the percent weight of residual ethyl acetate in the drug–polymer films using thermogravimetric analysis (TGA). Films at various stages of production (solvent evaporation, desiccation, or lyophilization) were removed from the contact lens material and cut into quadrants. Under a flow of nitrogen at 20 L per minute, film sections were heated from 30 °C to 350 °C at an increase of 10 °C/min and analyzed using a thermogravimetric analyzer (Pyris 1, Perkin Elmer, Waltham, MA). Four samples were run for each time point.

Gas chromatography (GC) was used to determine the amount of residual solvent released from the CLs at various time points. CLs were incubated in PBS as described previously. Release media was collected daily for analysis and it was combined with dichloromethane in a 1:1 weight ratio to extract ethyl acetate. A constant amount of dodecane (2000 parts per million) was added to each extraction sample to correct for any injection volume variability. Extraction samples were analyzed using an Agilent 7890A gas chromatograph with 10 m DB-1 column. One μL was injected and heated at 35 °C for 2 min and then raised to 250 °C at a rate of 50 °C/min. Retention time was measured using a flame ionization detector. Ethyl acetate in release media was calculated by comparing the ratio of the area under the curve (AUC) for the ethyl acetate peak to the AUC for dodecane in the sample and comparing this ratio to that of reference standards. Latanoprost solution (0.005%) was extracted and analyzed in the same manner.

2.7. Cytotoxicity

Latanoprost, residual solvent, or polymer breakdown products could potentially result in local toxicity. Therefore, cell culture studies were conducted to investigate this possibility prior to performing animal studies. Immortalized human corneal limbal epithelial (HCLE) cell lines were a generous gift provided by Dr. Ilene Gipson of Harvard Medical School. As previously described [14], the HCLE cells were grown in T75 flasks and maintained in keratinocyte serum-free medium supplemented with 1% penicillin–streptomycin, 25 μg/mL bovine pituitary extract, 0.2 ng/mL epidermal growth factor, and 0.4 mM calcium chloride dihydrate (Life Technologies, Grand Island, NY).

HCLE were plated on the bottom surface of 3.0 μm pore size Transwell® plates (Corning Life Sciences, Corning, NY). Cell density in all experiments was 1×10^5 cells/mL. $CL_{85:15,\ 45}$ were sterilized by UV light and placed in the Transwell® inserts. Commercial contact lenses (Kontur Kontact Lens Company, Hercules, CA) composed of the same hydrogel (methafilcon) as the $CL_{85:15,\ 45}$ were purchased in a sterile condition and also placed in Transwell® inserts. After 24 h of exposure, cell viability was assessed using a commercially-available colorimetric assay (MTT viability assay kit, CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay, Promega Corporation, Madison, WI) after 24 h of exposure. Results were reported as means ± standard deviations of measured absorbance normalized to the absorbance for non-treated control cells (% normalized cell viability = 100 × absorbance for cells treated with a CL/absorbance for non-treated cells).

2.8. In vivo drug absorption and biocompatibility studies

Because *in vitro* drug release studies can be poorly correlated with *in vivo* drug release performance, we investigated the ability of the contact lens to elute latanoprost safely and effectively in New Zealand white rabbit eyes. This species is commonly used to study the safety of contact lenses given that the size and structure of the animals' eyes are similar to that of human eyes [15]. Since latanoprost does not induce a reduction in IOP in rabbits [11], we studied the drug flux from the CL into the aqueous humor of the eye.

Table 1
Polymer film characteristics.

Name	Lactide/glycolide mole ratio	Film thickness (μm)[a]
$CL_{65:35,\ 20}$	65:35	19.2 ± 4.4
$CL_{65:35,\ 40}$	65:35	38.5 ± 8.4
$CL_{85:15,\ 45}$	85:15	45.5 ± 6.4

[a] Measurements are means ± one standard deviation.