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Hyaluronan-based Sustained Delivery of the Antibiotic Besifloxacin to the Eye

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Abstract

Corneal ulcers can cause blindness if not effectively treated. Current treatments require topical antibiotics delivered via a rather inconvenient hourly, round-the-clock, and multiple-day administration. In addition, they are difficult to administer in a reliable manner, and can result in low availability of therapeutic agent due to tears and blinking. This study focuses on the development of a topical hyaluronic acid (HA)-based biodegradable film designed to deliver Besifloxacin on a sustained-released (SR) basis to overcome today's dosing challenges. Prototype films of cross-linked, thiolated carboxymethylated HA (CMHA-S) containing Besifloxacin were produced and evaluated for *in vitro* drug release. Films were fabricated in silicone molds using 16 mg/mL CMHA-S and poly(ethyleneglycol) diacrylate (PEGDA), as a cross-linker. The polymerized gel was dried at 37°C overnight to create thin films. Various amounts of Besifloxacin (up to 150 μg/film) were formulated into the polymer solution prior to cross-linking. The release rate was monitored in phosphate buffered saline (PBS) by measuring UV absorption at 293 nm. Released amounts were calculated from the Besifloxacin standard solution. Results show that Besifloxacin can be continuously released from films for up to 9 days (on Day 9, approximately 1 μg was released from 150 μg loading). *In vitro* efficacy tests on the released Besifloxacin are currently in progress. In conclusion, Jade Therapeutics' Besifloxacin-loaded films represent a promising alternative to hourly antibiotic eye drops, and have the potential to move forward into *in vivo* studies and ultimately become a clinically useful product. Furthermore, this polymer-based system can be expanded to deliver other antimicrobials to treat additional indications such as ophthalmic fungal or viral infections.

Keywords: Corneal Ulcers, Delivery, Hyaluronan

1. Introduction

Corneal ulcer, an infectious keratitis, is a potentially devastating ocular infection, which may result in perforation, endophthalmitis, and/or vision loss if left untreated.[1] Infectious keratitis can progress rapidly. It may induce corneal destruction within 24-48 hours.[2] Contact wear is the main risk for corneal ulcers. Over the past decade, contact lens use has significantly increased. According to the CDC, in 2014 an estimated 40.9 million persons in the US over the age of 18 wear contact lenses.[3] With an increased amount of contact wearing, the number of incidents of corneal ulcer has also increased.

The current standard of care involves compounded eye drops containing topical antibiotics (used off label), which has serious drawbacks associated with low bioavailability of the therapeutic agents, difficulty in formulating, difficulty of administration, and concomitantly poor patient compliance.[4] Jade Therapeutics' proprietary, flexible, biodegradable polymer hyaluronic acid (HA) film overcomes the shortcomings of current standard of care practice outlined above and addresses the unmet need for single-application, extended treatment of bacterial corneal ulcers, as well as other potential ocular corneal conditions.[4] It is designed to be placed directly and easily on the ocular surface to deliver high concentrations of antibiotics, locally, in a patient-friendly manner over the course of multiple days without the need for repeated hourly eye drop applications. Jade's therapy would overcome the limitations of topical eye drops, improve ocular tissues bioavailability, eliminate medication wastage with drop bolus, decrease drug systemic exposure, and effectively and rapidly eradicate the bacteria in these corneal infection emergencies, while the HA film provides additional lubrication and healing benefits during matrix degradation to actual HA on-eye.[4]

Hyaluronan, also known as HA, is a naturally occurring biopolymer. HA is a key component in the extracellular matrix and composed of repeated monomers of N-acetylglucosamine and glucuronic acid. Due to its biocompatibility properties, hyaluronan has been developed as a drug-delivering vehicle. However, the rapid degradation of HA *in vivo* precludes many clinical applications. Chemical modifications of HA have been generated derivatives in which the biophysical and biochemical properties, as well as the rates of enzymatic degradation *in vivo* have been manipulated and tailored for specific clinical needs.[5] Thiolated carboxymethylated-HA (CMHA-S) is one type of chemically modified HA that has been utilized in a variety of applications, including wound repair and adhesion prevention.[6,7] CMHA-S is synthesized by carboxymethylating the HA, followed by introduction of crosslinkable thiol residues.[8] CMHA-S can then be crosslinked with either a thiol-reactive crosslinker such as poly(ethylene glycol) diacrylate (PEGDA) (Figure 1A), or by oxidative disulfide bond formation to form hydrogels. Then, the hydrogels can be lyophilized to form sponges or air-dried to form thin films.[9,10] CMHA-S has shown the capability of delivering growth factors slowly over timescales of weeks to months both *in vitro* and *in vivo*.[11,12] When growth factors were continuously released from topical hydrogels into full-thickness wounds, wound healing was accelerated in various disease and injury models.[11,13,14]

Crosslinked CMHA-S has also shown to accelerate healing of corneal epithelial abrasion and alkali injuries in rabbits.[15] By combining the intrinsic scar-free wound-healing properties of CMHA-S hydrogels with the capability of delivering drugs, CMHA-S can be utilized to treat corneal and ocular wounds.[4] This study focuses on the development of a topical hyaluronic acid (HA)-based biodegradable film designed to deliver Besifloxacin to the eye on a sustained-released (SR) basis in order to overcome today's dosing challenges.

2. Methodology

2.1. Materials

Thiolated carboxymethylated hyaluronic acid (CMHA-S) and poly(ethylene glycol) diacrylate (PEGDA) MW 3500 were obtained from BioTime Inc. (Alameda, CA). Besifloxacin hydrochloride (Besi-HCl) was obtained from Sigma-Aldrich (St. Louis, MO). FlexiPerm® was purchased from Greiner Bio-One (Monroe, NC) and Cady Bv2 mold was developed by Dr. Nathaniel Cady at SUNY Polytechnic Institute (Albany, NY).

2.2. Film Fabrication Using CMHA-S And The Release Study

Prototype films of crosslinked CMHA-S containing Besifloxacin were produced and evaluated for *in vitro* drug release. Films were fabricated using 16 mg/mL CMHA-S and PEGDA as a cross-linker in two silicone molds, FlexiPerm® and Cady Bv2 Mold. The thiol to acrylate ratio of the solution was 1.5:1. Various amounts of Besifloxacin (up to 150 μg/film) were formulated into the polymer solution prior to cross-linking. The polymerized gels were dried at room temperature to create thin films. The films were incubated at 37˚ C in a microcentrifuge tube containing 100 μl of phosphate buffered saline (PBS). The UV absorbance value was measured at 293 nm. The released amounts were calculated from a Besifloxacin standard solution.

2.3. Zone Of Inhibition (ZOI) Study

The Besifloxacin film was sterilized with ethylene oxide (EtO), and the efficacy of released Besifloxacin was evaluated by ZOI study. EtO-sterilized film and pre-sterilized film were placed on S. aureus inoculated plates, incubated 18 hours, and ZOI was compared. In addition, the released medium was also evaluated by ZOI study. Filter discs (6 mm in diameter) were soaked in released samples, dried overnight, and placed on S. aureus inoculated plates, incubated 18 hours, and ZOI was compared.

3. Data

3.1. Film Fabrication In Flexiperm® Mold And The Release Study

For current study, Besifloxacin was chosen as a target drug since it is commonly prescribed for treating bacterial keratitis.[16] Besifloxacin is a fourth generation of fluoroquinolone that has a broad spectrum *in vitro* activity against a wide range of Gram-positive and Gram-negative ocular pathogens, and its structure is shown in Figure 1B. The eye drop formulation was approved by the Food and Drug Administration (FDA) in 2009 and has been marketed under the trade name, Besivance.[17]

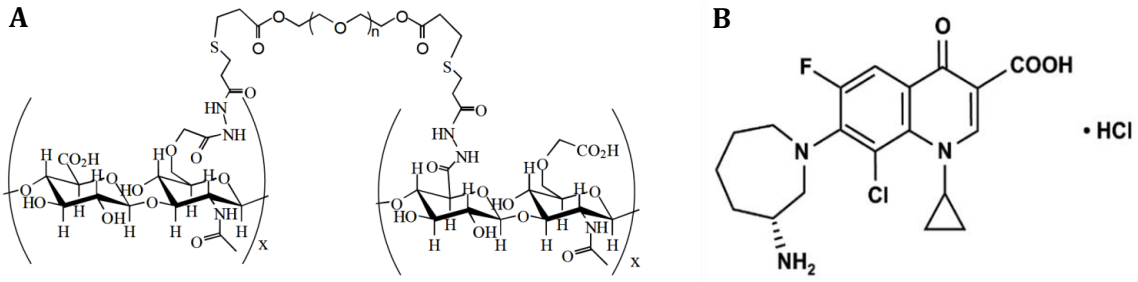

Figure 1. Chemical structures of crosslinked CMHA-S with PEGDA (A) and Besifloxacin hydrochloride (B)

Besifloxacin (50 µg to 150 µg) was encapsulated into CMHA-S and PEGDA formulation, and fabricated in FlexiPerm®, a silicone mold originally designed as a cell culture device (Figure 2A). After drying overnight, a thin Besifloxacin-loaded film was generated. The drug release was monitored in 100 µL of PBS for nine days. The result shows that Besifloxacin is continuously released *in vitro* for up to 9 days, with an initial burst (Figure 3). On day 9, approximately 1 µg was released from a 150 µg Besifloxacin loaded-film (Table 1). This is crucial because the perforation to the cornea could occur within 2-5 days if the eye is left untreated.[2]

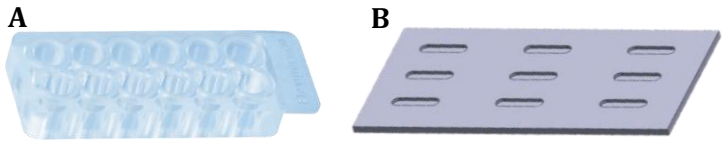

Figure 2. Two silicone molds used for fabricating films: FlexiPerm® (10 mm x 5 mm, radius) (A), Cady Bv2 mold (12 mm x 4 mm x 1 mm) (**B**)

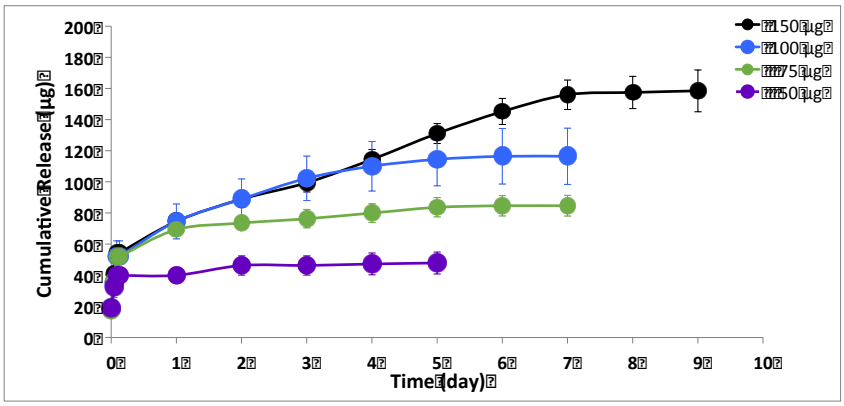

Figure 3. Release profiles from Besifloxacin films fabricated in FlexiPerm® mold. Various amounts of Besifloxacin (50-150 µg) were loaded in the films. Y-axis is the cumulative release of Besifloxacin (triplicate).

Table 1. Released Besifloxacin amount (µg) at each day up to day 9 (triplicate). The film was fabricated in FlexiPerm® mold. The amount was calculated from a Besifloxacin standard solution.

Day	0.00	0.04	0.08	1	2	3	4	5	6	7	8	9
50 µg	19	14	8	40	6	1	1	0				
75 µg	17	20	15	17	4	3	4	4	1	0		
100 µg	18	17	17	23	14	13	8	4	2	0		
150 µg	19	22	13	20	14	10	15	17	14	11	1	1

3.2. Film Fabrication In Cady Bv2 Mold And The Release Study

In order to continue *in vivo* study with rabbit, a new film shape needs to be designed. Various types of mold have been developed by Dr. Nathaniel Cady, and one of molds named as Cady Bv2 was utilized in this study (Figure 2B). The film fabrication process was same as described above except the mold. However, the process was much troublesome compared with FlexiPerm® as Cady Bv2 mold was shallower. On day 2, the film was ready for release study (Figure 4).

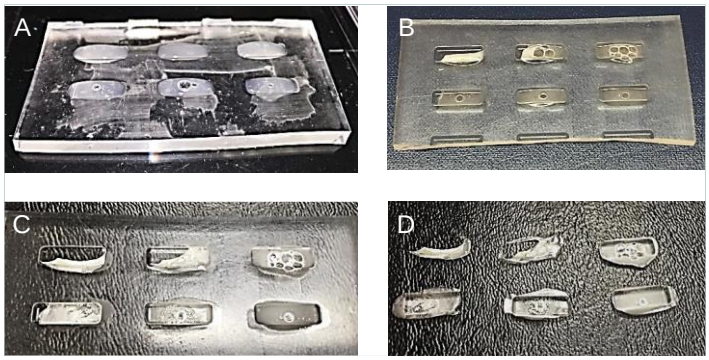

Figure 4. Film fabrication process with Cady Bv2 mold. On day 0, formulation in the mold (A); On day 1, films were being dried (**B**); On day 2, films were completely dried (**C**); On day 2, dried films were off the mold (**D**)

The release study shows that Besifloxacin is continuously released *in vitro* for up to 9 days (Figure 5), which is consistent with the previous release study with the films fabricated in the FlexiPerm® mold (Figure 4). On day 9, approximately 2 µg was released from a 150 µg Besifloxacin loaded-film (Table 2).

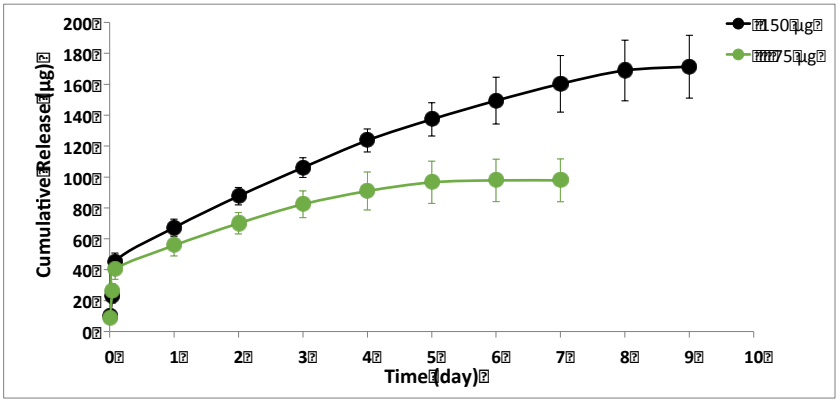

Figure 5. Release profiles from Besifloxacin films fabricated in the Cady Bv2 mold. Two different amounts of Besifloxacin (75 µg and 150 µg) were loaded in the films. Y-axis is the cumulative release of Besifloxacin (triplicate).

Table 2. Released Besifloxacin amount (µg) at each day up to day 9 (triplicate). The film was fabricated in Cady Bv2 mold. The amount was calculated from a Besifloxacin standard solution.

Day	0.00	0.04	0.08	1	2	3	4	5	6	7	8	9
75 µg	9	17	14	15	14	12	9	6	1	0		
150 µg	10	13	22	22	20	18	18	14	12	11	9	2

3.3 Zone Of Inhibition (ZOI) Study

The film was sterilized by ethylene oxide (EtO), which is widely used for the sterilization of healthcare devices and instruments. In order to evaluate the efficacy of released Besifloxacin after EtO sterilization, ZOI studies were performed. The most common pathogens associated with bacterial keratitis are *Staphylococcus Aureus* (*S. aureus*), gram-positive bacteria and *Pseudomonas aeruginosa* (*P. aeruginosa*), gram-negative bacteria.[17] Besifloxacin has shown to be potent against both bacteria, and the minimum inhibitory concentration (MIC_{90}) against *S. aureus* and *P. aeruginosa* is 0.12 µg/mL and 4 µg/mL, respectively.[17,18,19] For this current study, ZOI study has been completed only against *S. aureus*.

3.3.1 ZOI study with besifloxacin-loaded films

ZOI study was performed with the film itself against *S. aureus*. The film was placed in *S. aureus* inoculated plates, incubated 18 hours, and the ZOI was measured. The size of ZOI was comparable between pre-sterilized film and EtO-sterilized film, which suggests that the drug efficacy remains unchanged after EtO sterilization (Figure 6).

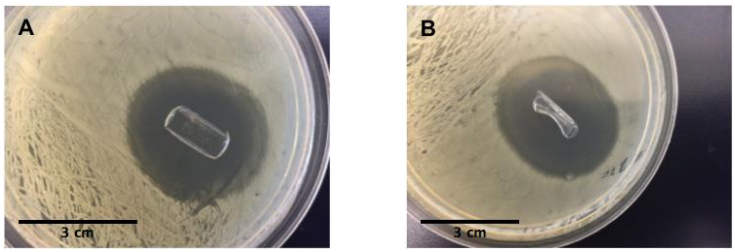

Figure 6. ZOI against *S. aureus* was compared between pre-sterilized Besifloxacin film (150 µg/film) (A) and EtO-sterilized Besifloxacin film (150 µg/film) (**B**). The drug efficacy remained unchanged after EtO sterilization.

3.3.2 ZOI study with the release samples

To determine the efficacy of daily released Besifloxacin, ZOI study was performed with the release samples. Filter disks was soaked in release media, and dried overnight. Next day, the dried filter disks were placed in *S. aureus* inoculated plates, incubated 18 hours, and the ZOI was measured. As shown in Figure 7, the efficacy of Besifloxacin remained unchanged after EtO sterilization. Furthermore, the released Besifloxacin on day 7 was still efficacious on ZOI study. Our ZOI study suggests that the Besifloxacin-loaded films have the potential to move forward into *in vivo* studies and ultimately become a clinically useful product.

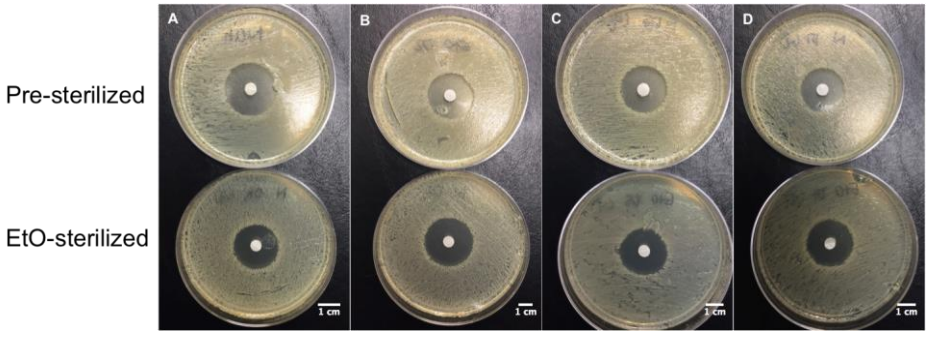

Figure 7. The filter disks were soaked in (two-fold diluted) release samples, dried, and placed on *S. aureus* inoculated plates for 18 hours. The ZOI is shown for the release samples on day 0 (**A**), day 2 (**B**), day 5 (**C**), and day 7 (**D**). On day 7, the released Besifloxacin is still efficacious on ZOI. Between the samples from pre-sterilized film (top) and the samples from EtO-sterilized film (bottom), the drug efficacy remained unchanged.

4. Conclusion

We have demonstrated that CMHA-S can deliver Besifloxacin on a sustained release manner over 9 days. As shown on ZOI study, the released Besifloxacin was efficacious until day 7, and EtO sterilization did not affect the efficacy. Besifloxacin-loaded films could represent a promising alternative to hourly topically administered antibiotic eye drops, and have the potential to move forward into *in vivo* studies and ultimately become a clinically useful product for treating corneal ulcers. Furthermore, this CMHA-S based system can be expanded to deliver other antimicrobials to treat additional indications, such as ophthalmic fungal or viral infections.

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