tvst

Article

Treatment of Corneal Infections Utilizing an Ocular Wound Chamber

Jennifer S. McDaniel[1], Laura L. F. Scott[2], Jennifer Rebeles[3], Gregory T. Bramblett[4], Elof Eriksson[5], Anthony J. Johnson[4], and Gina L. Griffith[4]

[1] Laulima Government Solutions, LLC, c/o Sensory Trauma, United States Army Institute of Surgical Research, San Antonio, TX, USA
[2] Epidemiology and Biostatistics Branch, United States Army Institute of Surgical Research, San Antonio, TX, USA
[3] BioAffinity Technologies, Science Research Laboratories (SRL) 1.424, San Antonio, TX, USA
[4] Department of Sensory Trauma, United States Army Institute of Surgical Research, San Antonio, TX, USA
[5] Applied Tissue Technologies, Hingham, MA, USA

Correspondence: Jennifer S. McDaniel, United States Army Institute of Surgical Research, Department of Sensory Trauma, Fort Sam Houston, TX 78234-6315, USA. e-mail: jennifer.s.mcdaniel7.ctr@mail.mil

Received: June 11, 2020
Accepted: July 31, 2020
Published: November 2, 2020

Keywords: wound chamber; pseudomonas; corneal wounds; bacterial keratitis; infectious keratitis

Citation: McDaniel JS, Scott LLF, Rebeles J, Bramblett GT, Eriksson E, Johnson AJ, Griffith GL. Treatment of corneal infections utilizing an ocular wound chamber. Trans Vis Sci Tech. 2020;9(12):4, https://doi.org/10.1167/tvst.9.12.4

Purpose: To demonstrate that the ocular wound chamber (OWC) can be used for the treatment of bacterial keratitis (BK).

Methods: A blepharotomy was performed on anesthetized, hairless guinea pigs to induce exposure keratopathy 72 hours before corneal wound creation and *Pseudomonas aeruginosa* inoculation. Twenty-four hours postinoculation, eyes were treated with an OWC filled with 500 µL 0.5% moxifloxacin hydrochloride ophthalmic solution (OWC), 10 µL 0.5% moxifloxacin hydrochloride drops (DROPS) four times daily, or not treated (NT). White light, fluorescein, and spectral domain optical coherence tomography (SD-OCT) images; ocular and periocular tissues samples for colony-forming units (CFU) quantification; and plasma samples were collected at 24 and 72 hours posttreatment.

Results: White light, fluorescein, and SD-OCT imaging suggests OWC-treated eyes are qualitatively healthier than those in DROPS or NT groups. At 24 hours, the median number of CFUs (interquartile range) measured was 0 (0–8750), 150,000 (106,750–181,250), and 8750 (2525–16,000) CFU/mL for OWC, NT, and DROPS, respectively. While 100% of NT and DROPS animals remained infected at 24 hours, only 25% of OWC-treated animals showed infection. Skin samples at 24 hours showed infection percentages of 50%, 75%, and 0% in DROPS, NT, and OWC groups, respectively. OWC-treated animals had higher moxifloxacin plasma concentrations at 24 and 72 hours than those treated with drops.

Conclusions: OWC use resulted in a more rapid decrease of CFUs when compared to DROPS or NT groups and was associated with qualitatively healthier ocular and periocular tissue.

Translational Relevance: The OWC could be used clinically to continuously and rapidly deliver antimicrobials to infected ocular and periocular tissues, effectively lowering bacterial bioburdens and mitigating long-term complications.

Introduction

Infection of the cornea or infectious keratitis (IK) is an ophthalmic emergency and can progress to blindness if not promptly and adequately treated. Caused by bacterial, viral, fungal, and parasitic agents, IK is a major contributor to corneal opacification and blindness worldwide.[1] Complications from IK develop quickly and can lead to ulceration of the cornea, neovascularization, corneal scarring and opacification, perforation, endophthalmitis, and, in the most severe cases, blindness and/or eye loss.[2,3] Due to the potential for these serious sequelae, IK remains a leading cause of blindness globally.[4–6]

Risk factors for the development of IK vary worldwide depending on geographical location. In developing countries such as Nepal and India, nonsurgical trauma, primarily related to agricultural work and outdoor occupations, accounts for 48.6% to 65.4%[7,8]

of all corneal ulcers. In contrast, only 27%[9] of corneal ulcers result from nonsurgical injuries in the United States.[10] While IK etiologies differ between developed and developing countries, bacterial keratitis (BK) is of particular concern in the United States due to widespread contact lens usage.[11,12] Recent data suggest that the cost of treating BK is between $377 to $857 million per year and is one of the major causes of ocular infections requiring immediate treatment to prevent vision loss.[13] Additional risk factors for developing BK include ocular surface disease, previous ocular surgery, and the use of immunosuppressive drugs.[4,14] Essentially, any damage to the corneal epithelium creates the potential for a bacterial superinfection and subsequent progression to corneal ulceration and permanent vision loss.

A number of causative organisms have been isolated from the infected corneas of patients with BK, including *Staphylococcus aureus*, *Streptococcus* spp., and *Pseudomonas aeruginosa*, which is the most commonly isolated species among contact lens wearers. In addition to being one of the most pathogenic agents and capable of causing corneal perforation in as a little as 72 hours,[4] patients infected with *P. aeruginosa* have also been shown to have worse visual acuity outcomes than those infected with other pathogens,[3] which is why we selected *P. aeruginosa* for this study.

Bacterial keratitis requires early and aggressive treatment in order to mitigate poor clinical outcomes. Due to the rapid corneal destruction observed with this disease, any presumptive cases of BK should be immediately treated as BK until a definitive diagnosis otherwise is made.[4] Treatments to manage BK are limited, with the primary course of therapy being topical, broad-spectrum antimicrobial drops plus occasional adjuvant steroid application to reduce the immune response.[15] As previously mentioned, BK can lead to blindness in as little at 72 hours if the progression of the disease cannot properly be controlled with antibiotic ophthalmic drops. Ophthalmic drops have to be administered frequently, as often as hourly in severe cases of BK, and patient compliance is often poor, especially in elderly populations or those with physical limitations that preclude the proper instillation of the drops. Furthermore, the majority of a drop instilled into an eye will be cleared from the eye as soon as the patient blinks, allowing short contact time of the drug on the ocular surface.[16]

To address the lack of effective therapeutic options to treat BK and to reduce the burden of care, we have developed and employed an ocular wound chamber (OWC) for use as a therapeutic delivery device that can be safely applied to the periorbital area of the eye. The OWC, originally adapted from the dermal platform wound device (PWD),[17–19] comprises a flexible, adherent base equipped with a transparent cover that surrounds the perimeter of the eye, protecting it from the external environment. A self-sealing port incorporated into the cover allows for the controlled delivery of therapeutics into the chamber space, creating a fluid-filled environment to promote reepithelialization of injured tissues, and also increases the residence time of drugs on the ocular surface and periocular tissues.[20,21] The OWC not only protects the eye and periocular tissues but also provides for the therapeutic agent to remain in contact with the eye for an increased period of time, allowing for the treatment of a variety of periocular and/or corneal surface injuries, including chemical/thermal burns and, as investigated in this study, ocular infection.

Previous studies from our laboratory have shown that the OWC can be safely and effectively used on corneal epithelial injuries and can also be used to prevent exposure keratopathy in our in vivo models of corneal epithelial wound healing and blepharoplasty, respectively.[20,21] Furthermore, other studies have demonstrated the use of dermal wound chambers to create a fluid-filled environment for skin injuries by augmenting reepithelialization of damaged tissue while also delivering topical antimicrobials to the area, thereby reducing bacterial loads and inflammation.[22,23] Given the ability of the OWC to provide a fluid-filled environment, this device has great therapeutic potential for a wide range of injuries/disorders, including keratitis, corneal ulcers, lagophthalmos, and periorbital trauma. However, it is not known if the OWC can be used as a drug delivery device to safely and effectively treat IK. In this study, we aimed to demonstrate that the OWC can be used as a novel treatment modality for BK by decreasing the bacterial burden more rapidly than ophthalmic drop delivery while simultaneously protecting surrounding periocular tissues.

Methods

Animals

Hairless, female Institute Armand Frappier guinea pigs (200–250 g, Crl:HA-Hr^{hr}) were acquired from Charles River Laboratories (Wilmington, MA) and acclimated for at least 72 hours prior to the beginning of the study. Randomly grouped animals ($n = 4$ per group) were anesthetized and administered analgesics as previously described.[20,21] Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals, National Research Council. The facility's Institutional Animal Care and Use Committee

approved all research conducted in this study. The facility where this research was conducted is fully accredited by the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All animals were maintained and handled according to institutional guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Blepharotomy

A blepharotomy was performed to remove the upper and lower eyelids as previously published.[21] Briefly, after general anesthetization of animals, a 25-gauge needle was used to inject 0.05 to 0.1 mL 2% lidocaine HCL with epinephrine 1:100,000 USP into the upper and lower eyelids to control for excess bleeding. Forceps with teeth (#12) were used to grip the upper and lower eyelids while Westcott scissors were employed to remove the entire upper and lower lid from the orbital rim. Hemostasis was accomplished with a high-temperature handheld cautery device as necessary. Animals were allowed to recover from the blepharotomy procedure for 72 hours prior to corneal epithelial wound creation and infection induction as described in this article.

P. aeruginosa Isolates

P. aeruginosa strain 27853 was obtained from American Type Culture Collection (Manassas, VA). Stock cultures were initially grown in Luria Bertani (LB) broth (Lennox, https://www.sigmaaldrich.com/catalog/product/sigma/l3022?lang=en&region=US&gclid=CjwKCAjwkJj6BRA-EiwA0ZVPVptExTEJZDJ1Tahda05QOohgGzhn8stSk612x-mY8SebpIbrk7b4lBoCcckQAvD_BwE) microbial growth medium (Sigma-Aldrich, St. Louis, MO) overnight at 37°C with shaking. For bacterial colony isolation, a 1-µL inoculation loop was used to inoculate a plain, Alfa Aesar LB Agar plate (Fisher Scientific, Waltham, MA). After overnight incubation at 37°C, a colony with a small morphology, shown to produce both fluorescein and pyocyanin, was isolated and subcultured on *Pseudomonas* isolation agar (Hardy Diagnostics, Santa Maria, CA). For experimental studies, a bacterial culture was grown overnight at 37°C with shaking in 5 mL LB broth from an isolated colony cultured on *Pseudomonas* isolation agar (Hardy Diagnostics). Spectrophotometric readings (520 nm; SmartSpec Plus Spectrophotometer; BioRad, Hercules, CA) were taken of overnight cultures followed by dilution to a bacterial concentration of 1×10^8 colony forming units (CFU)/mL. Bacterial dilutions were serially plated to confirm the CFU/mL placed on the eye.

P. aeruginosa Keratitis Model

Seventy-two hours after the blepharotomy procedure, a corneal epithelial wound was created using a corneal rust ring remover (Algerbrush II; Alger Company, Inc., Lago Vista, TX) as previously described.[20] Briefly, a trephine was used to demarcate a 4-mm area on the central cornea of left eyes followed by epithelium removal. Fluorescein staining was used to verify the uniform removal of the epithelium. Immediately, following creation of the corneal epithelial defect, 1×10^8 CFU/mL of *P. aeruginosa* 27853 was applied to the left of eye of each guinea pig. Animals were allowed to recover for 24 hours prior to commencement of treatment. Animals were then treated with an OWC loaded with 500 µL 0.5% moxifloxacin ophthalmic solution (Alcon, Fort Worth, TX) ($n = 4$; OWC), one 10-µL drop of 0.5% moxifloxacin ophthalmic solution ($n = 4$; DROPS) to the infected eye four times daily, or no treatment ($n = 4$; NT). For animals that received an OWC, the eye and surrounding tissues were prepared as previously described.[20] Following the removal of residual periorbital hair, the skin was cleaned with 70% alcohol and dried to ensure adherence of the OWC. After application of the OWC, 500 µL 0.5% moxifloxacin ophthalmic solution was injected into the OWC through the self-sealing silicone port using a 25-gauge 1-in. needle. The OWC and 0.5% moxifloxacin were replaced every 24 hours after assessments and imaging for the duration of the study.

Bacterial Quantification

At 24 and 72 hours posttreatment, animals were humanely euthanized, and whole eye globes and periocular tissue were collected and immediately placed on ice. Samples were placed in sterile tubes containing ceramic beads (MagNA Lyser Green Beads; Roche, Indianapolis, IN) and 1 mL sterile phosphate buffered saline (Gibco, Grand Island, NY). The samples were homogenized for 60 seconds at 5.5 m/s using a bead beater (MP BIOMEDICALSFastPrep-24 5G Instrument, Santa Ana, CA). Following homogenization, homogenate was plated at 1/10 and 1/100 dilutions on *Pseudomonas* isolation agar (Hardy Diagnostics). Plates were allowed to dry before being inverted and placed at 37°C overnight. After overnight incubation, colonies were manually counted. The CFU/mL was calculated using the following formula:

$$\frac{number\ of\ colonies \times dilution\ factor}{volume\ of\ culture\ plate}$$

White Light and Fluorescein Ocular Imaging

White light and fluorescein ocular imaging was performed as previously published.[20] Images were collected at specified time points using a surgical microscope equipped with a camera and a cobalt blue filter (OPMI VISU 200 S8; Carl Zeiss Surgical, Oberkochen, Germany). Sterile fluorescein sodium ophthalmic films USP (Fluorets; Chauvin Laboratory, Aubenas, France) were moistened with 100 µL balanced salt solution (BSS; Alcon), and a drop was placed onto the eye for 10 seconds before the eyes were rinsed with BSS (Alcon). Images of fluorescein uptake were acquired using the described surgical microscope under cobalt blue light, and analysis was performed using ImageJ (National Institutes of Health, Bethesda, MD). The data are reported as the area of fluorescein adherence in pixels.

Optical Coherence Tomography

To visualize corneal surface anatomy, images of infected eyes were obtained at the indicated time points using spectral domain optical coherence tomography (SD-OCT, Micron IV; Phoenix Research Laboratories, Pleasanton, CA) at the indicated time points as previously described.[20,21]

Liquid Chromatography–Tandem Mass Spectrometry

Blood samples were collected from anesthetized guinea pigs just prior to euthanasia via intracardiac puncture into EDTA-treated blood tubes. Samples were placed on ice and then centrifuged at 1000 *g*, 4°C, for 20 minutes. Plasma samples were aliquoted and stored at −70°C until ready to assay. Aliquots were thawed on ice for 30 minutes and subsequently centrifuged at 17,000 *g* for 20 minutes. Supernatants were spiked with internal standard vancomycin (Sigma-Aldrich) at 1 µg/mL prior to liquid chromatography–mass spectrometry–mass spectrometry (LC-MS-MS) analysis. LC-MS-MS analysis was conducted as previously described.[24,25] Briefly, antibiotics were bound to a 2.1 × 50-mm, 5-µm C18 column (Agilent ZORBAX Eclipse XDB 80Å; Agilent Technologies, Santa Clara, CA) at 40°C for 30 seconds with 100% mobile phase A. The column was eluted with a linear gradient from 0% to 90% mobile phase B for 90 seconds. Analysis time was 4 minutes for one injection. Moxifloxacin (Sigma-Aldrich) standards 0 to 2 µg/mL were used to generate a standard curve. Vancomycin (1 µg/mL; Sigma-Aldrich) served as the internal control.

Mass spectrometric analysis was carried out on an AB Sciex API 4000 mass spectrometer (AB Sciex, Framingham, MA) with an electrospray ionization interface and triple quadrupole mass analyzer. The mass spectrometer was operated in multiple-reaction monitoring mode[26] via the positive electrospray ionization interface using the mass charge ratios (*m*/*z*) of 725.5/144.0 (vancomycin) and 402.2/358.1 (moxifloxacin). For the MS/MS analysis of vancomycin, the declustering potential was set to 75 V, the collision energy 24 V, and collision cell exit potential 7.2 V with an *m*/*z* set at 725.5/144.0. For the MS-MS analysis of moxifloxacin, the declustering potential was set to 90 V, the collision energy 26.89 V, and collision cell exit potential 7.9 V with an *m*/*z* set at 402.2/358.1. Capillary voltage was set to 4.5 kV.

Statistical Analyses

A linear mixed model for repeated measures was used to analyze fluorescein staining data. In this regression model, time, treatment group, and the interaction of these two factors were used as fixed explanatory variables. A compound symmetry covariance structure was used for the model as determined using Akaike information criterion and Bayesian information criterion statistics. Differences among treatment groups in the number of CFUs from harvested eye and skin tissue as well as the concentration of plasma moxifloxacin were analyzed using the Kruskal-Wallis test, stratified by time point. The *P* values for all post hoc pairwise comparisons (p_{adj}) were adjusted according to the appropriate method (i.e., Dwass-Steel-Critchlow-Fligner or Tukey-Kramer). All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). Significance was evaluated using an experiment-wise alpha of 0.05. Fluorescein data are presented as means and 95% confidence intervals; CFU and moxifloxacin data are presented as medians and interquartile ranges (IQRs).

Results

Clinical Evaluation of Infected Ocular and Periocular Tissues

Clinical evaluation of white light imaging data (Fig. 1) qualitatively revealed less inflammation at all collected time points in periocular tissues of animals treated with the OWC when compared to those that received no treatment or were treated with 0.5% moxifloxacin ophthalmic drops. Furthermore, the corneal surfaces of eyes treated with drops or received

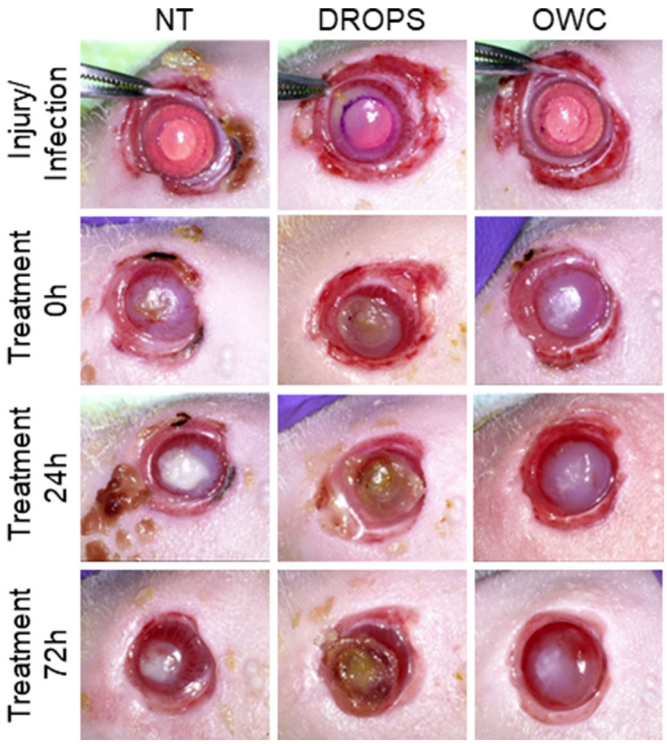

Figure 1. White light images of eyes taken immediately after corneal injury and infection induction and at 0, 24, and 72 hours posttreatment. Clinical examination showed less inflammation of the periocular tissues in animals treated with the OWC when compared to those that received no treatment (NT) or were treated with 0.5% moxifloxacin ophthalmic drops (DROPS) at all observed time points (0, 24, and 72 hours). Overall, less periocular inflammation and corneal desiccation were observed in animals treated with the OWC compared to other groups at all time points.

no treatment were more desiccated than eyes treated with the OWC. While qualitative, these clinically relevant findings are supported by previously published pathologic results showing significantly lower levels inflammatory cytokines as well as fibrosis in the skin samples taken from OWC-treated animals using the same blepharotomy guinea pig model.[21]

To assess the corneal anatomy and integrity of the corneal surface, fluorescein staining was conducted (Fig. 2A) and quantitated (Fig. 2B) at the time of inoculation with *P. aeruginosa* 27853 and at 0, 24, and 72 hours posttreatment. The effects of treatment group ($P < 0.01$), time ($P < 0.01$), and treatment group by time ($P < 0.05$) were all significant in the model. Specifically, significantly more fluorescein staining was observed at 72 hours in eyes treated with an OWC when compared to eyes that were untreated ($P_{adj} < 0.01$) or received 0.5% moxifloxacin ophthalmic drops ($P_{adj} < 0.01$; Figs. 2A, 2B). These data demonstrate a corneal surface that is nondesiccated and capable of fluorescein staining. Indirectly, the results suggest a healthier ocular surface structure when compared to the desiccated corneas observed in the NT and DROPS groups.

OCT imaging was conducted to further assess the integrity of the corneal surface anatomy (Fig. 3). These results supported the fluorescein staining observations and showed irregular corneal anatomy in the NT and DROPS groups. In eyes that were treated with an OWC, some stromal edema was noted, but otherwise a more normal corneal anatomy was observed.

Evaluation of Bacterial Load in Ocular and Periocular Tissues

While the clinical observations from this study are supported by our previous work demonstrating the OWC is safe, is effective, and results in less inflammation in periocular tissues,[20,21] the current study was intended to determine if the OWC could be used to decrease the bacterial bioburden in both the ocular and periocular tissues in our BK model. The number of CFUs measured in ocular tissue was significantly associated with treatment group at 24 hours ($P < 0.05$) but not at 72 hours ($P = 0.14$) (Figs. 4A and 4B, respectively). Although there were no statistically significant differences in CFU counts between each of the treatment groups at 24 hours after adjusting for multiple pairwise comparisons, there were clinically meaningful differences in both the median number of CFUs and the proportion of animals with active infections. At 24 hours, the median number (interquartile range [IQR]) of CFUs measured in the OWC group was 0 (0–8750) CFU/mL compared to 150,000 (106,750–181,250) CFU/mL for the NT group and 8750 (2525–16,000) CFU/mL for the DROPS group, with 100% of the animals in the NT and DROPS groups having active *Pseudomonas* infections and only 25% of the animals treated with an OWC having an active infection. By 72 hours, 25% of the animals in the DROPS group still had an active infection while all of the animals that received no treatment and 75% of the animals in the OWC group were still infected. Nevertheless, the median CFU counts measured at 72 hours in animals treated with the OWC were extraordinarily low relative to the NT group (150 [50–300] vs. 29,250 [2575–63,000] CFU/mL, respectively).

Statistical differences in CFUs were not observed among the three different treatment groups for periocular skin tissue harvested at either time point, 24 hours ($P = 0.34$) or 72 hours ($P = 0.48$) (Figs. 5A, 5B). From a clinical perspective, however, it is important to note that at 24 hours, 50% of skin samples taken from animals that received drops and 75% of animals that received no treatment showed continued microbial

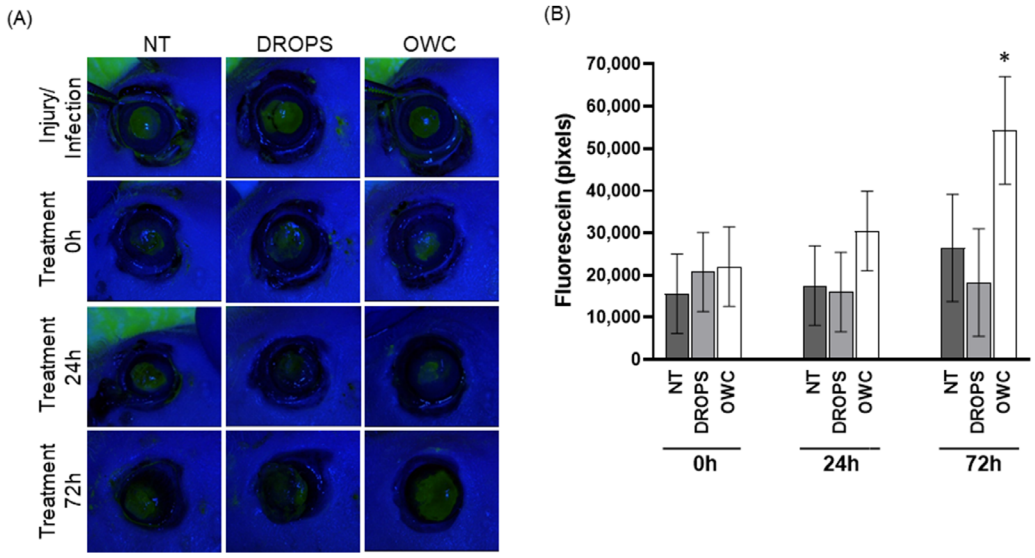

Figure 2. Fluorescein staining was conducted (A; representative images) and quantitated (B) at the time of corneal injury/infection induction and at 0, 24, and 72 hours posttreatment. Significantly more staining was observed at 72 hours posttreatment in eyes treated with an OWC when compared to eyes that were untreated (NT) or received 0.5% moxifloxacin ophthalmic drops (DROPS) (B). *$P_{adj} < 0.01$.

infection compared to 0% of animals receiving an OWC. At 72 hours, all animals treated with an OWC completed microbial clearance, while 50% of those that received no treatment and 25% of those in the DROPS group were still infected.

Evaluation of Therapeutic Antimicrobial Plasma Concentrations

There were no significant differences in moxifloxacin plasma concentrations between animals that received drops and those that received an OWC at either 24 hours ($P = 0.06$) or 72 hours ($P = 0.12$) posttreatment (Figs. 6A and 6B, respectively). However, the results have potentially meaningful clinical implications. At 24 hours posttreatment, the median (IQR) plasma moxifloxacin concentration for animals treated with an OWC (1.68 [0.47–4.05] µg/mL) was 24 times higher than for animals that received drops four times daily (0.07 [0.06–0.09] µg/mL). By 72 hours posttreatment, the median (IQR) moxifloxacin plasma concentration for animals treated with an OWC (0.50 [0.30–0.63] µg/mL) was approximately seven times higher than for those animals in the DROPS group (0.07 [0.05–0.15] µg/mL).

Discussion

Current therapies for the treatment and management of ocular surface and periocular injuries and infection are presently limited. In patients with periocular trauma, modified devices such as eye patches, swim goggles, or cellophane wrap have been used in combination with ophthalmic drops and lubricants to create moisture chambers, which often result in poor clinical outcomes.[20,21] Topical administration of eye drops is the current standard of care to treat most anterior segment diseases. However, due to the inherent barriers found within the ocular anatomy, including tear film, blinking, and drainage, drug delivery to the eye is clinically challenging.[27] After instillation of eye drops, most solutions are cleared within 15 to 30 seconds, resulting in little contact time between the ocular surface and the therapeutic. For patients requiring the use of frequent ophthalmic drop instillation for the treatment of ocular surface injury or infection, insufficient residence time of the drug on the eye and poor patient compliance lead to unsatisfactory results.[28] To circumvent these challenges, we sought to investigate the use of the OWC as a novel treatment modality to not only deliver drugs to the ocular surface but to also facilitate healing of damaged periocular tissues.

Several studies by our laboratory have demonstrated that the OWC can be safely applied to both uninjured and injured periocular tissue without further compromising ocular structures.[20,21] Furthermore, when used in our guinea pig models of corneal abrasion and exposure keratopathy, we observed a significant decrease in inflammation and fibrosis as based on molecular studies and histologic analyses.[20,21] While these studies have demonstrated the expanding utility of the OWC for the treatment of ocular disparities

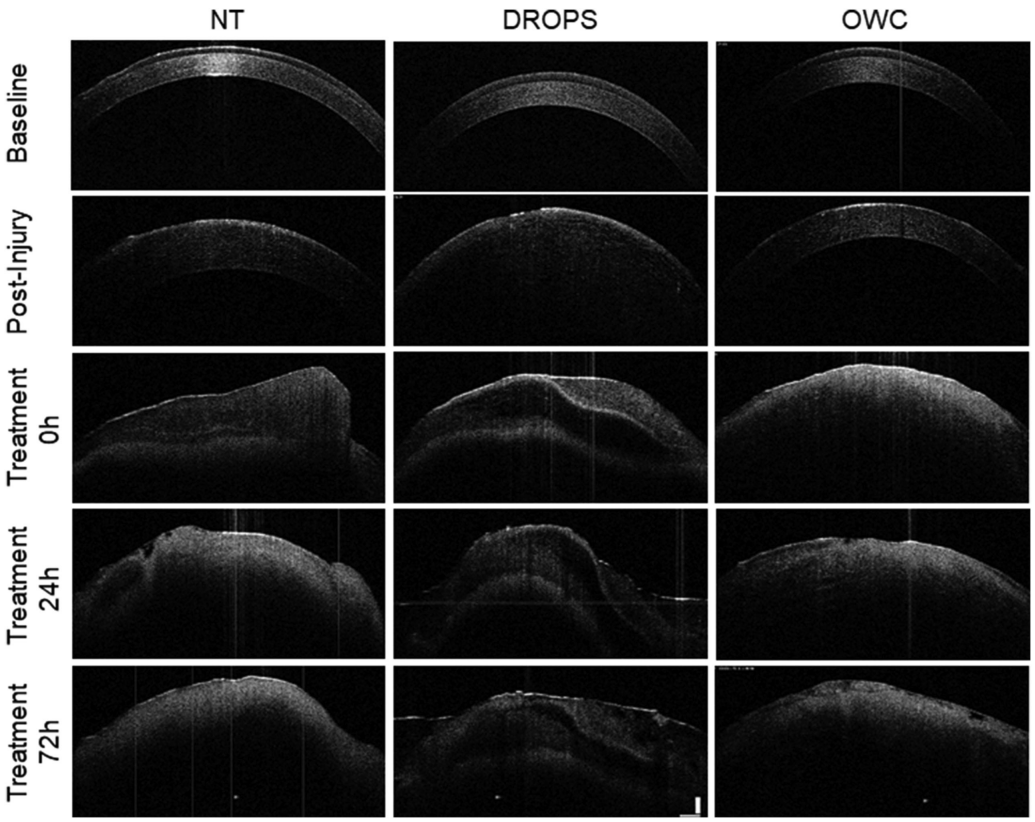

Figure 3. Representative corneal SD-OCT images of untreated (NT), 0.5% moxifloxacin ophthalmic drops (DROPS), and OWC-treated eyes prior to blepharotomy, corneal abrasion, and infection (baseline) at the time of injury/infection (postinjury) and at 0, 24, and 72 hours posttreatment. In eyes that were treated with an OWC, some stromal edema was noted, but otherwise, a more normal corneal anatomy was observed posttreatment. Abnormal corneal surface anatomy of NT and DROPS animals was observed at 24 and 72 hours posttreatment.

and damaged ocular/periocular tissues, no studies to date have investigated the use of the device for treating BK in conjunction with damaged periocular tissue. To address this gap in knowledge, we developed a guinea pig model of BK accompanied by periocular trauma to determine the effectiveness of delivering antimicrobials to the infected eyes via the OWC.

In the present study, we observed infected corneal surfaces and damaged periocular tissues through the use of white light, fluorescein, and OCT imaging. Gross clinical observations of injured skin tissue via white light imaging showed less inflammation in periorbital skin where the OWC had been applied, corresponding with what we have seen in previous studies. This is also consistent with results from our earlier studies showing that samples from injured periorbital skin treated with an OWC not only had lower levels of IL-13 and IL-15 cytokines but also had significantly lower levels of fibrosis compared to untreated controls.[21] These findings are also in line with work by others showing dermal wounds heal more efficiently in a moist or fluid-filled environment such as the one provided by the OWC.[17–19,29] Furthermore, studies conducted by Tsai et al.[30] have demonstrated that the application of a dermal PWD for delivery of topical antibiotics to infected porcine dermal tissue led to a rapid reduction in microbial counts in infected burns. In summary, the expeditious clearance of pathogens from infected tissue is essential for precipitating wound healing and mitigating downstream sequelae.

We acknowledge the limitations of using fluorescein staining as an indicator of corneal epithelial integrity in this animal model due to the extreme corneal desiccation experienced as a result of blepharotomy-induced exposure keratopathy, particularly in the NT and the standard-of-care (drops-only) animal groups. However, we believe relevant clinical findings can be gleaned from observations made during this study. For example, fluorescein staining is typically used to identify defects in the ocular surface, and adherence is restricted in healthy eyes.[31] In previous studies, we have used fluorescein staining as a means to monitor corneal epithelial wound closure over time; as fluorescein adherence decreases, wound closure increases.[20,21] Conversely, with this study, we found that due to the

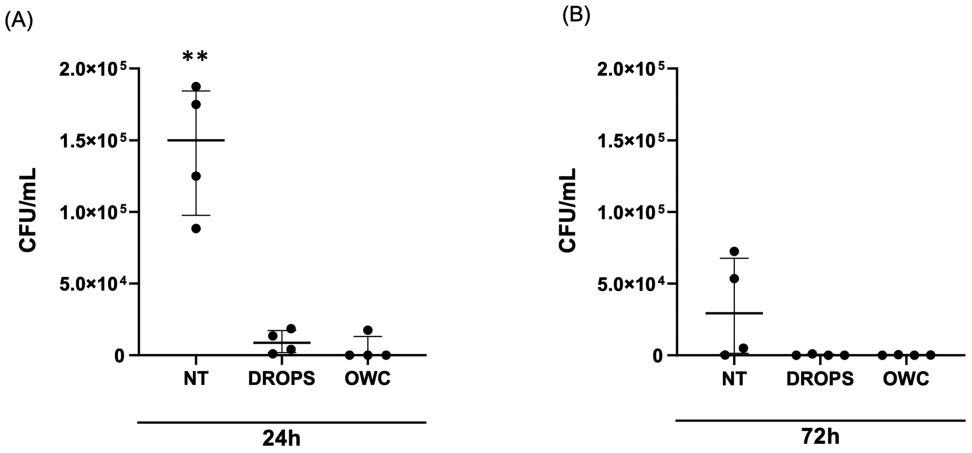

Figure 4. CFU counts were conducted at 24 hours (A) and 72 hours (B) posttreatment to assess the bacterial load of ocular tissue. The number of colony-forming units measured in ocular tissue was significantly associated with treatment group at 24 hours ($P < 0.05$) but not at 72 hours ($P = 0.14$). There were no statistically significant differences in CFU counts between each of the treatment groups at 24 hours after adjusting for multiple pairwise comparisons. However, the median number of CFUs (IQR) measured in the OWC group was 0 (0–8750) CFU/mL compared to 150,000 (106,750–181,250) CFU/mL for the NT group and 8750 (2525–16,000) CFU/mL for the DROPS group, with 100% of the animals in the NT and DROPS groups having active infections and only 25% of the animals treated with an OWC having an active infection. By 72 hours, 25% of the animals in the DROPS group still had an active infection while all of the NT animals and 75% of the OWC animals were still infected. Yet, the median CFU counts measured at 72 hours in animals treated with the OWC were extraordinarily low relative to the NT group (150 [50–300] vs. 29,250 [2575–63,000] CFU/mL, respectively).

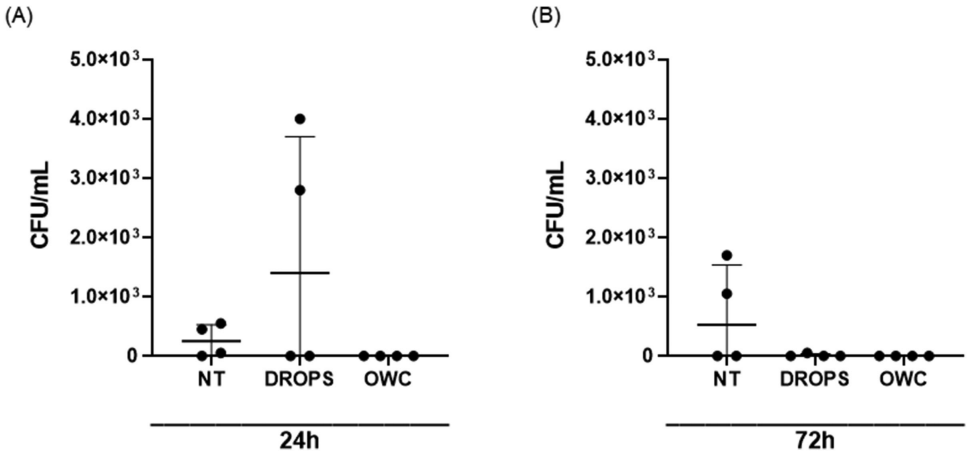

Figure 5. CFU counts were performed at 24 hours (A) and 72 hours (B) posttreatment to assess bacterial loads in periocular skin. Statistical differences in CFUs were not observed among the three different treatment groups at either 24 hours ($P = 0.34$) or 72 hours ($P = 0.48$). At 24 hours (A), 50% of skin samples taken from animals that were treated with 0.5% moxifloxacin ophthalmic drops (DROPS) and 75% of animals that received no treatment (NT) showed continued bacterial bioburden while all animals treated with the OWC showed complete microbial clearance. At 72 hours (B), 0% of skin samples from OWC group were still infected, while 50% of the NT group and 25% of the DROPS group remained infected.

desiccation of the eyes, an increase in fluorescein staining appeared to signal an increase in eye health in those animals treated with the OWC as compared to NT or DROPS simply because the desiccated eyes were unable to take up the fluorescein dye. Therefore, given the ability of the OWC to provide a fluid-filled environment to the eyes and prevent desiccation, the resulting corneal structure appeared to be more normal overall with less edema and irregular anatomy than that seen in the NT and DROPS groups. Taken together, gross white light observations, fluorescein staining, and OCT imaging all suggest that eyes treated with an OWC are qualitatively healthier than those treated with drops only or not treated at all.

Currently, the diagnosis of BK is limited to clinical observations, including case history, conjunctival

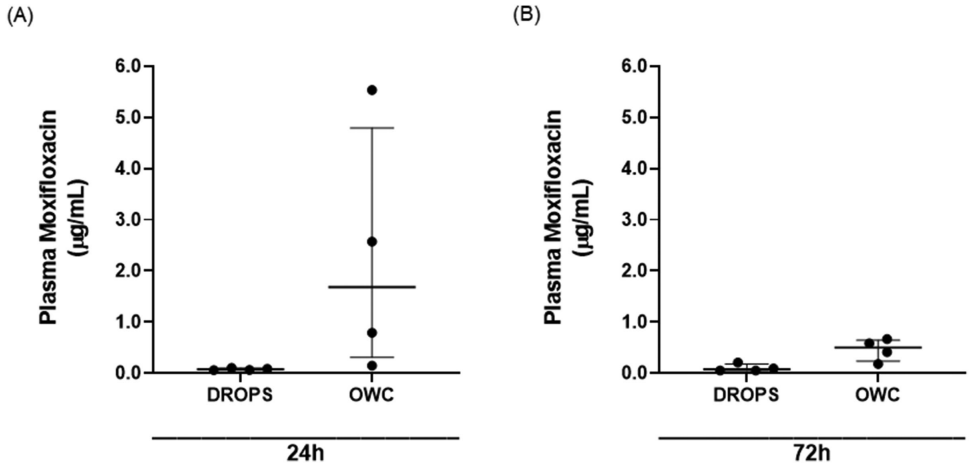

Figure 6. Plasma samples were taken at 24 hours (A) and 72 hours (B) posttreatment and analyzed for the presence of moxifloxacin. Plasma samples from animals that did not receive any treatment were used to normalize treatment groups. No significant differences were observed at either 24 hours ($P = 0.06$) or 72 hours ($P = 0.12$) posttreatment. However, at 24 hours posttreatment (A), the median (IQR) plasma moxifloxacin concentration for animals treated with the OWC (1.68 [0.47–4.05] mg/mL) was 24 times higher than for animals that received drops (0.07 [0.06–0.09] mg/mL) and, at 72 hours posttreatment, (B) approximately seven times higher in OWC-treated animals (0.50 [0.30–0.63] mg/mL) than for those animals that received drops (0.07 [0.05–0.15] mg/mL).

inflammation and discharge, and the presence of any corneal defects, infiltrate, or signs of corneal thinning/perforation confirmed via fluorescein staining and slit-lamp biomicroscopy.[6,32,33] For larger lesions or nonresponsive lesions, corneal scrapings are collected for microbiologic analyses. However, the commencement of treatment should not be delayed for identification due to the virulence and corneal destruction associated with many microorganisms. The current standard of care for the treatment of BK remains immediate administration of antibiotic therapy. As previously mentioned, due to the eye's unique physiologic and anatomic barriers, including tear turnover, drainage, blinking, and cellular junctions, drug delivery to the ocular surface is inadequate.[34] Studies by others have shown the PWD delivery of high concentrations of topical antimicrobials to infected wounds allows for the percutaneous absorption of therapeutics directly at the site of infection, rapidly reducing the bacterial bioburden and avoiding side effects often seen with oral or intravenous therapeutics.[22,23,30] As a result, we aimed to use our model of BK to demonstrate that the continuous delivery of an ophthalmic antimicrobial to infected eyes via an OWC, adapted from PWD technology, would result in rapid microbial clearance in infected tissue.

Previous studies from our laboratory used quantitative pathologic and molecular methods to study the safety and effectiveness of the OWC in our corneal epithelial wound healing and blepharoplasty models. In this study, a combination of qualitative and quantitative methods was used. Qualitative observations were used to analyze the clinical manifestations observed in white light, fluorescein, and SD-OCT images. CFU counts and plasma samples were obtained to quantitatively assess the persistence or absence of bacteria in the ocular and periocular tissues as well as the amount of moxifloxacin in the plasma. These studies demonstrate qualitatively that the OWC may be safely and effectively used to deliver ophthalmic antibiotics to infected ocular and periocular tissues. Through the qualitative analysis of CFU data, we were able to show that there was increased microbial clearance in infected corneal and periorbital tissue in animals treated with an OWC to deliver sustained, topical antimicrobial therapy. Higher amounts of moxifloxacin in the plasma of OWC-treated animals suggest that OWC use allows for the therapeutic agent to remain in contact with the ocular and periocular tissues for an increased period of time, resulting in increased percutaneous absorption of the therapeutics into the tissue. When taken together, these results indicate the potential clinically utility of the OWC to quickly reduce the bacterial bioburden in ocular and periocular tissues, thereby mitigating downstream sequelae and preventing vision loss.

Acknowledgments

Supported by the US Army Medical Research and Development Command (MRDC) Clinical and

Rehabilitative Medicine Research Program (PAD5) CRM0003. The views expressed in this article are those of the authors and do not reflect the official policy or position of the US Army Medical Department, Department of the Army, Department of Defense, or the US government.

Disclosure: **J.S. McDaniel,** None; **L.L.F. Scott,** None; **J. Rebeles,** None; **G.T. Bramblett,** None; **E. Eriksson,** Applied Tissue Technologies (F); **A.J. Johnson,** None; **G.L. Griffith,** None

References

1. Pascolini D, Mariotti SP. Global estimates of visual impairment: 2010. *Br J Ophthalmol*. 2012;965:614–618.
2. Alkatan HM, Al-Essa RS. Challenges in the diagnosis of microbial keratitis: a detailed review with update and general guidelines. *Saudi J Ophthalmol*. 2019;333:268–276.
3. Hilliam Y, Kaye S, Winstanley C. *Pseudomonas aeruginosa* and microbial keratitis. *J Med Microbiol*. 2020;691:3–13.
4. Al-Mujaini A, Al-Kharusi N, Thakral A, Wali UK. Bacterial keratitis: perspective on epidemiology, clinico-pathogenesis, diagnosis and treatment. *Sultan Qaboos Univ Med J*. 2009;92:184–195.
5. Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. *Bull World Health Organ*. 2001;793:214–221.
6. Upadhyay MP, Srinivasan M, Whitcher JP. Diagnosing and managing microbial keratitis. *Community Eye Health*. 2015;2889:3–6.
7. Upadhyay MP, Karmacharya PC, Koirala S, et al. The Bhaktapur eye study: ocular trauma and antibiotic prophylaxis for the prevention of corneal ulceration in Nepal. *Br J Ophthalmol*. 2001;854:388–392.
8. Srinivasan M, Gonzales CA, George C, et al. Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India. *Br J Ophthalmol*. 1997;8111:965–971.
9. Ormerod LD, Hertzmark E, Gomez DS, et al. Epidemiology of microbial keratitis in southern California: a multivariate analysis. *Ophthalmology*. 1987;9410:1322–1333.
10. Jeng BH, McLeod SD. Microbial keratitis. *Br J Ophthalmol*. 2003;877:805–806.
11. Bourcier T, Thomas F, Borderie V, Chaumeil C, Laroche L. Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. *Br J Ophthalmol*. 2003;877:834–838.
12. Collier SA, Gronostaj MP, MacGurn AK, et al. Estimated burden of keratitis—United States, 2010. *MMWR Morb Mortal Wkly Rep*. 2014;6345:1027–1030.
13. Smith AF, Waycaster C. Estimate of the direct and indirect annual cost of bacterial conjunctivitis in the United States. *BMC Ophthalmol*. 2009;9:13.
14. Green M, Apel A, Stapleton F. Risk factors and causative organisms in microbial keratitis. *Cornea*. 2008;271:22–27.
15. Austin A, Lietman T, Rose-Nussbaumer J. Update on the management of infectious keratitis. *Ophthalmology*. 2017;12411:1678–1689.
16. Urtti A. Challenges and obstacles of ocular pharmacokinetics and drug delivery. *Adv Drug Deliv Rev*. 2006;5811:1131–1135.
17. Junker JP, Caterson EJ, Eriksson E. The microenvironment of wound healing. *J Craniofac Surg*. 2013;241:12–16.
18. Breuing K, Eriksson E, Liu P, Miller DR. Healing of partial thickness porcine skin wounds in a liquid environment. *J Surg Res*. 1992;521:50–58.
19. Vranckx JJ, Slama J, Preuss S, et al. Wet wound healing. *Plast Reconstr Surg*. 2002;1107:1680–1687.
20. McDaniel JS, Holt AW, Por ED, et al. The utilization of an ocular wound chamber on corneal epithelial wounds. *Clin Ophthalmol*. 2018;12:903–911.
21. Holt AW, McDaniel JS, Bramblett GT, et al. Use of an ocular wound chamber for the prevention of exposure keratopathy in a guinea pig model. *Wound Repair Regen*. 2018;265:351–358.
22. Junker JP, Lee CC, Samaan S, et al. Topical delivery of ultrahigh concentrations of gentamicin is highly effective in reducing bacterial levels in infected porcine full-thickness wounds. *Plast Reconstr Surg*. 2015;1351:151–159.
23. Daly LT, Tsai DM, Singh M, et al. Topical minocycline effectively decontaminates and reduces inflammation in infected porcine wounds. *Plast Reconstr Surg*. 2016;1385:856e–868e.
24. Pranger AD, Alffenaar JW, Wessels AM, Greijdanus B, Uges DR. Determination of moxifloxacin in human plasma, plasma ultrafiltrate, and cerebrospinal fluid by a rapid and simple liquid chromatography–tandem mass spectrometry method. *J Anal Toxicol*. 2010;343:135–141.
25. Pan C, Zhang L, Yang B, et al. Determination of moxifloxacin in human plasma by UPLC-MS/MS and its application to a pharmacokinetic study. *Lat Am J Pharm*. 2015;346:1218–1223.

26. Chen W, Hwang YY, Gleaton JW, Titus JK, Hamlin NJ. Optimization of a peptide extraction and LC-MS protocol for quantitative analysis of antimicrobial peptides. *Future Sci OA*. 2019;51:FSO348.
27. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. *AAPS J*. 2010;123:348–360.
28. Katzman LR, Jeng BH. Management strategies for persistent epithelial defects of the cornea. *Saudi J Ophthalmol*. 2014;283:168–172.
29. Junker JP, Kamel RA, Caterson EJ, Eriksson E. Clinical impact upon wound healing and inflammation in moist, wet, and dry environments. *Adv Wound Care (New Rochelle)*. 2013;27:348–356.
30. Tsai DM, Tracy LE, Lee CC, et al. Full-thickness porcine burns infected with *Staphylococcus aureus* or *Pseudomonas aeruginosa* can be effectively treated with topical antibiotics. *Wound Repair Regen*. 2016;242:356–365.
31. Bron AJ, Argueso P, Irkec M, Bright FV. Clinical staining of the ocular surface: mechanisms and interpretations. *Prog Retin Eye Res*. 2015;44:36–61.
32. Mantopoulos D, Cruzat A, Hamrah P. In vivo imaging of corneal inflammation: new tools for clinical practice and research. *Semin Ophthalmol*. 2010;255–256:178–185.
33. Wipperman JL, Dorsch JN. Evaluation and management of corneal abrasions. *Am Fam Physician*. 2013;872:114–120.
34. Agrahari V, Mandal A, Agrahari V, et al. A comprehensive insight on ocular pharmacokinetics. *Drug Deliv Transl Res*. 2016;66:735–754.