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Diffusion of Antimicrobials Across Silicone Hydrogel Contact Lenses

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Abstract

Objectives—To measure the diffusion of topical preparations of moxifloxacin, amphotericin B (AmB), and polyhexamethylene biguanide (PHMB) through silicone hydrogel (SH) contact lenses *in vitro*.

Methods—Using an *in vitro* model, the diffusion of three antimicrobials through SH contact lenses was measured. Diffused compounds were measured using a spectrophotometer at set time points over a period of four hours. The amount of each diffused antimicrobial was determined by comparing the experimental value to a standard curve. A biological assay was performed to validate the contact lens diffusion assay by testing antimicrobial activity of diffused material against lawns of susceptible bacteria (*Staphylococcus epidermidis*) and yeast (*Saccharomyces cerevisiae*). Experiments were repeated at least two times with a total of at least 4 independent replicates.

Results—Our data show detectable moxifloxacin and PHMB diffusion through SH contact lenses at 30 minutes, while amphotericin B diffusion remained below the limit of detection within the 4 hour experimental period. In the biological assay, diffused moxifloxacin demonstrated microbial killing starting at 20 minutes on bacterial lawns, whereas PHMB and amphotericin B failed to demonstrate killing on microbial lawns over the course of the 60 minute experiment.

Conclusions—*In vitro* diffusion assays demonstrate limited penetration of certain anti-infective agents through silicone hydrogel contact lenses. Further studies regarding the clinical benefit of using these agents along with bandage contact lens use for corneal pathology are warranted.

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There is no conflict of interest to disclose.

Keywords

silicone hydrogel contact lens; bandage contact lens; drug diffusion; antibiotic; antifungal

Introduction

There are a multitude of therapeutic indications for soft contact lenses in the management of ocular surface disorders. Increasingly, silicone hydrogel (SH) materials are used for bandage lens purposes due to their higher oxygen permeability. Silicone hydrogel bandage contact lenses (BCLs) are employed to protect the cornea, increase patient comfort, facilitate healing, and provide mechanical support.^{1–3} In clinical practice, therapeutic indications for BCLs include persistent epithelial defects (PEDs), primary disorders of the corneal epithelium, corneal dystrophies, post surgical care of corneal refractive surgery or keratoplasty, trauma related surface issues, bullous keratopathy, exposure keratopathy, and infectious keratitis^{4–10}.

The most common and convenient method for drug delivery to the eye is topical administration. Of all ophthalmic medications, 90% of these are applied to the ocular surface in the form of drops.^{11, 12} Conventionally, an eye dropper is used to instill one drop into the inferior conjunctival cul-de-sac, where it resides for 3–5 minutes.^{13–15} Most of the drug leaves the ocular surface via the lacrimal drainage system, and thus the majority is systemically absorbed through the nasal mucosa. Only a small fraction of topically applied drug penetrates the ocular surface. Ghate and Edelhauser reported that only 1–7% of topical drug instilled into the conjunctival sac is absorbed by the eye and reaches the aqueous humor.¹⁶ The corneal epithelium provides the largest biological barrier to penetration of applied topical drugs. The bioavailability of the drug is further reduced by precorneal factors including lacrimal and accessory secretion, tear drainage, evaporation, and conjunctival absorption.¹¹

In the management of corneal ulcers, therapeutic soft contact lenses are sometimes used to promote growth and healing of persistent epithelial defects, as well as to decrease patient suffering from painful infections.¹⁷ In the context of concurrent use of hydrogel contact lenses, the lens itself acts as a barrier, and post-contact lens tear film debris, as well as tear exchange under the lens, also affect how much time the drug is in contact with the eye.¹⁸ Antimicrobial eye drops are used concordantly with the BCLs, but there remains uncertainty as to how much and how quickly the drug penetrates through newer silicone hydrogel materials, and thus how much medication is bioavailable to treat infection with the lens in place.

In a study published in 1984, McCarey et al. determined that gentamicin, an aminoglycoside antibiotic and ophthalmic agent, is able to diffuse across traditional hydrogel bandage lenses.¹⁹ There is little known, however, about the penetration of other classes of antibiotics, including fluoroquinolones, which are frequently used in the treatment of bacterial corneal ulcers. Furthermore, there is no experimental or anecdotal evidence that topical antifungal and antiamoebic drug preparations are able to diffuse across BCLs. The issue of penetration

of anti-infectives through BCLs has considerable clinical significance given the prevalence of their use in the management of bacterial, fungal, and amoebic infectious keratitis.

The purpose of this study was to investigate the diffusion of anti-infectives used in the treatment of and prophylaxis against microbial keratitis across SH contact lenses *in vitro* using UV spectrophotometry (primary outcome), and a biological assay (secondary outcome).

Materials and Methods

Contact lenses

Lotrafilcon A (Air Optix® Night and Day™ Aqua, CIBA Vision, Duluth, Georgia) silicone hydrogel contact lenses with the following specifications were used: base curve = 8.60, power = -0.25, diameter = 13.8 mm.

Antimicrobials

Representative antibacterial, antifungal, and anti-amoebic agents commonly used as ocular therapeutics^{20–22}, respectively, used in this study are: moxifloxacin (LKT Laboratories #M5794) dissolved in water with glacial acetic acid (6%) prepared at a concentration of 50 mg/ml, amphotericin B (AmB) (Sigma #A2411) dissolved in water at a concentration of 5 mg/ml, and polyhexamethylene biguanide (PHMB) (Baqua Spa, Zeneca, Inc.) was used directly from the purchased stock solution at a concentration of 200 mg/ml.

Contact lens diffusion model assay

The CL diffusion model is depicted in Figure 1. Using sterile forceps, lotrafilcon A silicone hydrogel contact lenses were placed, concave side up, in the upper compartment of the Transwell® six-well plates with 0.4 µm pore size filter (Costar® 3412, Corning Incorporated) allowing fluid exchange between the upper and lower chambers. One milliliter (ml) of phosphate buffered saline (PBS) was added to the well of the test multiple well plate which fills the lower chamber and saturates the filter, but does not submerge the lens. Forty microliters (the approximate average volume contained in one drop of ophthalmic solution¹³) of the test antimicrobials were added to the inner concavity of the contact lens. At set time points (1, 5, 10, 30, 60, 120, 240 minutes), the transwell insert was removed, the PBS solution remaining in the well was mixed, and 20 µl of the solution was transferred to the 96 plate UV well (Costar® 3635, Corning Incorporated) into wells containing 180 µl of PBS. The solution was then assayed using a BioTek Synergy™ 2 microplate reader where absorbance for the solution was measured at the known UV absorbance maximum (Table 1) for the test antimicrobial using PBS as a blank. The amount of diffused antimicrobial was determined with a standard curve; in all cases the R-value was greater than 0.98, and measured values were within the linear range of the assay. Spectrophotometric analysis supported that in this system moxifloxacin had a linear range of 0.5 – 50 µg/ml with a limit of detection 0.5 µg/ml. PHMB had a linear range of 0.2 – 200 µg/ml with a limit of detection equal to 0.2 µg/ml. Amphotericin B was measured with a linear range from 0.61 – 625 µg/ml with a limit of detection of 0.61 µg/ml. Excel software was used to establish a concentration-absorbance equation that was then used to determine experimental drug concentrations.

At least four independent replicates of the experiment were performed over two days for each of the drugs yielding similar results. All data and statistical analysis was done with Microsoft Excel and Graphpad Prism software.

Biological assay

A biological assay was designed to verify that tested antimicrobials were penetrating the contact lens and retained functionality. The contact lens diffusion model was performed as described previously (Figure 1); however, at 10 minute intervals over a 1 hour period, 10 μ l aliquots of the with diffused antibiotic were spotted onto lawns of susceptible test organisms. In this assay *Staphylococcus epidermidis* strain RP62A was used for moxifloxacin and PHMB, and *Saccharomyces cerevisiae* InvSc1 (Invitrogen) was used for amphotericin B. Microbial cultures were grown overnight in Tryptic Soya Broth (TSB) or yeast extract, peptone, and dextrose broth (YPD). Five microliters of overnight culture was added to 150 μ l of PBS and plated onto nutrient rich agar plates as a lawn and dried briefly before addition of the test aliquots. The plates were incubated overnight at 37°C for *S. epidermidis* and 30°C for *S. cerevisiae*. Zones of growth inhibition were visually assessed. Prevention of microbial growth indicated that the antimicrobial was capable of diffusing through the SH contact lens applied directly onto the lawns. The sensitivity of the assay was established using a 2-fold serial dilution series of the test antimicrobials. The limit of detection was 4.88 μ g/ml for moxifloxacin, 48.8 μ g/ml for PHMB, and 39.1 μ g/ml for amphotericin B.

Results

Three antimicrobials were chosen for use in the study based on several criteria: 1) common prescription practice in the management of infectious keratitis, 2) availability, 3) those with known maximum absorbance in UV spectra that were verified in prior experimental investigations, and 4) one representative drug from each class: antibacterial, antifungal, and antiamoebic.

Using the contact lens diffusion model assay, the penetration of moxifloxacin, PHMB, and amphotericin B through silicone hydrogel contact lenses was measured over time (Figure 2). Moxifloxacin and PHMB diffusion were detectable at the 30 min time point and increased over time, whereas amphotericin B diffusion remained below the limit of detection within the 4 hour experimental period (Figure 2). Based on the starting and final concentrations, the percentage of drug that diffused through the contact lens was calculated. It was determined that $10.6 \pm 4.1\%$ of the moxifloxacin, $5.1 \pm 1.5\%$ of the PHMB, and $>0.1\%$ of the amphotericin B diffused through the contact lens. For the 4 hour time point there was no statistical difference between the percentage of moxifloxacin and PHMB diffusion ($p = 0.21$, 2-tailed, unpaired Student's T-test, Excel software).

The biological assay was then used as an additional test to validate that functional antimicrobials pass through the silicone hydrogel contact lenses. Moxifloxacin demonstrated microbial killing starting at 20 minutes on bacterial lawns (Figure 3b), whereas PHMB and amphotericin B did not reach sufficient concentrations to create zones of clearance on susceptible lawns of bacteria and yeast, respectively, over the 60 minute period that samples

were taken (Figure 3c and 3d). However, a small zone of inhibition, but not clearance, was seen with PHMB at 60 minutes (Figure 3c arrow). This might be expected with the predicted 30 µg/ml of PHMB measured at 60 minutes (Figure 2) coupled with the sensitivity of the biological assay at 49 µg/ml. Additionally, smaller molecular weight (MW) polymers of PHMB are less antimicrobial than larger MW molecules.^{23, 24} It is quite possible that low MW polymers are passing through the CLs more readily accounting for the low levels of antimicrobial activity.

Discussion

The influence of a contact lens on the surface of the eye is multifactorial; biochemical changes take place which alter the composition of the surface²⁵, and there are biophysical interactions between the lens and tear film which alter the precorneal dynamics.²⁶ It is reasonable to assume that anything altering the surface dynamics of the ocular surface will also affect ocular penetration and bioavailability of the drug. As previously discussed, silicone hydrogel contact lenses are often used in the treatment of ocular surface disease, including infectious keratitis, in conjunction with topical antimicrobials. Infections of the cornea, however, sometimes persist despite frequent and aggressive topical anti-infective therapy in the presence of a BCL. In this study, *in vitro* diffusion assays demonstrated delayed and/or limited penetration of commonly used anti-infective agents through silicone hydrogel contact lenses.

According to our data, moxifloxacin and PHMB diffuse through silicone hydrogel contact lenses at detectable levels no sooner than 20 min and 30 min, respectively, after the adding the drug to the contact lens. This is significant given that ophthalmic drugs without a contact lens purportedly remain on the ocular surface only 3–5 min.^{14, 15} Amphotericin B diffusion was undetectable at 4 hours using our experimental assays. In this *in vitro* study, the starting concentrations of each compound exceeded the therapeutic doses, in order to maximize the sensitivity of the assay. Moxifloxacin was used at 10 times higher than the most common commercial formulation (Vigamox®), PHMB was used at 20% rather than the therapeutic dose of 0.02%, and amphotericin B was used at 5000 µg/ml compared to a typical standard dose of 1500 µg/ml. Even with these higher concentrations, the concentrations of drug that penetrated the contact lenses varied with respect to the standard therapeutic concentrations. Whereas, the diffused concentration of PHMB achieved 411 µg/ml, approximately 2 times higher than the therapeutic concentration, both moxifloxacin (diffused concentration of 212 µg/ml versus standard concentration of 5000 µg/ml) and amphotericin B (no measurable diffused drug compared to the standard concentration of 200 µg/ml) failed to achieve the levels found in topical formulations. The concentration of amphotericin B that diffused through the contact lens was undetectable - far below the standard therapeutic concentration for this drug (1500 µg/ml). As noted in Table 1, the mass of amphotericin B (923 daltons) was larger than that of moxifloxacin (401 daltons) or certain length polymers of PHMB (440–9000 daltons), and this may account for the reduced diffusion amphotericin B.

One limitation of this study was that we were not able to use clinical dosing concentrations of the antimicrobials, because the concentrations had to be high enough for detection in the PBS-filled chamber below the lens. We expect that the lower concentrations used clinically

would be expected to show even less diffusion. Additional limitations of this model are that it detects diffusion across CLs, but does not directly address the concentrations of antibiotics achieved in the post-lens tear film, nor does it consider transport of antimicrobials around the lens and mixing with the post-lens tear film.

Our study examined the diffusion of antimicrobials across SH contact lenses *in vitro*. Prior research using a kinetic model and computer generated data to examine gentamicin distribution on the eye in the presence of a contact lens, found that the amount of gentamicin that permeated through the contact lens was only 0.002% of the amount of gentamicin on the posterior aspect of the lens 10 minutes after topical application of the antibiotic.¹⁹ This *in vitro* experimental model suggests that topical ophthalmic drugs, when applied in the presence of a contact lens, will move around the lens, and that the diffusion through the lens may not be as important.¹⁹ This model, however, fails to account for variation in fitting characteristics of a lens on the surface of the eye. Further studies *in vivo* are needed to explore the pharmacokinetics of topically administered medications when used in the context of bandage contact lens use.

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Figure 1.

In vitro contact lens diffusion model. A) Add 1 ml PBS and transwell to multi-well plate well. B) Apply contact lens to transwell. C) Apply therapeutic to contact lens. D) Incubate to allow diffusion through lens. E) Measure diffusion through lens by UV absorbance of aliquots of fluid below transwell.

Figure 2. Diffusion of antimicrobials across Air Optix® Night and Day™ Aqua contact lenses (n = 4 lenses per time point) from 3 separate experiments (mean and standard deviation are shown).

Figure 3.

CL diffusion bioassay. Therapeutics were added to contact lenses (n = 4) as described in methods. Samples from the PBS below the filter insert were collected at 10 minute intervals and spotted onto blood agar containing lawns of *Staphylococcus epidermidis* (b–c) or yeast extract, peptone, dextrose (YPD) agar (d) containing lawns of *Saccharomyces cerevisiae* and compared to the control region where undiluted drug was spotted. A) Plate key for spotted drug controls and collected samples for bioassays with the following drugs: B) moxifloxacin, C) PHMB, and D) amphotericin B. White arrow denotes region of inhibited microbial growth. The dark spot at the control location on the PHMB plate was not microbial growth, rather it was a precipitate formed whenever very high levels of PHMB were applied to agar plates.

Table 1

Characteristics of drugs used in this study.

Drug	Use	UV absorbance maxima ^a	Molecular Mass
Moxifloxacin	antibacterial	293 ²⁵	401
PHMB	antiamoebic	236 ²⁶	440–9000
Amphotericin B	antifungal	385 ²⁷	923

Abbreviations: PHMB, Polyhexamethylene biguanide

^a Citation for absorbance maxima determination is noted as a superscript.