INTRODUCTION

Contact lenses can act as vectors for the transmission of microorganisms (bacteria, fungi, viruses), to the ocular surface and cause infection [1]. Disinfection of contact lenses and the cases in which they are stored is essential for proper contact lens and ocular hygiene. This is especially true for reusable, contact lens trial sets. The International Organization for Standardization (ISO) defines reusable contact lenses as 1) contact lenses only used by a practitioner or fitter for the purpose of selecting the appropriate contact lens parameters for the intended wearer (Trial Contact Lens or Diagnostic Contact Lenses) and 2) trial contact lenses permitted to be used on more than one person (Multi-patient Use Trial Contact Lenses) [2]. However, there is no standardized procedure to disinfect these fitting lenses between patients.

The American Optometric Association’s Health Policy Institute and the Contact Lens and Cornea Section recommends that hard, rigid gas permeable (RGP), and soft diagnostic or trial fitting lenses be disinfected with commercially available hydrogen peroxide contact lens disinfecting systems approved for contact lenses [2]. The drawback of these systems is the length of time needed for disinfection, which can take several hours.

A possible alternative procedure for disinfecting these lenses more quickly between patients could be the use of an antiseptic that is FDA-approved for ocular use. The most common antiseptic used for surgical prophylaxis in the eye is povidone-iodine. However, this agent cannot be used as it would stain the lenses. Another possible antiseptic which could be considered may be a stabilized formulation of 0.01% hypochlorous acid which is FDA-approved for eyelid hygiene. Hypochlorous acid is a naturally occurring antimicrobial agent. It is produced during human immune response by polymorphonuclear leukocytes responding to pathogens [3]. We previously demonstrated that 0.01% hypochlorous acid produced rapid bactericidal decreases of several species of ocular bacterial pathogens contained in biofilms [4].

This led us to the current “proof of concept” study for which the goal was to evaluate a potential new procedure for the rapid disinfection of contact lenses, cases, and other multi-patient contact lenses used during the trial fitting of contact lenses.
disinfection of contact lenses that may be used on reusable, contact lens trial sets between patients. Specifically, we tested the in vitro disinfecting efficacy of 0.01% hypochlorous acid (HyClear™, Contamac®, Grand Junction, CO, USA) on hybrid rigid gas permeable contact lenses fitted with silicone hydrogel skirts and single-well contact lens cases contaminated with common ocular bacterial, fungal, and viral pathogens. These single contact lenses represent both contact lens materials used in reusable, contact lens trial sets.

MATERIALS AND METHODS

Test Organisms

Clinical ocular strains of Staphylococcus aureus (SA), Coagulase-negative Staphylococcus (CNS), Pseudomonas aeruginosa (PA), Serratia marcescens (SM), Streptococcus pneumoniae (SP), Achromobacter xylosoxidans (AX), Candida albicans (CA), adenovirus type 19/64 (HAdv19/64), and HSV-1 were isolated from patients at the Charles T. Campbell Ophthalmic Microbiology Laboratory at the UPMC Eye Center, Department of Ophthalmology, University of Pittsburgh, School of Medicine in Pittsburgh, Pennsylvania. All isolates were identified using standard microbiological assays. The adenovirus isolate type was determined by serum neutralization and was determined to be type 19. Recently, HAdv19 isolates have been designated to be type 19. Recently, HAdv19/64 isolates have been designated at HAdv19/64 [5]. For the purpose of this study, we call the isolate HAdv19/64.

These de-identified isolates were retrieved from frozen stocks as part of a microbial collection used for validation of new diagnostic tests. The bacterial and yeast isolates were grown on trypticase soy agar (TSA), containing 5% sheep’s blood plates (Remel, Lenexa, KS). High titer stocks of HAdv19/64 were prepared from A549 human lung carcinoma cells while HSV-1 stocks were prepared in Vero cells. A549 cells were used for the determination of HAdv19/64 and HSV-1 titers. The A549 cells were grown and maintained in tissue culture media containing Eagle’s MEM supplemented with 10% fetal bovine serum (Sigma Cell Culture Reagents, St. Louis, MO).

Test Agents

Commercial spray bottles of HyClear™ (hypochlorous acid 0.01%), and HyClear™ vehicle without hypochlorous acid were provided by Contamac®, Grand Junction, CO, USA.

Contact Lenses and Cases

Hybrid RGP lenses with silicone hydrogel (soft), skirts (roflufocon D with 50% water silicone hydrogel skirts), and single well, hard plastic (polypropylene) contact lens cases were provided by Contamac®. The contact lenses were non-sterile and were stored in multipurpose contact lens solution upon arrival. The nonsterile contact lens cases were sterilized after exposure to 254 nm UV irradiation from a light source for 30 minutes.

Experimental Assay

The bacterial and CA strains were grown overnight at 37°C on TSA blood plates. On the day of the experiment, the bacteria and CA strains were suspended in sterile PBS to a 0.5 McFarland Standard, containing approximately 5 x 10^6 colony forming units per milliliter (CFU/ml), of bacteria. Fifty µl of these standards were added to 5 ml of sterile PBS to produce an inoculum of approximately 5 x 10^6 CFU/ml. Previously enumerated frozen stocks of HAdv19/64 and HSV-1 were thawed and diluted in tissue culture medium to produce an inoculum containing approximately 10^6 plaque forming units per milliliter (PFU/ml). The target inoculation count was approximately 1000 organisms for each lens or case.

Single trials of 2 replicate lenses and cases were performed for each treatment. In the case of HSV-1, a second trial was similarly performed. Four hybrid contact lenses for each organism were washed 3x in sterile PBS to remove any residual lens disinfection solution. After the final rinse and removal of excess PBS, the lenses were transferred to separate disposable Petri dishes, each containing a sterile 9 mm O-ring gasket. The lenses were placed on these gaskets to allow them to keep their shape with their concave sides up. The 4 lenses were then inoculated with 200 µl of the appropriate bacterial, CA, or viral inoculum. This optimal volume was determined in a preliminary study. The inoculum completely covered the RGP portion of the lenses and most of the silicone hydrogel skirts. A larger inoculum volume than 200 µl reduced the integrity of the lenses causing spillage of the inoculum. The lenses were incubated with the inoculum at room temperature for 30 minutes for the bacterial and CA strains to allow adhesion to the lenses, while the viruses were incubated for 5 minutes. Following incubation, the inoculum from the lenses was removed with a pipette and discarded. Of the 4 lenses, 2 lenses were sprayed with 0.01% hypochlorous acid and 2 lenses were sprayed with vehicle without hypochlorous acid. The spray bottles were held approximately 2 inches away from the lenses at an approximate angle of 45° while the 0.01% hypochlorous acid or vehicle was applied. The dishes were rotated 90° and a second spray was applied. The same procedure was performed for two additional sprays for a total of 4 sprays. Rotating the lenses 360° assured that all areas of the concave sides of the lenses were exposed to the 0.01% hypochlorous acid or vehicle. After completing the sprays, the lenses were transferred to 17 x 100 mm tubes containing 3 ml of sterile PBS using sterile forceps. The tubes were sonicated and vortexed to remove the bacteria, CA, or viruses from the lenses. Immediately afterward, standard colony count determinations (bacteria and CA) or viral plaque assays were performed to determine the amounts of live bacteria, CA, or viruses contained on the 0.01% hypochlorous acid or vehicle treated lenses.

The assay for the disinfection of the 4 single well contact lens cases per organism was carried out similarly to the lenses with the following exceptions: 1) after the 0.01% hypochlorous acid or vehicle treatments, 1 ml of sterile PBS was added to the cases; 2) after sonication, the lids were closed and the cases vortexed to remove the virus from the case surfaces; 3) the 1 ml of PBS was immediately transferred to a separate tube for bacterial, CA, and viral enumeration.

Colonies Count Determination (Bacteria and CA)

Colony counts were performed on undiluted samples along with 1:100 and 1:10,000 dilutions using the Eddyjet 2 spiral plating system (Neutec Group Inc., Farmingdale, NY), on 5% TSA with sheep's blood plates. The plates were incubated at 37°C for
24-48 hours, depending on the species, the colonies were counted, and the numbers of bacteria and CA determined after 0.01% hypochlorous acid or vehicle treatments using the automated Flash and Grow colony counting system (Neutec Group). Since the lenses were placed in 3 ml of PBS after treatment, the number of CFU/ml determined for the lenses was multiplied by 3 to determine the final number of CFU/lens. The CFU/case was the same as the number of CFU/ml since 1 ml of PBS was added to the cases after treatment.

**RESULTS**

The results of the disinfection of the contaminated contact lenses are presented in Table 1 while the results of the disinfection of the contaminated lens cases are presented in Table 2. One minute of exposure to 0.01% hypochlorous acid completely eradicated all bacterial and CA strains from both hybrid lenses tested except for *S. aureus*, which was still present on 1 lens. For the viral contaminants, one minute of exposure to 0.01% hypochlorous acid completely eradicated HAdv19/64 from both lenses whereas HSV-1 was not eradicated. There was less than a 1-Log_{10} decrease in HSV-1 titers for both of the lenses. This result was surprising since HSV-1 was completely eradicated from the cases with 1 minute of exposure to 0.01% hypochlorous acid. This prompted us to do a second trial with HSV-1 using both lenses and cases. Similar results were obtained in this second trial demonstrating little decrease of HSV-1 on the lenses but complete eradication from the cases with 1 minute of exposure to 0.01% hypochlorous acid.

The 0.01% hypochlorous acid completely eradicated all test organisms (bacterial, yeast, and viral) from the single well cases after a 1-minute exposure. These results include both trials of HSV-1.

**DISCUSSION**

This study evaluated the potential use of a commercially available solution of 0.01% hypochlorous acid (HyClear®, Contamac®, Grand Junction, CO, USA), for disinfecting contact lenses and cases contaminated with common ocular pathogens of bacteria, yeast, and viruses. Hypochlorous acid 0.01% is an FDA cleared treatment of blepharitis and for eyelid hygiene in patients [4]. It has been shown previously that hypochlorous acid is non-irritating when instilled into eyes [6].

The bacterial species selected are common causes of contact lens associated keratitis (PA,SM), keratitis (SA, CNS, CA), conjunctivitis (SA, SP), and a common contaminant of contact lens cases (AX). Adenovirus type 19/64 was chosen as a representative of adenoviruses, the most common cause of ocular viral infections worldwide and because it is a cause of the highly contagious epidemic keratoconjunctivitis (EKC). HSV-1 was included as it is a major ocular pathogen. Furthermore, these viruses represent the two major classifications of viruses, enveloped (HSV-1), and non-enveloped viruses (adenovirus).

**Determination of Viral Titers (Plaque Assay)**

The undiluted, 1:10, and 1:100 diluted samples were inoculated onto duplicate wells of 24-well multiplates containing A549 cell monolayers. After adsorption onto the monolayers for 3 hours, the cells were overlaid with tissue culture media containing 0.5% methylcellulose. After 5-9 days of incubation at 37°C and 5% CO_{2}, the cells were stained and fixed with 0.5% gentian violet containing formalin. The number of viral plaques were then counted, and the titers were then calculated and expressed as PFU/ml. The number of PFU/lens and PFU/case were determined as outlined for the bacteria and CA.

**Data Analysis**

The bacterial and CA colony counts + 1 were expressed as per lens or case (CFU+1/Lens or CFU+1/Case). The viral titers + 1 were expressed as plaque forming units per lens or case (PFU+1/Lens or PFU+1/Case). The colony counts + 1 and viral titers + 1 were Log_{10} converted. The mean ± standard deviation (SD), of the 2 replicates were calculated for each organism, treatment, and device. Total eradication of the organism by 0.01% hypochlorous acid was considered if the bacterial and CA counts and viral titers were below the limit of detection for each assay (20 CFU/ml for bacteria and CA, 5 PFU/ml for viruses). This study aimed for total eradication of the organisms.
Disinfectants have been previously shown to have differing effectiveness against enveloped and non-enveloped viruses with the enveloped viruses being more susceptible to disinfection [7].

The targeted number of organisms for this study was approximately 1000 per lens and case. This is greater than the number of bacteria cultured from lenses with and without patient handling [8,9]. We wanted to use an inoculum that would provide a greater challenge of bioburden on the lenses than has been previously demonstrated.

Disinfection of contact lenses and cases can be performed using a variety of methods. However, we wanted to test a practical and quick disinfection method that could be used by a practitioner in the office setting. Since 0.01% hypochlorous acid is available in commercially available spray bottles, it was decided to use spraying of the lenses and cases as the method of applying the disinfectant. It was determined that 4 sprays would be sufficient to cover the surfaces of the lenses and cases.

It has been determined previously that hypochlorous acid is a rapid killer of bacteria [6]. Therefore, an exposure time of 1 minute was chosen to make this potential technique of reusable, multi-patient fitting lenses and case disinfection practical for use between patients in an office setting.

The contact lenses used in this study were hybrid RGP lenses with silicone hydrogel (soft) skirts. These single contact lenses represent both lens materials used in reusable, contact lens trial sets. The advantage of using these hybrid lenses as a surrogate for lenses of both lens material types is that if the 0.01% hypochlorous acid is effective in eradicating the contaminating organisms, it can be postulated that the 0.01% hypochlorous acid would be effective on both types of lenses. However, if the 0.01% hypochlorous acid is ineffective in eradicating the organisms, it could not be determined whether one or both lens materials, or the interface between the two materials had a negative effect on the efficacy of the 0.01% hypochlorous acid. Nevertheless, based on all of the factors listed above, we believe that this assay would be an accurate representation of the clinical disinfection procedure.

The results of this study demonstrated that a 1-minute exposure to 0.01% hypochlorous acid was effective in disinfecting hybrid contact lenses and cases contaminated with bacteria, a yeast, and adenovirus. Among the bacterial and CA strains, all were completely eradicated from the lenses except for S. aureus, which was eradicated from 1 of 2 lenses. Overall, 0.01% hypochlorous acid performed well in disinfecting hybrid contact lenses contaminated with bacteria and C. albicans.

As for the viruses, a 1-minute exposure to 0.01% hypochlorous acid completely eliminated the non-enveloped HAdv19/64 from the lenses but had little to no effect against the enveloped virus, HSV-1. This is in total contrast to the results produced for the disinfection of the contact lens cases contaminated with HSV-1. A 1-minute exposure to 0.01% hypochlorous acid completely eradicated HSV-1 from the lens cases. This result surprised us and prompted a second trial with HSV-1 which produced the same results. The reason for the lack of efficacy of 0.01% hypochlorous acid against HSV-1 on the lenses is unknown. Perhaps there was some interaction between the enveloped virions and the lens materials (RGP and silicone hydrogel) or their interface that protected the virus from disinfection. It does not appear that the lens materials have an inhibitory effect on the 0.01% hypochlorous acid since a 1-minute exposure was effective against multiple types of organisms in this study. There is the possibility that a longer exposure time to the 0.01% hypochlorous acid could provide more effective disinfection against HSV-1. It was beyond the scope of this study to determine the mechanisms by which the 0.01% hypochlorous acid was ineffective against HSV-1 in contaminated hybrid lenses and whether increased exposure time would increase effectiveness. This can be the focus of a future study.

There was no ambiguity within the results for the disinfection of the hard plastic contact lens cases. The 0.01% hypochlorous acid completely eliminated all test organisms from the contact lens cases after only 1 minute of exposure. These results demonstrate that 0.01% hypochlorous acid is an effective disinfectant against all of these test organisms.

There are several limitations of this study. In this proof of concept study, the numbers of contact lenses and cases available to us were limited. Since we wished to test as many relevant organisms as possible, we had to sacrifice replicates for each organism. Therefore, we could only test the hypochlorous acid treatments on 2 replicate lenses and cases per organism. Unfortunately, with only 2 replicate lenses and cases per organism,

### Table 2: Bacterial, Fungal, (CFU/Case) and Viral Counts (PFU/Case) after Treatment of Contact Lens Cases (Log$_{10}$Mean ± Standard Deviation)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Vehicle Count</th>
<th>HA Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>4.09 ± 0.12</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Coagulase-negative Staphylococcus</td>
<td>3.86 ± 0.17</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>2.68 ± 0.24</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>3.39 ± 0.13</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>3.19 ± 0.09</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Achromobacter xylosoxidans</td>
<td>3.06 ± 0.28</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>2.95 ± 0.04</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Adenovirus Type 19/64</td>
<td>3.48 ± 0.67</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>HSV-1 (Trial 1)</td>
<td>3.37 ± 0.06</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>HSV-1 (Trial 2)</td>
<td>4.34 ± 0.01</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>
a statistical analysis was not feasible. However, since the aim of the study was to determine total eradication of the organisms, this result is more qualitative. We did demonstrate total eradication of all organisms on the cases and 7/9 organisms on the lenses. Another limitation of the study was the lack of neutralization of the hypochlorous acid after the 1-minute treatment period. We are unaware of any agent that will neutralize the hypochlorous acid on contact. Instead, we used dilution and immediate plating of the solutions onto the appropriate growth medium in order to minimize the prolonged effect of the hypochlorous acid on the organisms.

The results of this study demonstrated that 0.01% hypochlorous acid (HyClear™) was effective in disinfecting hybrid contact lenses and hard plastic lens cases contaminated with bacteria, a yeast, and adenovirus. The 0.01% hypochlorous acid was effective in eliminating HSV-1 from cases but not the lenses.

CONCLUSIONS

In conclusion, 0.01% hypochlorous acid has the potential to be used as a rapid disinfecting agent for reusable contact lens trial sets and their cases. Longer 0.01% hypochlorous exposure times should be evaluated for HSV-1 on lenses as well as disinfection testing on actual fitting lenses.

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REFERENCES

2. The American Optometric Association’s Health Policy Institute and the Contact Lens and Cornea Section 2018.