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Ability of Two Multipurpose Disinfecting Solutions to Kill Microbe's Adherent to Contact Lenses

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Abstract

Aim: The aim of this project was to determine the ability of two contact lens disinfecting solutions to kill bacteria and fungi attached to lenses.

Methods: *Pseudomonas aeruginosa* PA181, *Stenotrophomonas maltophilia* 006 and *Fusarium solanii* ATCC 36031 were allowed to adhere to contact lenses (comfilcon A and somofilcon A) for one hour. The lenses were washed and then placed into contact lens cases of the two disinfecting solutions (cleadew soft containing povidone-iodine, or OPTIFREE RepleniSH containing polyquaternium-1 and myristamindopropyl dimethylamine) and disinfected for the manufacturers recommended time. Lenses are then washed and any viable bacteria removed and grown on agar plates. Both contact lens types that had been worn for a minimum of 6 hours were also disinfected, and any remaining viable microbes were cultured.

Results: After adding microbes to the lenses in the laboratory study, no viable microbes grew from the comfilcon A lenses, but 1-6 colony forming units of bacteria could be grown from the somofilcon A lenses after either type of disinfecting solution was used. After wear, bacteria could be cultured from both lens types after disinfection, with slightly more bacteria being cultured from the front vs. the back surface of the comfilcon A lenses (average cfu/lens 15-190 vs. 5-10).

Conclusions: There was no difference in the ability of the disinfecting solutions to kill bacteria adherent to lenses. The finding of viable bacteria remaining on lenses after disinfection reinforces the need to rub and rinse lenses after wear to remove some of these adherent bacteria..

Introduction

Daily wear of contact lenses requires the lenses to be cleaned and disinfected when not being worn. There are several types of disinfecting solutions, those that have an oxidative disinfecting process such as hydrogen peroxide or iodine, and those that use disinfectants such as polymeric poly-quaternary ammonium compounds (QACs). All of these disinfecting solutions must pass standard tests to demonstrate their ability to kill a set of microbes.

These standard tests include the International Organisation for Standardisation (ISO) 14729 "Ophthalmic Optics—Contact Lens Care Products—Microbiological Requirements and Test Methods for Products and Regimens for Hygienic Management of Contact Lenses"[1]. In this set of tests, standard microbial strains are used initially in suspension and the ability of the disinfectants to reduce the viable number of these microbes, during the manufacturers recommended disinfection time, is measured. If a solution fails to meet thest and-alone disinfection criteria (at least a 3 $\log_{10}$reduction in bacteria and a 1 $\log_{10}$ reduction for fungi), it can be tested under the regimen criteria as long as it met, at the manufacturer's recommended soaking time, stasis for the fungi and an average of 5 $\log_{10}$ reduction in all bacteria, with at least 1 $\log_{10}$ occurring for each bacterium. The regimen test includes adding the microbes to the contact lenses and undergoing the manufacturers recommended cleaning steps such as rubbing and rinsing. From an inoculum of 5 $\log_{10}$ organism, no more than 10 viable organisms should remain viable on the lenses after the regimen test.

Another test that is recommended is ISO 18259 "Method to Assess Contact Lens Care Products with Contact Lenses in a Lens Case, Challenged with Bacterial and Fungal Organisms"[2]. In this test, specific bacteria or fungi are placed into a contact lens case containing a contact lens, and the multipurpose disinfecting solution is added for the manufacturer's recommended disinfection time. After this time, the lenses are removed and any viable microbial cells remaining in the case are cultured. The other test that can be performed is ISO 19045-2:2024 "Method for evaluating disinfecting efficacy by contact lens care products using trophozoites of Acanthamoeba species as the challenge organisms"[3]. This test measures the ability of trophozoites of *Acanthamoeba* to be killed by disinfecting solutions. There are no pass criteria for each of these tests, but obviously the greater kill the better.

With the exception of the regimen test, all others focus on the solution rather than the lenses. Whilst the importance of a rub and rinse with the disinfecting solution to remove adherent microbes has been shown in laboratory studies [4],only 7% of contact lens wearers reported they would rub and rinse their lenses after wear and before adding to a disinfecting solution [5]. Therefore, the current study was designed to examine whether two contact lens disinfecting solutions, one oxidative solution containing iodine and one solution containing QACs, were able to kill adherent microbes in the absence of a rub and rinse regimen.

Materials and Methods

In vitro investigation

Microbes and growth: *Pseudomonas aeruginosa* PA181, isolated from a case of microbial keratitis and resistant to piperacillin with intermediate resistance to imipenem and ceftazidime [6], and *Stenotrophomonas maltophilia* 006, isolated from a contact lens case

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at the time of microbial keratitis [7], were retrieved from the culture collection of the School of Optometry and Vision Science, UNSW Sydney. The strains were grown overnight in Trypticase soy broth (TSB; Oxoid, Basingstoke, UK) at 30°C, and then resuspended in sterile PBS pH 7.4 (NaCl 8 g L^{-1} , KCl 0.2 g L^{-1}, Na_2HPO_4 1.15 g L^{-1}, KH_2PO_4 0.2 g L^{-1}) to an optical density at 660nm of 0.1 ($1x10^8$ colony forming units (CFU) mL^{-1}), then diluted a further 100 fold in PBS to yield final concentration of $1x10^6$ CFU mL^{-1}.

Fusarium solanii ATCC 36031 was also retrieved from the culture collection, was grown on Sabouraud's Dextrose Agar (SDA) (Oxoid, Hampshire, UK) at 25°C for 10 to 14 days, and then harvested by scraping off the agar surface. Approximately $1x\ 10^8$ CFU mL^{-1} were suspended in PBS and vortexed for 2 to 3 min. Retrospective plate counts were performed to ensure that $1\ x\ 10^8$ CFUmL^{-1} were present. This was then diluted further in PBS to achieve a final concentration of $1x10^6$ CFU mL^{-1}.

Contact lens disinfecting solutions: Two contact lens disinfecting solutions were used (Table 1).

Adhesion of bacteria to lenses and disinfection: Contact lenses (Biofinity, comfilcon A), and clariti 1 day (somofilcon A, both from CooperVision, Pleasanton, CA, USA; -1.00 D) were used in the study. Whilst the comfilcon A lenses are routinely used on a daily wear schedule and so would be exposed to a disinfection cycle each night when not being worn prior to disposal, the clariti lenses are designed for daily disposable use, and so would not routinely be exposed to a disinfection cycle as they are disposed on each day after wear. However, as 20% of daily disposable lens users may actually reuse their lenses and use a contact lens case [8], and reuse of daily disposable lenses can increase the risk of corneal infection by 5.4 times [9], the authors thought it would be of interest to compare these lenses.

Contact lenses were removed from their packaging solutions and placed into 2 mL of PBS. The lenses were then rinsed in the PBS three times, and then placed into a final concentration of $1x10^4$ cfu of each microbe separately. The microbes were allowed to adhere to the lenses for one hour, then lenses were washed once in PBS to remove loosely adherent microbes. The lenses were then placed into the contact lenses cases supplied with each contact lens solution, and the manufacturer's recommended amount of each disinfectant added, and the lenses disinfected for the manufacturer's recommended disinfection time.

After disinfection, the lenses were removed and placed into 2 mL of PBS, then vortexed on high speed for 1 minute to release adherent microbes from the lenses. An aliquot of the solution (50 µL) was then plated in triplicate onto TSB containing agar at 15 g L^{-1}, and lecithin at 7 g L^{-1} and polysorbate 80 at 5 g L^{-1} as neutralising agents [10]. After overnight incubation at 37°C, the number of CFUs was counted.

Table 1: Contact lens disinfecting solutions

Disinfectant brand (manufacturer)	Disinfectants	Manufacturer's recommended minimum disinfection time (hours)
Cleadew (Ophtecs corp., Kobe, Japan)	Povidone-iodine	4
OPTIFREE RepleniSH (Alcon labs., Fort Worth, TX, USA)	Polyquaternium-1 and myristamindopropyl dimethylamine	6

***In vivo* test**

Contact lenses (comfilcon A and somofilcon A) were retrieved at the end of use (at least six hours wear for daily disposable lenses) from established contact lens wearers, selected as they had previously given informed consent to be contacted for future tests in prior clinical trials. As these lenses would have been discarded, and were not intended for further human use, the ethics committee of UNSW Sydney did not require ethical approval of the study. Lenses were collected, using sterile gloves, added to appropriate lens cases with 2 mL of sterile PBS and de-identified before further use.

A sample size calculation was performed based upon the data in a previous study that evaluated the number of staphylococci (the most commonly isolated bacterium from the normal ocular surface[11] in populations of lens wearers in Sydney Australia. There were an average of 3.29 ± 3.27 cfu from a contact lens[12]. If contact lens disinfecting solutions reduce this average number to ≤0.5 then eleven contact lens wearers for each contact lens type were required. Furthermore, prior studies had shown that 73% of contact lenses were contaminated during wear [13]. Assuming this is reduced to 10% after disinfection, a sample size of eight contact lenses would be needed t show a significant difference.

Upon receipt of the lenses in the laboratory, they were subjected to disinfection as per the manufacturer's recommendations. After disinfection, lenses were washed once in PBS and then each side was swabbed with a sterile cotton wool swab, by rubbing over the surface three times. The side (concave or convex) that was swabbed first was randomised. The cotton swabs were then added to sterile microtubes containing sterile PBS and vortexed on high speed for one minute. After vortexing, 100 µL of the solution was placed onto each of three chocolate agar plates for aerobic, anaerobic and 5% CO_2 enrichment growth and SDA for fungal growth. The plates were incubated at 37°C for 48 hours for aerobic and CO_2 growth and 72 hours for anaerobic growth, and 7 days at 21°C for fungal growth. After incubation, the number of microbial colonies were counted and back calculated to obtain the number of cfu/lens side. Gram staining was performed to determine whether bacteria were gram-positive or gram-negative and their cellular morphology (coccus or rod). Fungi were identified as moulds or yeasts based upon colony morphology.

Results

***In vitro* investigation**

The number of CFU of each microbe recovered from the contact lenses after disinfection is given in (Table 2).

Overall, the disinfecting solutions removed any live bacteria or fungi from the comfilcon A lenses. However, for somofilcon A, there remained some live bacteria on lenses after disinfection, with 4-12 cfu for cleadew and 20-24 cfu for RepleniSH (Table 2). However, there were no overall statistical differences between the disinfecting solutions.

***In vivo* test**

Unfortunately, the study was unable to obtain each type of contact lens from eleven individuals; only eight people were willing to donate comfilcon A lenses, and only four people were willing to donate

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Table 2: Number of microbes recovered from contact lenses disinfected with two disinfecting solutions

Microbial type	Contact lens	Disinfecting solution	Average CFU/lens after disinfection
P. aeruginosa PA181	Comfilcon A	Cleadew	0
		RepleniSH	0
	Somofilcon A	Cleadew	12
		RepleniSH	20
S. maltophilia 006	Comfilcon A	Cleadew	0
		RepleniSH	0
	Somofilcon A	Cleadew	4
		RepleniSH	24
F. solanii ATCC 36031	Comfilcon A	Cleadew	0
		RepleniSH	0
	Somofilcon A	Cleadew	0
		RepleniSH	0

somofilcon A lenses. This partly reflects the lens types commonly worn in the Sydney region, with only 22% of daily wearers using comfilcon A [14] and most (≥80%) daily disposable wearers using etafilcon A, delefilcon A, or comfilcon A (in-house data).

The numbers of viable bacteria (there were no fungi grown) remaining on lenses after wear and after disinfection are given in (Table 3).

No microbes could be grown from most contact lenses after disinfection with either of the solutions. When microbes (bacteria only) were grown the most commonly cultured were Gram-positive cocci, which upon gram staining and on inspection of colony morphology resembled staphylococci. Gram-negative bacteria were rarely cultured; a gram-negative rod was cultured from a comfilcon A lens that had been disinfected with RepleniSH in large numbers (1320 CFU/lens) from the front surface, and a Gram-negative coccus was cultured from the back surface of a comfilcon A lens disinfected with cleadew (40 CFU/lens). From somofilcon A lenses, a Gram-negative coccus was cultured from the back surface (40 CFU/lens) after disinfection with cleadew, and a Gram-negative rod (40 CFU/lens) was cultured from the front surface after disinfection with RepleniSH. Statistical analysis of either the CFU/lens or percentage of lenses with contamination after disinfection did not find any difference between the disinfecting solutions.

Discussion

This study has shown that two contact lens disinfecting solutions, one oxidative (povidone-iodine) and one using QACs were generally able to kill all bacteria that had adhered to lenses either in a laboratory study or during wear.

The laboratory study showed that disinfection effectivity could be dependent on the lens polymer, with somofilcon A lenses having live bacteria remaining on them after disinfection. While the somofilcon A lenses are designed for daily disposable use and are not routinely disinfected, 20% of daily disposable lens users may reuse their lenses and store them in a contact lens case [8], and reusing daily disposable lenses can increase the risk of corneal infection by 5.4 times [9]. It would be interesting to determine if other polymers used for daily disposable lenses face a similar issue.

The clinical test demonstrated that bacteria could be cultured

Table 3: Number of bacteria cultured from the front or back surface of contact lenses after wear and disinfection

50	Disinfectant	Front surface	Back surface
		Colony forming units (mean ± SD; % of lenses contaminated)	
Comfilcon A	Cleadew	15 ± 42 (12.5%)	5 ± 14 (12.5%)
	RepleniSH	190 ± 458 (50%)	10 ± 19 (25%)
Somofilcon A	Cleadew	20 ± 23 (50%)	20 ± 23 (50%)
	RepleniSH	10 ± 20 (25%)	20 ± 40 (25%)

from some lenses after wear and subsequent disinfection. The study did not include a rub and rinse procedure after lens removal. Had this been in place, this may have removed all or most of these adherent bacteria [4], and the authors remind readers of the need to reinforce this procedure to all contact lens wearers if they reuse their lenses. However, the authors do not recommend this for daily disposable lens wearers, with reinforcing the requirement to discard lenses after each day of wear being an appropriate message. For comfilcon A lenses, there tended to be more bacteria remaining viable on the front of contact lenses after disinfection, regardless of the disinfecting agent. A previous study examining the number of microbes in contact lens cases during daily wear of lenses had shown that OPTIFREE RepleniSH left greater numbers and frequency of viable Gram-negative bacteria such as *S. Maltophilia* in the lens cases [15]. This is similar to the current data, where disinfection with OPTIFREE RepleniSH left great numbers of viable Gram-negative bacteria on lenses.

It would be useful in future studies to increase the numbers of worn lenses collected, include other lens types, and other disinfecting solutions. This may also help to determine whether the differences could reach statistical significance. Using the data from the current study, for the in vitro tests, 7 lenses in each group may be able to show significant differences between CFU/lens with somofilcon A lenses; for the in vivo tests, the minimum number of people required to see differences in front surface contamination with comfilcon A lenses with percentage contamination would be 21, but with CFU/lens would be 108, per group. In conclusion, overall, the study has demonstrated equivalence of the disinfecting ability of the two contact lens disinfecting solutions on microbes adhered to lenses.

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