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Antimicrobial efficacy of a novel povidone iodine contact lens disinfection system

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ABSTRACT

Purpose: Contact lens (CL) wear is a risk factor for the acquisition of microbial keratitis. Accordingly, compliance to manufacturers' recommended hygiene and disinfection procedures are vital to safe (CL) use. In this study we evaluated a novel povidone-iodine (PI) (CL) disinfection system (cleadew, Ophtecs Corporation, Japan) against a range of bacterial, fungal and *Acanthamoeba*.

Methods: Antimicrobial assays were conducted according to ISO 14729 using the recommended strains of bacteria and fungi, with and without the presence of organic soil. Regrowth of bacteria and fungi in the disinfection system was also examined. The activity on biofilms formed from *Stenotrophomonas maltophilia* and *Achromobacter* sp. was evaluated. Efficacy against *A. castellanii* trophozoites and cysts was also investigated.

Results: The PI system gave > 4 $\log_{10}$ kill of all bacteria and fungi following the manufacturer's recommended disinfection and cleaning time of 4 h, with or without the presence of organic soil. No regrowth of organisms was found after 14 days in the neutralized solution. In the biofilm studies the system resulted in at least a 7 $\log_{10}$ reduction in viability of bacteria. For *Acanthamoeba*, > 3 $\log_{10}$ kill of trophozoites and 1.1–2.8 $\log_{10}$ kill for the cyst stage was obtained.

Conclusions: The PI system effective against a variety of pathogenic microorganisms under a range of test conditions. Strict compliance to recommended CL hygiene procedures is essential for safe CL wear. The use of care systems such as PI, with broad spectrum antimicrobial activity, may aid in the prevention of potentially sight threatening microbial keratitis.

1. Introduction

It has been estimated that there are some 140 million contact lens wearers worldwide [1]. With the exception of daily wear disposable lenses, all require some form of chemical disinfection for their safe use [2]. Failure to ensure good lens hygiene practices can result in sight threatening infection of the cornea (keratitis) by bacteria, fungi and the free-living amoeba *Acanthamoeba*. Microbial keratitis is a rare but significant risk among soft contact lens wearers with a reported incidence of 4 cases per 10,000 users per year [1]. In keratitis due to the free-living amoebae *Acanthamoeba*, almost 90% of cases occur in contact lens wearers and the reported incidence varies from 1 to 2 cases per million in the USA or 17–20 in the UK [3].

Typically, contact lens disinfection is achieved using multipurpose disinfectant solutions (MPS) or hydrogen peroxide (H_2O_2). The majority of MPS are based on the preservative polyhexamethylene biguanide (PHMB) but other formulations use quaternary ammonium compound polyquaternium-1 (PQ-1) alone or in combination with the amidoamine myristamidopropyl dimethylamine (MAPD), PHMB or alexidine [4]. With H_2O_2 disinfectants, the 3% solution is neutralized during disinfection through the use of a platinum disc inside the case or the separate addition of a catalase tablet [5].

Most recently has seen the introduction of an alternative contact lens disinfection method based on the chemical povidone iodine (PI). The system comprises of a contact lens storage case, diluent and a tablet containing the PI at 500 ppm, giving 50 ppm active iodine (cleadew, Ophtecs Corporation, Japan). The tablet is composed of two components: the first contains the PI and an inner core of ascorbic acid and the enzyme protease. Lenses are placed into the storage case which is filled with a suitable volume of diluent (8 ml). The tablet is then added which immediately begins to dissolve, releasing the PI from the outer layer and turning the solution a dark brown colour. After 5 min, the ascorbic acid is then released which neutralizes the PI turning the solution clear to indicate disinfection is complete which occurs after approximately 20 min. Finally, the protease layer is released which acts to remove proteins from the lens surface. To ensure complete disinfection and

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Table 1
Microbial Efficacy of cleadew System against Bacteria, Fungi and *Acanthamoeba*.

Microbiology test	Log10 kill (4 h)							
	P. aeruginosa (ATCC 9027)	*S. marcescens* (ATCC 13880)	*S. aureus* (ATCC 6538)	*C. albicans* (ATCC 10231)	*F. solani* (ATCC 36031)	*A. castellanii* (ATCC 50370 trophozoites)	*A. castellanii* (ATCC 50370 cysts: NNA)	*A. castellanii* (ATCC 50370 cysts: Neff)
Biocidal ("stand alone")	> 4	> 4	> 4	> 4	> 4	≧5	1.1	2.8
Biocidal ("stand alone") +0.03% "organic soil"	< 1	< 1	< 1	< 1	< 1	NT[a]	NT	NT
Regimen	< 1	< 1	< 1	< 1	< 1	< 1	< 1	NT
Regimen + 0.03% "organic soli"	< 1	< 1	< 1	< 1	< 1	< 1	< 1	NT
Biocidal: tap water (Leicester) as diluent	> 4	> 4	> 4	> 4	> 4	> 4	NT	NT
Biocidal: Japanese tap water (soft water) as diluent	> 4.5	> 4.5	> 4.3	> 4.5	> 4.3	> 4.5	NT	NT
Biocidal: Japanese drinking water (hard water: cortex) as diluent	> 4.5	> 4.9	> 4.5	> 4.4	> 4.2	> 4.5	NT	NT

[a] NT = not tested.

removal of protein, the manufacturer's recommended soaking time is 4 h. The diluent contains 40 ppm hydrogen peroxide which reacts with I^- formed in the neutralisation stage to generate additional I_2 to further enhance the biocidal activity of the system. Hydrogen peroxide at this concentration is not toxic to the cornea, as 100 ppm is required before ocular sensitivity occurs [5,6].

In this study we determined the antimicrobial efficacy of the PI system against a range of microbes, including those commonly associated with keratitis. The system showed good activity against all bacteria, fungi and *Acanthamoeba* according to international standards, including ISO 14729 and FDA 510(k) and has prompted this report [7,8].

2. Methods

For the ISO:14729 microbiological analysis, the following organisms were studied: *Pseudomonas aeruginosa* (ATCC 9027), *Serratia marcescens* (ATCC 13880), *Staphylococcus aureus* (ATCC 6538), *Candida albicans* (ATCC 10231) and *Fusarium solani* (ATCC 36031) conidia. Testing was conducted in accordance with the international standards ISO 14729 and FDA 510(k). For the bacteria and fungi both stand-alone (biocidal) and regimen assays were performed according to ISO 14729 [7]. Briefly, in the stand-alone procedure the test solutions or Dulbeccos's Phosphate Buffered Saline (DPBS), negative control, were challenged with 1×10^6/ml organism and the number of survivors determined by culture viability after 0 and 4 h using the WASP Spiral Plater and ProtoCOL colony counter system or pour-plate methods [9]. At each time point the test samples were first diluted 1:10 into neutralizing broth consisting of sodium thiosulfate (0.6%), plant catalase (500 U/ml) and Tween 80 (0.05%) in DPBS, before determining the number of surviving organisms. The experiments were also repeated in the presence of organic soil consisting of a final concentration of approximately 3000 heat-killed (100 °C for 10 min) *Saccharomyces cerevisiae* (ATCC 9763) in 0.03% heat-inactivated foetal bovine serum (from stock of approximately 3×10^6 cells in 100% serum).

In some experiments, the challenged storage cases were left at 25 °C for 14 days before re-culturing to determine whether bacterial or fungal regrowth occurred. In others, the storage cases were sampled after 20 min as this is the time by which neutralization of the PI will have occurred.

In the regimen assay, three sets of the following lenses were tested per organism: Acuvue® Advance® (galafilcon A, Vistakon), O_2 OPTIX® (lotrafilcon B, CIBA Vision) and PureVision® (balafilcon A, Bausch & Lomb). Although not required by the protocol, 0.03% organic soil (see above) was also included in the organism challenge inoculum. Briefly, three lenses of each test type were inoculated with approximately 2×10^6 organisms and left to adhere for 5 min at 25 °C in a humidified chamber. Each lens was then subjected to the manufacturer's recommended lens care protocol. At the end of the regimen procedure, each test lens and soaking solution was placed into neutraliser followed by filtration and culture of the lens and membrane to determine the presence of surviving organisms. For the *Acanthamoeba,* the lenses were observed microscopically for any remaining, adhered trophozoites or cysts.

To test the efficacy of the system against biofilm formed bacteria, *S. maltophilia (*ALC01), *Achromobacter* sp. (S4) were suspended in 8 ml 0.01% trypticase soy broth and inoculated into 6 well microtiter plates (Nunc) for incubation, in air, at 32 °C for 24 h. Following rinsing of the wells three times DPBS, the wells were subjected to disinfection using the PI system with DPBS acting as a control. After 4 h, the biofilm was disrupted by sonication (50% amplitude for 3 s × 3) and the number of surviving bacteria determined as described above. Control wells were treated with DPBS only.

Acanthamoeba castellanii (ATCC 50370) biocidal efficacy was conducted as described previously using both the trophozoite and cyst form of the organism [10]. Cysts were produced using starvation of trophozoites plated on 2.5% non-nutrient agar (NNA) or Neff's chemically defined encystment medium [10,11]. The efficacy of the system against trophozoites using bathroom tap water (Leicester) and tap water and drinking water (Japan) was also tested to represent non-compliant use. Here, the tap water, in place of the system diluent, was challenged with 1×10^5 trophozoites and the povidone iodine tablet added. After 4 h the solution was centrifuged at 500 × *g* for 5 min, the supernatant discarded and the remaining pellet inoculated on a 1.5% NNA plate seeded with *Escherichia coli* (ATCC 8739) or into proteose-peptone-yeast extract (PYG) medium. The plates or culture flasks were incubated in air at 32 °C and inspected microscopically for 7 days for the presence of trophozoite growth and replication. Replacing the tablet diluent with tap or drinking water was also tested against the ISO:14729 panel of bacteria and fungi using the stand-alone protocol, as described above.

In addition, the capacity of the neutralized PI system to induce trophozoite encystment was investigated during incubation at 32 °C for 24 h, as described previously [12]. Neff's encystment medium containing 0.5% propylene glycol was used as a positive control.

The biocidal efficacy of the diluent only against *S. maltophilia* (ALC01), *Achromobacter* sp. (S4), *P. aeruginosa* (W9298), *P. aeruginosa* (WBI) and *Elizabethkingia* sp. (3AS) was also tested after 4 and 24 h exposure. The *P. aeruginosa* strains were isolated from keratitis patients and the others from contact lens storage cases [13].