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Bacterial biofilm in silver-impregnated contact lens cases

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ABSTRACT

Purpose: This study investigated the efficacy of pre-conditioning lens cases on bacterial biofilm formation and removal.

Methods: Silver impregnated (MicroBlock / ProGuard™ & i-Clean) and control storage cases were pre-conditioned for 24 h with their respective multipurpose solutions (MPDSs). Cases were then inoculated with 2 ml of 10^6 CFU/mL of ocular isolates of either *P. aeruginosa* or *S. aureus* and incubated for 48 h. Cases were subsequently disinfected (4 − 6 hours) as per the manufacturer's recommended disinfecting time (MRDT) followed by the recommended case hygiene procedures - recapping wet (MicroBlock / ProGuard™ cases only) or rinse and air-dry or rinse, tissue-wipe and air dry (mechanical disruption). Surviving bacteria were enumerated using standard techniques.

Results: Pre-conditioning the MicroBlock / ProGuard™ cases with MPDS significantly reduced biofilm formation (-1.1 $\log_{10}$ CFU, $p < 0.01$ for *P. aeruginosa* & -1.3 $\log_{10}$, $p < 0.001$, CFU for *S. aureus*) compared to the i-Clean lens cases. Maintaining the MicroBlock / ProGuard™ lens cases wet after the MRDT resulted in partial removal of bacterial biofilms (-2.9 $\log_{10}$ CFU, $p < 0.001$ for *P. aeruginosa* and -2.6 $\log_{10}$ CFU, $p < 0.001$ for *S. aureus*). Air-drying of all three types of lens storage cases after MRDT significantly reduced the bacterial biofilm (-5.4 $\log_{10}$ CFU, $p < 0.001$ for *P. aeruginosa* and -3.5 $\log_{10}$ CFU, $p < 0.001$ for *S. aureus*). Mechanical disruption produced the greatest reduction in the levels of bacterial biofilm in all 3 types of lens cases tested (-6.8 $\log_{10}$ CFU, $p < 0.001$ for *P. aeruginosa* and -4.5 $\log_{10}$ CFU, $p < 0.001$ for *S. aureus*). Synergi MPDS was significantly better than AQuify MPDS in removing bacterial biofilm from all 3 lens case types for case hygiene treatments with an air-drying step.

Conclusion: Pre-conditioning of silver-impregnated ProGuard™ lens cases inhibited initial bacterial biofilm formation. Synergi MPDS was more effective than AQuify MPDS in removing bacterial biofilm in silver impregnated cases and tissue-wiping significantly improved biofilm removal.

1. Introduction

Microbial contamination of contact lens storage cases has been implicated in corneal infections and inflammation [1–3]. Contamination of lens cases is common (30–80%) among lens wearers [4–7] and is observed even in the presence of good lens case hygiene practices [3,8]. Silver impregnated lens storage cases have been introduced to reduce microbial contamination by releasing silver ions from the lens case material [9,10]. Silver is a well-known antimicrobial agent affecting bacteria on contact by interference with DNA, cellular respiration, sulfhydryl groups, and enzyme conformation [9]. Recent *in vitro* and *in vivo* studies indicate that these silver impregnated cases reduce but do not completely eliminate microbial contamination [9,11–13]. Biofilm formation can occur following microbial adhesion to storage case surfaces [14] and current manufacturer's recommendations for lens storage case hygiene may be inadequate to remove microbial biofilm from regular as well as silver impregnated lens storage cases [11,12,15].

Current recommendations for lens storage case hygiene are inconsistent, with most recommending rinsing of wells (with lens care products or saline) followed by air drying (well and lid orientation unspecified) [16]. The MicroBlock / ProGuard (Alcon, Fort Worth, TX) case has been shown to release silver when maintained wet [12] and understandably the manufacturers of the MicroBlock / ProGuard lens cases suggest recapping the lens cases wet following rinsing with the MPDS [9]. Manufacturers of the i-Clean storage cases (CooperVision, Pleasanton, CA) recommend air drying following rinsing with either MPDS or saline. The lens case hygiene guidelines for silver impregnated

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lens cases may be confusing for the contact lens wearer. Recent studies have shown that mechanical disruption is most effective in removing bacterial biofilm from silver impregnated [11] and non-silver contact lens storage cases [17]. Furthermore, many people do not realise that MPDS and lens cases made by the same manufacturer have often been optimised for use together, and mismatching lens case and disinfecting solution has been shown to be a risk factor for lens case contamination. [18]

The objectives of the current study were to evaluate the ability of silver impregnated contact lens storage cases to inhibit biofilm formation following pre-conditioning with their complementary and non-complementary lens care products, to evaluate the impact of non-complementary lens care products and to compare the manufacturer's recommendation for storage case hygiene to the mechanical disruption of robust biofilm. A commercially available non silver lens storage case (CooperVision, Pleasanton, CA) was used as the control.

2. Materials and methods

2.1. Bacterial strains and media

P. aeruginosa 071 or *S. aureus* 031 isolated from cases of microbial keratitis in Australia were used in this study. Stock cultures of bacteria stored at −80 °C were streaked on a chocolate agar plate (Oxoid Australia, Sydney, NSW, Australia) and incubated at 37 °C for 24 h. The bacterial cells were collected and washed once by centrifugation in phosphate buffered saline (PBS, 8 g/l NaCl, 0.2 g/l KCl, 0.2 g/l KH_2PO_4, 1.15 g/l Na_2HPO_4, pH 7.4). Bacteria cells were re-suspended in Tryptone Soy Broth (TSB, 17 g/l Casein, 3.0 g/l Soybean Meal, 2.5 g/l Glucose, 5.0 g/l NaCl, 2.5 g/l K_2HPO_4,) diluted with PBS (1:10 for *S. aureus* and 1:100 for *P. aeruginosa*). The concentration of the bacterial suspension was adjusted to 0.1 at 660 nm wavelength using a spectrophotometer (Heliosβ, Unicam Instruments, Cambridge, UK; approximately 1.0×10^8 colony forming units per ml; CFU/ml). The suspension was serially diluted in the appropriate media to obtain the final culture concentration of 1.0×10^6 CFU/ml.

2.2. Contact lens storage case preconditioning and biofilm formation

Two commercially available silver impregnated contact lens storage cases MicroBlock / ProGuard™ (Alcon, Fort Worth, TX) and i-Clean (CooperVision, Pleasanton, CA), and one non silver contact lens storage case (CooperVision.) were used. Lens cases were preconditioned with one of 2 multipurpose disinfecting solutions (MPDS), one containing polyhexanide (AQuify; Alcon) and the other containing Oxipol™ (Synergi; CooperVision [withdrawn from the market]) as follows; each well of the lens case was filled with 2 ml of the MPDS, recapped and left in a static incubator at 25 °C for 24 h. Following this, the MPDS was discarded and each well was inoculated with 2 ml freshly prepared bacterial suspension, loosely capped and incubated at 37 °C in a digital agitator at 120 rpm for 24 h. After 24 h incubation, bacterial suspension was discarded, and wells gently rinsed with PBS once. All the wells were then refilled with 2 ml of freshly prepared medium (1:10 for *S. aureus* and 1:100 for *P. aeruginosa*) and all the storage cases were re-incubated with agitation for a further 24 h. Following 48 h of incubation, the media was discarded, and wells were gently washed with PBS twice to remove loosely adherent bacterial cells.

Treatment of contact lens storage cases: Following growth of the biofilm, the lens cases were treated as follows:

1 Untreated control (n = 54; 22 MicroBlock / ProGuard™, 16 i-Clean & 16 Control cases): No hygiene or disinfection treatment (immediately assayed for numbers of bacteria). MicroBlock / ProGuard™ cases were subject to the manufacturer recommended treatment "Disinfect, Rinse and Recap Wet" and hence required the 6 extra untreated control cases.
2 Disinfect with MPDS, rinse and re-cap wet (n = 40 MicroBlock / ProGuard™ cases only; as per manufacturer's instructions [9]): Lens case wells were filled with 2 ml of the MPDS and disinfected for 4 − 6 hours. The solution was then discarded, wells re-filled with 2 ml of the MPDS and cases shaken gently for 10 s. The solution was discarded and cases recapped for 18 h.
3 Disinfect with MPDS, rinse and air-dry face down (n = 120; 40 MicroBlock / ProGuard™, 40 i-Clean & 40 non-silver control cases; as per manufacturer's instructions for i-Clean cases): Lens case wells disinfected as described in treatment 2. Solution then discarded, wells re-filled with 2 ml of the MPDS and shaken gently for 10 s. The solution discarded and cases air dried face down on a clean facial tissue (Kimberly-Clark Australia Pty; Milsons Point, Australia) for 18 h at room temperature.
4 Disinfect with MPDS, rub & rinse, tissue wipe and air-dry face down (n = 120; 40 MicroBlock / ProGuard™, 40 i-Clean & 40 non-silver control cases): Lens cases wells were disinfected as described in treatment 2. The solution was then discarded, wells re-filled with 2 ml of the test solution and rubbed clockwise and anti-clockwise for 5 s with a gloved finger (Schiffa powder-free Latex Gloves, Icon Supplies Pty Ltd; Merrylands, Australia), capped and gently shaken for 10 s. The solution was discarded and cases air dried face down on a wire rack (Bel-Art, New Jersey, USA) for 18 h at room temperature.

Biofilm Recovery: Following treatments, 2 ml of PBS was added to each well along with a sterile magnetic stirring bar and vortexed for 1 min to dislodge the bacterial biofilm. Tenfold serial dilutions of the dislodged bacterial biofilm were performed in Dey-Engley neutralizing broth. Aliquots of the dilutions were inoculated in triplicate on nutrient agar plates and enumerated following 18 h of incubation at 37 °C.

Statistical Analysis: Prior to data analysis, $\log_{10}$ transformation of the bacterial data was performed and values from both wells of a lens case were averaged and used for analysis. Analysis was performed separately for *P. aeruginosa* and *S. aureus*. Data was analysed using SPSS version 21 (IBM, Chicago, IL). Data were modelled as multifactorial general linear model (3-way ANOVA). Initially an overall model with the main factors; lens cases, MPDS and case hygiene treatment and all interactions were assessed. If significant interactions were present, the analysis was repeated at each level of the interacting factor. This was performed until there were no more significant interacting factors. The level of significance was set at 5 % and post hoc multiple comparisons were adjusted using Bonferroni correction.

3. Results

Both the test bacterial strains formed robust biofilms in the 2 silver impregnated and the control contact lens storage case after 48 h. *S. aureus* biofilms were more resistant to the case hygiene treatments compared to the biofilms formed by *P. aeruginosa*.

3.1. Storage case treatments

Preconditioning Only: Biofilm formation by *P. aeruginosa* in the MPDS pre-conditioned MicroBlock / ProGuard™ cases was significantly lower ($p < 0.01$) compared to MPDS pre-conditioned silver impregnated i-Clean (-1.1 $\log_{10}$ CFU) and non-silver control cases (-1.1 $\log_{10}$ CFU) (Fig. 1). MicroBlock / ProGuard™ lens cases pre-conditioned with MPDS had significantly lower numbers of *S. aureus* ($p < 0.001$) compared to MPDS pre-conditioned silver impregnated i-Clean (-1.4 $\log_{10}$ CFU) and non-silver control cases (-1.6 $\log_{10}$ CFU) (Fig. 1).

Refill, rinse and re-cap wet: Recapping the MicroBlock / ProGuard™ lens cases and keeping wet following rinsing with MPDS significantly reduced the *P. aeruginosa* (-2.9 $\log_{10}$ CFU vs. untreated lens cases; $p < 0.001$) and *S. aureus* biofilms (-2.6 $\log_{10}$ CFU vs. untreated lens cases; $p < 0.05$) (Fig. 1).

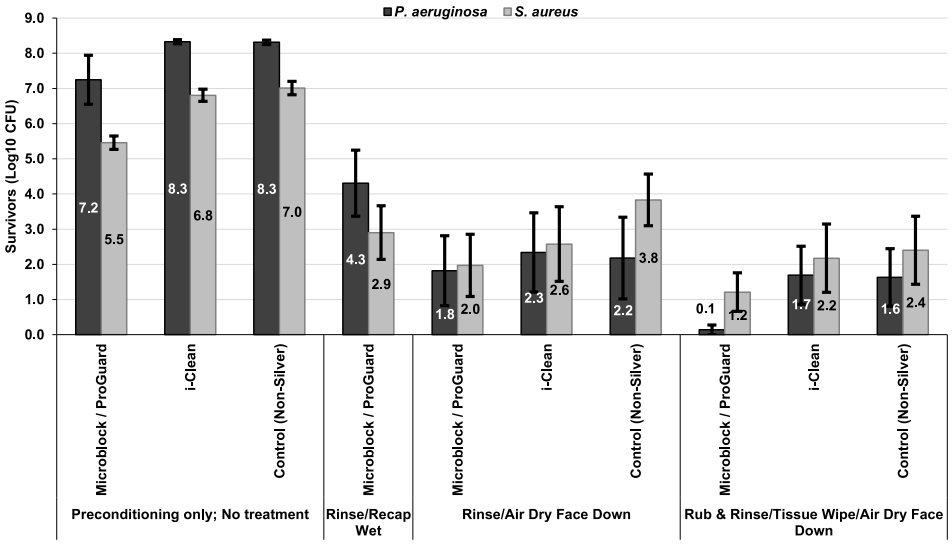

Fig. 1. Bacterial recovery (Mean ± 95 % CI) from silver impregnated (MicroBlock / ProGuard™ & i-Clean) and control (non-silver) lens storage cases following lens case hygiene procedures irrespective of the MPDS used.

Refill, rinse, air dry face down: Air drying of the cases following rinsing significantly reduced the levels of *P. aeruginosa* biofilm in all 3 lens cases (-5.4 $\log_{10}$ CFU vs. untreated cases; $p < 0.001$) (Fig. 1). There were no significant differences in biofilm removal between the silver impregnated MicroBlock / ProGuard™, i-Clean cases or the control case for this treatment ($p = 0.300$). Air drying the lens storage cases following a rinse with MPDS significantly also reduced *S. aureus* biofilm (-3.6 $\log_{10}$ CFU vs. untreated lens cases; $p < 0.001$) (Fig. 1).

Refill, rub & rinse, tissue wipe and air dry face down: The addition of the tissue-wipe step further improved *P. aeruginosa* biofilm removal from all three lens storage cases (-1.0 $\log_{10}$ CFU vs. air dried only; $p < 0.05$); however the greatest biofilm reduction was observed in the MicroBlock / ProGuard™ storage cases for this treatment ($p < 0.001$; Fig. 1). Similarly, the addition of tissue-wiping step further improved *S. aureus* biofilm removal from the lens storage cases (-0.8 $\log_{10}$ CFU vs. air-dried lens cases; $p < 0.001$) (Fig. 1).

3.2. Comparison of MPDS

For storage case treatments that included an air-drying step, the Synergi MPDS was more effective than AQuify MPDS in removing both *P. aeruginosa* and *S. aureus* biofilm from the silver-impregnated cases.

P. aeruginosa: There was no difference in biofilm formation of *P. aeruginosa* after pre-conditioning with either of the MPDS in the silver impregnated MicroBlock / ProGuard™, i-Clean cases or the control case (Preconditioning only, No treatment in Fig. 2). There was no significant difference ($p > 0.05$) in *P. aeruginosa* biofilm recovered from the MicroBlock / ProGuard™ lens cases rinsed with either MPDS and maintained wet i.e. recapped (Fig. 2). Air drying of all the 3 lens case types after rinsing with Synergi MPDS significantly reduced the level of bacteria compared to AQuify MPDS (-4.2 $\log_{10}$ CFU; $p < 0.001$) for this treatment (Fig. 2). Tissue wiping after rinsing with Synergi MPDS removed significantly more bacteria from the i-Clean (-3.2 $\log_{10}$ CFU; $p < 0.001$) and the non-silver control cases (-3.3 $\log_{10}$ CFU; $p < 0.001$) (Fig. 2) compared to rinsing with AQuify MPDS. There was no difference between the rinsing MPDS for this treatment in removing *P. aeruginosa* biofilm from the MicroBlock / ProGuard™ lens cases (Fig. 2). Tissue wiping following rubbing and rinsing produced the most reduction in *P. aeruginosa* biofilm of the three treatments tested from all 3 lens storage cases.

S. aureus: MicroBlock / ProGuard™ lens cases pre-conditioned with AQuify MPDS had lower levels of *S. aureus* biofilm (-0.6 $\log_{10}$ CFU; $p < 0.001$) compared to the cases pre-conditioned with Synergi MPDS (Preconditioning only, No treatment in Fig. 3). Rinsing and recapping the MicroBlock / ProGuard™ lens cases with Synergi MPDS resulted in a greater reduction of *S. aureus* biofilm (-2.8 $\log_{10}$ CFU vs. AQuify MPDS; $p < 0.001$) (Fig. 3). Rinsing with the Synergi MPDS followed by air drying improved removal of *S. aureus* biofilm compared to rising with AQuify MPDS from the MicroBlock / ProGuard™ (-3.5 $\log_{10}$ CFU vs. AQuify MPDS; $p < 0.001$), i-Clean (-4.3 $\log_{10}$ CFU vs. AQuify MPDS; $p < 0.001$) and the non-silver control cases (-2.3 $\log_{10}$ CFU vs. AQuify MPDS; $p < 0.001$) (Fig. 3). With the addition of a tissue-wiping, Synergi MPDS was again better at removing biofilm from MicroBlock / ProGuard™ (-2.1 $\log_{10}$ CFU vs. AQuify MPDS; $p < 0.001$), i-Clean (-4.0 $\log_{10}$ CFU vs. AQuify MPDS; $p < 0.001$) and the non-silver control cases (-3.9 $\log_{10}$ CFU vs. AQuify MPDS; $p < 0.001$) (Fig. 3). However, AQuify MPDS was significantly better at removing *S. aureus* biofilm from the complementary MicroBlock / ProGuard™ lens cases than similarly treated i-Clean (-1.9 $\log_{10}$ CFU; $p < 0.001$) and the non-silver control cases (-2.1 $\log_{10}$ CFU; $p < 0.001$) (Fig. 3).

4. Discussion

This study has demonstrated that a robust bacterial biofilm that resists the normal disinfection cycle [19] can be formed in silver impregnated contact lens storage cases pre-conditioned with multi-purpose disinfection solution. Pre-conditioning of all the storage cases with MPDS was performed to replicate regular use of a new and unused storage case and subsequent bacterial contamination during handling. The robust hard to remove biofilm formed by this method may therefore provide a better comparison of the effectiveness of different lens storage case hygiene methods. The results of this study show that current manufacturer's recommendations did not completely remove robust bacterial biofilm from the silver impregnated contact lens storage cases. The addition of a tissue wipe step to the current recommendation was the most effective method of removing bacterial biofilm.

The MicroBlock / ProGuard™ cases were more effective than the i-Clean cases in reducing the initial biofilm formation, and these results are in agreement with earlier studies [11,12]. The higher levels of bacteria (especially over double the levels of *P. aeruginosa*) recovered from the MicroBlock / ProGuard™ cases in comparison to those in the study by Wu et al., [11] demonstrates that the protective effect offered by silver ion release [12] may be inadequate if the level of biofilm is high. Pre-conditioning the MicroBlock / ProGuard™ cases was beneficial and reduced levels of *P. aeruginosa*; an earlier study performed without pre-conditioning reported significantly higher levels of *P.*

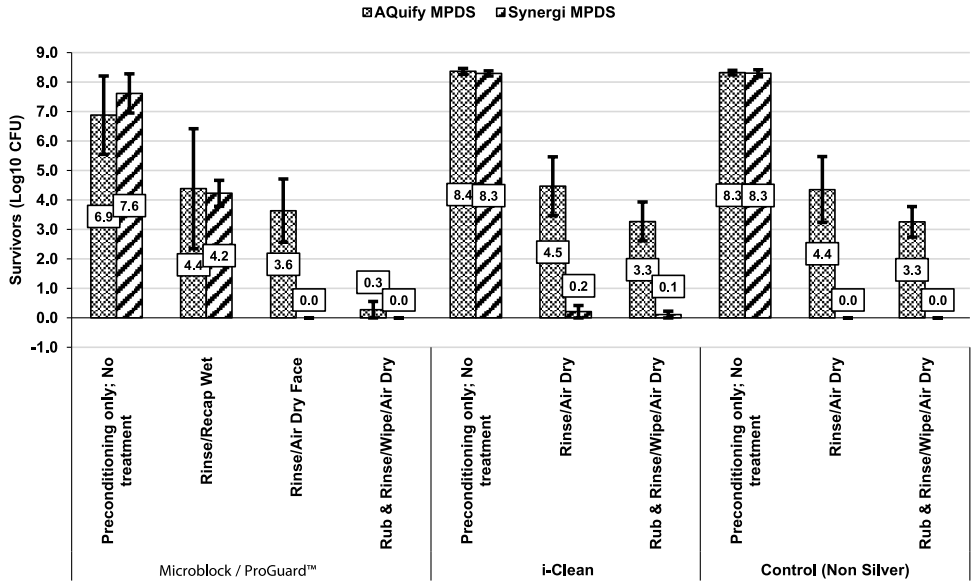

Fig. 2. Comparison of MPDS in reducing levels of *P. aeruginosa* (Mean ± 95 % CI) from silver impregnated (MicroBlock / ProGuard™ & i-Clean) cases and control (non-silver) lens storage cases following lens hygiene treatments.

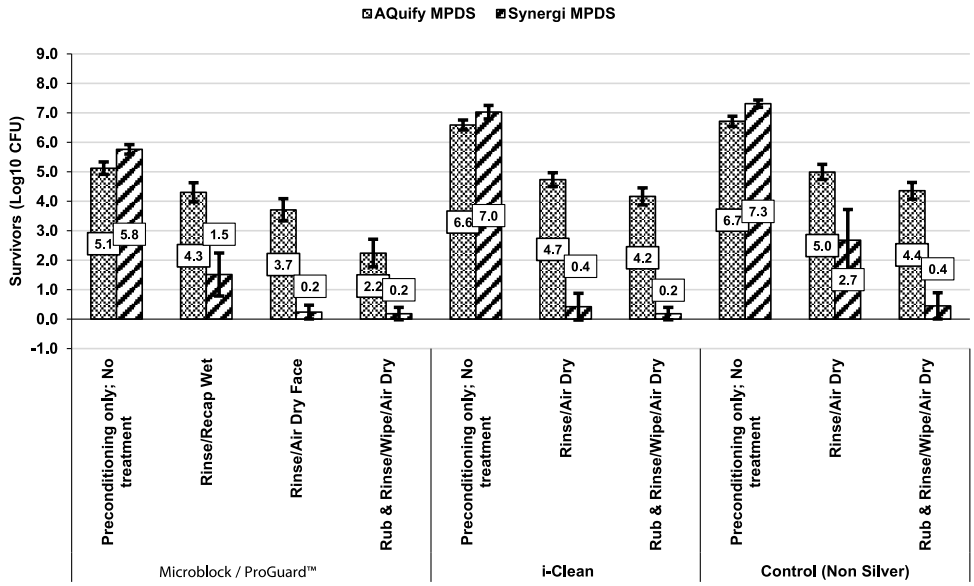

Fig. 3. Comparison of MPDS in reducing levels of *S. aureus* (Mean ± 95 % CI) from silver impregnated (MicroBlock / ProGuard™ & i-Clean) cases and control (non-silver) lens storage cases following lens hygiene treatments.

aeruginosa than on non-silver control cases [20]. The pre-conditioning MPDS had an impact on *S. aureus* biofilm formation as all cases pre-conditioned with AQuify MPDS showed reduced levels in comparison to cases pre-conditioned with Synergi MPDS. Since MicroBlock / ProGuard™ cases have decreased efficacy against *S. aureus* [11,21] and the reduction was observed in all AQuify pre-conditioned lens cases, it it likely that the effect is predominantly driven by AQuify MPDS which has higher antimicrobial activity against *S. aureus* [22].

Rinsing and recapping the lens cases following disinfection significantly reduced the levels of both bacterial strains. The bacterial biofilms in this study were resistant to disinfection with MPDS [19,23], therefore it is probably the longer duration of the treatment (6 h vs. overnight) with increased exposure to the silver ions [12] that contributed to the decrease. Air drying the lens cases following disinfection was more effective in reducing bacterial biofilm than recapping the lens cases wet. An earlier study with shorter duration of air drying did not show significant improvement over recapping in reducing bacterial biofilm in MicroBlock / ProGuard cases [11]. The longer duration of drying was the most likely factor for this improvement as there were no significant differences between the survivors for both the bacterial strains in the MicroBlock / ProGuard™ as well as the i-Clean and the non silver control lens cases. There was a significant difference between the the two MPDS but not for the lens case type in reducing the levels of either bacterial strains following air drying. Air drying reduces bacterial viablility and significantly enhances the antimicrobial efficacy of biocides in the MPDS [24], it is possible that the longer drying time enhanced the antimicrobial efficacy of the oxidative MPDS Synergi.

Mechanical disruption of the bacterial biofilm through additional steps of rubbing and rinsing followed by tissue wiping demonstrated greatest reduction of the biofilm. These results are similar to those published by Wu et al., [11,15,17]. Another study has shown that rubbing and rinsing with the MPDS is not as effective as tissue wiping in removing bacterial biofilm from the storage cases [19]. Regardless of the lens case hygiene treatment performed, the Synergi MPDS was

effective than AQuify MPDS in reducing bacterial biofilm. While other authors have examined the antimicrobial efficacy of the Synergi MPS used with the barrel storage cases [25], to our knowledge this is the first study that has evaluated the antimicrobial efficacy of Synergi in flat pack cases. The solution was particularly effective when there was an air drying step in the case treatment. The antimicrobial agents in Synergi MPDS are sodium hypochlorite and hydrogen peroxide [26] both of which have strong oxidising properties. An air drying step improved the antimicrobial efficacy. The decreased efficacy against both the bacterial strains tested in the rinse and recap treatment validates the importance of the air drying step for Synergi MPDS. The significant improvement in *S. aureus* biofilm removal from the control cases achieved by Synergi MPDS following the addition of mechanical disruption strengthens the argument for including this step to the current lens case hygiene recommendations. Another factor that may have contributed to the biofilm removal may be the increase in the air flow due to rack drying. Air drying face up was better than face down for biofilm removal [19] and rack drying provides a safer alternative to drying face up which carries the risk of environmental contamination [27].

The Synergi MPDS demonstrated excellent antimicrobial activity in both silver and non-silver lens storage cases against the 2 bacterial strains used in this study. Similarly, in another in-vitro study the Synergi the MPDS showed significant antimicrobial activity against a number of gram positive and gram negative bacteria in silver impregnated and control storage cases [25]. However, these results cannot be generalised for all the strains that may contaminate contact lens storage cases and might not translate to real-world results. A clinical study comparing the silver impregnated i-Clean and control cases using the Synergi MPDS did not find significant differences in the rate on contamination of these 2 cases [28]. Studies examining contact lens storage case contamination are usually performed using their complementary lens care solution. In this study, we examined the impact of using non-complementary MPDS on the biofilm removal during case hygiene procedures as the use of non-complementary lens care products has been shown to be associated with greater level and frequency of contamination of storage cases [18]. Previous studies evaluating the efficacy of storage case hygiene methods using regular and silver impregnated storage cases with their complementary MPDS [11,15,17,19,29] or hot water [19] have shown that mechanical disruption i.e. the tissue wipe is most effective in removing bacterial biofilm and reducing lens storage case contamination [27]. The results of this study demonstrate that even when performed with the non-complementary MPDS, the tissue wipe step significantly improved contact lens storage case hygiene. However, the results of this study need to be validated in clinical trials prior to advocating the use of non-complementary lens care products.

Patient factors such as compliance with hygiene procedures [7,30–32], age and gender [33] hand washing prior to handling the lenses [30,34] can influence lens case contamination. While patient compliance cannot be controlled as even written instructions have been shown to be insufficient [35,36], if care guideliness are simple consistent and effective, there is a better chance of compliance [27] which will add to the effectiveness of antimicrobial lens cases and multipurpose solutions. The tissue-wipe step offers a significant decrease in bacterial contamination of lens storage cases and therefore should be included in the lens care hygiene regime. Contact lens case hygiene should be standardised to include tissue wiping prior to air drying in line with the recommendations by the CDC [37] to maximise microbial removal.

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Competing interests

None.

References

[1] A.K. Bates, et al., 'STerile' corneal infiltrates in contact lens wearers, Eye (Lond) 3 (Pt 6) (1989) 803–810.
[2] M.S. Mayo, et al., Antibiograms, serotypes, and plasmid profiles of *Pseudomonas aeruginosa* associated with corneal ulcers and contact lens wear, J Clin Microbiol 24 (3) (1986) 372–376.
[3] F. Stapleton, et al., Epidemiology of *Pseudomonas aeruginosa* keratitis in contact lens wearers, Epidemiol Infect 114 (3) (1995) 395–402.
[4] P. Devonshire, et al., Microbial contamination of contact lens cases in the west of Scotland, Br J Ophthalmol 77 (1) (1993) 41–45.
[5] M.D. Willcox, et al., Contact lens case contamination during daily wear of silicone hydrogels, Optom Vis Sci 87 (7) (2010) 456–464.
[6] Y.T. Wu, et al., Profile and frequency of microbial contamination of contact lens cases, Optom Vis Sci 87 (3) (2010) E152–8.
[7] M.S. Yung, et al., Microbial contamination of contact lenses and lens care accessories of soft contact lens wearers (university students) in Hong Kong, Ophthalmic Physiol Opt 27 (1) (2007) 11–21.
[8] L.A. Wilson, et al., Microbial contamination of contact lens storage cases and solutions, Am J Ophthalmol 110 (2) (1990) 193–198.
[9] C.F. Amos, M.D. George, Clinical and laboratory testing of a silver-impregnated lens case, Cont Lens Anterior Eye 29 (5) (2006) 247–255.
[10] B.J. Hall, L. Jones, Contact lens cases: the missing link in contact lens safety? Eye Contact Lens 36 (2) (2010) 101–105.
[11] Y.T. Wu, et al., Impact of cleaning regimens in silver-impregnated and hydrogen peroxide lens cases, Eye Contact Lens 37 (6) (2011) 365–369.
[12] J. Dantam, H. Zhu, F. Stapleton, Biocidal efficacy of silver-impregnated contact lens storage cases in vitro, Invest Ophthalmol Vis Sci 52 (1) (2011) 51–57.
[13] J. Dantam, et al., In vivo assessment of antimicrobial efficacy of silver-impregnated contact lens storage cases, Invest Ophthalmol Vis Sci 53 (3) (2012) 1641–1648.
[14] J. Dart, The inside story: why contact lens cases become contaminated, Cont Lens Anterior Eye 20 (4) (1997) 113–118.
[15] Y.T. Wu, et al., Removal of biofilm from contact lens storage cases, Invest Ophthalmol Vis Sci 51 (12) (2010) 6329–6333.
[16] Y. Wu, et al., Contact lens and lens storage case cleaning instructions: whose advice should we follow? Eye Contact Lens 36 (2) (2010) 68–72.
[17] Y.T. Wu, et al., The effectiveness of various cleaning regimens and current guidelines in contact lens case biofilm removal, Invest Ophthalmol Vis Sci 52 (8) (2011) 5287–5292.
[18] Y.T. Wu, M.D. Willcox, F. Stapleton, The effect of contact lens hygiene behavior on lens case contamination, Optom Vis Sci 92 (2) (2015) 167–174.
[19] A.K. Vijay, et al., Contact lens storage case hygiene practice and storage case contamination, Eye Contact Lens 41 (2) (2015) 91–97.
[20] P.B. Vermeltfoort, et al., Bacterial transmission from lens storage cases to contact lenses-Effects of lens care solutions and silver impregnation of cases, J Biomed Mater Res B Appl Biomater 87 (1) (2008) 237–243.
[21] P.B. Vermeltfoort, et al., Bacterial transmission from contact lenses to porcine corneas: an ex vivo study, Invest Ophthalmol Vis Sci 46 (6) (2005) 2042–2046.
[22] C. Scheuer, et al., Multipurpose solutions and rates of biocidal efficacy, Eye Contact Lens 35 (2) (2009) 88–91.
[23] M.D. Willcox, H. Zhu, A.K. Vijay, Effect of a warming device on contact lens case contamination, Eye Contact Lens 38 (6) (2012) 394–399.
[24] L.A. Wilson, A.D. Sawant, D.G. Ahearn, Comparative efficacies of soft contact lens disinfectant solutions against microbial films in lens cases, Arch Ophthalmol 109 (8) (1991) 1155–1157.
[25] A. Datta, M. Willcox, F. Stapleton, In vitro antimicrobial efficacy of silver Lens cases used with a multipurpose disinfecting solution, Transl Vis Sci Technol 8 (3) (2019) 52.
[26] M.E. Cavet, et al., In vitro biocompatibility assessment of multipurpose contact lens solutions: effects on human corneal epithelial viability and barrier function, Cont Lens Anterior Eye 35 (4) (2012) 163–170.
[27] Y.T. Wu, et al., Impact of Lens case hygiene guidelines on contact Lens case contamination, Optom Vis Sci 88 (10) (2011) 8.
[28] J. Dantam, Anti-microbial efficacy of the silver-impregnated contact lens storage cases, Optometry & Vision Science, Faculty of Science, University of New South Wales, Sydney, 2011, p. 133.
[29] Y.T. Wu, et al., Impact of air-drying lens cases in various locations and positions, Optom Vis Sci 87 (7) (2010) 465–468.
[30] A.M. Yung, et al., The effect of a compliance enhancement strategy (self-review) on the level of lens care compliance and contamination of contact lenses and lens care accessories, Clin Exp Optom 90 (3) (2007) 190–202.
[31] P. Cho, M. Boost, R. Cheng, Non-compliance and microbial contamination in orthokeratology, Optom Vis Sci 86 (11) (2009) 1227–1234.
[32] S. Hickson-Curran, R.L. Chalmers, C. Riley, Patient attitudes and behavior regarding hygiene and replacement of soft contact lenses and storage cases, Cont Lens Anterior Eye 34 (5) (2011) 207–215.
[33] Y. Jiang, et al., Risk factors for microbial bioburden during daily wear of silicone hydrogel contact lenses, Eye Contact Lens 40 (3) (2014) 148–156.
[34] C.F. Radford, E.G. Woodward, F. Stapleton, Contact lens hygiene compliance in a university population, J Br Contact Lens Assoc 16 (3) (1993) 105–111.
[35] T. Kuzman, et al., Lens wearers non-compliance - is there an association with lens case contamination? Cont Lens Anterior Eye 37 (2) (2014) 99–105.
[36] D. Tilia, et al., The effect of compliance on contact Lens case contamination, Optom Vis Sci 91 (3) (2014) 262–271.
[37] Centers for Disease Control and Prevention, Contact Lens care systems & solutions, (2016) [cited 2019 31/10/2019]; Available from: https://www.cdc.gov/contactlenses/care-systems.html.