**The Effectiveness of Various Cleaning Regimens and Current Guidelines in Contact Lens Case Biofilm Removal**

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**Purpose.** Lens case hygiene is important for safe contact lens wear. However, there are no evidence-based data to suggest optimum hygiene regimens. This in vitro study aimed to evaluate and compare the effectiveness of manufacturers’ guidelines and several other regimens in removing biofilm using various types of contact lens cases and disinfecting agents.

**Methods.** Biofilms of *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* 122 were formed on two types of unused contact lens cases. Subsequently, each contact lens case was subjected to one of four cleaning regimens using two different multipurpose disinfecting solutions or distilled water: “rinse and air-dry (guidelines),” “rub, rinse and air-dry,” “tissue-wipe and air-dry,” and “rub, rinse, tissue-wipe, and air-dry.” The levels of residual biofilm were then quantified using viable counts and compared.

**Results.** The manufacturers’ guidelines resulted in 1 to 2 log CFU reduction of either biofilm. “Rub, rinse, tissue-wipe, and air-dry” was the most effective cleaning regimen (P < 0.001), capable of removing 4 to 6 log CFUs of bacteria; higher levels of biofilm were removed by mechanical friction from nonridged cases than that of ridged cases (P < 0.001). Biofilm removal varied with multipurpose solution tested.

**Conclusions.** Current manufacturers’ guidelines are not adequate in eliminating microbial contamination. Simply incorporating a rubbing/wiping step in daily case hygiene reduces viable organism recovery. Factors such as the cleaning regimen, antimicrobial potency of multipurpose solution, and the topography of the lens case may impact the surface detachment of biofilm during the cleaning process. *(Invest Ophthalmol Vis Sci. 2011;52:5287–5292)* DOI:10.1167/iovs.10-6785

Contact lens storage case contamination is often associated with contact lens-related microbial keratitis1–4 and inflammation.5 The contact lens case may be a potential reservoir for the microorganisms responsible for microbial keratitis, as identical strains of bacteria have been isolated from the corneal ulcer in microbial keratitis and the lens storage case of affected patients.5

Despite the use of contact lens disinfecting solutions, microbial contamination of lens cases is high, ranging from 30% to 80% of cases.6–10 Once bacteria enter the lens case, they may adhere to the case and switch from being planktonic cells to a sessile biofilm phenotype which is less susceptible to disinfectants.11 This biofilm represents a challenge in maintaining sterile storage cases,12,13 and simply soaking lens cases in disinfecting solutions may not be adequate to eliminate potentially virulent microorganisms. Additional cleaning regimens may be advisable to reduce biofilm levels in lens storage cases.

The most easily accessible and commonly followed lens case cleaning instruction is to rinse lens cases with multipurpose disinfecting solutions (MPDS), and air-dry.14 This information is available on the Food and Drug Administration (FDA) official website (http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm048893.htm) as well as the product inserts that accompany purchase of MPDS. However, the effectiveness of such cleaning regimen has not yet been evaluated. This presents a challenge for contact lens practitioners and wearers in the selection of an optimal care regimen for lens storage cases.

A previous study15 evaluated the various steps in the cleaning regimen and found that rubbing and tissue-wiping separately were effective at reducing levels of biofilm in lens cases, and soaking alone reduced the level of viable biofilm to a level comparable to the median level of case contamination in the community. However, the level of residual biofilm varied when different multipurpose disinfecting solution types were used. Based on this, we designed the series of experiments in the present study omitting the soaking step to specifically assess the impact of each sequential hygiene practice against a robust and consistent biofilm. Omitting the soaking step allowed the in vitro performance of the practices in the two studies to be directly compared. The present study examined these cleaning methods in sequence and in combination using different types of lens cases and rinsing agents to better represent the real life situation and compared with the effectiveness of manufacturers’ guidelines. In addition, the impact of the lens case surface topology on the ease of biofilm removal was also examined.

The hypothesis was that a combination of these most effective single cleaning methods will remove significantly more biofilm from the cases, greater than that achieved from adhering current manufacturers’ guidelines.

**Materials and Methods**

Two commercially available multipurpose disinfecting solution and lens cases were used in this study.

**Product A:** solution (Opti-Free Replenish; Alcon Ltd, Fort Worth, TX) includes polyquaternium-1 (Polyquad) 0.0001% and myristamido-propyl dimethylamine (Aldox) 0.0005% as disinfectants, surfactants and chelating agents (Tearglyde and Tetronic 1304), nonanoyl ethylendiaminetriacetic acid, and a borate buffer system (sodium borate, sodium citrate, and sodium chloride). It is accompanied by a ridged lens case (Alcon Ltd), with 16 ridges in the inner surface well. Lens cases are molded from polypropylene (Fig. 1A).
P. aeruginosa approximately 10^6 cells per mL by serial dilution in PBS with 1% Luria Broth. Subsequently, the concentration of each inoculum was adjusted to approximately 10^8 colony-forming units (CFU)/mL) at wavelength 660 nm. Photometrically to achieve an optical density (OD) of 0.1 (approximately tryptone, 5.0 g/L yeast extract and 5.0 g/L NaCl) and adjusted spectrophotometrically to achieve an optical density (OD) of 0.1 (approximately 10^6 colony-forming units (CFU)/mL) at wavelength 660 nm. Subsequently, the concentration of each inoculum was adjusted to approximately 10^6 cells per mL by serial dilution in PBS with 1% Luria Broth.

Bacterial Strains

Staphylococcus aureus ATCC 6538 and Pseudomonas aeruginosa 122 were selected as the challenge bacteria. S. aureus ATCC 6538 is a standard-type strain used as a test strain in International Standards Organization 14729, whereas P. aeruginosa 122 was a corneal isolate from contact lens-related microbial keratitis. These strains were chosen for their ability to form adequate biofilms on lens storage cases. Each strain was obtained from a bacterial stock stored at -80°C and streaked on a heated blood (“chocolate”) agar plate (Oxoid; Adelaide, SA, Australia) for recovery. Plates were incubated in O2 at 37°C for 24 hours. After 24 hours, colonies were harvested and suspended in phosphate buffered saline (PBS) with 1% Luria Broth (PBS-LB; 10.0 g/L tryptone, 5.0 g/L yeast extract and 5.0 g/L NaCl) and adjusted spectrophotometrically to achieve an optical density (OD) of 0.1 (approximately 10^6 colony-forming units (CFU)/mL) at wavelength 660 nm. Subsequently, the concentration of each inoculum was adjusted to approximately 10^6 cells per mL by serial dilution in PBS with 1% Luria Broth.

Biofilm Formation on Lens Storage Cases

Each lens case well was inoculated with 2 mL freshly prepared bacterial suspension (10^6 CFU/mL) for each bacterial strain individually. To facilitate biofilm formation, lids of the lens cases were loosely recapped and lens cases were incubated in a 37°C digital agitator at 120 rpm for 24 hours. Bacterial suspensions were then discarded, and all wells were rinsed with distilled water twice to dislodge planktonic cells.

For quantification of biofilm, the number of viable bacterial cells was enumerated by sampling each well with a sterile calcium alginate swab and lens cases were air-dried face down on a clean facial tissue (Kimberly-Clark Australia Pty; Milsons Point, Australia) for six hours.

3. Rub, rinse, and air-dry: treated lens cases were filled to 80% volume with MPDS or distilled water, rubbed clockwise and anticlockwise for 5 seconds with a finger of a hand wearing a disposable aseptic glove (Gelttek powder-free Latex gloves; Livingstone International Pty, Ltd; Rosebery, Australia). The solution was then discarded, and the wells air-dried face down on a clean facial tissue for six hours.

4. Tissue-wipe: a facial tissue was used to wipe the interior side of the case well in circular motions and the wells then air-drying face down on a clean facial tissue for six hours.

5. Rub, rinse, tissue-wipe, and air-dry: the rub and rinse steps described above were performed followed immediately by the tissue-wipe and air-drying steps.

In the guideline group (2), lens cases were rinsed with the complementary lens care solution from the same manufacturer as specified in the guideline16 (case A with solution A, case B with solution B). Lens cases in other regimen groups (3, 4, and 5) were rinsed with all three rinsing agents (solution A/solution B/water) respectively to evaluate the effect of rinsing lens cases with MPDS from different manufacturers.

The number of remaining biofilm cells (CFUs) were determined as described above. The effectiveness of each regimen was expressed as log CFU reduction from the controls (no cleaning).

Statistical Analysis

The total number of viable organisms for each swab was recorded as log CFU per mL. Student’s t-tests were used to compare the initial biofilm attachment between the two bacterial strains, and between the two types of contact lens cases. Two-way ANOVA was used to investigate the efficacy of rinsing agents and lens case types used in the each cleaning regimen group in reducing final CFU counts. The effectiveness of cleaning regimens was compared by one-way ANOVA. A Games-Howell adjustment was used for post hoc multiple comparisons. P < 0.05 was considered statistically significant. All statistical analyses were performed using computer statistical software (SPSS v.18; SPSS Inc., Chicago, IL).

RESULTS

Effectiveness of the Manufacturers’ Guideline Regime

For the control condition, there was greater P. aeruginosa biofilm (6.89–7.19 log CFU) on lens cases compared with S. aureus (6.80–6.86 log CFU) (P < 0.001), particularly in case B than in case A (P < 0.001; Fig. 2). There were no differences in S. aureus biofilm on cases A and B (P = 0.28), however there were higher levels of P. aeruginosa biofilm on case B than on case A (P < 0.001).

After the rinse and air-dry regime (manufacturers’ guidelines), irrespective of the bacterial strains tested, the overall reduction in biofilm was only 0.8–2.3 log units; the viable counts from the remaining biofilm ranged from 4.55 to 4.62 log units for case A and from 5.41 to 6.06 log units for case B. There were fewer remaining bacteria after rinsing with product A (P < 0.001).

Effectiveness of Rubbing, Rinsing, and Air-drying

Irrespective of the bacterial strain or lens case type, cases rubbed and rinsed with solution A showed a significantly greater reduction in bacterial numbers compared with those lens cases in which solution B or distilled water were used (P > 0.001; Fig. 3). A greater number of S. aureus cells were removed by using product B than those rinsed with distilled water (P < 0.001). No significant differences were found.
between solution B and distilled water in removing *P. aeruginosa* after rubbing and rinsing of lens cases (*P* > 0.05). Overall, *S. aureus* detached more readily from lens cases than *P. aeruginosa* (*P* < 0.0001) when adding a “rub” step into manufacturers’ guidelines.

**Effectiveness of Tissue-wiping**

When tissue-wiping was carried out alone, significantly more biofilm was removed from case B than from case A (*P* < 0.001, Fig. 4). Within each lens case type, no significant difference in the ease of detachment was found between strains (*P* > 0.5).

**Effectiveness of Rubbing, Rinsing, Tissue-wiping, and Air-drying**

Solution A removed significantly higher levels of *S. aureus* than either solution B or distilled water irrespective of lens case type (*P* < 0.05) (Fig. 5).

For case A, no significant differences were found in the level of *P. aeruginosa* removed using solution A or B, and both removed greater numbers of bacteria than distilled water (*P* < 0.001). For case B, under the tested regimen there were no significant differences in the level of *P. aeruginosa* biofilm reduction among these three rinsing agents (*P* > 0.79).

**Comparison of Efficiencies between Different Cleaning Regimens**

The levels of biofilm reduction after each cleaning regimen were shown in Figure 6. Overall, regardless of the rinsing agents and bacterial strains tested, each cleaning regimen showed a significant reduction of biofilm compared with the control (*P* < 0.05).

The most effective cleaning regimen in case A lens cases was the “rub, rinse, tissue-wipe, and air-dry” (*P* < 0.001). The level of biofilm removed using “rinse and air-dry (manufacturers’ guidelines),” “tissue-wipe,” or “rub, rinse, and air-dry” was not significantly different (*P* = 1.00).

For case B, the most effective cleaning regimens were the “rub, rinse, tissue wipe, and air-dry” (*P* < 0.001) and the “tissue-wipe alone” (*P* < 0.001); there was no significant difference between these two cleaning methods (*P* = 0.69). The next most effective cleaning regimen was “rub, rinse, and air-dry” (*P* < 0.001), which was more effective than the “rinse and air-dry” regime (manufacturers’ guideline) (*P* < 0.001).
Irrespective of the rinsing agents (MPDS or water), significantly more biofilm was removed from case B than that of case A after including rubbing and/or tissue-wiping regimen ($P < 0.001$). Particularly, using the combination of solution A and case B, after “rubbing, rinsing, tissue-wiping, and air-drying” showed to be the most effective in all the regimens ($P < 0.001$).

**DISCUSSION**

This study has established that rubbing and rinsing with MPDS followed by tissue-wiping and air-drying is the most effective cleaning regimen in removing biofilm cells of *S. aureus* or *P. aeruginosa* from the surface of contact lens cases. This cleaning regimen is substantially better than the currently recommended manufacturers’ guidelines.

Relatively small amounts of biofilm were removed after the manufacturers’ guidelines (rinsing and air-drying) (reductions from 0.8 to 2.2 log CFU). As a previous study has shown that the level of lens case contamination can be as high as 10⁶ CFU, and that the lens case is often the most contaminated item of all lens accessories, this marginal reduction in bacterial load after rinsing and air-drying may not be adequate to remove contaminants in lens cases. This suggests that cleaning steps additional to the current guidelines are necessary. Therefore, the present study further incorporated rubbing and tissue-wiping in a sequential manner and evaluated the effect of using different rinsing agents, solution A and solution B, which are commonly used by lens wearers.⁶

Consistently fewer bacteria were recovered from cases cleaned with MPDS containing solution A than from the cases cleaned with other rinsing agents tested (Figs. 2 and 3), and this is consistent with a study assessing the effect of disinfecting solutions against biofilm formed on contact lenses.¹¹ Notwithstanding these findings, the cleaning and antibacterial potency of rinsing agents was less relevant if frictional forces, such as rubbing and tissue-wiping (Fig. 5) were applied. This implies that abrasion is useful for removing biofilm from lens storage cases.

As mechanical friction and shear-induced detachment are likely to be decreased in rough surfaces,¹⁷ the effect of interior surface design/shape of lens cases on the effectiveness of biofilm detachment was further explored. The data indicate that significantly more biofilm was removed from tissue-wiping in non-ridged/smooth lens cases (case B) than from ridged lens cases (case A) (Fig. 4), in which the grooves are hard to reach and could shelter the bacteria. Hence, topography of the lens case well made a difference to its ease of cleaning. This finding is reflected in the overall comparison of cleaning regimen tested in Figure 6 which shows that neither rubbing nor tissue-wiping regimen had any significant greater effect in removing biofilm than rinsing alone in ridged lens cases. A significant reduction in biofilm was only found if rubbing and wiping are carried out sequentially in ridged lens cases. On the other hand, tissue-wiping alone in smooth surface cases (case B) showed a significant effect of biofilm reduction even in the absence of rinsing (Fig. 6). The multifiber and absorbent quality of tissue may enhance the removal of bacteria and excess water, hence, showing less bacterial recovery. It is conceivable that tissues may not be sterile; however, we did not recover any bacterial species other than the challenge bacteria. The potential limitation of the study is that rubbing was carried out while wearing gloves, and this was to ensure sterile technique in the laboratory. Despite this limitation, the study has established the principle that mechanical friction was the most efficient in reducing biofilm, especially in smooth-surfaced case wells.

It is speculated that had these lens cases in our experiment been soaked in multipurpose disinfecting solution for a recommended time, the biofilm might detach or dislodge more readily. The rationale of not involving a soaking step in the present study was that soaking alone reduced the viable biofilm¹⁵ to a level that did not allow sufficient discrimination between subsequent mechanical cleaning and drying practices. Hence, we designed the series of experiments in the present study to specifically assess the impact of each sequential hygiene practice against a more robust and consistent biofilm. This allows the cleaning steps to be better compared in vitro. The lens cases in this study were inoculated with an initial concentration of 2 × 10⁶ bacteria to develop biofilm on lens wells and the final bacterial recovery from the control group in both lens cases in this study were inoculated with an initial concentration of 2 × 10⁶ bacteria to develop biofilm on lens wells and the final bacterial recovery from the control group in both lens storage cases.
case types was approximately $7 \times 10^9$ (Fig. 2). This is consistent with the literature that the maximum bacteria recovered from used lens cases can be as high as $10^5$ to $10^6$ CFU.\textsuperscript{5,9,18}

There was greater *P. aeruginosa* biofilm formation than *S. aureus*, particularly with nonridged cases (case B). This is in line with another study in which *P. aeruginosa* demonstrated greater adhesion than *S. aureus* and also greater attachment on a smooth surface.\textsuperscript{19} It is suggested that *P. aeruginosa* may produce extensive slime and therefore a relatively better protection in its biofilm mode of growth,\textsuperscript{20} which may explain why *P. aeruginosa* biofilm is more difficult to remove by rubbing and rinsing. This is also similar to the adhesion of these bacterial types to contact lenses.\textsuperscript{21,22}

The study has shown that the level of lens case contamination is determined by several factors such as the cleaning regimen, the potency of disinfecting solutions used to rinse lens case wells, and the internal lens well design (which affects the adhesion and ease of detachment of bacteria). It is recommended by lens care practitioners and manufacturers that lens wearers use a disinfecting solution with its accompanying lens case (i.e., from the same manufacturer). However, our data (unpublished) has shown that 11% of lens wearers mismatched their multipurpose disinfecting solution with the lenses cases they used. It is expected that an ideal cleaning system would consist of a high potency disinfecting solution and an easy to clean lens case. Yet, this combination may not always be available or used by lens wearers. This emphasizes the importance of developing an evidence based cleaning regimen that removes biofilm effectively under any combination of MPDS and lens case type to ensure a contamination-free lens case during wear.

While rinsing in vitro biofilms with hydrogen peroxide may be an effective positive control, a report suggests that the frequency of case contamination during wear is similar for hydrogen peroxide case systems and multipurpose care systems.\textsuperscript{23} Reducing case contamination in hydrogen peroxide systems is a different challenge to multipurpose care systems because of the very different case design, namely a larger solution volume, deep barrel design, basket lens holder, and with some means of neutralizing system. Designing appropriate case hygiene recommendations for hydrogen peroxide systems is the subject of further study.

We acknowledge that this study is not a comprehensive review of all technologies or approaches regarding biofilm removal as there are suggestions regarding the use of microwave and boiling lens cases. However, these methods are no longer recommended by practitioners, the Food and Drug Administration, nor the industry.\textsuperscript{16} It is unlikely that new practices which require additional equipment would be adopted by lens wearers. Some modern cases are unstable to increases in temperature. The use of a microwave also is very difficult while traveling or in the usual setting for lens handling (a bedroom or bathroom). Therefore we have primarily focused on methods that are accessible and can be carried out easily by lens wearers and which require minimum additional paraphernalia. This has important relevance for improving compliance in contact lens wearers.

Based on the in vitro data, this study has suggested a new cleaning regimen that is simple, practical, and better than the currently available guidelines.\textsuperscript{16} This study demonstrated that rinsing and rubbing lens cases with MPDS followed by tissue-wiping can remove a greater bacterial bioburden than rinsing and air-drying cases only (manufacturers’ guidelines) regardless of lens case type and rinsing agents. For ease of biofilm removal by shearing force, the lens case well structure should be taken into consideration. Further studies should determine the performance of the proposed cleaning regimen in vivo to better evaluate its capacity in reducing biofilm formation in real life situations.

### References

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