Clinical Trials

In Vivo Assessment of Antimicrobial Efficacy of Silver-Impregnated Contact Lens Storage Cases

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PURPOSE. To evaluate microbial contamination in silver-impregnated contact lens (CL) storage cases while establishing the effect of “wet” and “dry” case maintenance and to determine its association with clinical signs, symptoms, and compliance.

METHODS. Two noncontemporaneous prospective studies were conducted. Regular storage cases in study 1 (n = 40) and silver-impregnated cases in study 2 (n = 41) were used in conjunction with CL solution and CLs (balafilcon A). Cases were replaced monthly and collected at 1, 3, and 4 (for silver-impregnated cases only) months. Regular cases and the fourth-month silver-impregnated cases were maintained dry, and the other cases were maintained wet between uses. At collection, storage cases were sampled and cultured for microbial identification and enumeration. Ocular clinical findings, subjective responses to CL wear, and compliance were recorded at each visit.

RESULTS. The percentages of microbial contamination for silver-impregnated and regular cases were 71% and 82% respectively. There were significantly (P < 0.005) fewer organisms in silver-impregnated cases (1.7 log CFU per well) than in regular cases (4.1 log CFU per well). In particular, silver-impregnated cases showed lower levels of Gram-negative bacteria (P = 0.04), Gram-positive bacilli (P = 0.03), and fungi (P = 0.006). Maintaining the silver-impregnated cases wet resulted in a lower percentage of contamination (71%; P < 0.01) than maintaining them dry (94%). There was no association between any clinical signs, symptoms, or compliance and microbial contamination of storage cases.

CONCLUSIONS. More than 70% of the storage cases used in daily wear CL care for a month was contaminated irrespective of the types of cases. However, silver-impregnated cases were colonized by reduced levels of Gram-negative bacteria. (www.anzctr.org.au number, ACTRN1260900165280.) (Invest Ophthalmol Vis Sci. 2012;53:1641–1648) DOI:10.1167/iovs.11-8197

Microbial keratitis is a rare, devastating ocular complication associated with contact lens (CL) wear.1–6 There are approximately 125 million CL wearers in the world,7 and approximately 5 per 10,000 wearers per year develop microbial keratitis.1,5,8–10 Microbial contamination of the CL care system11–15 has been identified as a risk factor for CL-associated microbial keratitis. Other established factors include soft CL1 and an extended-wear schedule.4,8,10,16–18 Among contact lens care products, contamination occurs most frequently19 and is highest in CL storage cases.20 Recommendations to wearers for lens and case hygiene vary among practitioners, the US Food and Drug Administration, and the industry,21,22 and inadequate case cleaning has been observed in 61%22 to 72%,23 of CL wearers. The frequency of microbial contamination of storage cases can range from 24% to 81% of wearers.14,24–35 Unlike CL contamination, which is usually bacterial in nature, storage case contamination is often polymicrobial.25,27 Microbial isolates recovered from the contaminated storage cases are often identical with those isolates recovered from the cornea in microbial keratitis.15,34

Storage cases may be reservoirs for pathogenic microorganisms, including Pseudomonas aeruginosa,3,5,8,35,36 Serratia marcescens,3,12 fungi14,20,27 and protozoa.25–28 Contaminants derived from the environment, multipurpose solutions, and the hands/fingers of the CL wearers might be introduced into the CL storage cases and eventually to the CLs. This would facilitate access of microorganisms to the ocular surface.37 The transmission of Staphylococcus aureus from the CL storage cases to porcine corneas, mediated by CLs,38 has been reported. The transmission of microbes from the storage case to CLs was not significantly different for the type of bacteria (Gram-negative and Gram-positive)39; however, the surface properties of CL (hydrophilic or hydrophobic) and the type of bacteria can influence microbial transmission from CL to cornea.37 In such circumstances, a pre-existing insult or compromise of the ocular surface might allow opportunistic pathogens to initiate infection. Limiting storage case contamination would seem to be an overlooked but important part of safe CL wear.53

The suggested methods of reducing case contamination include the development of antibacterial cases, appropriate case hygiene, and frequent replacement of cases.21 The latter two are dependent on patient compliance with instructions. Even then, contamination of storage cases occurs.49 Information on the performance of antibacterial storage cases is sparse. In vitro studies evaluating antimicrobial efficacy of silver-impregnated storage cases demonstrated their robust activity against Gram-negative bacteria41 and increased killing efficacy against P. aeruginosa in biofilm when used in combination with the lens care solution.59 However, there are limited available data on the clinical performance of silver-impregnated storage cases. The objectives of this study were to compare the microbial contamination percentages and levels between silver-impregnated and regular cases in clinical studies and to establish appropriate maintenance conditions for the silver-
impregnated cases. This study also evaluated the association between microbial contamination of cases with ocular clinical signs, symptoms of wearers during lens wear, and compliance of wearers with hygiene instructions.

MATERIALS AND METHODS

Contact Lenses Storage Cases, Contact Lenses, and Lens Care Products

Regular storage cases (CIBA VISION Corporation, Atlanta, GA) without silver impregnation and silver-impregnated CL storage cases (MicroBlock; CIBA VISION Corporation) were tested. Balafilcon A (PUREVISION; Bausch & Lomb, Rochester, NY) lenses were used in conjunction with multipurpose solution (AQ; CIBA VISION Corporation) for the lens care.

Clinical Study Design

Two noncontemporaneous prospective, single-group, bilateral-design, open-labeled clinical studies were conducted at the Brien Holden Vision Institute under good clinical practice to evaluate the percent-ages and levels of microbial contamination of regular (study 1) and silver-impregnated (MicroBlock; study 2) storage cases during use. The study protocols were reviewed and approved by the Human Research Ethics Committee and were performed in accordance with the guidelines of the Declaration of Helsinki for Experimentation on Humans (1975, revised in 1983). Informed consent was given by participants before start of lens wear.

Subjects older than 18 years with normal ocular signs, no systemic or ocular contraindications to CL wear, and with or without previous lens wear experience were recruited either by email or from the database of the Brien Holden Vision Institute. Assuming the case contamination percentage of 80% with regular cases, a sample of 40 participants in each study using either storage case (regular or MicroBlock cases) with multipurpose solution (AQ) collected at two study visits was required to demonstrate a significant reduction of 50% in case-contamination percentages (80% with regular cases vs. 40% with MicroBlock cases). The sample size for both studies was estimated at the 5% level of significance and with 90% power, assuming a 20% dropout percentage. Forty and 41 subjects were enrolled in studies 1 and 2, respectively. CLs and storage cases were replaced every month.

Subjects in study 1 were instructed to maintain their regular CL storage cases “dry.” Subjects in study 2 were instructed to maintain their silver-impregnated cases “wet” between uses for up to 3 months and dry for the fourth month to evaluate the effect of maintenance condition on case contamination. To say cases were maintained in a dry state refers to a condition in which, after lens insertion, the old solution was discarded from the lens cases; cases were rinsed with multipurpose solution (AQ) and then air dried. In wet case maintenance, similar steps were followed except that the storage cases were immediately capped without air drying after rinsing with multipurpose solution (AQ) after removal of lenses for wear. All subjects were asked to return the used CL cases for microbial analysis at the end of 1-month and 3-month (for both studies 1 and 2) and 4-month (for study 2 only) follow-up visits.

Clinical Procedures

At each scheduled (baseline, dispensing, 2-week, 1-month, 3-month, and 4-month) visit, visual acuity and ocular signs and symptoms were assessed. Slit lamp biomicroscopy was performed to evaluate corneal and conjunctival (limbal, bulbar, and palpebral) redness, staining, and palpebral roughness using the CCLRU grading scale. A compliance questionnaire was administered during regular visits to monitor the CL wearer’s habits toward the care regimen during the course of lens wear. The compliance questionnaire consisted of questions regarding the care of CLs and cases.

Microbial Procedures

After collection, the CL storage cases were processed for microbial analysis. The interior surfaces of the lens cases and lids (both wells of regular cases and right wells of silver-impregnated cases) were swabbed with a calcium alginate swab. The swab was then placed into 2 mL sterile phosphate-buffered saline (PBS; containing 1% wt/vol sodium hexametaphosphate). After vortexing for 30 seconds at 1200 rpm, the sample suspensions were inoculated (0.4 mL/plate) on each of the three “chocolate” blood agar plates for bacterial recovery and on a Sabouraud dextrose agar (SAB) with chloramphenicol (Oxoid, Basingstoke, United Kingdom) for fungal recovery. All the CLB plates were incubated at 37°C, one in O2 for 48 hours, another in 5% CO2 for 48 hours, and the third in anaerobic conditions (ANO2) for 4 days. SAB with chloramphenicol plates were incubated at 25°C in O2 for 7 days. The microbial colonies recovered from the culture plates were enumerated as colony-forming units (CFU) and were identified as described earlier. Briefly for bacteria, after preliminary classification of the organism by Gram’s stain, appropriate biochemical tests and API bacterial identification systems (bioMérieux, Marcy l’Etoile, France) were used for the identification of the isolates. Fungi (yeasts and moulds) were identified by the morphology of their colonies on agar plates and of their conidia.

After processing the right well for microbial analysis, the left well of the silver-impregnated cases was evaluated for total biofilm formation using crystal violet staining (5%, wt/vol). Briefly, each case was air-dried to remove residual moisture and then filled with 4 mL of 5% crystal violet staining solution for 45 minutes at room temperature. The crystal violet solution was then discarded, and the well of each case was washed gently by immersing in a beaker filled with running tap water. After air drying, the biofilm color was extracted with 95% ethanol. Finally, the solubilized biofilm extracts (2 mL) were removed into 24-well microtiter plates (Greiner Bio-One, Frickenhausen, Germany), and absorbance at 595 nm was measured using spectrophotometry to quantify the relative density of biofilm formation.

Data Analysis

Microbial contamination percentages of the storage cases were compared between two studies using logistic regression analysis with robust estimate of variance and Fishers exact test. Levels of microbial contamination (CFU) of cases were log transformed for the data analysis. A linear mixed model was used to compare the levels of contamination between different storage cases and for different storage conditions after adjusting for any intra-subject correlations.

A linear mixed model was used to compare the ocular signs of subjects from the two studies and to determine any association with the microbial contamination. χ2 tests were used to compare subjective symptoms between the studies and to verify the association of symptoms with the microbial contamination. To ascertain the association of compliance with the microbial contamination, each question was assessed a score and was categorized under lens care and case care and was compared using a linear mixed model. Statistical software (SPSS, version 16; SPSS, Chicago, IL) was used for data analysis.

RESULTS

Twenty-nine and 34 subjects completed studies 1 and 2, respectively. There were 11 permanent discontinuations for study 1 and eight for study 2; the reasons are listed in Table 1. Of the total subjects recruited, 35% were women and 65% were men in study 1, and 58% were women and 42% were men in study 2. These sex differences between the two studies were significant (P < 0.05). The mean ages of the subjects for studies 1 and 2 were 28 ± 10 (mean ± SD) years and 31 ± 12 years, respectively.

Fifty-nine regular cases and 75 silver-impregnated CL storage cases (MicroBlock) were sampled, which was 74% and 91% of the expected samples, respectively. The 28% dropout rate of
TABLE 1. Reasons for Discontinuation of Subjects during the CL Wear in Studies 1 and 2

<table>
<thead>
<tr>
<th>Reason for Permanent Discontinuation</th>
<th>Regular (%)</th>
<th>Silver-Impregnated (MicroBlock) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discomfort</td>
<td>7 (18)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Relocated</td>
<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Revocation of consent</td>
<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Lost to follow-up</td>
<td>1 (3)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Biomicroscopy</td>
<td>1 (3)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Unacceptable fit</td>
<td>0</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>0</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>11/40 (28)</td>
<td>8/41 (20)</td>
</tr>
</tbody>
</table>

In Vivo Assessment of Silver-Impregnated CL Cases

The sample size in study 1 did not reduce the statistical power beyond 80% at the 5% level of significance.

Microbial Results

Overall, the percentage of microbial contamination did not vary between silver-impregnated (71%, maintained wet as recommended) and regular (82%, maintained dry as recommended) cases. However, the level of microbial contamination (Fig. 1) was significantly ($P < 0.005$) lower in silver-impregnated cases (average, 1.7 log CFU per well) than in regular cases (average, 4.1 log CFU per well).

The comparison of bacterial and fungal species recovered from the regular and silver-impregnated cases revealed differences in the microbial spectrum between the two lens cases (Table 2). From the regular cases a range of Gram-negative bacteria were recovered, including *P. aeruginosa* and *S. marcescens*. Conversely, most of the isolates obtained from the silver-impregnated cases were Gram-positive cocci; the commonest were *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, and *Micrococcus* spp. Overall, the silver-impregnated cases showed significantly lower percentages of recovery for Gram-negative bacteria (11% vs. 25%, $P = 0.04$), Gram-positive bacilli (33% vs. 53%, $P = 0.03$), and fungi (4% vs. 20%, $P = 0.006$) than regular cases (Fig. 2).

To establish the appropriate storage regimens for the silver-impregnated cases, two conditions (wet and dry) of storage were nominated for the evaluation of percentage of microbial contamination. The wet state of maintenance was selected in accordance with the manufacturer’s recommendation, and the dry state of maintenance was chosen based on the guidelines of most manufacturers and the FDA for regular cases. The practice of appropriate storage condition was monitored through a compliance questionnaire. Additionally, the condition of the storage case, either wet or dry, was noted after it had been collected from the subjects. For the wet storage condition, 93% of cases were received as recommended (wet), whereas 100% of the storage cases were found dry, when the subjects were instructed to air-dry cases. The comparison of the different maintenance conditions of the silver-impregnated cases showed that the cases maintained wet had a significantly lower percentage of contamination (71%) than those maintained dry (94%, $P = 0.01$). Additionally, the wet cases had a significant ($P < 0.03$) reduction in Gram-positive cocci (from 87% to 64%) and fungi (from 18% to 4%) compared with the dry cases. For the silver-impregnated cases, one well was processed for the recovery of viable microorganisms, whereas the other well was assessed for the quantification of total biofilm formation. The comparison of biofilm formation with the silver-impregnated cases under different storage conditions showed that the CL storage cases maintained wet (absorbance of 0.1 ± 0.04 nm) had significantly lower biofilm formation ($P < 0.05$) than those maintained dry (absorbance of 0.14 ± 0.1 nm).

Further, the number of different species of bacteria and fungi recovered from each well of the lens case was compared between the regular and silver-impregnated storage cases. Approximately, 10% (6 of 59) of the regular cases showed a maximum of five different species of microorganisms per well (Fig. 5). However, only one silver case each maintained wet (1%) and dry (3%) showed contamination with ≥5 species of microorganisms. The lower number of contaminants recovered from the silver-impregnated storage cases maintained wet, is suggestive of their efficacy against a range of microorganisms in the storage cases.

Clinical Results

The subjective responses to the symptoms questionnaire revealed slightly but not significantly less ocular discomfort ($P = 0.06$) in the CL wearers using silver-impregnated cases. However, there was no direct association between symptoms (lens awareness, burning/stinging sensation, dryness, redness, itching, and discomfort) and signs (limbal, bulbar, and palpebral conjunctival redness, corneal vascularization, corneal and/conjunctival staining, and tarsal conjunctival abnormalities) with microbial contamination of the storage cases. In addition, there was no significant difference in the occurrence of ocular adverse events between studies 1 (37%) and 2 (27%) (Table 3).

Data analysis for the total compliance scores showed that there was no significant difference between the groups using regular and silver-impregnated CL storage cases (MicroBlock) and no difference between men and women irrespective of subject group. Total compliance scores were also not associated with the microbial contamination of storage cases. The practice information questionnaire for lens care (Table 4) revealed that regardless of group, approximately 56% of subjects rinsed their lenses, 31% rinsed and rubbed their lenses, and 13% did nothing after removing lenses from their eyes. Of those who rinsed or rubbed lenses or who did both, 82% used the disinfecting solution and 5% used saline. CLs were placed in the storage case for 8 to 12 hours by 80% of the subjects. After the storage period, 50% did nothing to the lens before insertion, while 33% rinsed the CLs with disinfection solution and 16% rinsed with saline. The solution in the case was
changed daily by 94% of the subjects, and 96% never topped up
the solution in the cases. Only 59% of the subjects reported
that they cleaned their storage case each day.

### DISCUSSION

Silver-impregnated CL storage cases have been introduced to
limit storage case contamination. The present study compared
the percentages and levels of microbial contamination be-
tween regular storage case (without silver) and silver-impreg-
nated cases and their association with the clinical findings and

#### Table 2. Microorganisms Recovered from the Regular and Silver-Impregnated (MicroBlock; maintained in wet and dry storage conditions) Cases

<table>
<thead>
<tr>
<th>Group</th>
<th>Organism</th>
<th>Regular Dry n (%)</th>
<th>Wet n (%)</th>
<th>Dry n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive coccis</td>
<td>Micrococcus spp.</td>
<td>3/59 (5)</td>
<td>10/75 (13)</td>
<td>9/32 (28)</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1/59 (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Serratia marcescens</em></td>
<td>5/59 (8)</td>
<td>5/75 (7)</td>
<td>3/32 (9)</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus lugdunensis</em></td>
<td>0</td>
<td>3/75 (4)</td>
<td>3/32 (9)</td>
</tr>
<tr>
<td></td>
<td><em>Staphylococcus saprophyticus</em></td>
<td>12/59 (20)</td>
<td>14/75 (19)</td>
<td>16/32 (50)</td>
</tr>
<tr>
<td>Gram-positive bacilli</td>
<td><em>Bacillus</em> spp.</td>
<td>6/59 (10)</td>
<td>5/75 (7)</td>
<td>3/32 (9)</td>
</tr>
<tr>
<td></td>
<td><em>Corynebacterium</em></td>
<td>4/59 (7)</td>
<td>1/75 (1)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Propionibacterium</em> spp.</td>
<td>31/59 (54)</td>
<td>19 (25)</td>
<td>6/32 (10)</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td><em>Aeromonas</em> spp.</td>
<td>2/59 (3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Burkholderia cepacia</em></td>
<td>0</td>
<td>1/75 (1)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Delftia acidovorans</em></td>
<td>1/59 (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Enterobacter cloacae</em></td>
<td>1/59 (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Enterobacterium sakazakii</em></td>
<td>0</td>
<td>2/75 (3)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella oxytoca</em></td>
<td>2 (3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas fluorescens</em></td>
<td>0</td>
<td>2/75 (3)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas putid</em></td>
<td>1/59 (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1/59 (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Raoultella terrigena</em></td>
<td>1/59 (2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Serratia liquefaciens</em></td>
<td>3/59 (5)</td>
<td>1/75 (1)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Serratia marcescens</em></td>
<td>5/59 (8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>4/59 (7)</td>
<td>2/75 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Fungus</td>
<td><em>Fungi</em></td>
<td>8/59 (14)</td>
<td>1/75 (1)</td>
<td>4/32 (13)</td>
</tr>
<tr>
<td></td>
<td><em>Yeast</em></td>
<td>4/59 (7)</td>
<td>2/75 (3)</td>
<td>2/32 (6)</td>
</tr>
</tbody>
</table>

**FIGURE 2.** Percentages of all subjects using regular cases (n = 59) and silver-impregnated cases (MicroBlock) maintained in wet (n = 75) and dry (n = 32) storage conditions contaminated with Gram-positive coccis, Gram-positive rods, Gram-negative bacteria and fungus. Because more than one group of microorganisms may be recovered from each sample, the total percentage for each of the three case and storage conditions may be >100%.

**FIGURE 3.** Percentages of cases showing multiple bacterial and fungal contaminants in regular cases (n = 59) and silver-impregnated cases (MicroBlock) maintained in wet (n = 75) and dry (n = 32) storage conditions. 0 = contamination-free cases; 6 = 6 species per case.
subject compliance. The study showed that though there was no significant difference in microbial contamination percentages between regular and silver-impregnated cases, there was a substantial decrease in the level of microbial contamination in the silver-impregnated cases. Further, the silver-impregnated cases maintained wet between uses performed better than those maintained dry. There were no associations between symptoms, signs, and compliance scores and the level or type of microbial contamination.

In the present study, we evaluated the clinical performance of antibacterial storage cases containing silver, a well-known, broad-spectrum antimicrobial agent. Silver is believed to exert its antimicrobial effects by various mechanisms, including interference with DNA and RNA replication, disruption of the cell membrane, interference with cell respiration, and inactivation and alteration of enzymes. The manufacturer of this silver-impregnated case claims that the slow, sustained release of silver ions from these storage cases contributes to the antimicrobial activity of the cases. Silver ions are incorporated into amorphous zones of polymeric material of the storage cases. The silver ions migrate to the surface of the lens case when an imbalance of vapor pressure demands equalization, providing a consistent supply of antimicrobial agent at the surface of the lens case.

Irrespective of the type of storage case, >70% of the storage cases were contaminated. These results are consistent with a previous study that has reported 90% contamination with the silver-impregnated CL storage cases (MicroBlock) and 100% contamination in regular cases. However, another study demonstrated significant reduction in the contamination with silver-impregnated CL storage cases (MicroBlock), showing either 26% or 38% under different storage conditions. These differences in the percentage of contamination could be attributed to differences in the processing time for the samples, which ranged from 4 hours to a few days (not specified); in the present study, the difference was 2 hours. A delay between the collection of storage cases and processing may result in prolonged contact between silver ions and microbes at the surface, leading to an overall decrease in contamination or a reduction in the survival of fastidious organisms. The location in sampling of the storage case may also influence the reported percentage of contamination. In addition, the levels of case contamination vary with the use of different disinfecting solutions.

In the present study, a lower level of Gram-negative bacteria was observed with the silver-impregnated cases. Additionally, similar to previous reports, there was no recovery of P. aeruginosa and S. marcescens, which are often associated with CL-related microbial keratitis. It has been reported that silver nanoparticles were effective against fungi and Gram-positive cocci. Our results confirm that storage cases is unknown and is beyond the scope of the present study. Further studies are needed to determine whether the silver-impregnated cases offer some margin of safety against adverse events associated with these potential pathogens.

Fungal contamination decreased from 18% to 4% with the use of silver-impregnated cases under the manufacturer’s recommended conditions. This is consistent with an earlier report in which the fungal contamination was 24%, with regular cases when used with different disinfection systems. This is the first clinical study to report reduced fungal contamination in the silver-impregnated cases. Previous work has demonstrated a small but significant reduction of Fusarium solani ATCC 36031 in the silver-impregnated cases in vitro. The toxicity of silver against Fusarium oxysporum has been reported. Another study established that silver nanoparticles were effective against Candida spp. In the present study, we did not classify the fungal isolates recovered, and the samples were not screened for Acanthamoeba spp., which may be important to consider in future studies given the recent outbreak of atypical CL-related keratitis and increased environmental exposure to Acanthamoeba. However, Acanthamoeba spp. were not recovered in a large sample of cases from wearers recruited from the same center (n = 373).

Our results revealed that the silver-impregnated cases demonstrated lower contamination levels under wet storage conditions. This is in accordance with a previous study, in which the activity of silver-impregnated cases was reported for different levels of moisture. Further, when the cases were air dried, there was an overall increase in contamination (94%), particularly for fungi and Gram-positive cocci. Our results confirm that silver-impregnated cases are most effective when maintained wet between use cycles.

The comparison between the storage conditions revealed weaker biofilm formation in the cases maintained in wet condition. It is possible that the silver ion release from the surfaces of the storage cases might have limited biofilm formation. Earlier reports proposed that the efficacy of the silver ions would be best at the interface of storage cases and biofilm, leading to a low viability of biofilms in silver-impregnated cases.
There were no associations between symptoms/signs and the levels or types of microbial contamination of cases. A previous study has suggested that the bacterial bioburden of CL is associated with discomfort, whereas the contamination of storage cases has been implicated in CL-related problems such as itching, redness, and dryness. However, in the present study, there was no direct association between symptoms with microbial contamination. Despite the repeated emphasis of care instructions, variable practices (Table 4) were observed among subjects toward CL care both after and before CL wear and in the routine care of the storage cases. This highlights the inconsistent practices common with the care regimen because of the lack of standard guidelines and even within a carefully controlled study. Eye care practitioners and the industry must be very specific with their instructions for the use of CLs and their care products. CL care contamination has been identified as a significant public health concern because it may lead to sight-threatening microbial keratitis; hence, there is need for evidence-based guidelines for the CL care regimen. In the present study, the total compliance scores were not different between men and women and were not associated with the microbial contamination of the storage cases. This suggests that reduction in the microbial contamination observed with the silver case is a robust effect rather than simply a reflection of better compliance.

In conclusion, this study has demonstrated that the percentages of contamination for regular cases and silver-impregnated cases were not significantly different. However, silver-impregnated cases maintained wet showed lower numbers of microbes, particularly in Gram-negative bacteria such as P. aeruginosa and S. marcescens. For the silver cases, wet storage between appears to reduce biofilm formation more effectively than does dry storage. Further studies are required to establish whether the use of such cases might limit the CL adverse responses associated with microbial contamination and to determine whether such benefits are maintained in community studies.

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