Factors Affecting Microbial Contamination on the Back Surface of Worn Soft Contact Lenses

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SIGNIFICANCE: The results of this study demonstrate that Smart Touch Technology packaging, which is designed to reduce and simplify contact lens handling before insertion, is effective in reducing the frequency of bacterial contamination of the back surface of contact lenses after short-term wear.

PURPOSE: The purpose of this study was to investigate the effect of lens packaging type, chelating agent, and finger contamination on microbial contamination on the back surface of worn soft contact lenses.

METHODS: Twenty-five subjects completed each contralateral lens wear comparison in this randomized study: Smart Touch Technology versus conventional blister packaging for (1) silicone hydrogel lenses with ethylenediaminetetraacetic acid (EDTA) and (2) hydrogel lenses without EDTA in the packaging, and (3) silicone hydrogel lenses without EDTA versus hydrogel lenses with EDTA both in Smart Touch Technology packaging. Participants washed hands, underwent finger swabs, and inserted the lenses. After 45 minutes, lenses were removed aseptically and the posterior lens surfaces cultured.

RESULTS: Thirty-eight subjects (average age, 30.9 ± 12.5 years) participated in this study. Overall, the level of back surface contamination was low for both lens materials, ranging from 0 to 43 colony-forming unit (CFU)/lens for the silicone hydrogel and 0 to 17 CFU/lens for the hydrogel lenses. Contact lenses from conventional packaging containing EDTA had 3.38 times increased risk (95% confidence interval [CI], 1.02 to 11.11; $P = .05$) of contamination being present compared with lenses from Smart Touch packaging with EDTA. Contact lenses from conventional packaging without EDTA had 3.4 times increased risk (95% CI, 1.02 to 11.36; $P = .05$) of contamination being present compared with Smart Touch packaging without EDTA, and silicone hydrogel lenses had a 6.28 times increased risk (95% CI, 1.65 to 23.81; $P = .007$) of contamination being present compared with hydrogels. The median (interquartile range) number of bacteria isolated from fingers used to perform lens insertion after handwashing but before lens insertion was not significantly different between the silicone hydrogel and hydrogel lenses (63.7 [204.2] vs. 59 [84.5], $P = .09$). Finger contamination was not significantly associated with lens contamination in the presence or absence of EDTA.

CONCLUSIONS: Smart Touch Technology packaging was effective in reducing the proportion of contaminated lenses. Although silicone hydrogel lenses were more likely to be contaminated, the presence of EDTA ameliorated this effect. Finger contamination was not associated with lens contamination.

By eliminating contact with the post-lens surface during lens handling, it would be expected that the risk of contamination and adverse events should be reduced. An in vitro study demonstrated that daily disposable contact lenses removed from a flat packaging design (whereby only the outer/front surface of the lens is handled), with fingers contaminated with Staphylococcus aureus, showed no colony formation on the inner surface of the lenses. In the same study, several colonies were found on the inner and outer surface of lenses of all conventional blister-packed lenses, which required handling with both of the hands contaminated with S. aureus, to determine correct lens orientation. The effect of the flat packaging design on lens contamination has not been evaluated in vivo. Furthermore, handling new sterile lenses after handwashing with soap,
rinsing with tap water, and drying using a paper towel has been shown to transfer a minimum of 12 to a maximum of 5700 colony-forming units per lens. Therefore, it was considered worthwhile to evaluate the effect of two different lens packaging types and the effect of preservative and chelating agent ethylenediaminetetraacetic acid, which is often present in contact lens packaging solution, and explore associations between contact lens and finger contamination.

The purpose of this study was to investigate the effect of lens packaging type on microbial contamination on the inner (back) surface of worn soft contact lenses after handling and short-term wear. Microbial contamination rates on the back surface of worn hydrogel and silicone hydrogel contact lenses removed from novel Smart Touch (flat) packaging with and without ethylenediaminetetraacetic acid were compared with lenses removed from conventional blister packs with and without ethylenediaminetetraacetic acid.

**MATERIALS AND METHODS**

**Study Design**

This prospective, contralateral, randomized, investigator-masked study consisted of three nondispensing study visits, conducted with a minimum washout period of 48 hours between study visits. Participants were randomly allocated to the lens material type to be worn at the first two visits and the eye to which lenses removed from the Smart Touch Technology or conventional lens packaging were to be inserted:

- **Visit 1**: bilateral wear of silicone hydrogel or hydrogel lens material and lens extracted from Smart Touch Technology or conventional lens packaging to be inserted on which eye;
- **Visit 2**: crossover to bilateral wear of the alternate lens material and lens extracted from Smart Touch Technology or conventional lens packaging to be inserted on which eye.

At visit 3, the effect of ethylenediaminetetraacetic acid in the Smart Touch Technology packaging solution was evaluated. Participants were randomly allocated to wear lenses removed from the Smart Touch Technology packaging—silicone hydrogel lens in one eye and the hydrogel lens in the other eye. Visit 3 was slightly delayed because of manufacturing constraints. A flow diagram of the study visits is shown in Fig. 1.

This study was registered on clinicaltrials.gov (NCT03253393). Participants were recruited from the local population at the institutional site (School of Optometry and Vision Science, University of New South Wales) by posting the approved study advertisement on University of New South Wales noticeboards and Web sites. All procedures were conducted in accordance with the Declaration of Helsinki and were approved by the University of New South Wales Human Research Ethics Committee (HC17791). Written informed consent was obtained from all participants before conducting any study-related procedures.

**Study Participants**

A total of 38 participants who met the inclusion/exclusion criteria and gave informed consent were enrolled in the study. However, only 25 participants were required to complete each lens wear comparison. The sample size of 25 subjects to complete each lens wear comparison was estimated, as at the time of designing the study, it was uncertain as to whether differences in microbial contamination rates on the back surface of worn contact lenses could be detected. Subjects were eligible to participate if they met all of the inclusion criteria and none of the exclusion criteria. Inclusion criteria included the following: minimum of 18 years of age and experienced soft contact lens wearer as well as willing to refrain from wearing contact lenses for 24 hours before the study visits. Exclusion criteria included any active ocular or medical disease that would affect safe contact lens wear.

**FIGURE 1.** Study visit flow diagram. EDTA = ethylenediaminetetraacetic acid.
wear, use of systemic or topical medications that may alter ocular findings, eye surgery within 12 weeks before enrollment, enrollment in another clinical trial, and pregnancy.

**Study Procedures**

Study participants attended up to three study visits. At each visit, participants practiced removing lenses from the Smart Touch Technology packaging with the same hand used for lens insertion. Once the participant was proficient with this technique, they were instructed to wash their hands before handling the contact lenses. The thumb and two index fingers of the hand routinely used to conduct contact lens insertion were swabbed by the study investigator using a sterile cotton swab (Multigate Medical, Villawood, New South Wales, Australia) moistened with sterile preservative-free saline (Pfizer Inc., Bentley, Western Australia, Australia) for the evaluation of skin microbiota. The swab was rolled over each finger twice—once in a forward direction and once in the reverse direction. Participants were then instructed to follow the manufacturer's guidelines for lens insertion (https://www.menicon.com/ifu/) and insert the contact lens randomly assigned for the right eye followed by the left eye.

After 45 minutes of wear, the contact lenses were removed aseptically by a masked investigator, wearing sterile latex gloves (Livingstone International, Rosebery, New South Wales, Australia). All contact between the contact lens with the eyelids and eyelashes during lens removal was avoided. The back surface of each collected contact lens was carefully placed on molten (45°C) nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) using sterile forceps and held in place using sterilized forceps until the agar solidified. The finger swabs were transferred to the microbiology laboratory for microbial sampling. In brief, the finger cotton swabs were placed in 1 mL of sterile preservative-free saline and vortexed for 60 seconds. A 500-μL aliquot of this finger swab solution was transferred to a nutrient agar plate. The finger swab and contact lens agar plates were placed in an incubator (Thermoline Scientific, Wetherill Park, New South Wales, Australia) at 35 to 37°C, and the number of colony-forming units was enumerated after 24 hours.

**Study Contact Lenses**

The silicone hydrogel lens material evaluated in this study was midafilcon A, and the hydrogel lens material was hioxifilcon A. All lenses were fitted in a single base curve (8.4 mm), diameter (14.2 mm), and back vertex power (−0.50 D). Further details of the study lenses are shown in Table 1 and Figs. 2 and 3. The lenses that were not commercially available in Australia were evaluated under Clinical Trials Notification CT-2017-CTN-04235-1 with ethics approval. Participants were advised to wear their habitual spectacles over the study lenses to achieve satisfactory vision, if required.

**Statistical Analysis**

Data analysis was performed using SPSS 22.0 (SPSS Inc., Chicago, IL). Normality of data was assessed using Shapiro-Wilk test, and median ± interquartile ranges were reported. For further analysis, contact lens and finger contamination were expressed as colony-forming units (continuous variables) or as a proportion of samples with zero contamination (categorical variables) per lens or finger swab respectively. Multiple comparison tests, such as ANOVA and χ² tests, along with corresponding post hoc analyses were conducted to evaluate differences in colony-forming units/lens and the proportion of lenses with zero contamination, respectively, between the different types of lens packaging and ethylenediaminetetraacetic acid.

| Visit | Contact lens | Material type | Material | EDTA |
|-------|--------------|---------------|----------|------|
| 1 or 2 | Miru 1 day UpSide in Smart Touch* | Silicone hydrogel | Midafilcon A | Yes* |
| 1 or 2 | Miru 1 day UpSide in conventional | Silicone hydrogel | Midafilcon A | Yes |
| 1 or 2 | Miru 1 day Menicon Flat Pack in Smart Touch* | Hydrogel | Hioxifilcon A | No* |
| 1 or 2 | Miru 1 day Menicon Flat Pack in conventional | Hydrogel | Hioxifilcon A | No |
| 3     | Miru 1 day UpSide in Smart Touch | Silicone hydrogel | Midafilcon A | No |
| 3     | Miru 1 day Menicon Flat Pack in Smart Touch | Hydrogel | Hioxifilcon A | Yes |

*Commercially available lens. EDTA = ethylenediaminetetraacetic acid.
RESULTS

Twenty-five subjects were originally enrolled and completed visits 1 and 2 of the study. Because of the delay in obtaining the lenses for visit 3 from the manufacturer and, therefore, given that some of the original study subjects were no longer available to participate, a further 13 subjects were enrolled to enable 25 subjects to complete visit 3, bringing the total number of enrolled participants to 38. Fourteen male and 24 female subjects with average age of 30.9 ± 12.5 years (range, 18 to 69 years inclusive) participated in the study, and no adverse events were reported. The median ± interquartile range (range) number of bacteria isolated from the fingers of the hand routinely used to conduct insertion for each package type are shown in Table 2. There was no significant difference in the number of bacteria isolated from hydrogel lenses removed from Smart Touch Technology (with ethylenediaminetetraacetic acid; 36%); ANOVA post hoc, $P = .04$). The proportion of silicone hydrogel lenses with zero contamination was significantly higher for lenses removed from the Smart Touch Technology (with ethylenediaminetetraacetic acid; 64%) compared with both the conventional lens packaging (with ethylenediaminetetraacetic acid; 36%; ANOVA post hoc, $P = .03$) and the Smart Touch Technology packaging (no ethylenediaminetetraacetic acid; 16%; ANOVA post hoc, $P = .02$).

The median and interquartile ranges for the number of bacteria isolated from the hydrogel lenses in Smart Touch (no ethylenediaminetetraacetic acid), in conventional packaging (no ethylenediaminetetraacetic acid), and in Smart Touch (with ethylenediaminetetraacetic acid) and proportion of lenses with zero contamination removed from each package type are shown in Table 3. There was no significant difference in the number of bacteria isolated from hydrogel lenses removed from Smart Touch Technology packaging (no ethylenediaminetetraacetic acid), conventional packaging (no ethylenediaminetetraacetic acid), or Smart Touch Technology packaging (with ethylenediaminetetraacetic acid) (ANOVA, $P = .52$). However, the proportion of hydrogel lenses with zero contamination was significantly higher for lenses removed from Smart Touch (no ethylenediaminetetraacetic acid) compared with conventional packaging (no ethylenediaminetetraacetic acid) (56% vs. 28%; ANOVA post hoc, $P = .03$). The addition of ethylenediaminetetraacetic acid to the Smart Touch packaging did not significantly change the proportion of uncontaminated lenses (64% vs. 56%).

Binary logistic regression showed that, in the presence of ethylenediaminetetraacetic acid in the packaging solution, conventional lens packaging was associated with a 3.38-times increased risk (95% confidence interval, 1.02 to 11.36; $P = .05$) of lens contamination compared with Smart Touch packaging (Table 4), whereas lens material was not significantly associated with lens contamination ($P < .72$).

In the absence of ethylenediaminetetraacetic acid in the packaging solution, conventional packaging was associated with a 3.41-times increased risk (95% confidence interval, 1.02 to 11.36; $P = .05$) of lens contamination compared with Smart Touch packaging.
but silicone hydrogels were associated with a 6.28-times increased risk (95% confidence interval, 1.65 to 23.81; \( P = .007 \)) of lens contamination compared with hydrogels (Table 5). However, finger contamination was not significantly associated with lens contamination in the presence or absence of ethylenediaminetetraacetic acid.

### DISCUSSION

This study is the first to demonstrate that eliminating handling the back surface of contact lenses before insertion is effective in reducing microbial contamination rates on the back surface of worn contact lenses. Smart Touch Technology packaging was associated with a threefold lower risk of back surface lens contamination compared with conventional packaging, whether or not ethylenediaminetetraacetic acid was incorporated in the packaging solution. However, silicone hydrogel materials were six times more likely to be contaminated than hydrogel materials when there was no ethylenediaminetetraacetic acid, it seems more likely that the silicone hydrogel lens material evaluated in this study is more prone to bacterial adhesion compared with hydrogel lens materials.5-14 The generally low number of microorganisms isolated may also be attributable to the best-case scenario being examined in this study, whereby participants washed their hands with soap before extracting fresh contact lenses from sterile packaging for insertion on eye. Handling is a major source of lens contamination,4,15 but consistently washing hands before lens handling reduces the risk of contact lens–related microbial16-18 and sterile keratitis.17 The finger swab may have also potentially removed bacteria that might otherwise have transferred to the contact lens. Furthermore, the number of microorganisms on contact lenses is significantly reduced after lenses are worn on eye, and it has been hypothesized that worn lenses are less likely to be contaminated because of antimicrobial properties of the tear film.7 The level of contamination after 5 hours of lens wear has been shown to be 22 to 65 times lower than initial contamination levels induced by lens handling alone.7

The proportion of commercially available silicone hydrogel lenses (with ethylenediaminetetraacetic acid) and hydrogel lenses (no ethylenediaminetetraacetic acid) removed from the Smart Touch Technology packaging with zero contamination after wear ranged from 56 to 64% compared with 28 to 36% for the conventional lens packaging. Nomachi et al.6 also demonstrated diminished microbial contamination for hydrogel contact lenses when removed from Smart Touch Technology packaging compared with lenses extracted from conventional blister packs in an in vitro handling study. Hovding19 previously reported that 34.9 to 53.9% of hydrophilic HEMA lenses and 44.4 to 66.7% of Siltex silicone lenses displayed zero growth on the posterior surface of the lenses after wear. However, differences in lens wearing time (3 to 8 hours), lens collection technique (conducted by participants after thorough handwashing), and/or culture techniques (swabs of the posterior lens surface were streaked on agar plates) make direct comparisons between the relatively low contamination rates in Hovding’s study tenuous.

Ozkán et al.20 reported that the risk of developing a contact lens–related corneal inflammatory event increased by 2.78 times for every 1-log increase in colony-forming units/mL contact lens contamination level. Given that the proportion of contaminated lenses was lower when removed from Smart Touch Technology packaging, it is possible that this may lead to a corresponding reduction in the incidence of inflammatory adverse events. However, this warrants further exploration in longer-term dispensing studies.

Ethylenediaminetetraacetic acid has been shown to cause lysis, loss of viability, and increased sensitivity of planktonic bacteria to antibacterial agents by chelating calcium and magnesium ions21 and has activity against biofilm formation of gram-positive bacteria.22 Therefore, ethylenediaminetetraacetic acid is widely used as a preservative in many products, including multipurpose solutions for contact lens care.22,23 However, in this study, ethylenediaminetetraacetic acid showed no effect on microbial recovery in hydrogel lenses but reduced the number of organisms and increased the proportion of lenses with zero contamination for the silicone hydrogel lenses. It was thought that this could be attributed to differences in the volume of packaging fluid, as the Smart Touch Technology packaging for the silicone hydrogel lenses has a greater volume (approximately 0.10 mL) compared with the hydrogel lens material. Nomachi et al.6 demonstrated diminished microbial contamination for hydrogel contact lenses when removed from Smart Touch Technology packaging compared with lenses extracted from conventional blister packs in an in vitro handling study. Hovding19 previously reported that 34.9 to 53.9% of hydrophilic HEMA lenses and 44.4 to 66.7% of Siltex silicone lenses displayed zero growth on the posterior surface of the lenses after wear. However, differences in lens wearing time (3 to 8 hours), lens collection technique (conducted by participants after thorough handwashing), and/or culture techniques (swabs of the posterior lens surface were streaked on agar plates) make direct comparisons between the relatively low contamination rates in Hovding’s study tenuous.

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### TABLE 4. Binary logistic regression model for presence of contact lens contamination—with EDTA in the packaging solution

| With EDTA | Exp(B) | P   | Odds ratio | 95% CI |
|-----------|--------|-----|------------|--------|
| Finger swab (CFU count) | 1.002 | .103 | 1.00 | — |
| Packaging (Smart Touch)* | 0.296 | .05 | 3.38 | 1.02–11.11 |
| Material (Hydrogel)* | 1.247 | .720 | 1.39 | — |

*Referent. CFU = colony-forming unit; CI = confidence interval; EDTA = ethylenediaminetetraacetic.

### TABLE 5. Binary logistic regression model for presence of contact lens contamination—no EDTA in the packaging solution

| No EDTA | Exp(B) | P   | Odds ratio | 95% CI |
|---------|--------|-----|------------|--------|
| Finger swab (CFU count) | 0.999 | .24 | 1.00 | — |
| Packaging (Smart Touch)* | 0.293 | .05 | 3.41 | 1.02–11.36 |
| Material (Hydrogel)* | 0.159 | .007 | 6.29 | 1.65–23.81 |

*Referent. CFU = colony-forming unit; CI = confidence interval; EDTA = ethylenediaminetetraacetic acid.
microbial contamination on the back surface of the lenses. Although a culture-based approach identifies viable and culturable bacteria, it is highly dependent on culture conditions, unlike culture-independent methods such as gene sequencing, which are more appropriate methods to identify a large proportion of bacterial diversity. Identification of bacteria could have helped in determining the likely pathogenicity. However, this was a preliminary study to determine whether differences in back surface contamination could be detected between the two types of lens packaging. Now that proof of principle has been demonstrated, future studies could incorporate more sophisticated lens culture techniques. Appropriately powered longitudinal studies to determine the impact of reduced lens contamination on the incidence of adverse events and other potential benefits are warranted. Another consideration is that, because of a delay between visits 2 and 3, 13 of the 25 participants completing visit 3 were new participants who did not complete visits 1 and 2.

Different participants may present with differences in bacteria on their fingers, ocular surface, or tear film, which may impact the study findings. However, the range of hand and lens contamination observed was comparable across the three visits, and therefore, the study population did not appear to substantially impact the results.

Limiting the transfer of bioburden from the skin to the contact lens and, subsequently, the eye is important. This study showed that Smart Touch Technology packaging reduces back surface contact lens contamination after a short period of wear. Although silicone hydrogel lenses were more likely to be contaminated, the presence of ethylenediaminetetraacetic acid ameliorated this effect. Future studies should examine the impact of reduced lens contamination after longer wear periods and the potential benefits of Smart Touch Technology packaging for reducing adverse events associated with contact lens wear.

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