Antimicrobial efficacy of a povidone iodine (PI) and a one-step hydrogen peroxide contact lens disinfection system

Simon Kilvington*

Department of Infection, Immunity and Inflammation, University of Leicester, Medical Sciences Building, P.O. Box 138, University Road, Leicester LE19HN, UK

Abstract

The antimicrobial efficacy of a novel povidone iodine (PI) contact lens disinfection system (Clencide) was compared with a one-step hydrogen peroxide system (AoSept). The PI system showed rapid killing of organisms with a 4.5–6.0 log reduction in bacteria, yeast, mould and Acanthamoeba trophozoites within 5 min and a 2.8–3.6 log kill of Acanthamoeba cysts after 2–4 h. The one-step peroxide gave a 4.0–6.0 log kill of bacteria in 0.5–1 h, 2.0–5.0 log for yeast after 2–6 h and 1.8 log for mould at 6 h. Acanthamoeba polyphaga trophozoites were reduced by 3.6 log at 1 h but cysts by only 1.2 log after 6 h. The study demonstrates that the PI system is an effective disinfection method for contact lenses.

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Keywords: Povidone iodine; Hydrogen peroxide; Contact lens disinfection; Acanthamoeba

1. Introduction

Contact lens wear is a predisposing factor in some 50% of reported cases of microbial keratitis [1–3,12]. Bacterial keratitis is the most common form, with staphylococci, Pseudomonas aeruginosa and Serratia spp. the usual infectious organisms [1,2,12,22]. Although less common, keratitis due to the free-living amoeba Acanthamoeba is almost exclusively associated with contact lens wear, which accounts for 90% of reported cases, with an incidence in the UK some 15 times that of the USA and 3 times the rest of Europe [17–19,21,23]. It has long been recognised that poor contact lens hygiene enables microorganisms to colonise the lens storage case [13]. This can then result in biofilm production that protects potentially pathogenic microbes from disinfectant action [14]. Such organisms can then adhere to the contact lenses for inoculation on to the cornea [3,5,16]. Protein deposits also interfere with oxygen diffusion across the lens, and other debris on the lens surface may cause corneal abrasions resulting in discomfort or initiation of infection [9–11,15,16] thus, the regular cleaning and disinfection of contact lenses is fundamental to both the comfort and the safety of the wearer. Currently, contact lens disinfection is achieved through the use of either multipurpose solutions (MPS) or hydrogen peroxide systems. The MPS utilise a single solution for disinfecting, rinsing and storing the lenses; with most MPS having an inherent cleaning system.

Hydrogen peroxide (3%) is a powerful disinfectant and has been shown to be effective against the highly resistant cyst stage of Acanthamoeba, giving a 3 log reduction in viability provided an exposure time of at least 4–6 h is allowed prior to neutralisation which is essential before lens wear to avoid pronounced discomfort and possible corneal damage [7]. To avoid these problems and simplify use, one-step hydrogen peroxide systems are available which do not require separate neutralisation. Here, neutralisation is achieved in the storage case during disinfection using a platinum-coated disc or soluble catalase tablet which catalyses the decomposition of hydrogen peroxide to water and oxygen [7].

Recently, a novel povidone iodine (PI) contact lens disinfection and cleaning system has been introduced to the market. The system comprises a disinfectant/proteolytic enzyme stage with simultaneous neutralisation of the PI
through the addition of a separate reagent. Disinfection and neutralisation are complete within 10 min but the lenses are left in the solution for at least 4 h to effect the enzymatic cleaning. In this present study, the efficacy of the system in comparison with a one-step hydrogen peroxide contact lens disinfectant was evaluated for known ocular pathogenic bacteria, fungi and Acanthamoeba.

2. Materials and methods

2.1. Test organisms

The bacteria, fungi and Acanthamoeba spp. tested were: Pseudomonas aeruginosa (ATCC 9027), Staphylococcus aureus (ATCC 6538), Serratia marcescens (NCTC 10211), Candida albicans (ATCC 10231), Fusarium solani (ATCC 36031) and Acanthamoeba polyphaga (Ros), the latter isolated from a case of Acanthamoeba keratitis which occurred in the United Kingdom. The bacteria and fungi were cultured according to recommended protocols for contact lens disinfectant efficacy testing [8]. The F. solani conidia were prepared as described previously [6]. A. polyphaga (Ros) trophozoites were maintained in axenic culture as described previously [7]. Cysts were produced from the trophozoite cultures using Neff’s constant pH encystment medium as described previously [7].

2.2. Test solutions

The PI contact lens disinfectant system (Clencide) was supplied from the manufacturer (Ophtecs Corporation, Osaka, Japan). The system comprises a sachet of disinfectant/proteolytic enzyme containing the PI (0.05 mg) and Bacillus subtilis protease (8 mg), a neutralisation tablet (sodium sulfite 2.4 mg) and a diluting/rinsing solution (sodium borate, sodium chloride and EDTA). The diluting/rinsing solution (8 ml) is added to the contact lens storage case and the disinfectant/proteolytic enzyme granules and neutralising tablet added. The lenses are then placed inside the case. The disinfection and neutralisation process is complete within 10 min (a colour indicator in the system changes from orange to clear to show that the disinfection stage is complete) but the lenses are left in the solution for at least 4 h to effect the enzymatic cleaning. The lenses are then removed from the case and rinsed with the remaining diluting/rinsing solution before wearing.

The one-step hydrogen peroxide contact lens disinfectant (AoSept, Ciba Vision, Atlanta, USA) was obtained locally and comprises a 3% (v/v) hydrogen peroxide solution and a contact lens storage case containing a platinum coated neutralising disk. As with the PI system, the disinfection and neutralisation process occurs simultaneously and the lenses can be worn after 6 h.

Phosphate buffered saline (PBS) was used in place of the disinfectant solutions in control studies. Dey–Engley Neutralising Broth (Difco, MI, USA) was used in the PI studies and 0.02% (w/v) bovine liver catalase (Sigma Chemical Company, Dorset, UK) for the hydrogen peroxide system.

2.3. Test methods

The systems were used exactly according to the manufacturers’ recommendations and challenged with the test organisms in a volume ≤0.1% of the disinfectant solution volume. The challenge test assays for the bacteria and fungi were conducted exactly in accordance with recommended procedures [8].

The method for the Acanthamoeba trophozoites and cysts was as described previously [7]. Sample time-points for the PI system were after 0, 5 min and 1, 2, 3, 4 h exposure with PI, and 0, 0.5, 1, 2, 4 and 6 h with the hydrogen peroxide system. Experiments were conducted in triplicate.

In one experiment, the PI system was activated and left for 10 min before being challenged with P. aeruginosa. Viable bacterial counts were then conducted over a 4-h period.

3. Results

3.1. PI disinfection system

Activity of the PI system against the test organisms is shown in Table 1. Complete kill (5–6 log reduction)
occurred with all bacteria and fungi tested by the first time point of 5 min. With *A. polyphaga*, complete kill (4.5 log) of trophozoites occurred within 5 min. For the cysts, a 2.8 log reduction occurred after 2 h and 3.6 log by 4 h.

In the experiment where the system was challenged with *P. aeruginosa* following a delay of 10 min after combining the PI disinfectant, enzyme granules and neutralisation tablet, no killing of the bacterium was detected over a 4-h period; indicating that the disinfectant capacity of the system is fully neutralised within 10 min.

No loss in organism viability was found in control studies in which the test organisms were inoculated into PBS or the Dey–Engley Neutralising Broth (results not shown).

### 3.2. One-step peroxide system

The efficacy of the hydrogen peroxide system against the bacteria, fungi and *A. polyphaga* is shown in Table 2. The system gave a 6 log kill of *P. aeruginosa* and *s. marcescens* by 0.5 h (total kill for these bacteria) and a 4 log kill of *S. aureus* at 1 h. With *C. albicans*, a 1 log kill was obtained after 0.5 h, 2 log by 1 h and 5 log by 6 h. For *F. solani* conidia only a 1.8 log kill was obtained by 6 h. *A. polyphaga* trophozoites were reduced by 3.6 log (total kill) at 1 h but only a 1.2 log reduction in cyst viability was found at 6 h.

Again, no change in organism viability was found in control studies in which the test organisms were inoculated into PBS or catalase neutraliser (results not shown).

### 4. Discussion

The suitability of contact lens disinfectant systems for human use is determined by the Food and Drug Administration (FDA) and the International Organization for Standardization [18]. In the “Stand Alone Test” (ISO 14729) employed here, a disinfectant must be capable of least a 5 log reduction in bacteria and 4 log reduction in fungi species by 3 log (99.9%) and 1 log (90%), respectively within the disinfection time recommended by the product manufacturer [8]. However, there is no requirement to demonstrate activity against *Acanthamoeba* [8].

In this study, we compared the disinfectant efficacy of a novel PI contact lens disinfectant system with that of a one-step hydrogen peroxide method. Although both products surpassed the requirements of the “Stand Alone Test” for bacteria and fungi, the PI system showed greater efficacy in the rate of disinfection and activity against *Acanthamoeba* cysts.

In the PI system the disinfectant is mixed with the proteolytic cleaning granules in the lens storage case. A neutralising tablet is added. Although the manufacturer recommends a 4-h contact time, this is to enable the proteolytic cleaner to work; it was found that the disinfectant stage is complete within 10 min. Under these conditions, the PI system produced at least a 5 log reduction in bacteria and 4 log reduction in fungi by the first sample point of 5 min. The system also demonstrated good activity against *Acanthamoeba*, giving a 4.5 log trophozoite kill by 5 min and a 2.8 or 3.6 log kill of cysts within 2 and 4 h, respectively. It is not clear why killing of the cysts continues, even after PI neutralisation is complete. Possibly, the cysts take up the PI during the disinfection process with lethal effect on the trophozoite within and that this process continues progressively after the external disinfectant has been neutralised. The efficacy of PI against *Acanthamoeba* spp. has been reported elsewhere and is confirmed by the findings of this study [4].

The one-step hydrogen peroxide system studied here employs a platinum coated disc as part of the storage case lens basket, to catalyse the breakdown of the disinfectant into water and oxygen. Such a process offers the convenience of a single disinfection-neutralisation step and eliminates the painful consequence of inserting non-neutralised lenses into the eye that can occur with two-step systems. However, as has been previously described, the rapid neutralisation of the peroxide results in decreased efficacy against *Acanthamoeba* cysts in comparison with a two-step system [7]. The system was also found to be less effective than PI against fungi, giving only a 1.2 log and 1.8 log kill of *F. solani* conidia after 4 and 6 h exposure.

In conclusion, the PI system represents a new and effective contact lens disinfectant system efficacious against ocular pathogenic bacteria, fungi and *Acanthamoeba*. However, unlike multipurpose disinfectant systems, once

### Table 2

Efficacy of one-step hydrogen peroxide system against bacteria, fungi and *Acanthamoeba polyphaga*

<table>
<thead>
<tr>
<th>Time</th>
<th><em>P. aeruginosa</em> (ATCC 9027)</th>
<th><em>S. marcescens</em> (NCTC 10211)</th>
<th><em>S. aureus</em> (ATCC 6538)</th>
<th><em>C. albicans</em> (ATCC 10231)</th>
<th><em>F. solani</em> (ATCC 36031)</th>
<th><em>A. polyphaga</em> (Ros)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trophozoites</td>
<td>Cysts</td>
<td>Trophozoites</td>
<td>Cysts</td>
<td>Trophozoites</td>
<td>Cysts</td>
</tr>
<tr>
<td>0.5 h</td>
<td>6.4 (0.2)a</td>
<td>5.9 (0.3)</td>
<td>2.8 (0.3)</td>
<td>1.4 (0.2)</td>
<td>0</td>
<td>2.8 (0.3)</td>
</tr>
<tr>
<td>1 h</td>
<td>–b</td>
<td>4.4 (0.2)</td>
<td>1.9 (0.4)</td>
<td>0.7 (0.2)</td>
<td>3.6 (0.1)</td>
<td>0.9 (0.3)</td>
</tr>
<tr>
<td>2 h</td>
<td>4.7 (0.2)</td>
<td>2.1 (0.4)</td>
<td>0.95 (0.1)</td>
<td>0</td>
<td>–</td>
<td>0.9 (0.4)</td>
</tr>
<tr>
<td>4 h</td>
<td>2.5 (0.2)</td>
<td>1.2 (0.3)</td>
<td>–</td>
<td>0.7 (0.3)</td>
<td>–</td>
<td>0.7 (0.3)</td>
</tr>
<tr>
<td>6 h</td>
<td>5.5 (0.4)</td>
<td>1.8 (0.2)</td>
<td>–</td>
<td>1.2 (0.25)</td>
<td>–</td>
<td>1.2 (0.25)</td>
</tr>
</tbody>
</table>

a Mean (n = 3) log reduction in viable organisms with standard error of the mean in parenthesis.
b No viable organism detect at this time point.
neutralisation is complete there is no residual disinfectant activity for continued antimicrobial protection against organisms that may have survived the disinfection process or been introduced from the environment on opening the storage case [20]. Accordingly, lenses should be re-disinfected before wearing if they have been stored for more than 24 h when using the PI system.

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References

Clinical Evaluation of OPL78 a Povidone-Iodine Disinfection System with Silicone Hydrogel Lenses

Kiichi Ueda¹, Masarnaru Inaba², Yuko Miyamoto³, Yasutaka Kubota⁴, Naoki Iwasaki⁵, Katsuhide Yamasaki⁶ and Fumio Saitoh⁷

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OPL78, a chemical disinfection system for soft contact lenses that contains povidone-iodine as the active ingredient, was clinically evaluated with silicone hydrogel lenses (SHCL). The study included 65 patients (49 females, 16 males: mean age: 33±9.8 years) who used OPL78 to disinfect a 2 weeks frequent replacement SHCL (or 12 weeks, then rated the usefulness of OPL78. We conducted slit-lamp examination, observation of SHCL worn on eyes and microbiological examination after patients had completed a disinfecting procedure. Finally, we conducted a questionnaire survey. Anterior eye findings by slit-lamp examination did not change much during the clinical evaluation. Though scratches and deposits were found on SHCL in some cases, these did affect the wearing of the SHCL. The microbiological examination disclosed no problems. Furthermore, the questionnaire Survey showed that the majority of respondents experienced no problems while using OPL78 with SHCL. On the basis of these results, it is concluded that OPL78 is useful for disinfecting SHCL.

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Key words: OPL78, povidone-iodine, disinfection, silicone hydrogel, clinical evaluation

Introduction

Among the eye disorders caused by contact lenses (CLs), the most significant is corneal infection. Recently, the incidence of corneal infections has been increasing, especially in users of 2-week frequent-replacement soft contact lenses (SCLs), leading to a growing interest in disinfection of SCLs.¹,²) The chemical disinfectants currently used for SCLs include a multi-purpose solution (MPS) system consisting of a single solution of polidronium chloride or polyhexamethylene biguanide (PHMB) as the active ingredient and disinfectant systems that contain hydrogen peroxide or povidone-iodine as the active ingredient. Most SCL users use an MPS, which is less effective than other types of SCL disinfectants.³) Hydrogen peroxide-type disinfectants can cause cytotoxicity-associated ocular disorders if hydrogen peroxide neutralization is insufficient. The povidone-iodine-type disinfectant system has a broad antibacterial spectrum, is effective against fungi, viruses, and amebae, and has been reported to have high corneal safety.⁴⁻¹¹)

The use of 2-week frequent-replacement SCLs and one-day disposable SCLs has increased in recent years, along with a rapid increase in the use of silicone hydrogel as a CL material.

While the conventional povidone-iodine type system consists of disinfection granules, neutralization tablets, and a dissolution/rinse solution, the recently developed OPL78 system consists of only disinfection granules and the neutralization tablet combined into a single tablet. We conducted
a clinical study to evaluate the efficacy of OPL78 as a disinfectant for 2-week frequent-replacement silicone hydrogel contact lenses (SHCLs).

I. Subjects and Methods

1. Subjects

Sixty-five individuals were enrolled in the study from January 26, 2009, through June 30, 2009, after receiving an explanation about the purpose and content of the study. Informed written consent was obtained from all subjects prior to the study. The subjects consisted of 16 men and 49 women with a mean age of 33.0 ± 9.8 years. The subject characteristics are shown in Figure 1.

![Figure 1. Subject characteristics](image)

*Figure 1. Subject characteristics

*SCL: soft contact lens; **MPS: multi-purpose solution

2. Methods

a. Contact Lenses and Chemical Disinfectants

The subjects used four different 2-week frequent-replacement SHCL products. The ACUVUE® OASYS™ product was studied in 20 subjects with 40 eyes. The AIR OPTIX® product was studied in 15 subjects with 30 eyes. The Medalist® Premier product was studied in 15 subjects with 30 eyes. The 2week Menicon PremiO product was studied in 15 subjects with 30 eyes. The specifications of the evaluated SHCLs are shown in Table 1. In general, each SHCL was replaced every two weeks. Subjects were instructed to wash their hands before handling the SHCLs.
Table 1. Lens specifications

<table>
<thead>
<tr>
<th>Lens Name</th>
<th>Manufacturer</th>
<th>Polymeric</th>
<th>Dk/L *</th>
<th>Moisture content (%)</th>
<th>Base curve (mm)</th>
<th>Replacement Schedule</th>
<th>Manufacturer</th>
<th>Polymeric</th>
<th>Dk/L *</th>
<th>Moisture content (%)</th>
<th>Base curve (mm)</th>
<th>Replacement Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACUVUE® OASYS™</td>
<td>Johnson &amp; Johnson</td>
<td>Senofilcon A</td>
<td>147</td>
<td>38</td>
<td>8.4</td>
<td>2 weeks, daily wear</td>
<td>Bausch &amp; Lomb</td>
<td>Balafilcon A</td>
<td>101</td>
<td>36</td>
<td>8.6</td>
<td>2 weeks, daily wear</td>
</tr>
<tr>
<td>AIR OPTIX®</td>
<td>CIBAVision</td>
<td>Lotrafilcon B</td>
<td>138</td>
<td>33</td>
<td>8.6</td>
<td>2 weeks, daily wear</td>
<td>Menicon</td>
<td>Asmofilcon A</td>
<td>161</td>
<td>40</td>
<td>8.3, 8.6</td>
<td>2 weeks, daily wear/ 6 consecutive nights, continuous wear</td>
</tr>
<tr>
<td>Medalist®</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Premier</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2week Menicon PremiO</td>
<td>Bausch &amp; Lomb</td>
<td>Balafilcon A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Menicon</td>
<td>Asmofilcon A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Oxygen transmission rate = * 10^{-9} (cm/s) * (mL O2/mL-mmHg)

The constituents of the OPL78 povidone-iodine disinfectant system are shown in Figure 2. Proper usage of the OPL78 system is illustrated in Figure 3. In the OPL78 system, the active disinfectant and the neutralization/cleaning component have been combined into a single tablet, designated OPL78-I, which is dissolved in the dissolution/rinse solution, designated OPL78-II, before use. Although scrubbing is not required for lens cleaning according to the directions provided with the OPL78 system, subjects whose lenses had greater amounts of deposits were instructed to scrub their SHCLs with the OPL78-II dissolution/rinse solution.

OPL78-I (press-coated tablets)
Outer layer:
• povidone-iodine (disinfectant)
  Inner layer:
• sodium sulfite (neutralizer)
• proteolytic enzyme (cleaning agent)

OPL78-II (dissolution/rinse solution)
• sodium chloride, boric acid

Figure 2. Constituents of OPL78
b. Observation time-points and assessments

Subjects used the OPL78 system to disinfect their SHCLs for 12 weeks. Assessments performed in this study included slit-lamp microscopy for the anterior eye, inspection of the lens condition after use, microbiological examination, and a questionnaire survey (Table 2). The slit-lamp microscopy findings for the anterior eye and the subjective symptoms recorded in the questionnaire were scored according to the criteria shown in Table 3. After use, the lenses were inspected for deposits and scratches using a slit-lamp microscope. Twelve weeks after the start of the study, the subjects responded to a questionnaire regarding the usability of the OPL78 system (i.e., to what extent using the OPL78 system felt convenient), the cleaning ability of the OPL78 system (to what extent OPL78 appeared to remove deposits from the SHCL), and the comfortableness of the SHCLs (to what extent wearing the SHCL that was disinfected with OPL78 felt comfortable). The SHCLs were collected from the subjects 2 weeks after initiation of the study and microbiologically evaluated according to the procedure, methods, and criteria\(^\text{12}\) defined in Table 4. The presence of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Serratia* spp., which are often regarded as clinically relevant in ophthalmology, was evaluated and the OPL78 system was considered to have no efficacy if
at least one of these bacteria was detected. Bacteria detected only following an enrichment culture were considered not present (negative result)\(^2\).

### Table 2. Characteristics of each assessment

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Content</th>
<th>Baseline</th>
<th>After 2 weeks</th>
<th>After 4 weeks</th>
<th>After 8 weeks</th>
<th>After 12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior eye findings</td>
<td>Staining of the corneal epithelium, SEALs*, corneal edema, corneal infiltrate, corneal ulcer, neovascularization, conjunctival injection, and papillary hyperplasia</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Lens condition after use</td>
<td>Scratches, deposits, deformation, and discoloration</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Microbiological examination</td>
<td>See Table 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>●</td>
</tr>
<tr>
<td>Questionnaire</td>
<td>Foreign body sensation, dryness, itching, fogginess, and other items</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●**</td>
</tr>
</tbody>
</table>

* SEALs: superior epithelial arcuate lesions  
** At week 12, the questionnaire also assessed each subject’s overall impression of the OPL78 system.

### Table 3. Scoring criteria for anterior eye findings and subjective symptoms

#### 1) Criteria for anterior eye findings

**A) Corneal findings**

- **Corneal staining**
  - (Extent)
    - 0: No staining
    - 1: 1–25% of the corneal surface
    - 2: 26–50% of the corneal surface
    - 3: 51–75% of the corneal surface
    - 4: 76–100% of the corneal surface

- **SEALs**
  - 0: None
  - 1: Mild
  - 2: Moderate
  - 3: Severe

- **Corneal edema**
  - 0: None
  - 1: Epithelial edema
  - 2: Stromal edema (including Descemet membrane folds)
  - 3: Total edema of the cornea

- **Corneal neovascularization**
  - 0: None
  - 1: Less than 2 mm from the corneal limbus
  - 2: 2 mm or more from the corneal limbus
  - 3: 2 mm or more from the corneal limbus in multiple directions or stromal neovascularization

**B) Conjunctival findings**

- **Conjunctival injection**
  - 0: None
  - 1: Less than 1/2
  - 2: 1/2 or more
  - 3: Entire perimeter

- **Papillary hyperplasia of the upper eyelid**
  - 0: None
  - 1: Only the fornical conjunctiva
  - 2: The fornical conjunctiva + less than 1/2 of the tarsal conjunctiva
  - 3: The fornical conjunctiva + 1/2 or more of the tarsal conjunctiva

**Others**

- 0: None
- 1: Mild
- 2: Moderate
- 3: Severe
2) For subjective symptoms

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None (Feel no annoying symptoms)</td>
</tr>
<tr>
<td>1</td>
<td>Mild (Occasionally feel a symptom, but almost always feel good)</td>
</tr>
<tr>
<td>2</td>
<td>Moderate (Always feel a symptom, but no need for temporary discontinuation of use)</td>
</tr>
<tr>
<td>3</td>
<td>Severe (Always feels strong symptoms and cannot wear the lens)</td>
</tr>
</tbody>
</table>

Table 4. Procedure, methods, and criteria for the microbiological examination

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>After care with the OPL78 system, collect the CL in the lens case</td>
</tr>
<tr>
<td>[2]</td>
<td>Put each CL into a sterilized PP tube filled with 2 mL DPBS</td>
</tr>
<tr>
<td>[3]</td>
<td>Agitate the tube with a vortex mixer for 1 minute</td>
</tr>
<tr>
<td>[4]</td>
<td>After agitation, culture both CL and solution</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Culturing Methods</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1]</td>
<td>Trypticase soy agar with 5% sheep blood</td>
</tr>
<tr>
<td>[2]</td>
<td>Chocolate II agar</td>
</tr>
<tr>
<td>[3]</td>
<td>Thioglycollate broth</td>
</tr>
<tr>
<td>[4]</td>
<td>Soybean-casein digest agar</td>
</tr>
</tbody>
</table>

For [1] and [2], 200 μL of the solution was used after agitation. For [3], all of the solution was used after agitation.

Examination Criteria (2)

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Specific bacteria*</th>
<th>Total bacterial count (cfu/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly Effective</td>
<td>Not detected</td>
<td>0–&lt;10³</td>
</tr>
<tr>
<td>Effective</td>
<td>Not detected</td>
<td>10³–&lt;10⁵</td>
</tr>
<tr>
<td>Not Effective</td>
<td>Detected</td>
<td>10⁵ or more</td>
</tr>
</tbody>
</table>

* Specific bacteria: S. aureus, P. aeruginosa, E. coli, Serratia spp.

c. Statistical Method

Scores for the anterior eye findings and subjective symptoms at week 12 were compared with those at the study initiation using the Wilcoxon signed rank test with a significance level of 0.05.

II. Results

1. Anterior Eye Findings

At the initiation of the study, staining of the corneal epithelium, corneal neovascularization, conjunctival injection, papillary hyperplasia of the upper eyelid, pigmented slide, dimple veiling, and corneal scarring were seen in some patients, but all cases were mild in severity, so all subjects continued the study. The severity of these findings at week 12 was not different from the baseline severity. During the study, superior epithelial arcuate lesions (SEALs) and conjunctival injection were found in one eye and two eyes, respectively, and all cases were mild (Table 5).
<table>
<thead>
<tr>
<th>Symptom</th>
<th>Severity *1</th>
<th>0</th>
<th>105 eyes</th>
<th>97 eyes</th>
<th>94 eyes</th>
<th>104 eyes</th>
<th>103 eyes</th>
<th>p-value *2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal staining-extent</td>
<td>1</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.9094</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
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</tr>
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<td>3</td>
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</tr>
<tr>
<td>Corneal staining-density</td>
<td>1</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.8832</td>
</tr>
<tr>
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<td>2</td>
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<td></td>
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<td></td>
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</tr>
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<td>SEALs</td>
<td>1</td>
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<td>129</td>
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<td>129</td>
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</tr>
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<tr>
<td>Corneal infiltration/ulcer</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Corneal edema</td>
<td>1</td>
<td>126</td>
<td></td>
<td>128</td>
<td>128</td>
<td>128</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.1797</td>
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<tr>
<td></td>
<td>3</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
</tr>
<tr>
<td>Corneal neovascularization</td>
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<td>130</td>
<td></td>
<td>130</td>
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<td>130</td>
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</tr>
<tr>
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<td>3</td>
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<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Conjunctival injection</td>
<td>1</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
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<td></td>
<td>0</td>
<td>0</td>
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<tr>
<td>Papillary hyperplasia of the upper eyelid</td>
<td>1</td>
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<td></td>
<td>126</td>
<td>127</td>
<td>126</td>
<td>128</td>
<td>0.1797</td>
</tr>
<tr>
<td></td>
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<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Others *3</td>
<td>1</td>
<td>124</td>
<td></td>
<td>124</td>
<td>124</td>
<td>123</td>
<td>124</td>
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</tr>
<tr>
<td></td>
<td>2</td>
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<tr>
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<td></td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*1: See Table 3 for scoring criteria.
*2: The Wilcoxon signed rank test was used to compare scores at the study initiation and at week 12. -- Statistical test could not be carried out because the scores at the study initiation and at week 12 were both 0 or there were very few subjects.
*3: Other findings included pigmented slide, dimple veiling, and corneal scarring.

2. Lens Condition after Use

Deposits were found on the SHCLs for 22 eyes (16.9%) at the end of week 2, but found for only 9 eyes (6.9%) at week 12. Among the subjects for whom deposits were found on the SHCL after use, 4, 7, and 4 subjects were instructed to scrub the SHCLs at week 2, 4, and 8, respectively, because the deposit level was regarded as high. Scratches were found on the SHCL for 2–7 eyes (1.5%–5.4%) at week 2 and later, but all cases were mild, allowing all subjects to continue to use the SHCL. Metals and lacquer were each found on the SHCL for one eye (Figure 4). No deformation abnormalities were found during the study period.
3. Microbiological examination

Specific bacteria (S. aureus, P. aeruginosa, E. coli, and Serratia spp.) were not detected in any of the 130 samples evaluated. The total bacterial count was $0 - <10^3$ cfu/mL for 128 samples and $10^3 - <10^5$ cfu/mL for 2 samples. Therefore, the OPL78 system was highly effective for 98.5% of the samples and effective for 1.5% of the samples (Table 6). The bacteria detected in the two cases with bacterial counts of $10^3 - <10^5$ cfu/mL were Staphylococcus and Corynebacterium.

Table 6. Microbiological examination

<table>
<thead>
<tr>
<th>Category</th>
<th>Total Bacterial Count (cfu/mL)</th>
<th>$0 - &lt;10^3$</th>
<th>$10^3 - &lt;10^5$</th>
<th>$10^5$ or more</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n*</td>
<td>128</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Specific Bacteria</td>
<td>Not detected</td>
<td>Not detected</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Effectiveness</td>
<td>Highly effective</td>
<td>Effective</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

* Bacterium detected only with an enrichment culture were considered not present (negative result). (N = 130)

4. Questionnaire Survey

In the questionnaire to assess subjective symptoms, most of the subjects reported that they could generally use the lenses comfortably. The most frequently reported subjective symptoms were a feeling of dryness (20.8%–34.6% of eyes), foreign body sensation (3.9%–15.4%), and itching (0.8%–11.5%). The incidence of itching at week 12 was significantly different from the incidence at the start of the study ($p = 0.0128$), but no significant difference was observed for any other symptom (Figure 5). Most subjects (77%–80%) answered “very good” or “good” when asked about the comfortableness when wearing the lens, the cleaning ability of the OPL78 system, and the usability of the OPL78 system, and 68% of subjects showed willingness to continue to use the OPL78 system (Figure 6).
**Figure 5. Questionnaire survey (subjective symptoms)**

- **Feeling of Dryness**
  - Number of Eyes: Initiation - Week 2 - Week 4 - Week 8 - Week 12
  - Time Point: (Total number of eyes = 130)

- **Foreign-body sensation**
  - Number of Eyes: Initiation - Week 2 - Week 4 - Week 8 - Week 12
  - Time Point: (Total number of eyes = 130)

- **Itching**
  - Number of Eyes: Initiation - Week 2 - Week 4 - Week 8 - Week 12
  - Time Point: (Total number of eyes = 130)

- **Fogginess**
  - Number of Eyes: Initiation - Week 2 - Week 4 - Week 8 - Week 12
  - Time Point: (Total number of eyes = 130)

**Severity**
- 0: None
- 1: Mild
- 2: Moderate
- 3: Severe

**Figure 6. Questionnaire survey (overall evaluations)**

- **Impression regarding use**
  - Comfortableness when wearing the lens
  - Cleaning ability of OPL78
  - Usability of OPL78

- **Willingness to continue use**
  - Not want to use: 1%
  - May or may not use: 31%
  - Want to use: 68%

(N of subjects = 65)
III Discussion

In this study, 65 subjects (130 eyes) were observed for 12 weeks to evaluate the clinical benefit of the OPL78 system when it is used as a disinfectant system for SHCLs, which were introduced in recent years.

In aqueous solution, povidone-iodine, the disinfectant component of the OPL78 system, releases available iodine (I₂ and I₃⁻), which has high cell permeability and kills bacteria by oxidizing thiol groups in membrane proteins, enzymes, and nuclear proteins. Because povidone-iodine has a broad antibacterial spectrum, yet produces little skin irritation, it is widely utilized for various clinical purposes, including disinfection of mucosal surfaces, fingers, and skin. Povidone-iodine has also been developed as a chemical disinfectant for soft contact lenses and has been reported to have a high disinfection efficacy against bacteria, fungi, Acanthamoeba, and viruses, with a high level of safety⁴,⁵. In this clinical study, the four types of bacteria that are often regarded as clinically relevant, S. aureus, P. aeruginosa, E. coli, and Serratia spp., were not detected in any of the samples from the 130 eyes. The disinfectant system was determined to be highly effective for samples from 128 eyes and effective for those from two eyes. The bacteria detected in the samples from the two effective cases were mostly Staphylococcus and Corynebacterium, which were probably indigenous bacteria in the conjunctival sac and skin. We isolated and cultured the detected Staphylococcus and Corynebacterium bacteria and loaded the OPL78 disinfection solution with 10⁵–10⁶ cfu/mL of each type, and found that all bacteria were killed. Although the microbiological examination was conducted by collecting the lens case with the lens and the post-neutralization solution in it after lens care by the subject, bacteria attached on the inside surface of the case that had not been exposed to the disinfection solution may have been detected owing to incomplete filling of the solution into the case. Because of the results of the microbiological examination, as well as the lack of symptoms or findings of suspected infection during the study period, the OPL78 system was considered effective as a disinfectant for SHCLs.

The anterior ocular findings observed during the study period by slit-lamp microscopy were mild in severity and known to be relatively common in SCL or SHCL users. Corneal staining can occur in those who use SHCLs in combination with a multi-purpose solution containing PHMB¹³,¹⁴, because the disinfectant component absorbed into the lens could affect corneal epithelial cells. In addition, mechanical stimulation of the cornea by harder SHCLs is considered to have the possibility to affect corneal epithelial cells¹³. In this study, the extent and density of corneal staining did not change from the start of study through week 12, because the disinfectant was less likely to remain in the lens due to the disinfection process utilized with the OPL78 system, in which the disinfection component is neutralized and the lens is rinsed before wearing.

Clenside®, the first povidone-iodine disinfectant product developed in Japan, could cause delayed-onset drug allergy-like symptoms in some users of conventional SCLs¹⁵. The OPL78 system, utilizing the same components for disinfection, neutralization, and cleaning as were used in Clenside®, did not cause drug allergy-like symptoms in this study, in which subjects used SHCLs. Even if one does not consider the materialistic difference between conventional SCLs and SHCLs, late-onset allergic symptoms cannot be evaluated in a study of only 12 weeks, so caution should be exercised during long-term use of the OPL78 system.

During the study period, hordeolum was reported as an adverse effect in one eye, but was not considered a side effect of the OPL78 system, because the causal relationship with OPL78 use was unclear.
Some deposits were identified during examination of the condition of the lenses after use of the OPL78 system. Although the OPL78 system contains cleaning agents, including a protease, its cleaning ability could be insufficient for SHCLs on which lipids are more likely to deposit, in which case the user should be instructed to scrub the lens with the OPL78 dissolution/rinse solution. It was unclear why deposits were more likely to be observed at week 2 than at the other time points; however, a possible explanation for this finding is that eye secretions increased until the subjects were accustomed to the lens, because many of the subjects used SHCLs for the first time in this study.

The most common subjective symptoms reported in the questionnaire, in order of decreasing incidence, were a feeling of dryness, foreign body sensation, and itching. Although the severity of itching at week 12 was significantly different from the severity at baseline, most subjective symptoms were mild and no discontinuation of contact lens use occurred. The observed subjective symptoms were consistent with those described in clinical reports on other SHCLs. In a clinical study by Shinoda et al., in which subjects used a one-month replacement SHCL together with a hydrogen peroxide disinfectant for three months, the subjective symptoms reported in the three month observation period and their incidences were similar to those reported in the present study; the most common subjective symptoms were dryness (incidence: 10.3%–33.3%), foreign body sensation (10.3%–20.5%), and itching (5.1%–7.7%)\(^6\)). Moreover, the foreign body sensation reported in the present study may be due to deposits on the SHCLs and the mechanical stimulation of the SHCL mentioned above.

Contact lens users are required to strictly adhere to defined lens care procedures. There exists a need for a new povidone-iodine system that can be used with a simpler disinfection procedure than the conventional method that requires adding three agents\(^4\). To use the OPL78 system, one only needs to dissolve OPL78-I into OPL78-II. The questionnaire survey in this study showed that 77% of subjects responded that the usability of OPL78 was very good or good, while 80% of subjects responded that the cleaning ability of OPL78 and comfortableness when wearing the lens were very good or good. As a result, 68% of subjects showed willingness to continue using the OPL78 system, resulting in a good overall rating for the system.

The results of this study demonstrate that the OPL78 system is safe, highly effective, and easy to use. Therefore, the OPL78 system is an ideal disinfectant system for SHCLs with advantages over currently available disinfectant systems.
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Comparison of the Antibacterial and Antifungal Effects of a Contact Lens Solution Containing Povidone-Iodine and Three Other Solutions

Ryoji Yanai
Department of Biomolecular Recognition and Ophthalmology, Yamaguchi University School of Medicine and Department of Ophthalmology, Toyota Chuo Hospital

Kiichi Ueda
Department of Biomolecular Recognition and Ophthalmology, Yamaguchi University School of Medicine and Shimonoseki City (Ueda Eye Clinic)

Motoharu Tajiri, Toru Matsumoto, Shigeru Nakamura and Fumio Saito
Ophtecs Corporation Research Center

Teruo Nishida
Department of Biomolecular Recognition and Ophthalmology, Yamaguchi University School of Medicine
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Ophtecs Corporation Research Center

Teruo Nishida
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We compared the disinfectant effects of a solution for soft contact lens care that contained povidone iodine (PVP-I) with the effects of 3 other solutions for contact lens care, specifically solutions that contained hydrogen peroxide, polydronium, or polyhexanide as the active ingredient against bacteria and fungi. All disinfectant solutions demonstrated antibacterial activity against Pseudomonas aeruginosa, Staphylococcus aureus, and Serratia marcescens, and antifungal activity against Fusarium solani within the time recommended for disinfection using commercially available solutions. However, only the solution containing PVP-I was effective against Candida albicans. These results suggest that contact lens solutions that contain PVP-I are useful as disinfectant solutions for soft contact lenses.

(J Jpn CL Soc 47: 32-36, 2005)

Key Words: Povidone-Iodine (PVP-I), Chemical Disinfection, Lens Care, Antibacterial Activity, Antifungal Activity

Introduction

Soft contact lenses (SCLs), which have high moisture contents, are susceptible to attachment by pathogenic microorganisms, often resulting in serious CL-related eye disorders such as corneal ulcers. Thus, the use of SCLs requires regular disinfection of the lenses to remove microorganisms. The methods for SCL disinfection are roughly classified into 2 types: heat disinfection (boiling disinfection) and cold disinfection (chemical disinfection). Boiling disinfection methods were initially developed and widely used by SCL wearers. However, chemical disinfection was subsequently developed to overcome various problems including allergies to proteins denatured by heating, deterioration of SCLs, and failure or malfunction of the boiling apparatus. In Japan, solutions containing hydrogen peroxide as the active ingredient and multipurpose solutions, second- and third-generation chemical disinfectants, respectively, were introduced and spread rapidly in the 1990s.
However, chemical disinfectants are not effective against a large number of bacterial cells or fungi such as *Candida* and are thus considered to have a weaker disinfectant effect than boiling.1

Iodine-based disinfectants, which contain povidone-iodine (PVP-I), a halogen compound synthesized by Gershenfeld in 1957, exert a bactericidal effect via iodine (I$_2$) release. They are effective against a wide range of microorganisms including vegetative bacteria, spores, tubercle bacilli, viruses, and fungi$^{2-7}$. In addition, they cause less skin irritation as compared to alcohol and are nearly odorless. For these reasons, they are currently the most commonly used medical disinfectants for hand, surgical skin, and mucosal disinfection$^{8-10}$. Chemical disinfectants containing PVP-I as the active ingredient have recently been commercialized in Japan, and have the following 3 properties: a broad microbial spectrum, an immediate disinfectant effect, and safety with respect to the body.

In this study, we compared the antibacterial and antifungal effects of a chemical disinfectant containing PVP-I as the active ingredient with those of conventional disinfectants that contain hydrogen peroxide, polidronium chloride, or polyhexanide chloride.

**Methods**

1. **Test Solutions**

A PVP-I-based disinfectant product (Ophtecs, Kobe, Japan), a hydrogen peroxide-based disinfectant product (Abbott Medical Optics Japan, Tokyo, Japan), a polidronium chloride-based disinfectant product (Alcon Japan, Tokyo, Japan), and a polyhexanide chloride-based disinfectant product (Abbott Medical Optics Japan) were used as the test solutions.

Test strains were selected according to ISO 14729$^{11}$. For bacteria, *Pseudomonas aeruginosa* (13275; Institute for Fermentation Osaka [IFO], Japan), *Staphylococcus aureus* (13276, IFO), and *Serratia marcescens* (13880; American Type Culture Collection [ATCC], USA) were used, all of which are frequently reported as causative organisms for corneal infections related to CLs$^{12,13}$. For fungi, *Fusarium solani* (36031, ATCC) was selected as a representative filamentous fungi and *Candida albicans* (1594, IFO) as a representative yeast species.

2. **Methods for Inoculum Preparation**

1) **Bacteria**

The test strain was cultured on soybean-casein digest agar slants at 35°C for 18 to 24 hours, and then bacterial cells were harvested with Dulbecco's phosphate-buffered saline (DPBS).

2) **Fungi**

The *F. solani* test strain was cultured on potato dextrose agar plates at 25°C for 10 to 14 hours, and then spores were harvested with DPBS. The *C. albicans* test strain was cultured on Sabouraud dextrose agar slants at 25°C for 42 to 48 hours, and then bacterial cells were harvested with DPBS. The cell suspension was filtered through absorbent cotton and centrifuged at 2,000 $\times$ g for 10 minutes at 25°C. The resulting supernatant was discarded, and the precipitate was suspended in DPBS. The
turbidity of the suspension was measured with a turbidimeter. The concentration of the suspension was adjusted to $1.0 \times 10^7$ to $1.0 \times 10^8$ colony-forming units (cfu)/ml.

3. Methods of Testing for Disinfectant Effects

The inoculum was added to each test solution at final concentrations ranging from $1.0 \times 10^5$ to $1.0 \times 10^6$ cfu/ml and disinfected for a period of time corresponding to 25%, 50%, 75%, or 100% (1.25 minutes to 4 hours) of the recommended disinfection time for each test solution (Table 1). Disinfection was performed according to the methods described in the package insert for each test solution. To stop the disinfection reaction, the solution containing PVP-I was neutralized with 0.003% sodium sulfate solution and the solution containing hydrogen peroxide with 0.01% catalase solution. The solutions containing polidronium chloride or polyhexanide chloride were neutralized with Dey/Engley neutralizing broth, although neutralization is not required for normal lens disinfection. After neutralization, bacteria were cultured by the pour plate method with soybean-casein digest agar at 35°C for 4 to 6 days and fungi with glucose peptone agar at 25°C for 5 to 6 days. Colonies were counted to calculate the number of viable cells.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Recommended Disinfection Time for Each Chemical Disinfectant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Solution</td>
<td>Disinfection Time</td>
</tr>
<tr>
<td>Povidone-iodine-based solution</td>
<td>≥ 5 minutes</td>
</tr>
<tr>
<td>Hydrogen peroxide-based solution</td>
<td>≥ 10 minutes</td>
</tr>
<tr>
<td>Polidronium chloride-based solution</td>
<td>≥ 4 hours</td>
</tr>
<tr>
<td>Polyhexanide chloride-based solution</td>
<td>≥ 4 hours</td>
</tr>
</tbody>
</table>

4. Evaluation of the Disinfectant Effect

The disinfectant effect of each test solution was evaluated according to the stand-alone test described in ISO 14729. A disinfectant effect was defined as a reduction in the number of cells to less than 1/1000 the number of loaded cells for bacteria and to less than 1/10 for fungi.

Results

1. Antibacterial Activity

The antibacterial activities of the various chemical disinfectants were compared using linear regression. For *P. aeruginosa*, the number of the cells was reduced to below the detection limit within the recommended disinfection time by all of the chemical disinfectants, and thus they demonstrated effective antibacterial activity. In addition, the number of loaded cells was already reduced to less than 1/1000 in 25% of the recommended disinfection time, for all disinfectants. Specifically, effective antibacterial activity was achieved in 1.25 minutes for the PVP-I solution group, in 2.5 minutes for the hydrogen peroxide solution group, and in 1 hour for the polidronium chloride and polyhexanide chloride solution groups (Fig. 1).
For *Staphylococcus aureus*, the number of loaded cells was reduced to below the detection limit within the recommended disinfection time in the PVP-I, hydrogen peroxide, and polyhexanide chloride solution groups, but not in the polidronium chloride solution group. In addition, the number of loaded cells was reduced to less than 1/1000 in 25% of the recommended disinfection time for the PVP-I and polidronium chloride solution groups, in 50% for the polyhexanide chloride solution group, and in 75% for the hydrogen peroxide solution group. Therefore, effective antibacterial activity against *S. aureus* was achieved in 1.25 minutes for the PVP-I solution group, in 7.5 minutes for the hydrogen peroxide solution group, in 1 hour for the polidronium chloride solution group, and in 2 hours for the polyhexanide chloride solution group (Fig. 2).

For *Serratia marcescens*, similar to the results for *Staphylococcus aureus* described above, the number of loaded cells was reduced to below the detection limit within the recommended disinfection time in the PVP-I, hydrogen peroxide, and polyhexanide chloride solution groups, but not in the polidronium chloride solution group. The number of loaded cells was reduced to below the detection
limit in 25% of the recommended disinfection time for the PVP-I and polyhexanide chloride solution groups, in 50% for the hydrogen peroxide solution group, and in 75% for the polidronium chloride solution group. Therefore, effective antibacterial activity on *S. marcescens* was achieved in 1.25 minutes for the PVP-I solution group, in 5 minutes for the hydrogen peroxide solution group, in 1 hour for the polyhexanide chloride solution group, and in 3 hours for the polidronium chloride solution group (Fig. 3).

![Fig. 3 Antibacterial activity against *Serratia marcescens*](image)

2. Antifungal Activity

For *F. solani*, the loaded cells were all sterilized and thus the cell number was reduced to below the detection limit within the recommended disinfection time in the PVP-I, polidronium chloride, and polyhexanide chloride solution groups. There was limited antifungal activity, with a reduction to up to 1/100 of the number of loaded cells, within the recommended disinfection time in the hydrogen peroxide solution group. In addition, the number of loaded cells was reduced to less than 1/10 in 25% of the recommended disinfection time for all disinfectants. Therefore, effective antifungal activity was achieved in 1.25 minutes for the PVP-I solution group, in 2.5 minutes for the hydrogen peroxide solution group, and in 1 hour for the polidronium chloride and polyhexanide chloride solution groups (Fig. 4).
For C. albicans, sufficient antifungal activity was obtained for the PVP-I solution group; the number of loaded cells was reduced to below the detection limit in 25% of the recommended disinfection time. In contrast, the number of loaded cells was not reduced to less than 1/10 within the recommended disinfection time and thus no effective antifungal activity was obtained in the hydrogen peroxide, polidronium chloride, and polyhexanide chloride solution groups (Fig. 5).

**Discussion**

We compared the antibacterial and antifungal effects of a chemical disinfectant containing PVP-I as the active ingredient with those of 3 conventional chemical disinfectants. The PVP-I solution reliably exerted sufficient disinfectant effects against both bacteria and fungi more rapidly than the other chemical disinfectants. In addition, the solutions containing hydrogen peroxide, polidronium chloride, or polyhexanide chloride provided sufficient disinfectant effects against bacteria and F. solani, but
showed no antifungal effect against C. albicans within the study period (Table 2). Our study suggested that PVP-I solutions have a higher disinfectant effect than conventional chemical disinfectants.

Table 2  Antibacterial and Antifungal Activities of the Chemical Disinfectants

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Fungi</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PA</td>
<td>SA</td>
<td>SM</td>
<td>FS</td>
</tr>
<tr>
<td>Povidone-iodine solution</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrogen peroxide solution</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polidronium chloride solution</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polyhexanide chloride solution</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>


All chemical disinfectants studied had sufficient antibacterial effects. However, the polidronium chloride and polyhexanide chloride solutions required 1 hour or more, while the PVP-I solution was effective within 1 minute; therefore, it was characterized as having an immediate disinfectant effect. In addition, greater numbers of viable cells were observed for Staphylococcus aureus and Serratia marcescens than for P. aeruginosa after disinfection with the polidronium chloride or polyhexanide chloride solutions; therefore, these disinfectants were considered less likely to exert sufficient antibacterial effects against the former 2 bacterial species. These results raise concerns regarding the disinfectant effect of multipurpose solutions against a large number of bacteria, depending on the strain. Furthermore, multipurpose solutions promote adhesion of P. aeruginosa to corneal epithelial cells and thus potentially increase the risk of corneal infection. The PVP-I and hydrogen peroxide solutions rapidly exhibited sufficient bactericidal effects and thus were considered excellent disinfectants. However, since they exerted no disinfectant effect against microbial contamination after neutralization, re-disinfection of SCLs is required when a lens case is opened accidentally after disinfection, re-exposing SCLs to microorganisms. Multipurpose solutions do not typically require neutralization and provide a long-lasting disinfectant effect once the disinfection reaction occurs. For this reason, multipurpose solutions are considered suitable for long-term storage. In fact, polidronium chloride solutions have shown antibacterial effects against P. aeruginosa and Staphylococcus aureus even 3 days after disinfection.

For fungi, all of the disinfectants studied showed effective antifungal activity against F. solani, but only the PVP-I solution had a sufficient antifungal effect against C. albicans. The yeast C. albicans is the most frequent causative organism for keratonyctis, and thus a high antifungal activity against the species is an essential property of a chemical disinfectant. Based on the results of our study, C. albicans is difficult to control with hydrogen peroxide or multipurpose solutions. Since C. albicans is present in daily living spaces, there is a risk of SCLs contamination by fingers that are not fully disinfected with hydrogen peroxide or multipurpose solutions. Results similar to those of our study have been noted in a previous investigation by the National Consumer Affairs Center of Japan. C. albicans disinfection is a serious problem for SCL hygiene. With respect to the time required to disinfect fungi, the PVP-I solution provided sufficient effects within 1 minute; therefore, it was considered to have an immediate disinfectant effect against fungi.

PVP-I has disinfectant effects against a wide variety of microorganisms, such as viruses and Acanthamoeba as well as bacteria and fungi. We plan to investigate the disinfectant effects of PVP-I solutions for a broader sample of microorganisms. In addition, since PVP-I solutions contain various additives in addition to the disinfection component, further investigations are needed to determine
whether deterioration and deformation of SCLs could occur due to the reaction between such additives and polymers including SCL materials and a lens case. Additionally, more studies are required regarding the disinfectant effects of PVP-I solutions, for example, on the extent to which PVP-I can exert a disinfectant effect against a biofilm formed inside a lens case. Finally, the compliance of SCL users represents a very important issue. Compliance is worse for PVP-I solutions that require a total of 3 agents for SCL care than for single, multipurpose solutions that account for all procedures related to SCL care. Thus, development of a simple procedure for disinfection is desired in terms of the compliance of CL wearers.

文献

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Comparison of the Antiviral and Anti-Acanthamoeba Effects of a Disinfectant Solution Containing Povidone-Iodine and Three Other Solutions

Ryoji Yanai
Department of Biomolecular Recognition and Ophthalmology, Yamaguchi University School of Medicine and Department of Ophthalmology, Toyota Chuo Hospital

Kiichi Ueda
Department of Biomolecular Recognition and Ophthalmology, Yamaguchi University School of Medicine and Shimonoseki City (Ueda Eye Clinic)

Motoharu Tajiri, Toru Matsumoto, Shigeru Nakamura and Fumio Saito
Ophtecs Corporation Research Center

Teruo Nishida
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Teruo Nishida  
Department of Biomolecular Recognition and Ophthalmology, Yamaguchi University School of Medicine

We compared the disinfectant effects of a solution for contact lens care that contained povidone-iodine (PVP-I) with the effects of 3 other solutions for contact lens care, specifically those containing hydrogen peroxide, polydronium, or polyhexanide, for their effects against 2 species of *Acanthamoeba* and 2 viruses. The solution containing PVP-I demonstrated disinfectant effects equivalent to heat disinfection against *Acanthamoeba polyphaga*, *Acanthamoeba castellanii*, adenovirus type 8, and herpes simplex virus type 1. However, the solutions containing polydronium or polyhexamide had no effects against these organisms. The solution containing hydroxy peroxide showed antiviral effects against herpes simplex virus type 1. These results suggest that contact lens solutions that include PVP-I are optimal for disinfection of soft contact lenses because of their antimicrobial activity against *Acanthamoeba* and viruses.

*(J Jpn CL Soc 47:37-41,2005)*

Key Words : Povidone-Iodine(PVP-I), Chemical Disinfection, Lens Care, *Acanthamoeba*, Virus

**Introduction**

*Acanthamoeba* are free-living protozoa that are widely distributed in nature, including freshwater and soil environments. Keratitis caused by *Acanthamoeba* is an intractable corneal infection, and its incidence has been increasing since the first reported case in the UK in 1974. *Acanthamoeba* keratitis is frequently observed in individuals who wear contact lenses, and has been primarily attributed to the use of contact lens storage solution prepared using tap water and well water. In recent years, the use of multipurpose solutions is increasing and accordingly, disinfectant solutions for soft contact lenses with a reduced disinfecting capacity seem to increase the risk of *Acanthamoeba* infection. When a person develops *Acanthamoeba* keratitis, early diagnosis and treatment are important, requiring differential diagnosis of *Acanthamoeba* keratitis from herpes keratitis at the early stage. Initial infection by herpes
simplex virus occurs in childhood primarily in the lips, oral cavity, eyelids, and conjunctiva. Most adult individuals have latent infections, which are generally asymptomatic. Recurrent herpes keratitis occurs by reactivation of herpes virus under immunocompromised conditions, such as common colds, fever, and stress. This type of disease can be further classified into epithelial, parenchymal, and nutritional forms. One epithelial disease is dendritic keratitis, which requires careful diagnosis because it may be mistaken for pseudodendritic keratitis due to Acanthamoeba infection. Adenovirus is the most common cause of viral keratoconjunctival diseases encountered in routine practice, and is an important causative organism for epidemic ophthalmological infections. In the prevention of viral keratoconjunctival diseases, it is important to wash hands and disinfect tools, as in the prevention of other infections, and to take precautions against viral infection via contact lenses when appropriate.

Povidone-iodine (PVP-I) is an iodine antiseptic with a broad spectrum of antimicrobial action against proliferating bacteria, spores, tubercle bacilli, viruses, and fungi, and is commonly used to disinfect fingers and surgical field skin areas. Chemical disinfectant products containing PVP-I as the antiseptic component have recently become available for practical use in Japan. These products show superior disinfecting effects against bacteria and fungi, and also act quickly.

In the present study, we evaluated the efficacy of a chemical disinfectant product containing PVP-I against Acanthamoeba and viruses by comparing its disinfectant effect with the effects of a conventional hydrogen peroxide-based product and multipurpose disinfectant products containing polidronium chloride or polyhexanide chloride, as well as boiling disinfection by heating.

**Methods**

1. **Test solutions**

A PVP-I-based disinfectant product (Ophtecs Corp., Kobe, Japan), a hydrogen peroxide-based disinfectant product (AMO Japan Ltd., Tokyo, Japan), a polidronium chloride-based disinfectant product (Alcon Japan Ltd., Tokyo, Japan), and a polyhexanide chloride-based disinfectant product (AMO Japan Ltd.) were used as the test solutions.

Solutions of 0.003% sodium sulfite and 0.01% catalase were used to neutralize PVP-I and hydrogen peroxide, respectively, to stop the disinfecting reaction. While neutralization is not required for disinfectant products containing polidronium chloride or polyhexanide chloride in routine lens care, neutralization by LP solution [0.7% lecithin and 0.5% polysorbate 80 added to a 100-fold dilution of a saline solution for amoeba (12.0 g of NaCl, 0.35 g of KCl, 0.30 g of CaCl₂, 0.40 g of MgSO₄•7H₂O dissolved in 1 L of 50 mM Tris-HCl buffer solution, pH 6.8)] was used in the test against amoebae or an Eagle's Minimal Essential Medium containing 10% fetal bovine serum in the test against viruses to stop the disinfecting reaction.

2. **Testing the disinfectant effect against amoebae**

1) Test amoeba strains

The representative strains causing Acanthamoeba keratitis, Acanthamoeba polyphaga (ATCC 30461) and A. castellanii (ATCC 30868), which have been reported as corneal isolates, were selected.
*Escherichia coli* (IFO 3972) was used as a feed in the culture of *Acanthamoeba* strains, and the test results were analyzed as follows by reference to the stand-alone test described in ISO 14729\textsuperscript{11)}.

2) **Seed stock preparation**

*Acanthamoeba* cells were cultured by feeding with *E. coli* on agar plate medium containing 0.01% malt extract and 0.01% yeast extract (MY medium) for at least 4 weeks. After confirming cyst formation, amoeba cysts were collected using the saline solution for amoebae, washed by centrifugation, and resuspended to a concentration of $10^5$ to $10^6$ cysts/mL.

3) **Amoeba cell counting**

A 10-fold dilution series was prepared from the test amoeba solution using the saline solution for amoebae. To each well of a multi-well plate, an appropriate amount of *E. coli*-containing-MY medium and 100 μL of an amoeba dilution was added (4 wells per dilution). After incubation of the plate at 25°C for 14 days, amoeba growth in each well was checked under a microscope, and the number of viable cysts was calculated following the method of Reed & Muench.\textsuperscript{12)}

4) **Assay of disinfectant effect**

To each test disinfectant solution, seed stock was added to an amount equivalent to 1% of the test disinfectant solution to initiate disinfection. After the minimum disinfection time specified for each disinfectant elapsed (Table 1), the test disinfectant solution was collected and added to the neutralizing solution, and the number of viable amoeba cysts was determined in the neutralized solution. For boiling disinfection, an appropriate amount of the saline solution for amoeba-containing seed stock was added to a boiling case, which was sterilized in a boiler, and the number of viable amoeba cysts was determined.

As a control, the number of viable amoeba cysts was determined in the saline solution for amoeba-containing seed stock as the loaded cyst number.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Recommended disinfection time for chemical disinfectants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test disinfectant solution</td>
<td>Disinfection time</td>
</tr>
<tr>
<td>Povidone-iodine-based disinfectant product</td>
<td>$\geq$5 minutes</td>
</tr>
<tr>
<td>Hydrogen peroxide-based disinfectant product</td>
<td>$\geq$10 minutes</td>
</tr>
<tr>
<td>Polidronium chloride-based disinfectant product</td>
<td>$\geq$4 hours</td>
</tr>
<tr>
<td>Polyhexanide chloride-based disinfectant product</td>
<td>$\geq$4 hours</td>
</tr>
</tbody>
</table>

3. **The disinfectant effect against viruses**

1) **Test virus strains**

Adenovirus type 8 (ATCC VR-1368) and herpes simplex virus type 1 (HSV-1; ATCC VR-260) were selected as test virus strains and HeLa cells (RCB 0007) and Vero cells (RCB 0001) were used as host cells, respectively.
2) Seed stock preparation

For adenovirus type 8, monolayer HeLa cell cultures were infected with the virus and incubated in a CO₂ incubator until a cytopathic effect (hereinafter "CPE") was microscopically observed throughout the monolayer. The infected cells were collected as a suspension, which was centrifuged at approximately 2,000 × g for 10 minutes at 4°C. The pellet was resuspended in phosphate buffer, sonicated, and used as the adenovirus type 8 seed stock to test the disinfectant effect.

For HSV-1, monolayer Vero cell cultures on the bottom of cell culture flasks were infected with HSV-1 and incubated in a CO₂ incubator until CPE was microscopically observed throughout the monolayer. The infected cells were collected as a suspension, which was centrifuged at approximately 2,000 × g for 10 minutes at 4°C. The supernatant was diluted 10-fold in phosphate buffer, and used as the HSV-1 seed stock to test the disinfectant effect.

4. Assay of disinfectant effects

Each test disinfectant solution was mixed with the viral seed stock, allowed to react at 25°C for the recommended disinfection time (Table 1), and added to neutralizing solution to stop the disinfecting reaction. Subsequently, the 50% tissue culture infectious dose (TCID₅₀) of the residual viral titer was determined using the calculation method of Reed & Muench¹² based on the viral titer remaining in the test sample. For boiling disinfection, an appropriate amount of phosphate buffer and the seed stock was added to a boiling case, which was sterilized in a boiler, and the viral titer was determined.

Results

1. Disinfectant effect against amoebae

The log reduction data for A. polyphaga treated with various chemical disinfectants are presented in Figure 1. The PVP-I-based disinfectant product resulted in an approximately 2-log reduction in cell count, which was equivalent to the effect of boiling disinfection (2.06-log reduction). Hydrogen peroxide-, polidronium chloride-, and polyhexanide chloride-based disinfectant products resulted in slight reductions in cyst counts (0.61-, 0.72-, and 0.72-log reductions, respectively), demonstrating an insufficient disinfectant effect.
The PVP-I-based disinfectant solution also exhibited a disinfectant effect against *A. castellanii*, resulting in a 2.28-log reduction in cell counts, which was similar to the effect of boiling disinfection (i.e., a 2.28-log reduction). Other disinfectant solutions showed little or no disinfectant effects with a log reduction of 0 to 0.5 (Figure 2).

The PVP-I-based disinfectant solution showed a disinfectant effect against adenovirus type 8, resulting in a 1.94-log reduction in viral titers, which was equivalent to the level of viral inactivation observed for boiling disinfection (2.0-log reduction). However, hydrogen peroxide-, polidronium chloride-, and polyhexanide chloride-based disinfectant solutions demonstrated little or no viral inactivation in the minimum disinfection time, resulting in log reductions of 0.44, 0.0, and 0.0, respectively (Figure 3).
PVP-I- and hydrogen peroxide-based disinfectant solutions exhibited disinfectant effects against HSV-1 in the minimum disinfection time, resulting in log reductions of 2.22 and 2.67 for viral titers, respectively, similar to the effect of boiling disinfection (3.22-log reduction). However, polidronium chloride- and polyhexanide chloride-based disinfectant solutions did not show viral inactivation, resulting in 0.04 and 0.54 log-reductions, respectively (Figure 4).

In the present study, we compared disinfectant effects of a PVP-I-based chemical disinfectant solution against Acanthamoeba and viruses with those of conventional chemical disinfection methods. The PVP-I-based disinfectant solution demonstrated sufficiently high disinfectant effects against all tested microbial strains, i.e., A. polyphaga, A. castellanii, adenovirus type 8, and HSV-1, and the effect was equivalent to that of boiling disinfection, which uses heat to kill microbes (Table 2). In contrast, the hydrogen peroxide-based disinfectant solution and multipurpose solutions showed insufficient disinfectant effects against Acanthamoeba and viruses within the recommended disinfection time,
indicating that these solutions were inappropriate for the prevention of infection by these microorganisms (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Amoeba</th>
<th></th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AP</td>
<td>AC</td>
<td>ADV8</td>
</tr>
<tr>
<td>Povidone-iodine-based disinfectant product</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrogen peroxide-based disinfectant product</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Polidronium chloride-based disinfectant product</td>
<td>-</td>
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</tr>
<tr>
<td>Polyhexanide chloride-based disinfectant product</td>
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</tr>
</tbody>
</table>

AP = *Acanthamoeba polyphaga*, AC = *Acanthamoeba castellanii*, ADV8 = adenovirus type 8, HSV-1 = herpes simplex virus type1

*Acanthamoeba* keratitis is attributed to the use of contact lenses in more than 80% of cases reported in Japan, and the number of patients is increasing as the number of contact lens wearers is increasing. For this disease, there is no established treatment at present and the prevention of infection is very important. Individuals wearing soft contact lenses should kill *Acanthamoeba* to a level that does not cause infection in the daily disinfection of their contact lenses. However, chemical disinfectant products that are widely used are not necessarily tested against *Acanthamoeba*, and boiling disinfection is the most secure method for lens disinfection. PVP-I is an antiseptic reported to be useful for killing amoebae and inactivating viruses. In the present study, we also observed a high disinfectant effect of the PVP-I-based product, supporting its clinical effectiveness.

PVP-I is also recommended as an antiseptic for disinfecting fingers and tools in the prevention of adenoviral conjunctivitis, and its effectiveness has been clinically well established. Our study provides evidence for its effectiveness in soft contact lens disinfection against not only adenovirus but also herpes virus. We have previously reported the effectiveness of PVP-I disinfectant products in lens disinfection against bacteria and fungi. Combined with the results of the present study, PVP-I disinfectant products are highly effective in soft contact lens disinfection with a broad spectrum of antimicrobial action against bacteria, fungi, *Acanthamoeba*, and viruses.

In summary, the PVP-I-based chemical disinfectant product had a higher disinfectant effect against *Acanthamoeba* and viruses than conventional chemical disinfection methods. Combined with the results of our previous report, the PVP-I-based product is useful for disinfecting soft contact lenses contaminated with a wide range of microorganisms, including bacteria, fungi, *Acanthamoeba*, and viruses.

文献


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Evaluation of povidone-iodine as a disinfectant solution for contact lenses: Antimicrobial activity and cytotoxicity for corneal epithelial cells

Ryoji Yanai a,*, Naoyuki Yamada a, Kiichi Ueda a, Motoharu Tajiri b, Toru Matsumoto b, Keiji Kido b, Shigeru Nakamura b, Fumio Saito b, Teruo Nishida a

a Department of Biomolecular Recognition and Ophthalmology, Yamaguchi University School of Medicine, 1-1-1 Minami-Kogushi, Ube, Yamaguchi 755-8505, Japan
b Ophtecs Corporation Research Center, Toyooka, Hyogo, Japan

Abstract

Povidone-iodine (PVP-I) possesses broad-spectrum antimicrobial activity and is used clinically as a disinfectant. We evaluated the disinfectant properties and safety of PVP-I for use as a contact lens solution. The concentrations of PVP-I required to reduce the number of Staphylococcus aureus or Candida albicans by 3 log units were lower than were those of hydrogen peroxide, polyhexamethylene biguanide (PHMB), and benzalkonium chloride (BAK). The cytotoxicity of PVP-I for cultured human corneal epithelial (HCE) cells was less than that of the other three agents. The safety margin for PVP-I was thus greatest among the tested compounds. PVP-I appears suited for use as a contact lens disinfectant.

Keywords: Soft contact lens; Disinfection; Povidone-iodine; Hydrogen peroxide; Multipurpose solution; Safety margin

1. Introduction

Cleaning and disinfection of contact lenses are essential for their safe wearing [1]. Disinfection is especially important for soft contact lenses in order to avoid lens-related infection of the cornea [2–5]. The incidence of microbial keratitis induced by wearing of contact lenses is 3.5–14.0 cases per 10,000 individuals for daily-wear hydrogel lenses and 20.0–144.6 cases per 10,000 individuals for extended-wear hydrogel lenses [6–8]. Although the frequency of this condition is relatively low, it is sight threatening and can be avoided by proper lens handling and management [9]. The heat-based system was the first method approved for disinfection of soft contact lenses and has remained the most efficient [10,11]. This system has disadvantages, however, including its induction both of lens deformation and of deposition of denatured proteins [12]. To avoid such problems associated with heat, a chemical disinfection system based on hydrogen peroxide was introduced [13]. Although hydrogen peroxide is effective against a broad range of microbes, neutralization is required to avoid serious insults to the ocular surface. This neutralization step is a disadvantage in terms of compliance [14,15]. Multipurpose disinfectant solutions, which contain cleaning, rinsing, and disinfectant agents in a single solution, were developed to resolve such problems. Currently, multipurpose disinfectant solutions are used widely, but the antimicrobial activity of these solutions is not as great as that of heat- or hydrogen peroxide-based disinfection systems [16–18]. The development of a new disinfection system with an increased and broader spectrum antimicrobial activity, with a reduced impact on contact lens materials, and with a low cytotoxicity at the ocular surface is thus desirable to ensure the comfort and safety of soft contact lenses.
Povidone-iodine (PVP-I) is an iodinated polyvinyl polymer that has been used as a topical antiseptic to prevent infections of the skin or mucous membranes during surgery [19,20]. PVP-I possesses broad-spectrum antimicrobial activity against bacteria, yeasts, molds, other fungi, Acanthamoeba, and certain viruses [21–24], but it has a low toxicity to human cells and tissues [25]. On the basis of its low toxicity at the ocular surface, a 5% solution of PVP-I has been applied as an antiseptic before intraocular surgery and has been shown to markedly reduce the abundance of the conjunctival bacterial flora [26–29]. No toxic effects of such irrigation on the ocular surface were observed.

To evaluate the efficacy of PVP-I as a contact lens disinfectant, we have now examined both its antimicrobial activity against the bacterium Staphylococcus aureus and the fungus Candida albicans as well as its cytotoxicity toward cultured human corneal epithelial (HCE) cells. PVP-I was compared in these tests with hydrogen peroxide and the major chemical components of multipurpose solutions, polyhexamethylene biguanide (PHMB) and benzalkonium chloride (BAK).

2. Methods

2.1. Growth of microorganisms

S. aureus (Institute for Fermentation at Osaka (IFO) 13276) was grown on soybean-casein digest agar (Nihon Pharmaceutical, Kanagawa, Japan) at 35 °C for 18–24 h, and C. albicans (IFO1594) was cultured on Sabouraud dextrose agar (Becton Dickinson, Franklin Lakes, NJ) at 25 °C for 42–48 h. The microbial cells were harvested with Dulbecco’s phosphate-buffered saline (PBS), and the cell suspension was transferred to a sterile syringe attached to a sterile absorbent cotton pad and was centrifuged at 2000 rpm for 10 min at 25 °C. The pellet was resuspended in PBS, and the optical density at 660 nm of the resulting suspension was determined. The microbial suspensions were prepared in triplicate. The microbial suspensions were added to 9 ml of neutralizing solution (sodium sulfite [Kishida Chemical, Osaka, Japan] for PVP-I; catalase [Nagase ChemteX, Osaka, Japan] for hydrogen peroxide; D/E Neutralizing Broth [Becton Dickinson] for PHMB and BAK) and incubated for 10 min at room temperature. Portions (1 ml) of the mixtures were removed and serially diluted with PBS, and 1 ml of each dilution was then plated on culture agar (soybean-casein digest agar for S. aureus; glucose-peptone digest agar for C. albicans) in triplicate. The cultures were incubated for 5 days at 35 °C for S. aureus and at 25 °C for C. albicans, after which the numbers of colonies were counted and used to determine the log reduction in CFU per milliliter. According to the ISO 14729 guidelines [13], a reduction in live S. aureus of ≥99.9% (3 log units) and a reduction in live C. albicans of ≥90% (1 log unit) represent significant disinfectant activity. However, we determined the concentrations of each agent required to reduce the number of live S. aureus and C. albicans by 3 log units.

2.2. Culture of a human corneal epithelial cell line

An HCE cell line that had been transformed with a simian virus 40-adenovirus recombinant vector was kindly provided by Araki-Sasaki et al. [30]. The cells were cultured in supplemented hormone epithelial medium (SHEM), consisting of Dulbecco’s modified Eagle’s medium–Ham’s F12 (50:50, v/v) (Invitrogen, Carlsbad, CA) supplemented with 15% heat-inactivated fetal bovine serum (Invitrogen), human recombinant insulin (5 μg/ml) (Nacalai Tesque, Kyoto, Japan), cholera toxin (0.1 μg/ml) (Sigma–Aldrich, St. Louis, MO), human recombinant epidermal growth factor (10 ng/ml) (Sigma–Aldrich), 0.5% dimethyl sulfoxide (Katayama Chemical Industries, Tokyo, Japan), and gentamicin (40 μg/ml).

2.3. Measurement of antimicrobial activity

PVP-I (BASF Japan, Tokyo, Japan), hydrogen peroxide (Nipponperoxide, Kanagawa, Japan), PHMB (Sanyo Chemical Industries, Kyoto, Japan), and BAK (Tokyo Kasei Kogyo, Tokyo, Japan) were tested for antimicrobial activity against challenge organisms based on the current ISO guidelines [31,32]. PVP-I (0.05–50,000 ppm), hydrogen peroxide (3–300,000 ppm), PHMB (0.1–1000 ppm), and BAK (0.2–2000 ppm) at various concentrations in PBS (16 ml) were placed individually in test tubes, and 0.16 ml of a suspension of S. aureus (log[mean number of organisms ± S.D.]: 5.23 ± 0.058 for PVP-I, 5.47 ± 0.416 for hydrogen peroxide, 5.40 ± 0.300 for PHMB, 5.17 ± 0.321 for BAK) or C. albicans (log[mean number of organisms ± S.D.]: 5.83 ± 0.058 for PVP-I, 5.80 ± 0 for hydrogen peroxide, 5.97 ± 0.058 for PHMB, 5.87 ± 0.231 for BAK) was inoculated into the tubes at time zero. The tubes were incubated at 25 °C for 30 s–24 h; at various times, 1 ml of each solution was removed and added to 9 ml of neutralizing solution (sodium sulfite [Kishida Chemical, Osaka, Japan] for PVP-I; catalase [Nagase ChemteX, Osaka, Japan] for hydrogen peroxide; D/E Neutralizing Broth [Becton Dickinson] for PHMB and BAK) and incubated for 10 min at room temperature. Portions (1 ml) of the mixtures were removed and serially diluted with PBS, and 1 ml of each dilution was then plated on culture agar (soybean-casein digest agar for S. aureus; glucose-peptone digest agar for C. albicans) in triplicate. The cultures were incubated for 5 days at 35 °C for S. aureus and at 25 °C for C. albicans, after which the numbers of colonies were counted and used to determine the log reduction in CFU per milliliter. According to the ISO 14729 guidelines [13], a reduction in live S. aureus of ≥99.9% (3 log units) and a reduction in live C. albicans of ≥90% (1 log unit) represent significant disinfectant activity. However, we determined the concentrations of each agent required to reduce the number of live S. aureus and C. albicans by 3 log units.

2.4. Evaluation of cytotoxicity

Cytotoxicity of each disinfectant was examined by staining with neutral red, which is incorporated into the lysosomes of viable cells [33]. HCE cells were cultured under 5% CO₂ for 3 days at 37 °C in 48-well culture plates (Coster, Corning, NY) at a density of 2 × 10⁴ cells per well in SHEM. The medium was then removed, and the subconfluent cells were washed twice with SHEM before incubation for 5 s–30 min at 37 °C in the CO₂ incubator with various concentrations of PVP-I (10–50,000 ppm), hydrogen peroxide (30–30,000 ppm), PHMB (1–10,000 ppm), or
BAK (10–1,000 ppm) in 0.2 ml of PBS in triplicate. The cells were then washed twice with PBS and incubated for 3 h at 37 °C with 0.005% neutral red in SHEM (0.2 ml). After washing the cells twice with PBS, the dye was extracted for 15 min with 0.2 ml of a solution containing 1% acetic acid and 50% ethanol. The extract was mixed thoroughly to dissolve the neutral red crystals and its fluorescence intensity was rapidly measured at excitation and emission wavelengths of 530 and 580 nm. The concentration of disinfectant required to reduce the intensity of neutral red staining by 50% (NR50) relative to the level of staining observed with cells treated only with PBS was calculated [34].

2.5. Calculation of safety margin

To estimate the clinical efficacy of each chemical, we calculated the ratio of the NR50 to the concentration required to reduce the number of microorganisms by 3 log units for both S. aureus and C. albicans for incubations of 30 min.

3. Results

We first examined the disinfectant activities of PVP-I, hydrogen peroxide, PHMB, and BAK against S. aureus (Fig. 1) and C. albicans (Fig. 2). All four chemicals manifested antimicrobial activity in a concentration- and time-dependent manner. PVP-I achieved a complete kill (reduction in the number of viable microorganisms of 5–6 log units) within 30 s at 50 ppm for S. aureus (Fig. 1A) and at 500 ppm for C. albicans (Fig. 2A), but it did not show a disinfectant effect at 0.5 ppm for S. aureus or at 5 ppm for C. albicans during incubation for 24 h. Hydrogen peroxide achieved a complete kill within 30 s at 300,000 ppm for both microorganisms (Figs. 1B and 2B). PHMB at 1,000 ppm required 30 min for S. aureus and 1 h for C. albicans to achieve a complete kill (Figs. 1C and 2C). BAK achieved a complete kill within 30 s for S. aureus and within 5 min for C. albicans at 2,000 ppm, but it did not show a marked disinfectant effect at 2 ppm with either microorganism during incubation for 24 h (Figs. 1D and 2D).

All four chemical agents manifested cytotoxicity for HCE cells in a concentration- and time-dependent manner (Fig. 3). Whereas PVP-I exhibited antimicrobial activity against S. aureus and C. albicans at concentrations of ≥5 and ≥50 ppm, respectively, it did not affect the viability of HCE cells at concentrations up to 2,000 ppm; it was cytotoxic at concentrations of ≥4,000 ppm (Fig. 4). These results demonstrated that PVP-I is effective for disinfection of S. aureus and C. albicans at concentrations at which it is not cytotoxic for HCE cells.

Hydrogen peroxide showed antimicrobial activity for S. aureus and C. albicans at concentrations of ≥3,000 and ≥30,000 ppm, respectively, whereas it manifested cytotoxicity toward HCE cells at concentrations of ≥1,200 ppm (Fig. 5). Hydrogen peroxide was thus effective for disinfection of S. aureus and C. albicans, but, at the concentrations required for antimicrobial activity, it was also cytotoxic for HCE cells.

PHMB exhibited antimicrobial activity for S. aureus and C. albicans at concentrations of ≥10 and ≥100 ppm, respectively, whereas it was cytotoxic for HCE cells at concentrations of ≥100 ppm (Fig. 6). Thus, like hydrogen peroxide, PHMB was cytotoxic for HCE cells at concentrations required for disinfection of S. aureus or C. albicans.

BAK showed antimicrobial activity for S. aureus and C. albicans at concentrations of ≥20 and ≥200 ppm, respec-

Fig. 1. Antimicrobial activity of PVP-I (A), hydrogen peroxide (B), PHMB (C), or BAK (D) against S. aureus. Data are means ± S.D. of triplicates from a representative experiment.
atively, as well as cytotoxicity for HCE cells at concentrations of ≥20 ppm (Fig. 7). The concentrations of BAK necessary for antimicrobial activity were thus also cytotoxic for HCE cells.

PVP-I was the most potent of the tested compounds with regard to antimicrobial activity for both \textit{S. aureus} and \textit{C. albicans}, whereas hydrogen peroxide was the least potent (Table 1). The \( \text{NR}_{50} \) of PVP-I for HCE cells was also the highest among the tested agents, whereas that of BAK was the lowest. PVP-I thus showed the highest safety margin by far with regard to disinfection of both microorganisms and cytotoxicity toward HCE cells.

### 4. Discussion

The wearing of soft contact lenses can cause infection of the corneal or conjunctival epithelium with pathogenic microorganisms [35]. Efficient disinfection of the lenses is
thus essential for their safe use \[36,37\]. However, certain disinfectant components themselves have been found to induce keratitis \[38\]. It is therefore important to strike a balance between antimicrobial activity and biocompatibility in disinfection of soft contact lenses. We have now evaluated both the antimicrobial activity and cytotoxicity of PVP-I for this purpose in comparison with three chemical components of commercially available lens disinfectant solutions. PVP-I was not only the most effective disinfectant against both the tested bacterium and fungus but also the least cytotoxic, yielding the highest safety margin by far.

PVP-I has been used for many years as a broad-spectrum antimicrobial to disinfect medical materials and supplies as well as the human body \[39\]. Its microbicidal activity is mediated by reaction with enzymes of the respiratory chain and with amino acids of cell membrane proteins in the microorganisms \[40\]. We have now evaluated the antimicrobial activity of PVP-I in comparison with three chemical components of contact lens disinfectant solutions tested at various concentrations and times that include the concentrations present in the contact lens solutions (30,000 ppm for hydrogen peroxide; 1 ppm for PHMB) and the times recommended for incubation of lenses with these solutions (10 min for hydrogen peroxide; 4 h for PHMB). We determined the concentrations of PVP-I required to reduce the number of live microorganisms by 3 log units during 30 min to be 4.17 ppm for \textit{S. aureus} and 34.46 ppm for \textit{C. albicans}, values that are similar to those (0.1–1%) determined for the same organisms in a previous study \[40\]. Indeed, we have recently developed a contact lens disinfection and cleaning system containing 0.05% PVP-I (Clencide \textsuperscript{1}, and this system has been shown to be effective against bacteria, yeast, mold, and \textit{Acanthamoeba} \[41–43\].

According to the ISO 14729 guidelines \[31\], the effects of potential disinfectants for contact lens solutions on microorganisms should be investigated with a suspension test. We selected \textit{S. aureus} as a representative bacterium and \textit{C. albicans}, one of the most frequent microbial causes of keratitis \[2–6,44\], as a representative fungus for suspension tests of the antimicrobial activity of PVP-I. We have previously examined the effects of a contact lens solution containing PVP-I (Clencide \textsuperscript{1}) on all five challenge organisms recommended by the ISO 14729 guidelines: \textit{Pseudomonas aeruginosa}, \textit{S. aureus}, \textit{Serratia marcescens}, \textit{Fusarium solani}, and \textit{C. albicans}. Our previous study showed that the susceptibility of \textit{C. albicans} to this solution was lower than that of the other microorganisms \[42\]. In addition, both \textit{S. aureus} and \textit{C. albicans} derive their importance not only from the severity of the associated infections but also from their ability to develop resistance to antimicrobial agents \[45,46\]. We examined cytotoxicity toward cultured HCE cells as an indicator of potential injury to the corneal epithelium. Cytotoxicity was apparent at 125 ppm for PVP-I, 30 ppm for hydrogen peroxide, 10 ppm for PHMB, and 10 ppm for BAK during incubation with HCE cells for 30 min. To compare the safety margin of PVP-I with those of hydrogen peroxide,
Table 1
Antimicrobial potencies for *S. aureus* and *C. albicans*, cytotoxicity for HCE cells, and safety margins of the test compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antimicrobial potency a (ppm)</th>
<th>Cytotoxicity b (ppm)</th>
<th>Safety margin c</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em></td>
<td><em>C. albicans</em></td>
<td><em>S. aureus</em></td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td>PVP-I</td>
<td>4.17</td>
<td>34.46</td>
<td>2909</td>
<td>697.6</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>8357.18</td>
<td>20888.78</td>
<td>698</td>
<td>0.08</td>
</tr>
<tr>
<td>PHMB</td>
<td>235.98</td>
<td>442.87</td>
<td>58</td>
<td>0.25</td>
</tr>
<tr>
<td>BAK</td>
<td>15.23</td>
<td>120.74</td>
<td>16</td>
<td>1.05</td>
</tr>
</tbody>
</table>

a Values are concentrations required to reduce the number of live cells by ≥99.9% for both *S. aureus* and *C. albicans*.
b Values are NR50 concentrations for HCE cells.
c Values represent NR50/antimicrobial potency ratios.

PHMB, and BAK in the present study, we chose an exposure time of 30 min for both microorganisms and HCE cells on the basis of the time course data shown in Figs. 1–3.

The disinfection criteria of the ISO 14729 guidelines require a reduction in the number of live bacteria and fungi of 3 and 1 log units, respectively. In the present study, we instead determined the concentrations of the test agents required to reduce the number of both live *S. aureus* and *C. albicans* organisms by 3 log units, given that all four chemicals achieved a complete kill of the fungus during incubation for 30 min. PVP-I and BAK showed a greater antimicrobial potency than did hydrogen peroxide and PHMB. Although all four tested compounds showed a concentration-dependent cytotoxicity toward HCE cells, the NR50 values of PVP-I and hydrogen peroxide were much larger than were those of PHMB and BAK. Furthermore, the safety margin of PVP-I was much higher than those of the other three agents.

Overall, our data indicate that PVP-I, which possesses broad-spectrum antimicrobial activity and has been safely used for ocular disinfection, is suitable for disinfection of soft contact lenses than are other chemicals currently used for this purpose. However, there are multiple requirements for the use of disinfecting agents in contact lens solutions. Further characterization of PVP-I with regard to such issues as formulation, stability, ongoing disinfection ability, and compatibility with contact lenses is thus warranted.

Acknowledgments

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References


The improvement of CL discomfort by a novel povidone-iodine based disinfection system

Haruki Nakagawa, Kazuhiro Sasanuma, Katsuhide Yamasaki, Fumio Saitoh
Opttics Corporation, Kobe, Japan

Introduction

It is reported that more than half of SCL wearers have some discomfort feeling while wearing SCL (CL discomfort). The causes of CL discomfort seem to include psychological factors such as discomfort perception, protein deposition on SCLs, and environmental factors like the state of the tear film. Therefore, it is essential to evaluate protein deposits on CL during wearing. It is well known that patients mostly feel CL discomfort due to destabilization of tear film.

Materials and Methods

Purpose

The purpose of this study was to evaluate the influence of protein deposits on CL discomfort (CLD) from a perspective of lens materials and CL solutions.

Methods

Soft contact lens: Etafilcon A and Balafilcon A as an ionic lens, Senofilcon A and Comfilcon A as a non-ionic lens

Lenses care solution:
Contact lens care solutions used in this study are shown in Table 1. Povidone-iodine based disinfecting and cleaning system (PVP-I system) is comprised of the tablet containing PVP-I in the outer layer, a neutralizer and proteinase in its inner core, and a dissolving and rinsing solution. The tablet was put into the designated lens case, poured the dissolving and rinsing solution and then soaked the lenses. After more than 4 hours, rinsed the lenses by the dissolving and rinsing solution before wearing.

Criteria for CLD while wearing

Table 1. Contact lens care systems used in this study

Comparison with Lipocalin-1 deposits and dryness feeling by lens materials

Comparison with NIBUT while wearing ionic and non-ionic lenses

Comparison with NIBUT while wearing ionic lenses

Comparison with Lipocalin-1 deposits, NIBUT and dryness feeling while wearing ionic lenses with PVP-I system

Conclusions

References

1. The TOGS International Workshop on Contact Lens Discomfort: Executive Summary, JAVS, October 2013, Vol. 54, No. 11
3. T. James et al., Optometry and Vision Science, 2004, 81, 301–305

The relevance between CLD and proteins in tear fluid and on the lenses:

A positive correlation was observed between CLD, especially dryness feeling and amount of Lipocalin-1 in tear fluid on the SCLs. Meanwhile, no relevance was observed between Lysozyme and CLD. (Pearson correlation analysis) (Table 2)

The subjects who had moderate to severe dryness feeling were significantly got increased the amount of Lipocalin-1 in their tear fluid and the deposits on their SCLs compared to subjects with mild. (Mann Whitney U test) (Fig.1)

Lipocalin-1 deposits, NIBUT and dryness feeling while wearing ionic lenses with PVP-I system

It was suggested that concentration of Lipocalin-1 in tears of SCL wearers with dryness feeling is high. Thus Lipocalin-1 easily deposited on ionic lenses might be a cause of dryness feeling due to destabilization of tear film.

Choosing of non-ionic SCLs or using care solutions such as PVP-I system containing protease for SCL wearers with dryness feeling is highly recommended.

Contact: Haruki Nakagawa (kobe-research1@opttecs.co.jp)
Clinical Outcomes and Contact Lens Case Contamination Using a Povidone–Iodine Disinfection System

Jacqueline Tan, Ph.D., Ananya Datta, M.Phil., Katherine Wong, B.Optom., Mark D. P. Willcox, Ph.D., and Ajay K. Vijay, Ph.D.

Objective: To assess the incidence of adverse events during the use of a povidone–iodine disinfecting solution (cleadew) and the microbial contamination in contact lens cases.

Methods: A prospective, single-center, open-label, controlled study evaluating the use of cleadew cleaning and disinfecting system in existing daily wear soft contact lens wearers over a 3-month period was conducted. Ocular signs and symptoms during lens wear were assessed at baseline and after 1 and 3 months of using cleadew. Contact lens cases were assessed for the frequency of microbial contamination and the types of microbes, using traditional microbial culture, followed by identification of bacteria using 16S rRNA sequencing.

Results: Use of cleadew was well tolerated. There was reduction in corneal staining (0.5 ± 0.5 at 3 months of use) compared with the participant’s habitual multipurpose disinfecting system (1.1 ± 1.0); all other clinical signs were not significantly different. There were no cases of solution-induced corneal staining. There was a low adverse event rate of 0.8% per 100 participant-months. Contact lens case contamination was low, with 30% of cases having no culturable microbes. Comparison with previously published data showed that use of cleadew resulted in low frequencies of Gram-positive (49%) and fungal (8%) contamination and a low, but higher than significantly less frequently contaminated than for some other types of disinfecting solutions.

Conclusions: Cleadew cleaning and disinfecting system was associated with low levels of adverse events during use. Contact lens cases were significantly less frequently contaminated than for some other types of disinfecting solutions.

Key Words: Povidone–iodine—Contact lens storage case—Bacteria—Comfort—Adverse events.

(Eye & Contact Lens 2017;0: 1–7)

Almost a decade ago, global estimates indicated that there were more than 140 million contact lens wearers worldwide.1 Discontinuations from contact lens wear due to discomfort and dryness remain, even with contemporary lens types,2 and this has been identified as a factor hampering growth of the contact lens market.3 Nevertheless, the overall number of contact lens wearers is expected to rise, given the growth in use of contact lenses to control myopia progression in children and the uptake of multifocal contact lens corrections as the population ages.3,5 Contact lenses are generally considered to be a safe form of vision correction. However, adverse events do occur. Acute infectious and inflammatory/infiltrative complications associated with contact lens wear have been shown to present a considerable health and economic burden to both affected individuals and to public health systems. In addition to the severe pain and potential for loss of vision with microbial keratitis, the median treatment cost for microbial keratitis in Australia and New Zealand has been estimated at over AUS$1,200, which includes both the direct cost of health care, plus indirect costs related to time off work and costs associated with assistance from a care giver.6 Although corneal infiltrative events are less severe and typically associated with discomfort and inconvenience caused by discontinuation of contact lens wear,7 the cost per contact lens–associated corneal infiltrative event is high, ranging from US$1,003 to 1,496.8 Therefore, eliminating or minimizing the risk factors for developing lens-related complications is desirable for both affected individuals and society in general.

Microbial contamination of contact lens storage cases, used during storage of lenses when not being worn, is an important consideration given the potential for pathogenic microorganisms in the lens case to colonize contact lenses and be transmitted to the eye.9 Microbial colonization of contact lenses has been implicated in contact lens–induced corneal inflammatory events.10–12 Contact lens case contamination rates range from 18% to 85%.13 In a study examining the incidence of noninfectious keratitis during wear of silicone hydrogel contact lenses, Carnt et al.7 found that the rate was dependent on the contact lens multipurpose disinfecting solution combination, with the lowest levels of corneal infiltrative events being produced when silicone hydrogel contact lenses were used in combination with a one-step hydrogen peroxide disinfecting system. Analysis of the contact lens cases used during the clinical trials reported by Carnt et al.7 demonstrated that lens cases were contaminated during use,14 and a further analysis showed that the rate of corneal infiltrative events was correlated to the level of microbial contamination of lens cases.15

Povidone–iodine (a commonly used medical disinfectant) has been available as a contact lens care solution in Japan for several years15,16; however, no studies have reported the incidence rates of adverse events in contact lens wearers using povidone–iodine, nor on the rate of contamination of contact lens cases with this solution. Studies have demonstrated the excellent in vitro antimicrobial activity of 5% povidone–iodine against a number of clinical strains of Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus (including methicillin-resistant strains), Candida albicans,
and Acanthamoeba. Many of these studies had been conducted using the International Organisation for Standardization protocol 14729, which is a requirement for testing before sale of solutions in many countries. Versions of a povidone–iodine cleaning and disinfecting system have been also shown to be effective against Acanthamoeba and Fusarium when tested in vitro. The purpose of this study was to assess the incidence of adverse events during wear of contact lenses and use of a povidone–iodine disinfecting solution (cleadew cleaning and disinfecting system; Ophtecs Corp, Kobe, Japan), and the rate and level of contamination in contact lens cases.

MATERIALS AND METHODS

Clinical Trial
This was a prospective, single-center, open-label, controlled study evaluating the use of cleadew cleaning and disinfecting system in existing, frequent replacement daily wear soft contact lens wearers over a 3-month period. Details of the cleadew cleaning and disinfecting system are shown in Table 1. 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.

Silicone hydrogel and hydrogel lens wearers were included, but people using daily disposable or gas permeable lenses or those with self-reported iodine sensitivity were excluded. Forty participants who provided written informed consent were dispensed with cleadew cleaning and disinfecting solution for use with a fresh pair of their regular soft contact lenses. Participants were instructed to wear their contact lenses for a minimum of 4 days per week on average for the duration of the study and to replace the lenses according to the manufacturers recommended schedule. On removal of the lenses, participants were advised to place lenses directly into the appropriate case compartment and close the basket. The lens case was to be filled to the indicated line with cleadew dissolving and rinsing solution and a cleadew tablet added to the lens case. The lens case was to be capped and lenses left in the case for a minimum of 4 hr (the manufacturer’s recommended minimum disinfection and cleaning time). On removal of the contact lenses from the case, participants were instructed to rinse their lenses with the cleadew dissolving and rinsing solution before inserting lenses into their eyes. Solutions in the lens case were to be discarded, and the lens case (lens well and baskets) was to be thoroughly rinsed with the cleadew dissolving and rinsing solution and left to air dry face down on a clean tissue when not in use. Participants were instructed to use the lens cases for 1 month, to return the lens cases to the clinic at the scheduled 1- and 3-month visits, and to use a new lens case each month.

Clinical examinations of the participants’ eyes were conducted at the baseline, 1- and 3-month scheduled visits. At each visit, compliance with the minimum lens wear requirements and solution regimen were verified, and subjective symptoms and feedback were obtained through a self-administered visual analog scale questionnaire. Anterior ocular health, including bulbar and limbal conjunctival redness, extent of corneal and conjunctival staining, and palpebral redness and roughness were graded using the CCLRU grading scales using a Zeiss SL-120 biomicroscope (Carl Zeiss Meditec, Jena, Germany). The subjective ratings and ocular health variables measured at baseline were considered to represent the clinical performance of the participant’s habitual lens care product, whereas assessments conducted at 1 and 3 months were representative of the cleadew cleaning and disinfecting system with the participant’s habitual contact lenses. The clinical trial was conducted under a similar protocol to that reported previously with the exception that the participants used their habitual contact lenses rather than being supplied with a particular lens type.

Contact Lens Case Analysis
Lens cases were collected at the 1- and 3-month visits. The lens cases were transferred to the laboratory within 1 hr of collection for analysis. Microbial analysis of the collected cases followed the same protocol as described previously. For contact lens case sampling, swabs were taken from the inside of the contact lens case (the case well, lid, and basket) and suspended in sterile phosphate-buffered saline. After mixing thoroughly, 400 μL aliquots of the saline were plated onto 3 blood agar plates and 1 Sabouraud agar plate. The blood agar plates were incubated aerobically, anaerobically, or microaerophilically for 48 hr, and then the colonies examined. Colony morphology was recorded and each type of morphology was subjected to a Gram stain. The Sabouraud agar plates were incubated at 25°C for 7 days to culture for yeast and molds and the colony morphology recorded. The number of colony-forming units (CFU) was recorded for each colony type. For 11 participants, separate swabs were used for the different case compartments to determine whether there were differences between these sites in contamination rates or types of microbes.

Identification of the types of bacteria contaminating lens cases was performed using 16S rRNA sequencing. In brief, DNA of each sample was extracted (using a QIAamp DNA Mini kit; Qiagen, Valencia, CA) according to the manufacturer’s instructions. After extraction, amplification of the 16S rRNA sequence was performed in a total volume of 25 μL containing 1 μL of DNA template, 12.5 μL of EconoTaq Plus 2X Master Mix (Lucigen, Middleton, WI), 10.5 μL of DNAse-free water, and 1 μL each of 10 μM universal forward primer (F27 5’–AGAGTTTGATCCTGGCTCAG–3’) and reverse primer (R1492; 5’–CGG TTA CCT GTTACGACTT–3’). Genomic DNA of E. coli and DNAse-free water was used as the positive and negative controls, respectively. The polymerase chain reaction was performed in a thermal cycler (Bio-Rad, Hercules, CA) at the following settings: initial denaturing at 94°C for 5 min followed by 25 cycles of denaturing at 94°C for 30 sec, annealing for 30 sec at 56°C, extension at 72°C for 90 sec, and a final extension step at 72°C for 10 min. Electrophoresis of the amplified products was performed in a 1% agarose gel stained with 30 ppm of GelRed 10000X solution in dimethylsulfoxide (Biotium, Hayward, CA) to ensure single bands were obtained. The polymerase chain reaction products were then cleaned using Sephadex G-50 (GE Lifescience, Upplands, Sweden) columns and sequenced using forward and reverse primers separately with the BigDye

<table>
<thead>
<tr>
<th>TABLE 1. Details of Cleadew Cleaning and Disinfecting System</th>
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<tbody>
<tr>
<td>Disinfecting, Neutralizing, and Cleaning Tablet</td>
<td>Dissolving and Rising Solution</td>
</tr>
<tr>
<td>Povidone–iodine (4.0 mg/tablet)</td>
<td>Sodium borate</td>
</tr>
<tr>
<td>Ascorbic acid (2.0 mg/tablet)</td>
<td>Boric acid</td>
</tr>
<tr>
<td>Proteolytic enzyme</td>
<td>Sodium chloroide</td>
</tr>
<tr>
<td></td>
<td>Hydrogen peroxide (as a preservative)</td>
</tr>
</tbody>
</table>
Terminator v3.1 (Applied Biosystems, Austin, TX). The sequenced samples were purified using Sephadex G-50 columns and analyzed in an Applied Biosystems 3730 DNA Analyzer at the Ramaciotti Centre for Genomics (University of New South Wales, Sydney, NSW, Australia). The sequences were manually trimmed using Sequence Scanner v1.0 software (Applied Biosystems) and the forward and the reverse sequences assembled using DNA Baser v3.5.0 (Heracle BioSoft, SRL Romania). The Basic Local Alignment Search Tool (BLAST) of the National Center of Biotechnology Information (www.ncbi.nlm.nih.gov) database was used to identify the aligned sequences.

**Statistical Analysis of Data**

One-way analysis of variance was used to compare data across the three visits, and the level of significance was set at α = 0.05. Bonferroni correction was used and adjustments made for multiple comparisons where applicable. Overall contamination rates and contamination rates for different types of microorganisms were measured and tabulated.

**RESULTS**

**Clinical Performance of the Povidone–Iodine Solution**

A total of 40 participants (11 men and 29 women) with an average age of 30 ± 13 years (range 18–60 years inclusive) were enrolled and completed the study. However, two participants who presented to the 3-month visit reported not having worn their contact lenses for 1 week before the final visit. Therefore, the 3-month data for these subjects were excluded from the analysis. Thirty-four participants wore silicone hydrogel contact lenses, and the most common lens worn was senofilcon A (by 8 participants) followed by comfilcon A (by 8 participants). Four participants routinely used a peroxide disinfection system before the study, whereas the remainder used a variety of multipurpose solutions. Table 2 describes the lens/solution combinations used habitually by the participants before enrollment in the study.

Ocular variables were measured for both eyes, but as there were no differences between the eyes on statistical analysis, only data for the left eye have been presented (Table 3). No significant differences were found between participants’ habitual lens care product at baseline compared with cleadew at the 1- or 3-month visits for bulbar and limbal conjunctival redness, extent of conjunctival staining, and palpebral redness and roughness (P > 0.05). However, the extent of corneal staining was significantly lower when cleadew was used at the 3-month visit compared with participants’ habitual lens care product at baseline (P < 0.01).

No significant differences were found in subjective comfort or vision between participants’ habitual lens care product at baseline and cleadew over the course of the study (Table 3). However, there was a trend (P < 0.1) for participants to report a significant improvement in end-of-day comfort with cleadew at the 1- and 3-month follow-up visits compared with their habitual lens care product (Table 3) but also a trend for slightly more itchiness during use of the cleadew solution.

There was no case of solution-induced corneal staining with the cleadew cleaning and disinfecting system over the course of the study. A total of three adverse events occurred during the study but none were classified as serious adverse events (Table 4), and all were deemed to be unrelated to the use of the cleadew cleaning and disinfecting system based on histories of the events obtained by questioning the participants. There was one possible contact lens–related corneal infiltrative event (Table 4, subject 24), but as this participant reported not wearing their contact lenses on the day the symptoms started and did not wear their contact lenses for 2 weeks before the final 3-month visit, it was deemed unrelated to use of the solution. Nevertheless, should this adverse event be classified as lens related, the rate of corneal infiltrative events per 100 participant-months with cleadew was 0.83%. The adverse event rate of cleadew with senofilcon A lenses (which contain polyvinyl pyrrolidone), the most commonly used contact lens (N = 17 participants), was zero.

**Lens Case Contamination**

A total of 75 lens cases were collected from the 40 study participants (first month = 40 and third month = 35). Five subjects did not return their used lens cases to the final 3-month visit. One subject could not identify the 3-month lens storage case from the 2-month lens storage case, and one subject forgot to use a new lens storage case after the second month. Therefore, data for these lens cases were not included in the analysis. A total of 64 lens cases were used by participants in the study. A total of 75 lens cases were collected from the 40 study participants (first month = 40 and third month = 35). Five subjects did not return their used lens cases to the final 3-month visit. One subject could not identify the 3-month lens storage case from the 2-month lens storage case, and one subject forgot to use a new lens storage case after the second month. Therefore, data for these lens cases were not included in the analysis. A total of 64 lens cases were used by participants in the study.

**TABLE 2. Habitual Lens and Disinfecting Solutions Used by Participants Before Enrollment in the Study**

<table>
<thead>
<tr>
<th>Contact Lens Material (Manufacturer)</th>
<th>No. of Subjects</th>
<th>Disinfecting Solution (Main Disinfectant; Manufacturer)</th>
<th>No. of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senofilcon A (Johnson &amp; Johnson Vision Care, Inc., Jacksonville, FL)</td>
<td>17</td>
<td>AOS (hydrogen peroxide; CIBA Vision)</td>
<td>3</td>
</tr>
<tr>
<td>Comfilcon A (CooperVision, Pleasanton, CA)</td>
<td>8</td>
<td>Renu Easisept (hydrogen peroxide; Bausch + Lomb, Rochester, NY)</td>
<td>1</td>
</tr>
<tr>
<td>Lotrafilcon B (Alcon Laboratories Inc.)</td>
<td>5</td>
<td>AQuly (polyhexanide; CIBA Vision)</td>
<td>1</td>
</tr>
<tr>
<td>Enfilcon A (CooperVision)</td>
<td>1</td>
<td>Renu (polyaminopropyl biguanide; Bausch + Lomb)</td>
<td>9</td>
</tr>
<tr>
<td>Galafilcon A (Johnson &amp; Johnson Vision Care, Inc.)</td>
<td>1</td>
<td>Biotrue (polyaminopropyl biguanide and polyquaternium; Bausch + Lomb)</td>
<td>3</td>
</tr>
<tr>
<td>Etafilcon A (Johnson &amp; Johnson Vision Care, Inc.)</td>
<td>1</td>
<td>Complete (polyhexamethylene biguanide; Abbott Medical Optics, Santa Ana, CA)</td>
<td>2</td>
</tr>
<tr>
<td>Oculfilcon D (CooperVision)</td>
<td>1</td>
<td>OPTI-FREE Puremoist (polyquaternium-1 and myristamidopropyl dimethyamine; Alcon Laboratories Inc.)</td>
<td>10</td>
</tr>
<tr>
<td>Methafilcon A (CooperVision)</td>
<td>1</td>
<td>OPTI-FREE ReplensSH (polyquaternium-1 and myristamidopropyl dimethyamine; Alcon Laboratories Inc.)</td>
<td>7</td>
</tr>
<tr>
<td>Balafilcon A (Bausch + Lomb)</td>
<td>1</td>
<td>OPTI-FREE Express (polyquaternium-1 and myristamidopropyl dimethyamine; Alcon Laboratories Inc.)</td>
<td>1</td>
</tr>
<tr>
<td>Hilafilcon A (Bausch + Lomb)</td>
<td>1</td>
<td>Unknown</td>
<td>3</td>
</tr>
<tr>
<td>Other hydrogels (overseas/non-English label brands)</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
cases were excluded from the analysis. Of the 73 lens cases analyzed, no microorganisms were cultured from 22 lens cases (30%). There was no significant difference in the contamination rates of lens cases or the type of bacteria cultured between the two visits (percentage contamination 1 month = 72.5% and at 3 months = 66.7%, P = 0.617); hence, the data from both visits were pooled for analysis. The cases that were analyzed for microbial contamination of different compartments from 11 participants showed that, in each case, if one compartment was contaminated so was the other, and the contaminants were at similar levels and of similar colony types (morphology and Gram stain). Similar results have been published previously for other contact lens multipurpose solutions. Thus, the data were combined, and all data are presented for total lens case.

Of the 51 contaminated lens cases, 21 (42%) lens cases were contaminated with Gram-positive bacteria only, 12 (24%) lens cases with Gram-negative bacteria only, and 18 (35%) lens cases contained both Gram-positive and Gram-negative bacteria. Two or more bacterial strains were cultured from 35 (69%) of the 51 contaminated lens storage cases and 7, the maximum cultured, bacterial strains were cultured from 1 (3%) contaminated lens case. The rate of contamination of cases by different microbial types is shown in Figure 1. Staphylococcus epidermidis and Serratia marcescens were the most frequently cultured Gram-positive and Gram-negative bacteria, respectively, from the contaminated lens storage cases (Table 5).

Significantly higher numbers of Gram-negative bacteria were cultured from contaminated lens cases than Gram-positive bacteria (P < 0.05). S. aureus (299,293 ± 422,908 CFU/case) and Klebsiella oxytoca (226,256 ± 319,957 CFU/case) were cultured in the largest numbers for Gram-positive and Gram-negative bacteria, respectively. Fungi were isolated from 8% of the cases, but no further identification work was undertaken to identify these contaminants.

**DISCUSSION**

This study evaluated the cleadew cleaning and disinfecting system in daily wear silicone hydrogel and hydrogel contact lens wearers over 3 months. This study demonstrated that cleadew is well tolerated during contact lens wear, is associated with low levels of corneal infiltrative events, and no cases of solution-induced corneal staining occurred over 3 months of daily wear.

This is the first clinical study evaluating the rate of microbial contamination of contact lens cases using a povidone–iodine disinfection system. Approximately 30% of the lens storage cases had no culturable microorganisms and, of the contaminated lens cases, approximately 65% were contaminated by a microbial type.

Specific combinations of contact lenses and lens care products have been implicated in causing greater levels of corneal staining in both short-term evaluations after 2 to 4 hr of lens wear, and in studies of up to 3 months daily wear. In this study, no cases of solution-induced corneal staining were observed with cleadew in silicone hydrogel and hydrogel lens wearers over the 3-month period. This shows that the cleadew cleaning and disinfecting system has potential health benefits, given that solution-induced corneal staining has been potentially associated with a three times increased risk of corneal inflammatory events. Indeed, only a single possible contact lens–related corneal infiltrative event occurred during the study with cleadew (0.8% rate of corneal infiltrative events per 100 participant-months). This rate is lower than those reported for polyquad (3.6%) and polyhexamethylene biguanide multipurpose solutions (4.2%) and for a 1-step hydrogen peroxide–based system (2.2%), and is comparable with daily disposable lens wear (0%) in similar clinical trials. It should be noted that the reduction in corneal staining after use of cleadew compared with the participant’s habitual lens/solution combinations may be affected by differences in compliance to lens and disinfecting solution hygiene during habitual wear compared with in a clinical trial. Collins and Carney found that compliance affected corneal staining.

Participants tended to report a clinically significant improvement in end-of-day comfort with cleadew at the 1- and 3-month follow-up visits (average score 85–87) compared with their habitual lens care product (average score 78). This may be attributed to the lack of solution-induced corneal staining with cleadew, as previous studies have reported lower end-of-day comfort scores in participants with solution-induced corneal staining compared with those without.

Alternately, ocular comfort in symptomatic contact lens wearers over 8 hr of lens wear can be improved by the choice of contact lens and

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**TABLE 3. Objective and Subjective Variables Recorded at Each Visit to the Clinic**

<table>
<thead>
<tr>
<th>Clinical variable (scale = 0–4)</th>
<th>Baseline (n=40)</th>
<th>1 Month (n=40)</th>
<th>3 Months (n=38)</th>
<th>Analysis of Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulbar redness—nasal</td>
<td>1.9±0.4</td>
<td>1.9±0.4</td>
<td>1.7±0.4</td>
<td>0.06</td>
</tr>
<tr>
<td>Bulbar redness—temporal</td>
<td>1.9±0.4</td>
<td>2.0±0.4</td>
<td>1.8±0.5</td>
<td>0.18</td>
</tr>
<tr>
<td>Bulbar redness—superior</td>
<td>1.6±0.5</td>
<td>1.5±0.3</td>
<td>1.4±0.4</td>
<td>0.31</td>
</tr>
<tr>
<td>Limbal redness—nasal</td>
<td>1.5±0.4</td>
<td>1.5±0.3</td>
<td>1.4±0.4</td>
<td>0.17</td>
</tr>
<tr>
<td>Limbal redness—temporal</td>
<td>1.8±0.6</td>
<td>1.7±0.5</td>
<td>1.6±0.5</td>
<td>0.58</td>
</tr>
<tr>
<td>Limbal redness—superior</td>
<td>1.6±0.6</td>
<td>1.5±0.5</td>
<td>1.5±0.5</td>
<td>0.83</td>
</tr>
<tr>
<td>Conical staining—extent (worst case)</td>
<td>1.1±1.0</td>
<td>0.6±0.7</td>
<td>0.5±0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Conjunctival staining—extent (worst case)</td>
<td>1.5±1.1</td>
<td>1.8±1.1</td>
<td>1.7±1.1</td>
<td>0.54</td>
</tr>
<tr>
<td>Palpebral redness—upper lid</td>
<td>1.9±0.5</td>
<td>1.9±0.7</td>
<td>1.8±0.6</td>
<td>0.49</td>
</tr>
<tr>
<td>Palpebral roughness—upper lid</td>
<td>1.1±0.6</td>
<td>0.9±0.6</td>
<td>0.9±0.7</td>
<td>0.41</td>
</tr>
</tbody>
</table>

**Subjective rating (0–100)**

- Comfort—insertion           | 91±9           | 91±10         | 92±9            | 0.88                 |
- Vision—insertion            | 89±14          | 94±9          | 93±9            | 0.18                 |
- Burning/stinging—insertion  | 93±11          | 93±12         | 95±7            | 0.80                 |
- Itching—insertion           | 96±6           | 97±5          | 93±10           | 0.09                 |
- Comfort—end of day          | 78±25          | 87±16         | 85±15           | 0.08                 |
- Vision—end of day           | 87±17          | 89±16         | 90±12           | 0.76                 |

**Table:**

<table>
<thead>
<tr>
<th>Objective and Subjective Variables Recorded at Each Visit to the Clinic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical variable (scale = 0–4)</strong></td>
</tr>
<tr>
<td>Bulbar redness—nasal</td>
</tr>
<tr>
<td>Bulbar redness—temporal</td>
</tr>
<tr>
<td>Bulbar redness—superior</td>
</tr>
<tr>
<td>Limbal redness—nasal</td>
</tr>
<tr>
<td>Limbal redness—temporal</td>
</tr>
<tr>
<td>Limbal redness—superior</td>
</tr>
<tr>
<td>Conical staining—extent (worst case)</td>
</tr>
<tr>
<td>Conjunctival staining—extent (worst case)</td>
</tr>
<tr>
<td>Palpebral redness—upper lid</td>
</tr>
<tr>
<td>Palpebral roughness—upper lid</td>
</tr>
<tr>
<td><strong>Subjective rating (0–100)</strong></td>
</tr>
<tr>
<td>Comfort—insertion</td>
</tr>
<tr>
<td>Vision—insertion</td>
</tr>
<tr>
<td>Burning/stinging—insertion</td>
</tr>
<tr>
<td>Itching—insertion</td>
</tr>
<tr>
<td>Comfort—end of day</td>
</tr>
<tr>
<td>Vision—end of day</td>
</tr>
</tbody>
</table>

**Analysis of Variance:**

- Comfort—insertion: 91±9, 91±10, 92±9
- Vision—insertion: 89±14, 94±9, 93±9
- Burning/stinging—insertion: 93±11, 93±12, 95±7
- Itching—insertion: 96±6, 97±5, 93±10
- Comfort—end of day: 78±25, 87±16, 85±15
- Vision—end of day: 87±17, 89±16, 90±12

**Note:** Bold indicates significant difference (P < 0.05) between visits; Italic indicates trend (P < 0.1) in difference between visits.
lens care product. The magnitude of the difference between comfort at baseline compared with after 1- or 3-month use of cleadew was greater than five points (1–100 point scale). The minimum comfort difference that can be discerned by subjects on this scale has been determined to be five points, indicating that, with an increased sample size (50 in each group) there would likely be a statistical difference in comfort between the habitual multipurpose disinfecting solutions used and the cleadew solution. Although participants tended to report more itching on lens insertion when using cleadew at the 3-month visit compared with their habitual lens care product at baseline, the magnitude of difference was less than 5 on a subjective rating scale of 0 to 100 and so may not be clinically significant. To determine whether this difference in itchiness is statistically significant, a clinical trial involving approximately 175 participants in each group would need to be conducted. The difference in comfort between baseline and use of cleadew may be due to differences in compliance of participants habitually compared with while in a clinical trial. One study has reported that noncompliance to lens hygiene results in lower comfort.

This study is the first to report the rates of contamination of used contact lens cases while using a povidone–iodine–based disinfectant. Nearly 70% of the lens cases in this study were contaminated after use, which is similar to studies that have been conducted with currently available single and dual disinfectant or hydrogen peroxide–based multipurpose solutions using similar clinical trial protocols, or a recent report of participants in a trial where contact lens cases were cultured after 2 weeks of use. The clinical trial was run under almost the same protocol as others that have been published, including a minimum of 40 participants in each clinical trial, with the exception of not using a defined contact lens type. Given contact lens type did not affect the frequency of contamination of cases during use, and the culture of contact lens cases followed the same protocol as previous studies, the data from this study has been compared with previously reported data. This analysis used a test of proportions to compare between the levels of contamination of lens cases. The data demonstrated that the frequency of uncontaminated lens cases with cleadew was significantly higher than cases from participants using OPTI-FREE RepleniSH (P=0.0128; Alcon, Fort Worth, TX), AQuify (P<0.0001; CIBA Vision, Atlanta, GA), or the hydrogen peroxide solution CLEAR CARE (P=0.0151; Alcon) but not less than when using OPTI-FREE Express (P=0.215; Alcon). The frequency of contamination of lens cases by Gram-positive bacteria with cleadew was significantly less than with OPTI-FREE Express (P=0.0037), AQuify (P<0.0001), or CLEAR CARE (P=0.0006) but not less than when using OPTI-FREE RepleniSH (P=0.0574). The level of contamination of cases by fungi when using any of the disinfecting solutions is low, but there were significantly less fungi cultured from cleadew compared with CLEAR CARE lens cases (P=0.0385) only. However, the frequency of contamination of cleadew cases by Gram-negative bacteria was significantly higher than OPTI-FREE Express (P<0.0001) or CLEAR CARE (P=0.0091) but not OPTI-FREE RepleniSH (P=0.234) or AQuify (P=0.2543). When microorganisms isolated from the lens cases were classified as significant or nonsignificant based on their assumed pathogenicity, 73% of the contaminated lens cases were found to have nonsignificant levels of microbial contaminants. These results are significantly better

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Date</th>
<th>Visit</th>
<th>Diagnosis</th>
<th>Eye</th>
<th>Serious Adverse Events</th>
<th>Product Related</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>March 30, 2015</td>
<td>Unscheduled—postbaseline</td>
<td>Possible allergy or meibomian gland blockage in upper eyelid</td>
<td>Left</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>24</td>
<td>August 3, 2015</td>
<td>3 mo</td>
<td>Probable infiltrative keratitis</td>
<td>Left</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>40</td>
<td>August 31, 2015</td>
<td>Unscheduled—after 1 mo</td>
<td>Bulbar conjunctival laceration</td>
<td>Left</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

FIG. 1. Microbial contamination rates of cleadew compared with previously published data.
TABLE 5. Bacterial Types Isolated From Contaminated Cleadew Lens Cases

<table>
<thead>
<tr>
<th>Bacterial Identification</th>
<th>No. of Times Isolated (% of Lens Cases)</th>
<th>Colony-Forming Units/Case (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>1 (1)</td>
<td>1</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>1 (1)</td>
<td>5</td>
</tr>
<tr>
<td>Brachybacterium rhamnosum</td>
<td>1 (1)</td>
<td>10</td>
</tr>
<tr>
<td>Brevedibacterium amylolacticum</td>
<td>1 (1)</td>
<td>75</td>
</tr>
<tr>
<td>Enterobacter faecalis</td>
<td>1 (1)</td>
<td>113 ± 149</td>
</tr>
<tr>
<td>Kocuria kristinae</td>
<td>1 (1)</td>
<td>13</td>
</tr>
<tr>
<td>Kocuria palustris</td>
<td>1 (1)</td>
<td>15</td>
</tr>
<tr>
<td>Kocuria spp</td>
<td>1 (1)</td>
<td>13</td>
</tr>
<tr>
<td>Microbacterium paraoxydans</td>
<td>1 (1)</td>
<td>35</td>
</tr>
<tr>
<td>Microbacterium spp</td>
<td>1 (1)</td>
<td>1</td>
</tr>
<tr>
<td>Micrococcus luteus</td>
<td>5 (7)</td>
<td>119,062 ± 266,058</td>
</tr>
<tr>
<td>Micrococcus spp</td>
<td>6 (8)</td>
<td>69 ± 9</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>4 (5)</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>2 (3)</td>
<td>299,293 ± 422,908</td>
</tr>
<tr>
<td>Staphylococcus cohnii</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>9 (12)</td>
<td>33 ± 22</td>
</tr>
<tr>
<td>Staphylococcus homolacticus</td>
<td>3 (4)</td>
<td>38 ± 27</td>
</tr>
<tr>
<td>Staphylococcus pasteuri</td>
<td>5 (7)</td>
<td>65 ± 99</td>
</tr>
<tr>
<td>Staphylococcus saprophyllicus</td>
<td>3 (4)</td>
<td>46 ± 38</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>10 (13)</td>
<td>20 ± 20</td>
</tr>
<tr>
<td>Staphylococcus wameri</td>
<td>8 (11)</td>
<td>38 ± 41</td>
</tr>
<tr>
<td>Gram-negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinetobacter radioresistens</td>
<td>2 (3)</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>Acinetobacter spp</td>
<td>5 (7)</td>
<td>87,012 ± 194,531</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>1 (1)</td>
<td>4,783 ± 333</td>
</tr>
<tr>
<td>Citrobacter spp</td>
<td>1 (1)</td>
<td>428</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>5 (7)</td>
<td>81,214 ± 181,002</td>
</tr>
<tr>
<td>Flavobacterium lindanitolerans</td>
<td>1 (1)</td>
<td>5</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>2 (3)</td>
<td>226,256 ± 319,957</td>
</tr>
<tr>
<td>Pantoaea anthophila</td>
<td>1 (1)</td>
<td>8</td>
</tr>
<tr>
<td>Pantoaea spp</td>
<td>3 (4)</td>
<td>137 ± 274</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2 (3)</td>
<td>120 ± 163</td>
</tr>
<tr>
<td>Pseudomonas japonica</td>
<td>1 (1)</td>
<td>240</td>
</tr>
<tr>
<td>Propionibacterium spp</td>
<td>3 (4)</td>
<td>32 ± 8</td>
</tr>
<tr>
<td>Raoultella ornithinolytica</td>
<td>1 (1)</td>
<td>350,000</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>9 (12)</td>
<td>43,047 ± 128,857</td>
</tr>
<tr>
<td>Serratia spp</td>
<td>2 (3)</td>
<td>94 ± 126</td>
</tr>
</tbody>
</table>

(P<0.0178) than the 51% nonsignificant contamination rate of lens cases with OPTI-FREE RepleniSH, but not other lens cases (62%–85%).14

Willcox et al.14 demonstrated that the most common Gram-positive bacteria to contaminate contact lens cases were S. epidermidis (32%–61% of cases), Staphylococcus saprophyticus (18%–44%), S. aureus (0%–9%), and "viridans" streptococci (3%–13%), whereas the commonest Gram-negative bacteria were Delftia acidovorans (1%–2%), Stenotrophomonas maltophilia (2%–14%), S. marcescens (0%–5%), and Achromobacter sp. (0%–10%). Cleadew cases were contaminated with S. epidermidis (12%, P=0.0072) or S. saprophyticus (4%; P<0.0001) less frequently than other lens cases. There was no significant difference in contamination with S. aureus, but cleadew cases were contaminated less frequently with viridans streptococci than those of OPTI-FREE Express only (P=0.0047). Cleadew cases were contaminated less frequently with D. acidovorans (P<0.0001) or S. maltophilia (P=0.0011) than those of OPTI-FREE RepleniSH only. However, cleadew cases were contaminated more frequently with S. marcescens or Achromobacter sp. than those of OPTI-FREE Express (P=0.0025 and 0.0227, respectively), CLEAR CARE (P=0.0006 and 0.0196, respectively), or AQutify (P=0.0023 Achromobacter only). Cleadew has been shown to provide excellent activity against planktonic and biofilm forms of S. marcescens in vitro.34,35 Indeed, cleadew produced a 7.9 log10 reduction in the numbers of planktonic S. marcescens ATCC 13880 when tested under ISO 14729:2001 conditions.34 Cleadew produced >4 log reduction against adherent S. marcescens on lens cases in vitro.35 It may be that a combination of contact lenses in cases along with adherent S. marcescens on cases results in a reduced biocidal activity of cleadew, and this remains to be tested in future studies. It should be noted that the technique to identify the different bacterial types in this study (16S rRNA) was different from the traditional culture techniques used by Willcox et al.,14 and this may have affected the results for different species, but not for overall levels of Gram-positive or Gram-negative bacteria, as those methods were identical between the two studies (i.e., culture and Gram stain).

It is intriguing that there was such a low level of corneal infiltrative events with the use of cleadew, given the relatively high frequency of contamination of cases by Gram-negative bacteria. A previous study has shown a correlation between the frequency of contamination of lens cases by at least one or three Gram-negative bacteria (D. acidovorans, S. maltophilia, S. marcescens) and the number of corneal infiltrative events. Perhaps the reason for the low level of corneal infiltrative events with cleadew was the very low frequency of contamination with D. acidovorans, S. maltophilia, although there was a relatively high frequency of contamination with S. marcescens. Others have found that the risk of a corneal infiltrative event was increased with the numbers of coagulase-negative staphylococci isolated from lid margins of contact lens wearers.16 Perhaps the low frequency of coagulase-negative staphylococci in lens cases when cleadew was used is another reason for its low level of corneal infiltrative events. Another possible explanation involves the volume of solution in the lens cases. The cleadew lens case can hold a volume of up to 8 mL of solution, whereas the volume that can be accommodated in regular flat bottom lens cases of multipurpose solutions such as those used in the study by Willcox et al.14 (e.g., OPTI-FREE products) is a maximum of 4 mL. This volume difference may dilute the bacteria (or products such as lipopolysaccharide) such that they are not in high enough levels to cause corneal infiltrative events. As all four studies reported in Willcox et al.14 and this study used different participant groups, and this may affect rates of adverse events and contact lens case contamination, a more direct comparison between solutions using a cross-over study design could be used to provide further validation of the comparisons between solutions.

This study has some limitations and suggestions for further study. The study was conducted as an open-label study design which may introduce bias in subjects’ responses to the questionnaire. However, subjects completed a range of questions in regard to comfort and vision for various time points during the day, and the only noteworthy finding was subjective end-of-day comfort scores. Had subjective bias been the primary causative factor, then we may have expected clinically and statistically significant improvements across all the subjective responses. It may also be interesting to enroll symptomatic subjects into a trial using cleadew to determine whether the use of this solution can improve their symptomatology. Discomfort during lens wear is associated with levels of prolactin-induced protein in the tear film.32 It may be valuable to conduct further research to evaluate whether there are any corresponding changes to tear film proteins with use of...
cleadew which may be associated with the improved end-of-day comfort scores.

In conclusion, this study showed that the cleadew cleaning and disinfecting system is compatible with a variety of silicone hydrogel and hydrogel contact lenses and has the potential to improve subjective end-of-day comfort. Use of cleadew resulted in very low, if any, rates of corneal infiltrative events associated with lens wear. Almost three-quarters of contact lens storage cases demonstrated nonsignificant levels of microbial contaminants, and 30% of cases had no culturable microbes. Therefore, cleadew cleaning and disinfecting system may be of utility, particularly for use among symptomatic contact lens wearers, and to reduce the microbial burden and the potential risks of developing associated contact lens–related complications.

REFERENCES


