not apparent in control cysts that were not exposed to a disinfectant.

Discussion. Effective disinfection of Acanthamoeba is essential for safe lens wear. Clinical cases of Acanthamoeba keratitis in patients who wear rigid gas-permeable contact lenses, as well as the previously reported problems that originate from homemade saline solutions, underscore the importance of lens care formulations for effective cyst inactivation. The viable counting technique presented here is available to test lens care solutions in search of more effective chemical disinfection methods. The experimental data presented in this report show the applicability of this technique to produce quantitative data not accomplished by previously published methods.

Key words: Acanthamoeba enumeration, cyst inactivation, disinfection, rigid gas-permeable contact lenses, contact lens solutions

Acknowledgments. The authors thank Dr. Thomas J. Byers for advice and facilities, and Ms. Laurie Haldeman for typing the manuscript.

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References

Pseudomonas Attachment to Low-Water and High-Water, Ionic and Nonionic, New and Rabbit-Worn Soft Contact Lenses

Christiane A. Lawin-Brussel, Miguel F. Refojo, Fee-Lai Leong, and Kenneth R. Kenyon

The authors determined the attachment of a single strain of Pseudomonas aeruginosa to seven brands of hydrogel soft contact lenses (SCLs): nonionic, low-water (polymacon and crofilcon); nonionic, high-water (lidofilcon); ionic, high-water (bufilcon, etafilcon, and perfilcon); and surface-neutralized, high-water (bufilcon). The lenses were exposed to a 1 × 10⁸ colony-forming units (CFU)/ml P. aeruginosa suspension either when new and sterile or after 24 hr of continuous wear in rabbit eyes. Quantitative scanning electron microscopy showed that, regardless of lens type, significantly fewer bacteria attached to worn than to new SCLs (P < 0.05). The bacterial attachment on new, unworn SCLs was significantly lower (Wilcoxon rank-sum test) (P < 0.05) on polymacon and crofilcon than on all other lenses tested except perfilcon; on etafilcon than on bufilcon; and on perfilcon than on all SCLs tested except polymacon. The bacterial attachment on rabbit-worn SCLs was significantly lower (P < 0.05) on polymacon than on all other lenses tested except crofilcon and perfilcon; on crofilcon than on bufilcon; on lidofilcon and on surface-neutralized bufilcon.
than on crofilcon and perfilcon; on etafilcon than on crofilcon, bufilcon, and perfilcon; and on perfilcon than on crofilcon and bufilcon. The results did not show a consistent relationship between hydration and surface charge and P. aeruginosa adherence. Among the SCLs tested, no one lens had a decisive advantage over another, because all, both new and worn, can bind amounts of P. aeruginosa that could potentially produce bacterial keratitis on predisposed eyes. Invest Ophthalmol Vis Sc 32:657–662, 1991

Infective ulcerative keratitis is one of the most severe hazards of hydrogel soft contact lens (SCL) wear. Extended-wear SCLs carry the highest risk. In the literature on the mechanism of adherence of Pseudomonas aeruginosa to new and used soft contact lenses (SCLs), one encounters some apparent contradictions. Thus, Butrus et al found that P. aeruginosa adhered more to human-worn than to never-worn SCLs, and Miller et al reported that the adherence of the bacteria to SCLs presoaked in human tears was enhanced in some SCLs and inhibited in other SCLs obtained from different individuals. Dart and Badenoch observed that the degree of deposits on human-worn, heavily deposited SCLs had no effect on the adherence of P. aeruginosa.

In addition, although Stern and Zam reported that the treatment of the SCLs with proteins, and particularly mucin, enhanced the adherence of P. aeruginosa to the SCLs. Butrus et al found that the mucin-coated SCLs inhibited adherence of the bacteria to the lenses, but both groups agreed that adherence was increased when the SCLs were presoaked in protein solutions. Miller et al agreed with Stern and Zam that in general, the adherence of P. aeruginosa to SCLs was enhanced when the lenses were presoaked in solutions of mucin and proteins. Although these studies were conducted with different SCLs, different history of wear and material of construction, probably different bacterial strains, and different methods of bacteria quantification, it appeared that P. aeruginosa attached readily not only to previously worn lenses but also to unused, never-worn SCLs.

SCLs in the eye are readily coated with tear proteins, mucus, and lipids. Used SCLs can have high levels of grossly invisible protein coatings and heterogeneous, grossly visible deposits. In addition, P. aeruginosa adheres preferentially to the heterogeneous, grossly visible deposits. Therefore, if one examines bacterial adhesion to SCLs with heterogeneous deposits or to lenses with a diffuse protein coating, one might find different results in the amount of attached bacteria. The uncertainty remains in whether the coating that forms on all used lenses is a significant factor in SCL-induced P. aeruginosa keratitis. Aswad et al found that in the rabbit cornea stressed by lid closure, a significantly greater incidence of bacterial keratitis developed in eyes fitted with used contaminated SCLs than in eyes fitted with new contaminated SCLs. These results conflicted with those of Koch et al who, with a similar animal model, found no difference in the rate of infection of rabbit eyes fitted with P. aeruginosa-contaminated new or used SCLs.

The significance of the contact lens coating in relation to bacterial attachment and infectivity of SCLs is controversial, and the role of the SCL material and surface charges in SCL infectivity has not been well examined. Miller and Ahearn compared the adherence of P. aeruginosa to SCLs of various water content and polymer compositions with the use of presumably new SCLs that belong to the four groups within the Food and Drug Administration (FDA) classification. They found that degrees of bacterial adherence varied on the SCLs. There was no correlation between bacterial adherence and hydration of the SCLs, and bacterial adherence was lower on ionic than on nonionic SCLs. However, they did not evaluate their results statistically. Miller et al examined the effect of human tear coatings on adherence of P. aeruginosa to various SCLs and sometimes found apparently higher or lower numbers of bacteria on the used than on the new lenses. These results have additional uncertainty because of the lack of statistical evaluation of the results. We sought to determine the rate of attachment of P. aeruginosa on new and rabbit-worn SCLs of different groups in the FDA hydrogel SCL classification. For the in vivo tear coating of the SCLs before bacterial contamination, we used rabbit-worn SCLs under a tarsorrhaphy with the intent to diminish the variables encountered when contact lenses were obtained from human subjects. In the latter case, the lenses had been rejected due to premature spoilage, or were old, heavily deposited, or poorly tolerated SCLs.

Materials and Methods. We used six different brands of SCLs that represent three FDA classification groups and one lens (bufilcon-Elite) that does not fit into the FDA classification (Table 1). The SCLs were divided into five sets, each of which contained all seven brands: three sets of new SCLs and two sets of rabbit-worn SCLs.

A single strain of P. aeruginosa, harvested from a human corneal ulcer, was used for all experiments. The harvested bacteria were stored at −70°C in tryptic soy broth (Difco, Detroit, MD) and 5% glycerol (Sigma, St. Louis, MO) in 5-ml portions. Bacteria taken in five loops from one of the original portions of the bacterial suspension were grown overnight at 37°C without agitation in 100 ml of sterilized tryptic soy broth (3 g/100 ml distilled water), spun down at full speed for 10 min (Centrifuge Model HN, Interna-
### Table 1. SCLs used in the experiments

<table>
<thead>
<tr>
<th>FDA group</th>
<th>USAN*</th>
<th>Trade name</th>
<th>Chemical name†</th>
<th>% H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Low-water, nonionic</td>
<td>Polymacon</td>
<td>Sequence</td>
<td>Poly(hydroxyethyl methacrylate)</td>
<td>38.6</td>
</tr>
<tr>
<td>I Low-water, nonionic</td>
<td>Crofilcon A</td>
<td>CSI</td>
<td>Poly(glyceryl methacrylate-co-methyl methacrylate)</td>
<td>39.0</td>
</tr>
<tr>
<td>II High-water, nonionic</td>
<td>Lidofilcon A</td>
<td>Safflon 70</td>
<td>Poly(vinyl pyrrolidine-co-methyl methacrylate)</td>
<td>70.0</td>
</tr>
<tr>
<td>IV High-water, ionic</td>
<td>Bufilcon A</td>
<td>Hydrocurve II</td>
<td>Poly(hydroxyethyl methacrylate-co-diactone acrylamide-co-methacrylic acid)</td>
<td>55.0</td>
</tr>
<tr>
<td>IV High-water, ionic</td>
<td>Etafilcon A</td>
<td>Acuvue</td>
<td>Poly(hydroxyethyl methacrylate-co-sodium methacrylate)</td>
<td>58.0</td>
</tr>
<tr>
<td>IV High-water, ionic</td>
<td>Perfilcon A</td>
<td>Permalens</td>
<td>Poly(hydroxyethyl methacrylate-co-vinylpyrrolidine-co-methacrylic acid)</td>
<td>71.0</td>
</tr>
<tr>
<td>None High-water, ionic polymer, nonionic surface</td>
<td>Bufilcon A</td>
<td>Hydrocurve Elite</td>
<td>Same as Hydrocurve II</td>
<td>55.0</td>
</tr>
</tbody>
</table>

* U.S. Adopted Name.
† All polymers are cross-linked with minor amounts of one of various cross-linking agents.

...tional Equipment Co, Needham Heights, MA), and washed in double-filtered phosphate-buffered saline (PBS) (pH 7.4), while being mixed in a vortex (Vortex Genie, Scientific Industries, Springfield, MA) at full speed. This cycle was completed three times. The bacterial suspension was resuspended to an optical density of 0.150 at 590 nm (Beckman DU® Spectrophotometer), which is equivalent to a bacterial concentration of $1 \times 10^8$ CFU/ml. The bacterial suspension was freshly made before each experiment.

**Bacterial adherence to new SCLs:** Three sets of new SCLs were used in these experiments. Each lens was cut into four segments under sterile conditions. Each segment of each lens in every set was used to assay bacterial adherence. The pieces of sterile lenses were exposed to 5 ml of a $1 \times 10^8$ CFU/ml P. aeruginosa suspension for 1 hr at room temperature, rinsed again in a large volume of double-filtered PBS, and fixed in half-strength Karnovsky’s fixative. The lenses were cut in half after the fixation process and before critical point drying.

**Results.** The mean bacterial adherence for the various lenses are shown in Figure 1. Bacterial attachment was uneven over the lens surface and caused high standard deviations. The bacterial attachment was not a normal (Gaussian) distribution. The Wilcoxon rank-sum test was used for the statistical evaluation of the results. For all lenses, there was a statistically significant difference ($P < 0.05$) in bacterial attachment between the unworn lenses and the lenses worn for 24 hr. All lenses, regardless of type, showed significantly more bacterial attachment when they were unworn (new) (Fig. 2).
no large deposits, and the coating was never so thick that the surface pattern could not be visualized. The comparison of bacterial attachment on new and worn SCLs and the statistical differences, if any, at the significant level of $P < 0.05$, are shown in Table 2.

**Discussion.** Bacterial attachment to surfaces is believed to be dependent on the organism, the composition of the surface, and the substances that mediate binding. Klotz et al.\(^{19}\) stressed that different *P. aeruginosa* isolates can vary in hydrophobicity and that more hydrophobic organisms show a significantly increased tendency toward surface adherence. Van der Waals forces and Brownian motion, as well as net surface charge, have been reported to determine bacterial attachment.\(^6\)\(^{-20}\)\(^{-23}\) However, we found that *P. aeruginosa* attach indifferently to two hydrogel SCLs (bufilcon A and bufilcon-Elite) of the same chemical composition and hydration, regardless of surface charge and whether worn or unworn. We found higher bacterial attachment on new high-water nonionic lenses than on low-water nonionic lenses (e.g., lidofilcon vs polymacon or crofilcon). However, after the lenses were worn, this relationship is reversed on lidofilcon vs crofilcon. On the other hand, when we compared the three ionic lenses, we found fewer bac-

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**Fig. 1.** Mean bacterial adherence (±SD) to new and worn soft hydrogel contact lenses. Solid bar, worn lens; cross-hatched bar, new lens. *Significant compared with worn lens of the same type ($P < 0.05$).

**Fig. 2.** SEM polymacon Sequence (A, C) and bufilcon Hydrocurve Elite (B, D) soft contact lenses, new (A, B) and after 24 hr in rabbit eyes (C, D). Lenses were incubated in *Pseudomonas aeruginosa* suspensions of the same concentration and for the same time period.
The ionic surface was often lost after the lens was worn. In general, our results did not show a consistent relationship between SCL hydration and surface adherence. However, when the lenses were worn by the animal, fewer bacteria adhered to the lens with highest hydration (periflcon) compared to other lenses tested, except for polymacon. This finding agrees somewhat with the findings of Miller and Ahearn,16 who found fewer bacteria attached to apparently new, high-water, ionic lens (perfilcon) compared to the lenses in the columns at the left of the table. We suspect that the large amount of bacteria found on all lenses, worn and unworn, and exposure to the same high bacterial concentration could be sufficient to produce the large number of bacteria found on all lenses.

### Table 2. Difference on Pseudomonas aeruginosa counts on unworn (U) and on worn (W) soft contact lenses

<table>
<thead>
<tr>
<th></th>
<th>Polymacon</th>
<th>Croflcon</th>
<th>Lidofoflcon</th>
<th>Bufilcon</th>
<th>Bufilcon Elite</th>
<th>Etafilcon</th>
<th>Periflcon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>W</td>
<td>U</td>
<td>W</td>
<td>U</td>
<td>W</td>
<td>U</td>
</tr>
<tr>
<td>Polymacon</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Croflcon</td>
<td>+</td>
<td>+</td>
<td>(0.001)</td>
<td>(0.002)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>+</td>
</tr>
<tr>
<td>Lidofoflcon</td>
<td>•</td>
<td>•</td>
<td>—</td>
<td>+</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Bufilcon</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>+</td>
<td>=</td>
<td>=</td>
<td>=</td>
</tr>
<tr>
<td>Bufilcon Elite</td>
<td>(0.007)</td>
<td>(0.006)</td>
<td>(0.0001)</td>
<td>(0.001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0004)</td>
</tr>
<tr>
<td>Etafilcon</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>•</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>+</td>
</tr>
<tr>
<td>Periflcon</td>
<td>(0.04)</td>
<td>(0.01)</td>
<td>+</td>
<td>+</td>
<td>(0.0001)</td>
<td>(0.00004)</td>
<td>(0.00001)</td>
</tr>
</tbody>
</table>

* Indicates significant differences in pairwise comparison at the 0.05 significant level using the Wilcoxon rank-sum test, where the numbers of bacteria on the lenses in the row at the head of the table are significantly lower than on the lenses in the columns at the left of the table.

Key words: Pseudomonas aeruginosa, soft contact lenses, hydrogels, bacterial attachment.

Acknowledgments. The authors thank D.A. Kara for statistical evaluation and Dr. D. Korb and CooperVision for donating the lenses.

From the Eye Research Institute, the Department of Ophthalmology, Harvard Medical School, and Massachusetts Eye and Ear Infirmary, Boston, Massachusetts. Supported in part by grant 616/1-1 of the Deutsche Forschungsgemeinschaft (CAL-B), and grants EY00327 and EY05799, from the National Eye Institute, National Institutes of Health, Bethesda, Maryland. Submitted for publication: December 19, 1989; accepted October 9, 1990. Correspondence to: Miguel F. Refojo, DSc, Eye Research Institute, 20 Staniford Street, Boston, MA 02114.

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