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

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References


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

Christiane A. Lowin-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 $1 \times 10^8$ 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 crofili-
corn, bufilcon, and perfilcon; and on perficon than on crofi-
corn and bufilcon. The results did not show a consistent rela-
tionship between hydration and surface charge and *P. aereu-
ginosa* 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 poten-
tially produce bacterial keratitis on predisposed eyes.


Infective ulcerative keratitis is one of the most se-
vere hazards of hydrogel soft contact lens (SCL) wear.
Extended-wear SCLs carry the highest risk.\(^1\)\(^-\)\(^5\) In the
literature on the mechanism of adherence of *Pseudomo-
nas aeruginosa* to new and used soft contact lenses
(SCLs),\(^6\)\(^-\)\(^7\) one encounters some apparent contradic-
tions. Thus, Butrus et al\(^8\) found that *P. aeruginosa*
adhered more to human-worn than to never-worn
SCLs, and Miller et al\(^9\) 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 Baden-
och\(^10\) 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\(^11\) reported
that the treatment of the SCLs with proteins, and
particularly mucin, enhanced the adherence of *P. aereu-
ginosa* to the SCLs. Butrus et al\(^8\) found that the
mucin-coated SCLs inhibited adherence of the bacte-
ria to the lenses, but both groups agreed that adher-
ence was increased when the SCLs were presoaked in
protein solutions. Miller et al\(^9\) agreed with Stern and
Zam in general, that the adherence of *P. aeruginosa*
to SCLs was enhanced when the lenses were pre-
soaked 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.\(^12\)

SCLs in the eye are readily coated with tear pro-
teins, mucus, and lipids. Used SCLs can have high
levels of grossly invisible protein coatings and hetero-
geneous, grossly visible deposits. In addition, *P. aereu-
ginosa* adheres preferentially to the heterogeneous,
grossly visible deposits.\(^13\) Therefore, if one examines
bacterial adhesion to SCLs with heterogeneous de-
posits 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\(^14\) found that in the rabbit cornea stressed by lid
closure, a significantly greater incidence of bacterial
keratitis developed in eyes fitted with used contami-
nated SCLs than in eyes fitted with new contami-
nated SCLs. These results conflicted with those of
Koch et al\(^15\) 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 rela-
tion 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\(^16\) compared the adher-
ence of *P. aeruginosa* to SCLs of various water con-
tent and polymer compositions with the use of pre-
sumably new SCLs that belong to the four groups
within the Food and Drug Administration (FDA)
classification.\(^17\) They found that degrees of bacterial
adherence varied on the SCLs. There was no correla-
tion between bacterial adherence and hydration of
the SCLs, and bacterial adherence was lower on ionic
than on nonionic SCLs. However, they did not evalu-
ate their results statistically. Miller et al\(^17\) examined
the effect of human tear coatings on adherence of *P. aereu-
ginosa* 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 statisti-
cal 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 hy-
drogel SCL classification.\(^17\) For the in vivo tear coat-
ing 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 classifica-
tion 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 con-
tained 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 \(-7^\circ\)C in
tryptic soy broth (Difco, Detroit, MI) 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>Saurflon 70</td>
<td>Poly(vinyl pyrrolidone-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-diactetone 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-vinylpyrrolidone-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.

Table 1. SCLs used in the experiments

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 × 10⁸ CFU/ml P. aeruginosa suspension for 1 hr at room temperature, dipped five times in a large volume of double-filtered PBS to wash away nonadherent bacteria, and fixed in half-strength Karnovsky’s fixative. The bacterial suspension was freshly made before each experiment.

Bacterial adherence to worn SCLs: Two sets of worn SCLs were used in these experiments. The rabbit experiments adhered to the ARVO resolution on the use of animals in research. The nictitating membrane of both eyes of seven white New Zealand rabbits was removed under general anesthesia to obtain a good lens fit. Ten days later, under sterile conditions and general anesthesia, one eye per animal was fitted with a randomly chosen SCL and closed with six single sutures. Care was taken not to perforate the tarsus to avoid suture rubbing on the lens surface. Both eyes of the animal were used, but not at the same time. Therefore, the experiment was repeated twice per lens brand, for a total of 14 lenses. After 24 hr, the lenses were removed under sterile conditions, rinsed immediately in PBS, and exposed to 5 ml of a 1 × 10⁸ 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.

After alcohol dehydration and critical point drying, the lenses were viewed under a scanning electron microscope (SEM) (AMR, Model 1000 A, Bedford, MA) at 20 K^V and a fixed working distance of 12 mm. Twelve random areas of each lens piece were photographed at a magnification of ×1200. The bacteria attached to the lens in each of the 12 areas were counted and expressed as the number per unit area of hydrated lens. The lens shrinkage from the hydrated state (bacterial attachment) to the dehydrated state (bacteria counting) was corrected according to Refojo’s¹⁸ general relationship of dimension changes with hydrogel hydration as follows: 18.0% for polymacon and crofilcon, 27.0% for bufilcon, 29.0% for etafilcon, 38.0% for lidofilcon, and 38.5% for perfilcon.

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 soft hydrogel contact lenses. Solid bar, worn lens; cross-hatched bar, new lens. *Significant compared with worn lens of the same type ($P < 0.05$).

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.\(^1\) stressed that different \textit{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 \textit{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 (eg, 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-

![Fig. 1. Mean bacterial adherence ($\pm$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 ($P < 0.05$).](image1)

![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 \textit{Pseudomonas aeruginosa} suspensions of the same concentration and for the same time period.](image2)
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>Crofilcon</th>
<th>Lidofohc</th>
<th>Bufilcon</th>
<th>Bufilcon Elite</th>
<th>Etafilcon</th>
<th>Perfilcon</th>
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<tbody>
<tr>
<td>U</td>
<td>W</td>
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<td>W</td>
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<td>Polymacon</td>
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<td>Crofilcon</td>
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<td>Lidofohc</td>
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<td>Bufilcon</td>
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<tr>
<td>Bufilcon Elite</td>
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<td>Etafilcon</td>
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<tr>
<td>Perfilcon</td>
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</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.

The ionic surface was often lost after the lens was worn. In general, our results did not show a consistent change and relationship between SCL hydration and surface bacteria attached to the lens with highest hydration (perfilcon), but this relationship persisted only against bacterial concentration could be sufficient to produce corneal infection in equally predisposed corneas.

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

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