Extended wear of soft contact lenses is associated with an increased risk of *Pseudomonas aeruginosa* infection of the cornea. To assess the role of bacterial adherence in the pathogenesis of these infections, superficial corneal epithelial cells and leukocytes from ten patients who use extended-wear soft lenses and ten control eyes were compared for their propensity to attach *P. aeruginosa* in vitro. Cells were washed from the cornea by saline irrigation, incubated with a 10-ml solution containing $10^7$ colony-forming units/ml of bacteria at 35°C for 30 min, collected on a filter, and prepared using a modified acridine orange staining method. Fluorescence microscopy showed bacterial adherence to corneal epithelial cells, leukocytes, and ocular mucus. The mean number of bacteria adhering to epithelial cells was 2.6 for control eyes and 6.6 for the lens-wearing eyes ($P = 0.002$). The percentage of epithelial cells attaching ≥ four bacteria was higher for lens-wearing eyes than control eyes (57.4% versus 26.0%, $P = 0.0005$). There was no significant difference between contact lens-wearing eyes and control eyes in the number of leukocytes collected or in the number of bacteria attached to these cells. These results show that *P. aeruginosa* adherence to epithelial cells is enhanced in those who use extended-wear soft contact lenses, and this may contribute to the increased incidence of *P. aeruginosa* keratitis for this population. Invest Ophthalmol Vis Sci 33:2908-2916, 1992.
scribe a study of the effect of extended soft contact lens wear on \( P. \) aeruginosa adherence to human corneal epithelial cells.

**Materials and Methods**

**Bacteria**

A human corneal isolate of \( P. \) aeruginosa (strain 6294) was obtained from the Bascom Palmer Eye Institute (Miami, FL). This strain was determined to be Fisher immunotype 1, and as observed by transmission electron microscopy, it was nonpiliated, similar to many strains isolated from corneal ulcers. The bacteria were stored in trypticase soy broth at -70°C. Ten percent glycerol was added to many strains isolated from corneal ulcers. The bacteria were stored in trypticase soy broth at -70°C with 10% glycerol to prevent frost injury.

Washing gram-negative bacteria decreases adherence to epithelial cells, and after six washes, bacteria may no longer adhere. In particular, washing of \( P. \) aeruginosa before adherence assays significantly reduces bacterial adherence to some epithelial cells. This effect is thought to be a result of the loss of loose exopolysaccharide. Furthermore, centrifugation can decrease the viability of \( P. \) aeruginosa significantly. Washing of bacteria increases the chances of phagocytosis by leukocytes. These cells also were present in the adherence assay we describe. This problem was addressed in a previous study by using a dialysate of media to grow \( P. \) aeruginosa, eliminating the need to wash the bacteria before adherence assays. A modification of this procedure was developed to prepare bacteria for our use.

A single colony of bacteria grown overnight on trypticase soy agar (TSA) was resuspended in phosphate-buffered saline (PBS) and used to inoculate a second TSA plate covered with a 12,000–14,000 molecular weight cut-off dialysis membrane. Using this method, bacteria were separated physically from the media during the growth phase on the membrane, but they still were able to obtain essential elements for growth through the pores of the dialysis tubing. This method retained bacterial exoproducts that might be involved in adherence by limiting their diffusion into the media and eliminated the need to wash the bacteria before incubation with cells.

After incubation at 37°C for 19 hr, bacteria on the dialysis membrane were suspended in PBS to an optical density of 0.28 at 650 nm; this previously was calibrated to yield approximately \( 5 \times 10^8 \) colony-forming units (cfu)/ml. The suspension was diluted in PBS to \( 10^6 \) cfu/ml and used as the inoculum for the adherence assay.

**Subjects**

We selected ten young healthy subjects wearing cosmetic soft extended-wear contact lenses for at least 12 mo. The lenses were worn for at least two consecutive nights before cell collection. The control group consisted of ten age- and sex-matched subjects that had never worn contact lenses. Informed consent was obtained from subjects before irrigation of their corneas. A detailed history of aspects of lens wear and care were recorded for each lens wearer. Details about experimental and control subjects are presented in Tables 1 and 2, respectively. Nearly all subjects reported a previous history of contact lens-related complications or discomfort during extended lens wear. None of the subjects, however, had complications at the

| Table 1. Subject details for contact lens wearing group |
|---|---|---|---|---|---|
| **Subject** | **Age** | **Sex** | **Lens wear (yr)** | **EW† (yr)** | **Usual wear‡ (days)** | **Worn§ (days)** | **Lens care** | **History of complications** |
| 1 | 42 | M | 11 | 6 | 5 | 5 | D, H | Constant low-grade red eyes |
| 2 | 32 | F | 16 | 1 | 5–6 | 6 | None | Lens deposits, corneal abrasion |
| 3 | 23 | F | 3 | 1 | 0.3–7 | 3 | D, H, E | Past history of GPC |
| 4 | 33 | F | 9 | 4 | 7 | 8 | D, C, E | Red eyes, severe irritation |
| 5 | 23 | M | 8 | 7 | 3–5 | 5 | D, C | Occasional irritation |
| 6 | 30 | F | 12 | 8 | 7 | 2 | D, C, E | Past history of SPK, corneal abrasion |
| 7 | 31 | F | 7 | 5 | 7 | 6 | D, H, E | None |
| 8 | 24 | F | 8 | 4 | 2 | 5 | H, E | Early GPC |
| 9 | 24 | M | 10 | 3 | 2 | 2 | D, H, E | Recent episode of blur, diplopia |
| 10 | 22 | F | 4 | 4 | 2–3 | 2 | D, H, E | Early GPC, lens deposits, incomplete blinking |

\( \text{D, daily cleaner; H, hydrogen peroxide disinfection; C, chemical disinfection; E, enzymatic cleaner; GPC, giant papillary conjunctivitis; SPK, superficial punctate keratitis.} \)

\^ Extended wear.

\( \text{† Usual wearing time between removal.} \)

\( \text{‡ Days worn continuously before cell collection.} \)

\( \text{§ Disposable lenses.} \)

* Mean ± SD age = 28.4 ± 6.4 yr.
Table 2. Subject details for control group

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age*</th>
<th>Sex</th>
<th>Ocular history</th>
</tr>
</thead>
<tbody>
<tr>
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<td>31</td>
<td>M</td>
<td>Mild blepharitis</td>
</tr>
<tr>
<td>12</td>
<td>29</td>
<td>F</td>
<td>Past episodes of bacterial</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>conjunctivitis</td>
</tr>
<tr>
<td>13</td>
<td>40</td>
<td>F</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>26</td>
<td>M</td>
<td>—</td>
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<tr>
<td>15</td>
<td>39</td>
<td>M</td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td>24</td>
<td>M</td>
<td>—</td>
</tr>
<tr>
<td>17</td>
<td>33</td>
<td>M</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>43</td>
<td>F</td>
<td>Chronic mild blepharitis</td>
</tr>
<tr>
<td>19</td>
<td>32</td>
<td>F</td>
<td>—</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>F</td>
<td>—</td>
</tr>
</tbody>
</table>

* Mean ± SD age = 32.7 ± 6.2 yr.

time of this investigation that would be considered to contraindicate contact lens wear.

Cell Collection

After contact lens removal, corneal epithelial cells were collected from subjects using a noninvasive con-
neal irrigation chamber. This technique removes both viable and nonviable, surface or exfoliating cells.
The proportion of viable cells collected has been shown to increase if saline rather than buffered tear
solution is used for irrigation. Thus, with saline irri-
gation, at least some of the cells removed presumably
have been sloughed prematurely. To optimize viable
cell counts, sterile physiologic saline (0.9% NaCl, pH
7.2) was used for irrigation.

The eye-washing procedure was done immediately
after lens removal (always between 2:30 PM and 4:00
PM because, during these times, maximum numbers
of cells are collected). This also minimized variance
in the data caused by diurnal variation. The left cor-
nea of each subject was irrigated with 9 ml of sterile
saline delivered over 25 sec, and the supernatant was
collected in a beaker below the apparatus.

Adherence Assay

Because each sample contained leukocytes and mucus strands, in addition to a variable number of
epithelial cells, the most appropriate means for quant-
ifying adherence of bacteria to cells was considered to
be direct observation using microscopy. The 9-ml
eye-wash supernatant was mixed immediately after
collection with 1 ml of the bacterial suspension in a
syringe. The optimal time of incubation for maxi-
mum adherence of \( P. \ aeruginosa \) to corneal cells pre-
viously was found to be 30 min, and the surface
temperature of the cornea is approximately 35°C.
After a 30-min incubation period at 35°C, the solu-
tion was passed through a syringe filter holder con-
taining a 13-mm diameter, 5-μm pore-size polykar-
bonate filter (Nucleopore, Pleasanton, CA) to collect
cells and mucus strands. Each filter was washed and
simultaneously stained by passing 10 ml of sterile
physiologic saline containing 10\(^{-4}\) M acridine orange
(Sigma, St. Louis, MO) through the filter arrange-
tment. The syringe used for the adherence assay also
was used here to maximize the efficiency of cell col-
clection on the filter. This washing and staining step
was done three times. Because nonadherent bacteria
were smaller than the pores, most bacteria were
washed through the filter during this procedure.

After removal from the syringe holder, the filters
were placed on a microscope slide, allowed to dry, and
heated to 80°C in an oven for 10 min. Each filter
cooled and was restained by adding a drop of 10\(^{-4}\) M
acridine orange solution.

A Nikon Diaphot TMD inverted microscope (To-
kyo, Japan) with an epifluorescence attachment (510-
nm dichroic mirror, 450-490 nm excitation filter,
520-nm barrier filter) was used to examine each tissue
sample at \( \times 1000 \) magnification under oil immersion.
This method stains cells and bacteria separate and dis-
tinct colors. The total number of corneal epithelial
cells and leukocytes on each filter was recorded, as
was the number of bacteria attached to each epithelial
cell and leukocyte.

Data Analysis

Large numbers of cells were counted for bacterial
adherence. Therefore, the number of bacteria at-
tached to cells from each subject approached a nor-
mal distribution. Thus, it was appropriate to calculate
a mean for each subject. However, the number of sub-
jects in each group was small. The nonparametric
Mann-Whitney U test was used to examine whether
there was a significant difference in the calculated
mean values between contact lens-wearing and con-
trol eyes.

Adherence of Radiolabeled Bacteria to Rabbit
Corneal Cells

Radiolabeled bacteria were used in a supplemen-
tary experiment to confirm that the heat fixation and
restaining procedure did not remove bacteria that
were attached to corneal epithelial cells. The bacteria
were radiolabeled by inoculating 980 μl of modified
Mian's medium (7.5 mmol/l NaH2PO4, 16.8 mmol/l
K2HPO4, 10 mmol/l MgSO4, and 2% NaNO3 in dis-
tilled water) with 10 μl of modified Mian's medium
containing 100 mmol/l cold sodium acetate and 10 μl
of tritiated sodium acetate and \( P. \ aeruginosa \). The
bacteria were grown in this medium overnight at
37°C. This technique produced 1 count per min/3 cfu
bacteria. The bacteria were suspended in PBS to produce a solution containing $10^8$ cfu/ml. Epithelial cells were collected from rabbit cornea using a scalpel blade and suspended in PBS to a concentration of 100 cells in 9 ml. The epithelial cell suspension was incubated at 35°C for 30 min with 1 ml of the labeled bacteria; this provided an effective inoculum of $10^7$ cfu/ml. The cells then were collected on a filter and washed. Two filters were prepared simultaneously. Both filters were stained with acridine orange and allowed to dry. One of each pair was heat fixed and restained with acridine orange. Each filter was placed into a scintillation vial to which 1 ml of Protosol (New England Nuclear, Boston, MA) and 10 ml of Liqui-fluor-toluene (New England Nuclear) were added. The total radioactivity of each vial was measured with a scintillation counter (Packard Tri-carb 4530; Packard Instrument, Downers Grove, IL) to estimate the number of bacteria present in the sample. This experiment was done three times.

The adherence assay using radiolabeled bacteria was repeated with an inoculum of $10^8$ bacteria/ml, a tenfold increase in the inoculum. All samples were stained, heat fixed, and restained as described before the radioactivity was measured. Three experiments were done, and the average number of counts per minute was calculated. The Mann-Whitney U test was used to examine whether there was a significant increase in adherence to rabbit corneal epithelial cells with the higher inoculum.

**Results**

**Staining and Microscopy**

Conventional fluorescence microscopy of acridine orange-stained corneal epithelial cells with adherent bacteria produced a black background with green bacteria and green cells. However, if the sample was heated to 80°C for 10 min and restained, epithelial cells maintained their green coloration, but the bacteria appeared red (Fig. 1A). This modified technique for acridine orange staining improved the visibility of the bacteria attached to the cells compared with the one-step staining technique using this fluorochrome. The bacteria consistently appeared red, and epithelial cells and leukocytes (Fig. 1B) were green.

**Adherence of Radiolabeled Bacteria to Rabbit Corneal Epithelial Cells**

The results of the supplementary experiment using radiolabeled *P. aeruginosa* are shown in Table 3. An average of three bacteria attached to each cell using an inoculum of $10^7$ cfu/ml, whether or not the sample underwent heat fixation. This study showed that heat fixation followed by a second staining step did not remove bacteria that were adherent to cells on the filter after the initial staining procedure. In addition, an inoculum of $10^8$ cfu/ml produced a fivefold increase in adherence to corneal epithelial cells, suggesting that the inoculum of $10^7$ cfu/ml did not saturate
the binding sites on rabbit corneal epithelial cells (U = 0, P < 0.05).

Adherence of Pseudomonas aeruginosa to Epithelial Cells

Microscopic examination showed no relationship between epithelial cell morphology and the number of bacteria binding to each cell; however, there were different binding patterns. Some epithelial cells bound bacteria only peripherally, others displayed bacterial binding only to one particular area of the cell, and certain cells showed bacteria distributed randomly over the entire cell surface. These effects might be artifactual and related to epithelial cell orientation on the filter.

The mean number of bacteria attached to epithelial cells was 6.6 for lens wearers and 2.6 for the control group, reflecting a significant difference between these two populations (U = 4.5, P = 0.002, Table 4). The distribution of bacterial attachment to cells from the two groups is shown in detail in Figure 2. There was a significantly higher percentage of epithelial cells binding zero (U = 7, P = 0.0003) or one (U = 2, P = 0.0005) bacteria in the control group. Although this ratio was higher for the contact lens wearers (1.16), statistical analysis did not show a significant difference between the lens-wearing and control groups (U = 38.5, P > 0.1).

Mucus

Mucous strands were present in all samples (Fig. 1C). Although most strands had heavy bacterial adherence, some attached only a few bacteria. Unlike cells, mucus strands were highly variable in size; thus, it was difficult to quantify and compare adherence for the two groups of subjects.

Discussion

P. aeruginosa adherence to superficial human corneal epithelial cells removed by saline irrigation was at least four bacteria (U = 4, P = 0.0005) compared with control subjects.

Leukocytes

Leukocytes were seen in all samples, and most were found to attach bacteria (Fig. 1B). There was no difference in the number of leukocytes isolated from the two groups or in the number of bacteria attached to these cells (Table 4). The mean of the leukocyte-to-cell ratio for control eyes was similar to the results of previous studies (0.38). Although this ratio was higher for the contact lens wearers (1.16), statistical analysis did not show a significant difference between the lens-wearing and control groups (U = 38.5, P > 0.1).

Table 3. Total radioactivity of filters bearing “stained only” and “heat-fixed” corneal epithelial cells

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Stained only sample (counts per minute) 10^7 cfu/ml</th>
<th>Heat-fixed sample (counts per minute) 10^8 cfu/ml</th>
<th>Heat-fixed sample (counts per minute) 10^8 cfu/ml</th>
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<tr>
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<td>1078</td>
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<td>2</td>
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<td>3</td>
<td>892</td>
<td>906</td>
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Table 4. Results for eyes of contact lens-wearing subjects versus eyes of control subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cells*</th>
<th>Bact./cell†</th>
<th>Leuk. ‡</th>
<th>Bact./leuk. §</th>
<th>Leuk./cell¶</th>
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<tr>
<td>Contact lens-wearing subjects</td>
<td></td>
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<tr>
<td>1</td>
<td>59</td>
<td>10.9</td>
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<tr>
<td>2</td>
<td>164</td>
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<tr>
<td>3</td>
<td>179</td>
<td>4.9</td>
<td>37</td>
<td>2.3</td>
<td>0.21</td>
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<tr>
<td>4</td>
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<td>3.51</td>
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<table>
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<th>Subject</th>
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<th>Leuk. ‡</th>
<th>Bact./leuk. §</th>
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<td>Control subjects</td>
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<td>20</td>
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<td>2.0</td>
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<tr>
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<td>1.3</td>
<td>48</td>
<td>1.1</td>
<td>0.20</td>
</tr>
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</table>

* Total number of epithelial cells collected from subject.
† Mean number of bacteria attached to epithelial cells.
‡ Total number of leukocytes collected from subject.
§ Mean number of bacteria attached to leukocytes.
¶ Leukocyte-to-epithelial cell ratio for subject.

SD, standard deviation.
found to be enhanced among those who use extended-wear soft contact lenses compared with a matched control group of nonlens wearers. Previous studies found that receptors on cells in suspension in vitro retain a functionally similar form to immobilized cells and that there is a strong relationship between epithelial cell binding of gram-negative bacteria in vitro and susceptibility to colonization with these organisms.

In this report, we described an improved staining method that enhanced the visibility of bacteria attached to cells when viewed with fluorescence microscopy. This involved heat fixation and restaining of samples with acridine orange and caused red staining of \textit{P. aeruginosa} bacteria and green staining of epithelial cells, leukocytes, and mucus. Denaturation of RNA and/or DNA is thought to be responsible for the red staining of cells with acridine orange. Because higher temperatures or longer periods of heating progressively shifted epithelial cell staining toward the red end of the spectrum, the most likely explanation for this dual color effect was that \textit{P. aeruginosa} nucleic acid was denatured more readily. Radiolabeled bacteria were used in an experiment to ensure that heat fixation did not remove bacteria attached to rabbit corneal epithelial cells after ordinary staining with acridine orange.

The mean number of bacteria that adhered to corneal epithelial cells from nonlens-wearing eyes was 2.6. Previous studies of \textit{P. aeruginosa} adherence to corneal cells have reported figures of 10, 25–275, and 100 cfu/epithelial cell. Bacterial strain variability combined with the differences in methods probably explain the variations in these findings. In our study, a novel method of preparing the bacterial inoculum was used. The bacteria were removed directly from the growth medium and used in the adherence assay without further manipulation. Bacteria grown in different media and under different conditions in vitro show differences in binding to target cells. Epithelial cells were not washed before incubation with bacteria to minimize epithelial cell loss and prevent removal of structures or loosely attached cell components that might be involved in the initiation or inhibition of adherence. For this reason, components of the precorneal tear film also were present during incubation of cells with bacteria. Although the tear film was diluted by the irrigation solution, the effect of tear enzymes, antibodies, and proteases on adherence is not well understood. Furthermore, it is possible that the cells investigated in previous studies display different binding characteristics to superficial human corneal epithelial cells removed by irrigation.

Little is known about factors that promote or inhibit bacterial adherence to the cornea, so it is difficult to determine the mechanism for altering adherence to epithelial cells observed after lens wear. Subjects in this study group were age- and sex-matched to the control group, but they were not assigned randomly to one of the two groups. Because the issue of extended contact lens wear has received adverse publicity in recent years, those who choose this form of lens wear may represent a select or biased population. For example, contact lens wearers in this study may be more likely than members of the control group to adopt a life style that could result in adverse effects on the eye. Otherwise, there may be changes to epithelial cells as a result of contact lens wear that are responsible for this effect.

If cell distribution is altered during lens wear, deeper layer cell types, thought to favor bacterial adherence, may be collected more often by irrigation. Otherwise, enhanced adherence may reflect changes in the life cycle of corneal cells after lens wear.
wear. "Dark" corneal epithelial cells bind P. aeruginosa more avidly than "light" cells, which are thought to be younger. Contact lens wear may prolong cell residence time at the corneal surface, even though it is not known whether superficial corneal cells from lens wearers are functionally older than cells from other eyes. With the additional heat fixation step, it was not possible to determine the age distribution of epithelial cells collected from subjects in our study.

Epithelial cell studies using the irrigation chamber show that there is no difference in the number or distribution of viable cells collected after extended wear of hard gas-permeable contact lenses. Exfoliation rates of corneal epithelial cells after soft lens extended wear have not been published. Recently, it was demonstrated that extended soft lens wear in elderly aphakic patients causes a significantly larger mean surface area of superficial epithelial cells (measured by specular microscopy). Such a phenomenon would be expected to be associated with an increased number of bacteria binding to each cell if the number of bacteria binding per unit surface area remained the same. In young healthy subjects wearing extended-wear soft contact lenses, superficial epithelial cells are enlarged in surface area by only 16%. Such a change would not explain the twofold increase in bacterial adherence to cells on the basis of size alone.

The increased adherence to corneal epithelial cells after lens wear could reflect biochemical changes in the cells rather than physiologic alterations (such as an increase in the number or affinity of receptors on the cell surface or a disruption of factors that normally inhibit bacterial adherence). Others found that extended contact lens wear induces changes in carbohydrates present at the corneal surface. Because carbohydrates may be receptors for bacterial attachment, this phenomenon could contribute to the alteration in adherence after lens wear. Extended contact lens wear with partial tarsorrhaphy in rabbits increased the binding of concanavalin A, wheat germ agglutinin, and Maclura pomifera agglutinin to surface epithelium. These substances are lectin probes for D-mannose, sialic acid, and alpha-D-galactopyranoside, respectively. Sialic acid is thought to be a receptor for P. aeruginosa under certain circumstances, and some strains possess galactosophilic and mannosphilic lectins as components of the cell wall. Recent studies, however, show that P. aeruginosa adherence to the cornea may be more complicated than simple bacterial binding to a specific receptor molecule. Neuraminidase treatment of unwounded mouse pup cornea (which removes sialic acid residues from the surface) increased P. aeruginosa adherence. P. aeruginosa does not adhere well to intact trachea or cornea. However, this bacterium binds to tracheal and human corneal epithelial cells in vitro, as demonstrated in this and previous studies. This suggests that there may be some inhibitory factor(s) in both whole trachea and cornea that prevent adherence in vivo. Various antiadherence factors have been studied for mucous membranes. These include fibronectin (which is specific for gram-negative organisms) and nonspecific factors (such as secretory immunoglobulin A, surface-associated mucin, surface charge, and hydrophobicity). Antiadherence factors could exist as part of the corneal surface layer or on surface cells, either as part of the cell membrane or the cell glyocalyx. These factors might be removed or disrupted during lens wear.

The median leukocyte-to-epithelial cell ratio in samples collected from the control subjects for this study supported earlier results. The average total number of corneal epithelial cells and leukocytes collected, however, was much higher than previously reported. This might be related to diurnal variation or differences in methods (such as the composition, volume, and speed of delivery of the irrigation solution used or the dimensions of the irrigation tube). In our study, the syringe containing the eye-wash supernatant was washed through the filter four times in total; this may be a more efficient method of collecting cells. Others noticed that conjunctival biopsies from soft lens wearers had significantly higher neutrophil and lymphocyte cell counts than biopsies from subjects wearing hard lenses or control eyes. In our study, using the corneal irrigation chamber to remove cells, there was no significant difference in the number of leukocytes isolated or in the number of bacteria attached to leukocytes from contact lens wearers compared with control subjects. However, there was large intersubject variation in the number of leukocytes and epithelial cells collected from the lens wearers compared with control subjects (Table 4). This may explain why the leukocyte-to-epithelial cell ratios for the eyes of lens wearers were not significantly different from those in control eyes, even though the five highest ratios were from the ten subjects in the lens-wearing group. These results support the findings of an earlier investigation (using the corneal irrigation chamber) of subjects wearing a lens in only one eye. In seven of eight cases, irritation of both eyes revealed that there was a higher leukocyte-to-cell ratio in the lens-wearing eye. Perhaps a larger study group might show a statistically significant inflammatory response among certain contact lens wearers.

We found that P. aeruginosa was able to bind to ocular mucus. Adherence to mucus may act as a de-
fense against infection by facilitating bacterial clearance from the anterior eye because this layer of the tear film coats the surface of the eye and is exchanged during blinking. Mucus strands collected from all subjects were variable in their ability to bind bacteria. A recent study found that human respiratory mucins can be divided into components with variable ability to bind *P. aeruginosa*; the most glycosylated and sulfated varieties do not bind bacteria. In cystic fibrosis, a condition that predisposes patients to *P. aeruginosa* infection of the respiratory tract, there is an increase in sulfated fractions of respiratory mucins. Because sulfated fractions do not bind bacteria as effectively, microorganism removal by the mucociliary system may be impaired. Contact lens wear is associated with altered mucin production by conjunctival cells, including changes in the production of sulfated varieties. If these conjunctival mucin fractions behave similarly to their respiratory tract-derived counterparts, there may be a disruption in the effectiveness of ocular mucus to bind and clear *P. aeruginosa* from eyes during contact lens wear. In addition, clearance of bacteria bound to mucus could be impeded by the physical presence of the lens.

Bacterial attachment to mucins does not always reflect patterns of adherence to cells. For some types of gram-negative bacteria, there is an inverse relationship between binding to the two host structures. In our study, it was difficult to quantify and compare bacterial binding of mucus from lens wearers with counterparts, there may be a disruption in the effectiveness of ocular mucus to bind and clear *P. aeruginosa* from eyes during contact lens wear. In addition, clearance of bacteria bound to mucus could be impeded by the physical presence of the lens.

Bacterial attachment to mucins does not always reflect patterns of adherence to cells. For some types of gram-negative bacteria, there is an inverse relationship between binding to the two host structures. In our study, it was difficult to quantify and compare bacterial binding of mucus from lens wearers with control subjects because variable amounts of mucus were collected from the subjects.

In summary, we presented evidence that extended wear of contact lenses enhances adherence of *P. aeruginosa* to human corneal epithelial cells. There was no consistent inflammatory response (demonstrated by similar leukocyte-to-cell ratios for control and lens-wearing subjects). The basis for the enhanced adherence was not determined; however, additional studies of the mechanisms involved may identify strategies to prevent the occurrence of contact lens-related infectious keratitis.

**Key words:** bacterial adherence, *Pseudomonas aeruginosa*, infectious keratitis, contact lenses, cornea

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**References**


