The Role of Type III Secretion System and Lens Material on Adhesion of *Pseudomonas aeruginosa* to Contact Lenses

Elizabeth P. Shen,1,2,3 Ruey-Yug Tsay,4 Jean-San Chia,5 Semon Wu,6,7 Jing-Wen Lee,3 and Fung-Rong Hu3

**PURPOSE.** To determine the distribution of invasive and cytotoxic genotypes among ocular isolates of *P. aeruginosa* and investigate the influence of the type III secretion system (T3SS) on adhesion to conventional, cosmetic, and silicone hydrogel contact lenses (CL).

**METHODS.** Clinical isolates from 2001 to 2010 were analyzed by multiplex PCR for *exoS*, *exoU*, and *exoT* genes. Bacterial adhesion to etafilcon, nelfilcon (gray colored), balafilcon, and galyfilcon CL with or without artificial tear fluid (ATF) incubation were compared. Surface characteristics were determined with scanning electron microscopy (SEM).

**RESULTS.** Among 87 total isolates, 64 strains were from microbial keratitis cases. CL-related microbial keratitis (CLMK) isolates were mostly of the cytotoxic genotype (expressing *exoU*) (*P* = 0.002). No significant differences were found in bacterial adhesion to all types of CL between the genotypes under T3SS-inducing conditions. A trend for least bacterial adhesion of galyfilcon compared to the other CL was noted for both genotypes. Needle complex *psc*C mutants adhered less to all materials than the wild type (*P* < 0.05), indicating a role of the T3SS in contact lens adhesion. ATF-incubated CL had significantly more bacterial adhesion (*P* < 0.05). SEM showed most of the bacteria adhering on CL surfaces.

**CONCLUSIONS.** CLMK isolates were mostly of cytotoxic genotype. Different genotypes did not significantly differ in its adhesion to various CL. T3SS and other adhesins are involved in bacteria–contact lens adhesion through complex interactions. Contact lens materials may also play an important role in the adherence of both genotypes of *P. aeruginosa*. (Invest Ophthalmol Vis Sci. 2012;53:6416–6426) DOI:10.1167/iovs.11-8184

*Pseudomonas aeruginosa* is the most commonly isolated Gram-negative bacteria causing microbial keratitis (MK) in contact lens wearers.1–6 The incidence of contact lens (CL)-related MK (CLMK) is reported to be approximately 5.5 to 20.9 per 10,000 wearers, depending on the contact lens material and wearing schedules.2,6,7 This incidence is expected to rise with the increasing popularity of cosmetic color-tinted soft contact lenses for emmetropic individuals.8,9 Because CLMK often occurs in the younger population,10,11 there have been extensive efforts to prevent this sight-threatening complication and find the perfect contact lens.5,12 Research has shown that corneal hypoxia due to contact lens wear induces lipid raft formation which increases susceptibility to *P. aeruginosa* infection.13,14 Silicone hydrogel materials, which are highly permeable to oxygen, were developed to prevent possible corneal hypoxia induced by conventional hydrogel materials and thus hopefully reduce the incidence of CLMK. However, recent reports indicate that the annual incidence of CLMK for daily wear silicone hydrogel CL wearers is approximately 6 times greater than for daily wear or for daily disposable conventional soft CL wearers.15 Even after other risk factors are adjusted for, daily wearers of silicone hydrogels still showed a higher risk of infection than conventional hydrogel wearers, albeit not reaching a statistically significant difference (odds ratio, 2.6; 95% confidence interval [CI], 1.0–7.1).15 Clearly, increasing the oxygen permeability of contact lenses did not significantly lower the occurrence of CLMK. Other factors related to the contact lens material or bacterial and ocular surface interactions must also be considered.

Development of CLMK starts with bacterial contamination and subsequent bacterial adhesion to contact lenses, causing prolonged exposure of the cornea to microbial pathogens.14,16 The material of each type of contact lens displays various surface properties such as hydrophobicity, wettability, and roughness. These surface properties may influence the propensity of bacteria to adhere to contact lenses.17–19 The relatively hydrophobic silicone hydrogel contact lens balafilcon (Purevision; Bausch & Lomb, Taipei, Taiwan) was previously shown to have greater adherence of *P. aeruginosa* bacteria.20–22 However, other investigators reported contradictory results.23 These disparate results may be due to differences between bacterial serotypes and its growth conditions as previous reports had indicated (i.e., nutrient limitation, growth temperature, and surface hydrophobicity).21,24–26

Two phenotypes of *P. aeruginosa*, based on the secretion of certain type III secretion system (T3SS) toxins, were previously discovered.1 The T3SS is a specialized protein export system that forms a needle-like complex between bacterial and host cells for the transport and secretion of four exotoxins, ExoS,
ExoU, ExoT, and ExoY. The PscC protein is an essential structural component of the needle complex located on the outer membrane of P. aeruginosa cells. Mutation in the PscC protein results in loss of cytotoxicity and the T3SS exotoxin secretion. As most strains carry the exoT and exoY genes, P. aeruginosa strains that have the exoY gene encoding the protein ExoS but not the exoU gene can invade corneal epithelial cells and are, thus, known as invasive strains. Cytotoxic strains, on the other hand, carrying the exoU-positive (exoU⁺) and exoS-negative (exoS⁻) genotypes cause acute host cell lysis by the production of ExoU, a phospholipase. Invasive and cytotoxic phenotypes with their respective exoS and exoU genotypes were found to be mutually exclusive in nearly all strains. Previously, reports with a sufficient number of clinical P. aeruginosa isolates found a significantly higher prevalence of cytotoxic strains among contact lens wearers. Cytotoxic strains were also found to be highly correlated with antibiotic resistance. Interestingly, environmental and clinical isolates from lung, urinary tract, or burn wound infections were mostly invasive. Thus, the higher prevalence of the cytotoxic genotype specifically among CLMK isolates. We then compared the adhesion of different genotypes of P. aeruginosa to conventional hydrogel, cosmetic hydrogel lenses, and silicone hydrogel contact lenses and found less bacterial adhesion to non–surface-treated silicone hydrogel lenses. Strains with the needle complex PscC protein mutation with a different T3SS genotype background were shown to adhere significantly less to various contact lens materials than wild-type strains. A general increase in bacterial adhesion to all contact lenses incubated with artificial tear fluid (ATF) was also shown. Scanning electron microscopy (SEM) of CL surfaces demonstrated an association between CL surface morphology and bacterial adhesion.

**MATERIALS AND METHODS**

**Contact Lenses**

Four types of contact lenses used in this study were provided by their corresponding manufacturers. Their characteristics are listed in Table 1. Ethafilcon A (Acuvue 2, Vistakon; Johnson & Johnson, Taipei, Taiwan) is a conventional hydrogel contact lens material. Nelfilcon A (Freshlook; Ciba Vision, Taipei, Taiwan) is a color-tinted cosmetic hydrogel lens. All nelfilcon A lenses used in this study were tinted gray. Balafilcon A (Purevision; Baush & Lomb) and galyfilcon A (Acuvue Advance, Vistakon; Johnson & Johnson) are silicone hydrogel contact lenses.

**Artificial Tears**

Artificial tears were constructed with phosphate buffered saline (PBS; One-Star Biotechnology, Taipei, Taiwan), 0.3 mM CaCl₂, and the

| Table 2. Characteristics of Bacterial Strains Used in Bacterial Experiments |
|-----------------------------|-------------------|-----------------|------------------------|-------------------|
| **Strain** | **Genotype** | **Serotype** | **Presence of Flagella** | **Source or Reference** |
| PAK | Invasive | O6 | Yes | ref. 45 |
| 6294 | Invasive | O6 | Yes | ref. 28 |
| PAO1 | Invasive | O2/O5 | Yes | Laboratory collection |
| 2007AX44 | Invasive | O6 | Unknown | CLMK isolate |
| 2007AO1 | Invasive | O15 | Unknown | CLMK isolate |
| PA103 | Cytotoxic | O11 | No | ref. 46 |
| 6206 | Cytotoxic | O11 | Yes | ref. 28 |
| 2002AP68 | Cytotoxic | O11 | Unknown | CLMK isolate |
| 2007AD46 | Cytotoxic | O7 | Unknown | CLMK isolate |
| PAKΔpscC | Invasive | O6 | Yes | ref. 45 |
| PA103ΔpscC | Cytotoxic | O11 | No | TL Yahr |
The following proteins: lactoferrin (bovine colostrum, 1 mg/mL), lysozyme (chicken egg white, 1 mg/mL), γ-globulin (bovine, 1 mg/mL), mucin (bovine submaxillary gland, 0.1 mg/mL), and bovine serum albumin (0.1 mg/mL). All proteins were purchased from Sigma-Aldrich (St. Louis, MO).

**Table 3. Distribution of Type III Secretion Genes among *P. aeruginosa* Ocular Isolates**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Non-Contact Lens-Related MK</th>
<th>CLMK</th>
<th>Ocular Infections Other than MK</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>exoS</em>/<em>exoU</em>−</td>
<td>25 (71.4)</td>
<td>9 (31.0)</td>
<td>15 (68.2)</td>
</tr>
<tr>
<td>(invasive)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>exoS</em>/<em>exoU</em>+</td>
<td>7 (20)</td>
<td>18 (62.1)**</td>
<td>6 (27.3)</td>
</tr>
<tr>
<td>(cytotoxic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>exoS</em>/<em>exoU</em>+</td>
<td>0 (0)</td>
<td>2 (6.9)</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td><em>exoS</em>/<em>exoU</em>−</td>
<td>3 (8.6)</td>
<td>0 (0)</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>Total</td>
<td>35 (100)</td>
<td>29 (100)</td>
<td>23 (100)</td>
</tr>
</tbody>
</table>

* P = 0.002, Fisher exact test, CLMK versus non-contact lens-related MK.
† P = 0.02, Fisher exact test, CLMK versus ocular infections other than MK.

**Genotyping**

Multiplex PCR was performed with all clinical *P. aeruginosa* strains to determine the presence of the *exoU*, *exoS*, and *exoT* genes. Using primer pairs reported by Ajay et al., we amplified the following gene fragments: *exoU* (134 basepairs [bp]), *exoS* (118 bp), and *exoT* (153 bp). Bacteria were grown overnight at 37°C in TSB, and DNA was isolated by using a DNA purification kit according to the manufacturer’s protocol (Viogene, Taipei, Taiwan). The PCR mixture consisted of 1 μL of DNA template (500 ng), 1 μL of total PCR primers (MDBio, Inc., Taipei, Taiwan), a final 40 mM concentration of each primer, 12.5 μL of 2X GoTaq Green Master Mix (Promega Corp., Madison, WI), and 10.5 μL of sterile water. The negative control contained GoTaq Green Master Mix, no DNA, and 11.5 μL of sterile water. The standard reaction mixture included 1 μL each of bacterial DNA and GoTaq Green Master Mix and 10.5 μL of sterile water. PCR amplification was carried out as follows: initial denaturation at 94°C for 10 minutes; 35 cycles at 94°C for 45 seconds, 55°C for 45 seconds, and 72°C for 45 seconds; and a final extension step at 72°C for 7 minutes. The reaction was run in a 3% agarose gel (Sea Kem LE agarose; BMA, Rockland, MD) with 0.5 mg of ethidium bromide/mL (Sigma-Aldrich).

**Serotyping**

Clinical isolates were serotyped using antiserum from Denka Seiken Co. Live *P. aeruginosa* cells were used. The O antigen serotype was determined by agglutination reaction to specific antiserum. The Lany1 digital coding corresponding to the Japanese letter coding as specified by instruction manual was used.

**Detection of ExoS and ExoU mRNA by RT-PCR**

Total RNA was extracted from bacterial cells grown under inducing or noninducing conditions using Aurum Total RNA mini-kit (Bio-Rad, Hercules, CA). cDNA was transcribed with reverse transcriptase from 1 μg of total RNA following the manufacturer’s instructions (ImPromII reverse transcription system; Promega). A sample of 30 ng of DNA was washed once with 5 mL of normal saline and resuspended to various concentrations as required for experimentation.
**TABLE 4. Bacterial Adhesion to Various Contact Lens Materials Maintained under Inducing (EGTA +) and Noninducing (EGTA−) Conditions of Growth**

<table>
<thead>
<tr>
<th>Growth condition</th>
<th>Contact lens material</th>
<th>Etafilcon</th>
<th>Etafilcon + ATF</th>
<th>Nelfilcon</th>
<th>Nelfilcon + ATF</th>
<th>Galyfilcon</th>
<th>Galyfilcon + ATF</th>
<th>SD Mean Bacterial Adhesions $10^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inducing</td>
<td>8.5*</td>
<td>24.0</td>
<td>3.6</td>
<td>168.4</td>
<td>9.6 †</td>
<td>67.8</td>
<td>6</td>
<td>11.5†</td>
</tr>
<tr>
<td>Noninducing</td>
<td>2.2</td>
<td>163.3</td>
<td>6</td>
<td>7.6†</td>
<td>5.9*</td>
<td>125.8</td>
<td>6</td>
<td>11.6*</td>
</tr>
<tr>
<td>Inducing</td>
<td>8.7*</td>
<td>17.8</td>
<td>5.9†</td>
<td>209.6</td>
<td>2.5</td>
<td>41.0</td>
<td>6</td>
<td>2.4*</td>
</tr>
<tr>
<td>Noninducing</td>
<td>2007AX44</td>
<td>68.3</td>
<td>6</td>
<td>5.9*</td>
<td>17.8</td>
<td>254.8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Inducing</td>
<td>18.3*</td>
<td>31.0</td>
<td>6</td>
<td>5.9*</td>
<td>17.8</td>
<td>254.8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Noninducing</td>
<td>2007AD46</td>
<td>73.8</td>
<td>6</td>
<td>5.9*</td>
<td>17.8</td>
<td>254.8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Inducing</td>
<td>16.7*</td>
<td>25.5</td>
<td>6</td>
<td>5.9*</td>
<td>17.8</td>
<td>254.8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Noninducing</td>
<td>2007A01</td>
<td>130.0</td>
<td>6</td>
<td>5.9*</td>
<td>17.8</td>
<td>254.8</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

**SDS-PAGE and Immunoblotting**

*P. aeruginosa* were grown under inducing and noninducing conditions as specified above. Culture supernatant were collected and then precipitated with 55% ammonium sulfate at 4°C. Precipitated protein were collected by centrifugation (12,500g) for 15 minutes and washed with 50 mM Tris-HCl (pH 7.6) before being concentrated through a centrifugation filter device (Ultra Centrifuge tubes; molecular weight cutoff, 10,000; Amicon; Millipore, Billerica, MA). Protein concentrations were determined using DC protein assay kit (Bio-Rad) before equal concentrations of each sample were loaded onto a 10% SDS polyacrylamide gel. After electrophoresis, proteins were transferred onto polyvinylidene difluoride membranes (Bio-Rad) and incubated with blocking solution (5% non-fat dry milk in PBS) for 2 hours. Membranes were then incubated with hen anti-ExoY antibody (1:5000 dilution; Agrisera AB, Inc., Vännäs, Sweden) or anti-ExoU antibody (1:20,000 dilution; provided by Professor DW Frank, University of Wisconsin) overnight, followed by washing with Tris-buffered saline (TBS; 0.1 M Tris, 0.1 M NaCl, pH 7.5) and 0.05% Tween20. Goat anti-chicken immunoglobulin Y (IgY)-horseradish peroxidase (HRP) antibody (1:10,000 dilution; Immunology Consultants Laboratory, Inc., Portland, OR) or goat anti-mouse IgG HRP (1:10,000 dilution; Jackson Immunoresearch Laboratories, Inc., West Grove, PA) was used as secondary antibody and incubated for 1 hour. After final washes with TBS-0.05% Tween20, the signal was enhanced using ECL Plus chemiluminescent system (GE Healthcare; Bio-Sciences, Taipei, Taiwan). To verify the status of the T3SS during bacterial adhesion experiments, bacterial adhesion solutions with or without EGTA for inducing or noninducing conditions, respectively, were also collected for immunoblot analysis. All experiments were repeated at least three times.

**Bacterial Adhesion to Contact Lenses**

Assessment of *P. aeruginosa* adhesion to various contact lens materials was done by the viable cell culturing method. Characteristics of strains used in this experiment are listed in Table 2. Five invasive strains (PAK, 6294, 2007AX44, 2007A01, and PAK-jgspc) and five cytotoxic strains (PA103, 6206, 2002AP68, 2002AD46, and PA103-jgspc) were used in these experiments. Strains grown in inducing conditions were resuspended to an optical density at 660 nm (OD$_{\text{660}}$) of 0.1 $(\sim 10^8$ bacteria/mL) in normal saline supplemented with 2% TSB and 2 mM EGTA to maintain the bacteria under inducing conditions. For maintenance under noninducing conditions, bacteria grown under noninducing conditions were also resuspended to OD$_{\text{660}}$ of 0.1 $(\sim 10^8$ bacteria/mL) with normal saline supplemented with 2% TSB and 0.3 mM calcium. After contact lenses were removed from manufacturers’ storage case, they were aseptically cut in half. The lenses were then immersed in either 1 mL of PBS or ATF for 18 hours and kept in an incubator at 37°C with a rotatory shaker at 130 rpm. Lenses were transferred to a new tissue culture plate (24-well tissue culture plate; Sarstedt, Beaumont Leys, Leicester, UK) with the concave surface facing up, and 1 mL of bacterial solution was added. The plate was incubated at 37°C for 2 hours with a rotatory shaker at 130 rpm. After incubation, each lens was picked up with aseptic fine-tip forceps and washed 3 times by careful dipping in 3 mL of PBS. Each lens was then placed in 1 mL of PBS and homogenized with a tissue homogenizer (Polytron model PT 4000; Kinematica, Inc., New York, NY) and serially diluted and plated on Muller-Hinton agar plates (BD Biosciences). The
genes were nearly mutually invasive strains and that exoS was present in all mutants grown under inducing conditions were and xoS cytotoxic strains. Among the total isolates were 71% and 20% of the exoS pscC and PA103 0.05). The pscC and PA103 compare the bacterial adhesion between wild-type and IOVS strains were 31% and 62%, respectively. The and pscC P. aeruginosa isogenic mutants PAK exoS exoU and pscC pscC and PA103(cytotoxic genotype) with that of the bacterial adhesion between the wild-type strains PAK (invasive genotype) and PA103(cytotoxic genotype) and for all wild-type strains than for adhesion under noninducing conditions, indicating a possible role of the pscC and CLMK with other ocular isolates. A P value of less than 0.05 was considered statistically significant.

Scanning Electron Microscopy

Contact lenses were incubated for 2 hours with PAO1 and 6206, following the aforementioned protocol, and were fixed in half-strength Karnovsky fixative (2% paraformaldehyde and 2.5% glutaraldehyde) after the washing step. The fixative was removed, and distilled water was added for rinsing. Contact lenses without bacterial adhesion were removed from their containers and rinsed in distilled water before freeze drying. Rapid freezing was done by liquid nitrogen immersion for all lenses before lenses were placed under vacuum overnight. The concave side of the samples was mounted on aluminum stubs and sputter-coated with gold for examination under SEM (JSM model 5300; JEOL Ltd., Tachikawa, Tokyo, Japan) at 15 kV under various magnifications.

Statistics

Data were entered into a Windows Excel spreadsheet (Microsoft, Inc., Taipei, Taiwan) and analyzed with SPSS statistical software (version 11.0; SPSS, Inc., Armonk, NY). Because bacterial adhesion data were not normally distributed, the Mann-Whitney nonparametric test was used to compare adhesions between each group. Fisher’s exact test was used to compare the genotype frequencies between CLMK and noncontact lens-related MK and CLMK with other ocular isolates. A P value of less than 0.05 was considered statistically significant.

RESULTS

From August 2001 to December 2010, a total of 87 clinical ocular isolates of P. aeruginosa were collected. There were 64 isolates from cases with MK and 23 isolates from other ocular infectious diseases including endophthalmitis, scleritis, dacryocystitis, and conjunctivitis. Genotyping revealed that 56% of the total isolates were exoS’exoU’ invasive strains and that 36% were exoS’exoU’ cytotoxic strains. Among the total isolates, the exoS and exoU genes were nearly mutually exclusive with the exception of 3 isolates positive for both and 4 isolates negative for both. ExoT was present in all isolates.

Among the MK isolates, 35 isolates were from non-contact lens-related cases, while 29 isolates were from CLMK cases. Isolates from cases with non-contact lens-related MK were exoS’exoU’ and exoS’exoU’ strains in 71% and 20% of the isolates, respectively (Table 3). For CLMK isolates, exoS’exoU’ and exoS’exoU’ strains were 51% and 62%, respectively. The cytotoxic genotype was found more frequently in CLMK isolates than in isolates from non-contact lens-related cases (P = 0.002, Fisher exact test). The cytotoxic genotype was also significantly more common in CLMK cases than in isolates from cases of ocular infections other than MK (P = 0.02, Fisher exact test).

RT-PCR was used to detect ExoU and ExoS mRNA of each strain used in contact lens bacterial adhesion under both inducing and noninducing conditions. Under inducing conditions, the presence of ExoU or ExoS mRNA that corresponded to each strain’s T3SS genotype was detected for both wild-type strains and for the pscC needle complex mutants (Fig. 1A). However, pscC mutants grown under inducing conditions were unable to secrete exotoxins, indicating that the PscC needle complex protein is essential for exotoxin secretion (Fig. 1C). Under noninducing conditions, neither exotoxin gene expression nor secretion was detected (Figs. 1, 1D). The inducing and noninducing conditions of growth were maintained after transfer to respective bacterial adhesion solutions with or without EGTA as indicated in Figures 1E and 1F.

Table 4 compares the mean bacterial adhesion of each strain to four types of contact lens materials in bacterial adhesion solutions maintained under inducing and noninducing conditions. Under inducing conditions, significantly more bacterial adhesion was noted for all wild-type strains than for adhesion under noninducing conditions, indicating a possible role of the T3SS in bacterial adhesion. Figure 2 further compares the bacterial adhesion between the wild-type strains PAK (invasive genotype) and PA103(cytotoxic genotype) with that of the pscC isogenic mutants PAKΔpscC and PA103ΔpscC, respectively. Under inducing growth conditions, the pscC isogenic mutants of both genotypes adhered significantly less than bacteria for each type of contact lens material, suggesting that functional T3SS apparatus enhanced bacterial adhesion (P < 0.05, Mann-Whitney U test) (Figs. 2, 2B). As a negative control, under noninducing conditions, the differences in bacterial adhesion between wild-type and pscC mutants were diminished (Figs. 3, 3B). Under noninducing conditions, ATF incubation of contact lenses significantly increased bacterial adhesion of both genotypes for all lens materials, indicating tear fluid proteins may substantially influence bacteria-lens adhesion, even without T3SS activation (Table 4).

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932977/)
To demonstrate genotype differences and reduce interstrain variability among *P. aeruginosa* population, we averaged the mean bacterial adhesion of four wild-type strains for each genotype (invasive strains used were PAK, 6294, 2007AX44, and 2007A01; cytotoxic strains used were PA103, 6206, 2002AP68, and 2007AD46) (Fig. 4). Figures 4A and 4B show bacterial adhesion to various materials with strains grown under inducing and noninducing conditions, respectively. Compared to cytotoxic strains, invasive strains generally adhered in greater numbers to all types of contact lens material, although they did not reach a statistically significant difference (Fig. 4A). A general trend of the least bacterial adhesion to galyfilcon lenses compared to that of all other types of lenses was found. This trend was seen for both invasive and cytotoxic genotypes (Fig. 4). A statistically significant difference was noted while comparing bacterial adhesions between galyfilcon and balafilcon lenses for both genotypes (*P* < 0.05, Mann-Whitney *U*-test) (Fig. 4). With the lenses incubated in ATF and strains grown under noninducing conditions to mimic physiologic conditions, galyfilcon consistently showed a trend for the least bacterial adhesion (Fig. 5). Statistically significant differences in bacterial adhesions were found between ATF-incubated galyfilcon and balafilcon lenses for both genotypes (*P* < 0.05, Mann-Whitney *U*-test) (Fig. 5).

**DISCUSSION**

In this study, we collected clinical ocular isolates of *P. aeruginosa* and found that the *exoS* and *exoU* genes were
Strains used in bacterial adhesion
> reported better adherence of
P. aeruginosa
57,58
Cytotoxic strains cause rapid disrup-
U52
with the
Bacterial adhesion to etafilcon
Thus, the higher
P. aeruginosa
Mean bacterial adhesion of four wild-type
isolates
P. aeruginosa
Thus, the
26
36
P. aeruginosa
59
P. aeruginosa
<
53,54
27
P. aeruginosa
<
53,54
test,
Strains with lower O antigen complexity and without
IOVS
However, in the
53,54
test,
3
5.3
6
U
P. aeruginosa
5.3
6
U
P. aeruginosa
strains to contact lenses was proposed.
possibility of a stronger or higher adherence of cytotoxic
of the cytotoxic genotype among CLMK isolates.
claimed to be the first to note a significantly higher prevalence
prevalence of each genotype among
CL. Although many previous studies have studied the
prevalence of the cytotoxic genotype among
CLMK isolates. CLMK isolates had a
significantly higher prevalence of P. aeruginosa with the
cytotoxic genotype than isolates from patients who did not use
CL. Although many previous studies have studied the
prevalence of each genotype among P. aeruginosa isolates from clinical MK cases, only two reports compare the
genotype distribution between CLMK and non-CLMK isolates.
Among those studies, one with only nine isolates noted no
statistically significant difference in genotype distribution
between CLMK and non-CLMK isolates.52 However, in the
only other report with a larger number of isolates, the authors
claimed to be the first to note a significantly higher prevalence
of the cytotoxic genotype among CLMK cases.26 Thus, the
possibility of a stronger or higher adherence of cytotoxic
strains to contact lenses was proposed.26
Even though the cytotoxic genotype was more prevalent
among CLMK isolates, the degree of bacterial adhesion to the
same contact lens material under inducing conditions was
generally found to be similar between cytotoxic and invasive
strains. To our knowledge, we are the first to compare
adhesion differences between invasive and cytotoxic geno-
types for conventional, color-tinted, and silicone hydrogel
contact lenses. Of the two studies that compared adhesion
differences between strains of different genotypes, only
etafilcon lenses were used.53,54 Bacterial adhesion to etafilcon
lenses between cytotoxic (strain 6206) and invasive (Paer1,
Paer8, and 6294) strains was found to be not statistically
different between the two genotypes.53,54 Thus, the higher
prevalence of the cytotoxic genotype among CLMK isolates
may not be related to the quantity of bacteria attached to the
contact lens but more possibly associated with the virulence of
the cytotoxic strains themselves. As previously reported, cell
death due to infection from cytotoxic strains occurs faster than
from invasive strains.27 Cytotoxic strains cause rapid disrup-
tion of plasma membrane integrity and also loss of epithelial
barrier function.27,55,56
Although bacterial adhesion was not significantly different
between the two genotypes, mutation in the needle complex
protein PscC resulted in decreased adhesion to all type of
lenses, indicating a possible role of the T3SS in bacterial
adhesion to lens materials. Previously, reports have indicated a
possible cooperative association between the T3SS and other
adhesins such as lipopolysaccharide O antigens and flagel-
là.57,58 Strains with lower O antigen complexity and without
flagella seemed to promote expression of the T3SS.57,58
Although our study was not designed to address the association
or interaction among various adhesins with the T3SS in
bacterial adhesion to contact lenses. Further studies to clarify the
complex coordination of various adhesins with the T3SS and
contact lens material adherence under physiologic conditions
are required.
For the same genotype, we generally found the least
bacterial adherence to galyfilcon lenses than to the other
types of lenses under all conditions tested (Figs. 4, 5). In
conformity with other studies comparing pseudomonal adhe-
sion between silicone hydrogel lenses, balafilcon lenses
comparatively adhered greater numbers of bacteria than

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932977/ on 10/16/2017)

**Figure 5.** Mean bacterial adhesion of four wildtype P. aeruginosa strains maintained under noninducing conditions to four types of soft contact lens materials incubated with ATF (mean CFU/mm² ± SD). For both cytotoxic and invasive strains of P. aeruginosa, galyfilcon adhered significantly less bacteria than balafilcon (*Mann-Whitney U test, P < 0.05). For cytotoxic genotype, balafilcon lenses had significantly more bacterial adhesion than etafilcon lenses (Mann-Whitney U test, P < 0.05). A general trend of the least bacterial adhesion for galyfilcon lenses compared to the other three types of contact lenses was seen for both genotypes.

![Figure 6](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932977/ on 10/16/2017)

**Figure 6.** (A) SEM of new etafilcon contact lens exhibiting relatively wavy yet homogeneous polymeric structures (original magnification ×2000). SEM of etafilcon lens incubated for 2 hours with PAO1(B) and 6206 (C). Bacterial clumping was seen on the surfaces and not within the polymeric structure (original magnification ×2000).
galyfilcon.\textsuperscript{20,60} This may be due to the greater hydrophobicity of balafilcon lenses.\textsuperscript{21,61–63} The relatively hydrophilic hydrogel material etafilcon was also shown to have less bacterial adhesion than balafilcon lenses.\textsuperscript{21} To render the lens surface more hydrophilic, balafilcon lenses were surface treated in plasma-reactive chambers to transform the silicone surface components on the lenses into hydrophilic silicate compounds.\textsuperscript{48} Thus, glassy discontinuous silicate "islands" on the surfaces of balafilcon lenses were produced and seen by others\textsuperscript{48} and us by SEM (Fig. 8A). Nevertheless, this surface treatment does not completely shield the hydrophobic silicone exposed and therefore still creates a relative hydrophobic lens surface with poor wettability.\textsuperscript{60,64} Galyfilcon lenses, on the other hand, are non–surface-treated, and because of the incorporation of a new internal wetting agent (Hydraclear) a hydrophilic surface was created, decreasing the tendency for microbial adhesion.\textsuperscript{20,64,65} Thus, properties related to contact lens materials may play a crucial role in bacterial adhesion.

To mimic physiologic conditions, contact lenses were incubated with ATF prior to bacterial adhesion assay with bacteria cultivated under noninducing conditions to account for the high calcium conditions in tears. The artificial tears formula used in our study follows suggestions provided by Willcox et al.\textsuperscript{21} This formula, although simplified, contains essential antibacterial proteins (lactoferrin, lysozyme), glycoproteins (lactoferrin, mucin, $\gamma$-globulin), and calcium found in natural tears.\textsuperscript{21,46,47} Under noninducing conditions, we found a significant increase in pseudomonal adhesion to all types of contact lens material after protein incubation, indicating that tear proteins may more significantly affect bacterial adhesion than the T3SS. Because a mixture of proteins was used, we cannot determine which protein specifically mediated or promoted bacterial adhesion. Nevertheless, in comparison, the influence of tear fluid proteins in vivo was previously shown to affect bacterial lens adhesion. Worn hydrophobic balafilcon lenses were previously shown to attach significantly greater numbers of \textit{P. aeruginosa} than worn hydrophilic galyfilcon lenses or worn conventional hydrogel lenses.\textsuperscript{21,60} Although bacterial adhesion to worn lenses was not tested in our study, the artificial tear incubation of the lenses may provide clues to the extent and complexity of bacterial adhesion to various contact lens materials under in vivo conditions.

SEM is commonly used to analyze the surface structures of contact lenses.\textsuperscript{19,48} Etafilcon, nelfilcon, and galyfilcon lenses showed relatively homogeneous sponge-like polymeric structures over the entire surface, while balafilcon lenses had a combination of lines or cracks with oval pores. The striking difference in surface morphology between these lenses is most likely due to the plasma oxidation surface treatment of balafilcon A lenses that cause mosaic-like patterns, which was previously shown by others.\textsuperscript{48} Our study found that after 2 hours of incubation with the standard strains PAO1 and 6206, most of the bacteria were clumped together and were
distributed randomly on the lens surfaces. Although bacterial adhesion to nelfilcon lenses was similar to that of the other conventional hydrogel etafilcon, nelfilcon lenses had comparatively more bacteria within their relatively large porous structure. Thus, for individuals wearing contaminated contact lenses, corneal surfaces are in direct contact with the attached bacteria, increasing the probability of infection. Because the attached bacteria are mostly on the surfaces of these lenses, frequent and effective cleaning of soft contact lenses could remove surface-adhering bacteria to lower the risk of infection. The previously no-rub recommendation for contact lens cleaning should be discouraged. Daily disposable cosmetic contact lenses are recommended over planned disposables as bacteria tend to be embedded within the polymeric structure.

There are some limitations to our study. First, the viable cell culture method used in this study to enumerate bacterial adhesion to contact lenses depends on the removal of bacteria from the attached surfaces by maceration of the lens material, shaking, and/or ultrasonography. Although other methods such as phase-contrast microscopy may be used for direct enumeration of attached bacteria, it can only provide total counts and cannot determine the viability of these organisms, which is crucial in clinical situations. Second, further experimentation with bacterial adhesion with worn contact lenses will best mimic physiologic conditions and further consider the effects of lens surface structures during in vivo conditions.

CONCLUSIONS
In conclusion, isolates from CLMK cases were more commonly of the cytotoxic genotype than isolates from cases with non-contact lens-related MK. The invasive genotype dominated all other ocular isolates. A general and consistent trend of least bacterial adhesion to galyfilcon lenses for both genotypes and in both inducing and noninducing growth mediums was found. There were no genotype-related differences in bacterial adhesion to all contact lens materials studied. SEM showed a difference in surface morphology between surface-treated (balafilcon) and non–surface-treated lenses (etafilcon, nelfilcon, and galyfilcon), which may contribute to their differences in _P. aeruginosa_ adhesion. The correlation between the T3SS and pseudomonal adhesions to various contact lens materials may be through complex interactions with other adhesins and tear fluid proteins. Differences in contact lens material may crucially affect the adherence of cytotoxic and invasive strains of _P. aeruginosa_.

Acknowledgments

The authors thank Suzanne M. J. Fleiszig (School of Optometry, University of California at Berkeley) for providing _P. aeruginosa_ strains 6294 and 6206; Timothy L. Yahr (Department of Microbiology, University of Iowa) for providing _P. aeruginosa_ strains PA103 and PA103ΔpscC; and Stephen Lory (Department of Microbiology and Molecular Genetics, Harvard Medical School) for providing _P. aeruginosa_ strains PAK and PAKΔpscC. We also thank Dara W. Frank (Department of Microbiology and Molecular Genetics, Medical College of Wisconsin) for generosity in providing ExoU monoclonal antibodies.

References


