Lipopolysaccharide in Adherence of *Pseudomonas aeruginosa* to the Cornea and Contact Lenses

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**Purpose.** To determine the role of smooth or rough lipopolysaccharide on adherence of *Pseudomonas aeruginosa* bacteria to the rat cornea in vitro and on contact lenses of differing types.

**Methods.** Adherence of a smooth (AK.957) and isogenic rough strain (AK1012) of *P. aeruginosa* bacteria to rat corneas that were either normal, traumatized using a 20-gauge needle or treated for 15 min with 0.1N sodium hydrochloric acid was assessed by homogenization and viable counting. Adherence of these organisms to 43 unworn contact lenses representing the four Food and Drug Administration lens groups was also assessed using viable counts.

**Results.** Attachment to contact lenses was greater for the smooth strain for all four lens types (*P* < 0.001). No variation in adherence to the different lens types was observed. Smooth bacteria also adhered to the cornea to a greater extent than the rough strain, regardless of trauma type (*P* < 0.001). Adherence to traumatized corneas was greater than to nontraumatized corneas for both strains of *P. aeruginosa* bacteria (*P* < 0.01). Measurement of surface hydrophobicity of the two bacterial strains revealed that the smooth strain was more hydrophobic than the rough strain (*P* < 0.001), perhaps accounting for the adherence pattern.

**Conclusions.** These results indicate that bacterial surface characteristics may be important determinants of adherence and could explain the propensity of certain bacterial strains to infect the cornea. Invest Ophthalmol Vis Sci 1993;34:1930-1936.

Ulcerative keratitis is one of the most serious complications of contact lens wear, occurring in an estimated 1 in 500 persons using contact lenses for extended wear.1–3 The gram-negative bacterium, *Pseudomonas aeruginosa*, is the microorganism most commonly isolated from contact lens-related ulcerative keratitis.1,2,4,5 It causes a rapidly destructive ulcer, which often leads to scarring and vision loss in otherwise healthy persons.

The pathogenesis of corneal infection involves a number of steps. First, the bacterium must be inoculated into the eye. Several investigators have suggested that contact lenses may provide the vehicle whereby organisms are transferred from the environment to the anterior eye.6–8 The mechanism whereby *P. aeruginosa* attaches to the contact lens surface is not well understood and may be relevant to the infection process.

Conditions that enhance bacterial adherence, such as epithelial trauma,6–11 vitamin A deficiency,12...
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and contact lens wear\textsuperscript{13,14} are associated with an increased risk of keratitis. However, bacterial factors mediating adherence to the cornea are not well characterized.

A major component of the outer capsule of P. aeruginosa is lipopolysaccharide (LPS), which is a well-known virulence factor that promotes infection by interference with the host immune response.\textsuperscript{15,16} It is structurally composed of three parts: a hydrophobic biologically active lipid A molecule; core polysaccharide, rich in monosaccharides and phosphate residues; and an outer amino sugar-rich, immunologically reactive side chain region (O-side chain), which may vary in length and has been used as a method of serotyping P. aeruginosa strains.\textsuperscript{15} In disease, most of the properties of LPS may be attributed to the lipid A portion, in particular the lipid component. The majority of gram-negative bacteria isolated from infection are O-serotypeable suggesting that they possess an O-antigen attached to the lipid A rough-core region. Such strains are called smooth strains. Rough strains do not possess an O-side chain.\textsuperscript{17} The O-side chain of LPS may be involved in generating the overall hydrophobic charge of a bacteria and thus could influence attachment to surfaces.\textsuperscript{18}

LPS has various functions that increase the virulence of the organism. Most studies evaluating the pathogenicity of LPS have focused on the immune response and serum resistance. The role of LPS in adherence of gram-negative organisms to cells has been evaluated for several bacterial species. A decrease in the length of the O-side chain of Salmonella typhimurium leads to an increased tendency for this organism to bind to mammalian cells, granulocytes,\textsuperscript{19} HeLa cells\textsuperscript{20-22} and macrophages.\textsuperscript{23} Furthermore, attachment of Neisseria gonorrhoeae is inhibited by purified LPS,\textsuperscript{24} as is the adherence of Actinobacillus pleuropneumoniae to porcine trachea.\textsuperscript{25} Prior vaccination with LPS confers resistance to keratitis in rabbits,\textsuperscript{26} yet it is not known if LPS influences adherence of P. aeruginosa to corneal epithelial cells. In ocular disease, the majority of isolates recovered are smooth (serotypeable), suggesting that they possess long oligosaccharide side chains.\textsuperscript{27,28}

The aim of this study was to investigate the role that LPS plays in adherence of P. aeruginosa to unworn contact lenses of various types, as well as to intact and injured cornea.

MATERIALS AND METHODS

Bacteria

AK957 and AK1012 are isogenic mutant strains of P. aeruginosa that contain smooth and rough LPS, respectively (bacterial strains donated by Dr. Gerald B. Pier, Harvard Medical School, Boston Massachusetts). AK957 is serotype 7 and is a clone of PAO1 strain (originally derived by Prof. Bruce W Holloway, Monash University, Melbourne, Australia). AK1012 was selected by resistance to "smooth" specific bacteriophage E79.\textsuperscript{29}

Bacteria were stored at \(-70^{\circ}\text{C}\) in trypticase soy broth, frost protected by 10\% (vol./vol.) glycerol until used. Each strain was inoculated onto trypticase soy agar and incubated overnight at 35\(^{\circ}\text{C}\). One colony from each plate was then inoculated into trypticase soy broth and incubated for 19 hr statically at 35\(^{\circ}\text{C}\). Bacteria were washed by centrifugation three times with sterile phosphate buffered saline (PBS) for 3 min at 5000 g. The concentration of bacteria was adjusted, using a spectrophotometer set at 650 nm, to give an optical density of 0.25, which was calibrated to yield 2.0 \(\times\) 10\(^{8}\) colony forming units (cfu)/ml. The inoculum at this density was found to be the same for both strains of bacteria (data not shown). The bacterial strains were then masked so the experimenter did not know the identity of the strains used.

Animals

Forty-two female three-mo-old Buffalo rats were used in this study. All procedures were in accordance with the ARVO resolution on treatment of animals. After death, the eyes were enucleated carefully so as not to damage the corneas.

Contact Lenses

The United States Food and Drug Administration categorizes hydrogel lens materials as follows: Group I (low water content, nonionic); Group II (high water content, nonionic); Group III (low water content, ionic); and Group IV (high water content, ionic).\textsuperscript{30} Forty-three lenses representative of the four Food and Drug Administration groups were used in this study. These included ten lenses from Groups I, II, and IV and eight from Group III. A further five Group I lenses were used as controls and were not inoculated with bacteria. These are listed in Table 1.

The lenses were supplied in varying parameters. Nominal base curves and diameters were used to approximate total lens surface area by use of the following formula for a spherical shell:

\[
A = 2\pi r \left( r - \sqrt{r^2 - \frac{d^2}{2}} \right)
\]

where \(A\) is the area of a spherical surface of radius of curvature \(r\) over the chord diameter, \(d\). This area was
Adherence Assay

A viable count was performed. A pilot study revealed that the number of colonies recovered from the effluent after 10 or more washes is similar.

Hydrophobicity

The surface hydrophobicity of each strain was determined by a modification of the method of Rosenberg et al. Briefly, a 0.8 ml aliquot of bacteria was added to 2.0 ml of hexadecane (Sigma, Sydney, Australia) and vortexed in a glass vial for 2 min. The vial was allowed to stand for 30 min during which time the hexadecane separated from the aqueous phase. A 0.1 ml aliquot of the lower aqueous phase was then removed and a viable count performed. The hydrophobicity was expressed as the percentage of the number of organisms present at the hexadecane interface.

Statistical Analysis

There was considerable variation and an apparent departure from a Gaussian distribution in the spread of the number of bacteria adherent to rat cornea and contact lenses within the different groups. To normalize the distribution and enable parametric statistical analysis, all data was transformed logarithmically.

RESULTS

Attachment to Contact Lenses

Bacteria attached to all contact lenses incubated in the bacterial suspension. Smooth bacteria were significantly more likely to adhere to these lenses than the rough strain (ANOVA, F_{1,30} = 60.7, P = 0.0001). The reconverted mean (±SE) adherence for smooth bacteria was 28.1 (±7.2) x 10^2 cfu/mm^2 compared to 1.5 (±0.3) x 10^2 cfu/mm^2 for rough bacteria.

The mean number of adherent bacteria for each subgroup of contact lenses presented in Figure 1. There was no statistical difference in bacterial attachment to the various contact lens types. (ANOVA, F_{5,30} = 1.34, P = 0.27). The difference in binding between the smooth and rough strains was consistent statistically across the lens types as indicated by the absence of a significant interaction between lens type and bacterial strain (ANOVA, F_{3,30} = 1.24, P = 0.31).

Adherence to Corneas

P. aeruginosa adhered to all corneas incubated in bacteria. Bacteria were not isolated from control corneas that were incubated in PBS alone. The mean number of adherent bacteria per cornea for each type of treatment is given in Figure 2.

Adherence of the smooth strain to cornea was on average 12 times greater than adherence of the rough strain (ANOVA, F_{1,37} = 69.6, p < 0.0001). For non-traumatized corneas, the reconverted mean (±SE) adherence of smooth organisms to rat corneas was 19.5

### TABLE 1. Types of Contact Lenses Used to Determine Attachment of Smooth and Rough P. aeruginosa to Contact Lenses

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Lenses</th>
<th>Lens Type</th>
<th>Material/Water Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>Permathin</td>
<td>Tetrafilcon A 43%</td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>Permaflex</td>
<td>Surficon 74%</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>CM67</td>
<td>Xylofilcon 67%</td>
</tr>
<tr>
<td>IV</td>
<td>8</td>
<td>Sofimate</td>
<td>Bufilcon A 45%</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Permalens</td>
<td>Perfilcon 71%</td>
</tr>
</tbody>
</table>
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FIGURE 1. Attachment of smooth and rough *P. aeruginosa* to different contact lenses. I-IV denotes Food and Drug Administration lens types I-IV; Smooth = strain AK957; Rough = AK1012.

(±6.8) X 10^4 organisms, compared to 1.9 (±0.6) X 10^4 organisms for the isogenic rough strain.

Both scratching and acid injury significantly increased adherence to corneas compared to the intact corneas (ANOVA, F_{2,37} = 6.0, *P* < 0.01). Mean adherence after a needle scratch defect was 59.2 (±11.5) X 10^4 organisms and 4.5 (±1.0) X 10^3 organisms for the smooth and rough strain, respectively. After acid treatment, the mean number of smooth bacteria adhering was 102.1 (±8.3) X 10^4, whereas for the rough strain, 5.9 (±1.3) X 10^3 organisms attached. The difference in adherence with trauma was statistically consistent between the bacterial strains as demonstrated by the absence of a significant interaction (ANOVA, F_{2,37} = 0.2, NS).

Figure 2 demonstrates that adherence increases after scratch injury and acid treatment in comparison to intact cornea. However, the difference between trauma induced by a needle scratch and acid is not statistically significant. Post hoc statistical analysis with Scheffe’s F test confirmed these observations.

Hydrophobicity of Strains

The mean surface hydrophobicity (±SE) for the smooth and rough strains was 0.57 ± 0.06 and 0.10 ± 0.02 respectively, indicating that the smooth strain was more hydrophobic than the rough strain (ANOVA, *P* < 0.001).

DISCUSSION

Adherence of bacteria to surfaces and epithelia is known to be an important step in the pathogenesis of infection. Bacterial surface components, such as pili, outer membrane proteins, and flagella have been investigated as possible mediators of adherence.\(^{32-36}\) In this study, LPS was investigated for its role in adherence to contact lenses and the cornea. The results of this study show that a smooth strain of *P. aeruginosa* adheres better to unworn contact lenses and to the rat cornea than a rough isogenic mutant of the same strain.

The role of LPS in adherence to contact lenses may involve nonspecific physicochemical interactions because bacterial attachment is unlikely to occur via specific receptor binding sites. Surface hydrophobicity and surface charge are both believed to contribute to adherence of bacteria to surfaces.\(^{37}\) In this regard, interactions that may affect attachment include metal or other cations, polar group interactions, steric interferences, and specific reactions between functional groups.\(^{38-40}\) In our study, the LPS smooth strain, which was more hydrophobic, bound to a greater extent than the less hydrophobic rough strain. Others have found that certain piliated strains adhere in larger numbers to contact lenses than their less hydrophobic nonpiliated counterparts.\(^{37,41}\) Some form of relationship between hydrophobicity and adherence, although inconclusive, is suggested by these results. Factors related to surface charge may also be involved in the modulation of adherence by LPS, because the presence of particular amino acid sequences within the smooth LPS molecules may create an electrochemical charge that increases the affinity of the bacterium to specific charged molecules on the surface of the contact lens.

No difference between bacterial adhesion to the individual lens types was seen. Similarly, Duran et al
murium and verses noted in adherence of the smooth and rough drophobic rough strains have a greater affinity for a affinity of one strain of P. aeruginosa charide side chain increases hydrophobicity and the hydrophobicity.19 increasing water content of contact lenses.44 The results of those studies indicate that attachment may differ according to individual bacterial and contact lens properties.

Hydrophobic effects may contribute to the differences noted in adherence of the smooth and rough strains to the rat cornea. Previously, studies of S. typhi-

murium and Escherichia coli have shown that more hydrophobic rough strains have a greater affinity for a large number of different animal cells when compared to their more hydrophilic smooth counterparts.19-23,46 Our study has shown that possession of a long oligosac-

ccharide side chain increases hydrophobicity and the affinity of one strain of P. aeruginosa for the corneal epithelium as compared to a more hydrophilic rough mutant of the same bacteria. These results suggest that hydrophobicity may be a more important determinant than the type of LPS. Indeed, it is thought that LPS modulates non-specific adherence of S. typhimurium to polymorphonuclear cells by influencing hydrophobicity.19

It is widely recognized that trauma predisposes the cornea to infection and that P. aeruginosa adheres more to injured corneas. In this study adherence of both strains of P. aeruginosa increased 1.5-fold after needle scratch injury. The increased hydrophobicity of the corneas after injury may explain why adherence of both rough and smooth strains increased after trauma. Although trauma increased adherence, the increase was only small compared to the large differences seen between two strains.

Treatment of epithelial cells with mild acid has been shown to cause mild epithelial trauma characterized by removal of the mucin and the glycocalyx, and disruption of surface cells in the form of loss of fine structure (microplicae and microvilli) with minimal desquamation.47-51 Acid treatment has been found to enhance adherence of P. aeruginosa to tracheal epithelium.49-51 Our results show that P. aeruginosa also adheres more to cornea after acid treatment.

The results of this study suggest that LPS may modulate P. aeruginosa adherence to corneas and contact lenses. The difference in adherence of the rough and smooth strains to the cornea was larger than the difference between intact and injured corneas. This suggests that although trauma may predispose the cornea to infection, certain bacterial factors may also be important determinants in the pathogenesis of infec-

tion. Bacterial factors other than LPS are likely to be involved because the rough strain was still able to adhere to both cornea and contact lenses. To confirm the role of LPS in adherence, it will be necessary to perform competitive inhibition studies using LPS separated from the smooth strain to confirm the role of O-side chains in adherence to these surfaces. Investigation of the adherence of smooth and rough strains with similar hydrophobicity would also be valuable to test the relationship between hydrophobicity and LPS with respect to bacterial adherence.

Key Words
Pseudomonas aeruginosa, cornea, contact lens, infective keratitis, bacterial adherence, lipopolysaccharide

Acknowledgment
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