A Novel Antibiofilm Technology for Contact Lens Solutions

Bruce F. Farber, MD, Hsi-Chia Hsieh, PhD, Eric D. Donnenfeld, MD, Henry D. Perry, MD, Arthur Epstein, OD, Arlene Wolff, BS

**Purpose:** Nonsteroidal anti-inflammatory drugs, including sodium salicylate, inhibit extracellular bacterial biofilm production. The authors studied the effect of the addition of sodium salicylate on bacterial adherence and biofilm formation on contact lenses and cases and commonly used medical polymers.

**Methods:** The study was done in vitro with bacterial adherence and biofilm measured on lenses and cases that were exposed to saline contaminated with *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* with and without 1 and 3 mm sodium salicylate. Bacterial adherence to contact lenses was quantitated by a vortex assay and by scanning electron microscopy. Biofilm formation on contact lens cases and other polymers was measured by an optical density assay and a radiolabeling assay.

**Results:** Inhibition of biofilm formation was demonstrated on plastic contact lens cases in a dose-related manner with 1 and 3 mm sodium salicylate. A dose-related decrease in bacterial adherence also was noted. Assays with contact lenses also demonstrated less adherence in the presence of sodium salicylate. Electron micrographs of the contact lens showed less biofilm, most noticeable with 3 mm salicylate. Other studies demonstrated decreased adherence of *S. epidermidis* to polyethylene and polystyrene. Sodium salicylate also decreased biofilm on plastic tissue culture wells, but sorbic acid paradoxically increased deposition.

**Conclusion:** The authors found that the addition of low-dose sodium salicylate to saline decreased the adherence of *S. epidermidis* and *P. aeruginosa* to contact lenses and lens cases. Biofilm production also was decreased on the lens cases and on medical polymers used to make plastic cases. These studies suggest that sodium salicylate deserves additional study to determine its use in contact lens solutions.


Infectious keratitis remains a serious complication of contact lens use. Poggio et al estimated an annual incidence of infectious keratitis of 20.9 per 10,000 patients wearing cosmetic extended-wear contact lenses and 4.1 per 10,000 patients wearing daily wear cosmetic soft contact lenses. A conservative estimate based on approximately 20 million Americans wearing only daily wear soft contact lenses would create an annual incidence of 8200 cases of infectious keratitis. The majority of contact lens-related corneal ulcers are due to *Pseudomonas aeruginosa*. Many factors have been postulated to be important influences on the risk of infection. These factors include the lens material, wearing schedule, disinfection techniques, adherence of bacteria to the lens, and contamination of the lens care system and solutions.

Contact lens deposits or spoilage creates an undesirable interface between the contact lens and the conjunctiva and corneal surface. Contact lens deposit symptomatology includes decreased visual acuity, foreign body sensation, mucous discharge, tearing, redness, photophobia, and abnormal lens fit. Severe deposits may result in decreased oxygen permeability of the contact lens with the formation of superficial and deep corneal neovascularization, intrastromal lipid, and intracorneal hemorrhage. Protein deposits play an important role in the development of
contact lens-related allergy, including giant papillary conjunctivitis. Contact lens deposits harbor infectious organisms and increase the risk of infectious keratitis. These deposits include tear film proteins, sebaceous secretions, exogenous materials, mineral deposits, and bacterial biofilm.

Recent data have demonstrated that the attachment of microorganisms to medical devices is mediated by biofilm, or capsular polysaccharides which form a protective matrix around a device. Biofilm produces a scaffold which facilitates bacterial adhesion and colonization. In addition, biofilm protects and insulates bacteria from anti-infectives and disinfectant systems. Biofilm formation has been demonstrated on both contaminated lenses and in lens cases, even after appropriate sterilization. These bacteria likely serve as an inoculum for contact lens-related bacterial keratitis. Several authors have documented culturing organisms from the contact lens and contact lens case that were identical to the patients' infectious keratitis.

Nonsteroidal anti-inflammatory drugs, including sodium salicylate, inhibit extracellular bacterial biofilm production. These compounds inhibit bacterial attachment to a number of medical polymers, including silastic and polyurethane catheters. We investigated the addition of low-dose sodium salicylate to contact lens solutions. We attempted to determine whether the addition of sodium salicylate to contact lens solutions can decrease biofilm formation on contact lenses and their cases and bacterial attachment to contact lenses. We chose to study P. aeruginosa because it is the most common cause of contact lens-related infectious keratitis and Staphylococcus epidermidis because it is the organism most commonly cultured from contact lenses.

Methods

Strain

S. epidermidis strain 91 was used in these experiments. This is a slime-positive strain that has been described previously. A clinical isolate of P. aeruginosa also was used.

Lens Cases

Studies on biofilm formation were performed in plastic contact lens cases (Allergan, Irvine, CA). An overnight culture of the test organism (10^6 colony-forming units [CFU/ml] in trypticase soy broth (TSB) was added to saline with and without sodium salicylate in a 1:1 dilution. The bacterial suspension was incubated for 5 hours at 37°C, and the suspension was removed. In the experiments with P. aeruginosa, nonadherent film floating on the culture was removed with a cotton swab. The remaining biofilm was washed gently two times with 5 to 10 ml saline to remove nonadherent bacteria and debris. The adherent film was removed mechanically with a pipette and dissolved in 3 ml saline. The optical density was read on a Bausch and Lomb spectronic 601 at 686 nm (Rochester, NY). In addition, bacterial counts were done on the samples by serial dilution and plating onto Mueller-Hinton agar plates, which were incubated at 37°C for 18 hours.

Low-density Polyethylene

Low-density polyethylene was used as a primary reference material. It had been prepared under controlled conditions and has been used as a standard in experimental studies. The material was obtained from Abiomed, Inc (Danvers, MA), and has been described in detail. The sheets were cut into 10 × 26-mm rectangular sections. A bacterial suspension similar to that described previously was used. The polyethylene sheets were placed in the suspensions with and without sodium salicylate for 24 hours at 37°C. They were removed, and the white biofilm was mechanically removed with a no. 1014 brush and placed in 5 ml saline. The optical density then was read as previously described.

Polystyrene

Bacterial suspensions were prepared as previously noted and incubated with and without sodium salicylate at 37°C for 24 hours in 50 ml clear polystyrene tubes (Corning, NY). The supernatant was removed, and the white film on the walls of the tube was inspected. The biofilm then was dissolved in 5 ml saline by mechanically removing it with a plastic transfer pipette. The preparation was read at an optical density of 686 μm. In addition, similar experiments were performed, but 14C-sodium acetate (4 μCi) (1–14C) was added to all tubes before incubation. The supernatant was removed from the tubes, and 2 ml Aquasol was added. The biofilm was removed mechanically from the sides of the tube, resuspended in Aquasol and was counted in scintillation vials.

Contact Lenses

An overnight TSB culture was standardized to one-half optical density at 686 μm and diluted 1:100 in broth. The bacterial suspension then was added in a 1:1 dilution to saline with and without sodium salicylate. Acuvue (Johnson and Johnson, Raritan, NJ) contact lenses were incubated in the contaminated saline for 20 hours at 37°C. The lenses were removed and placed in 5 ml saline with gentle washing. They then were removed and placed in 5 ml fresh saline, vortexed at number 7 for 1 minute five times. The optical density of the saline was measured at 686 μm, and colony counts were performed by serial dilution. Lenses also were incubated with P. aeruginosa (10^6 CFU/ml) for 2 hours in saline alone or supplemented with sodium salicylate. The lenses were removed and washed in saline three times. They were incubated with 3H-leucine (2 μCi/ml) for 30 minutes and then washed and counted with Aquasol in scintillation vials. The results are expressed in counts per minute.

The effect of the addition of sodium salicylate to H2O2 during the disinfection of contact lenses was studied. Lenses were placed in lens cases with H2O2 supplemented
with 1 and 3 mmol/(mm) salicylic acid. The H₂O₂ was neutralized with catalase. The lens were rinsed in saline and incubated in *P. aeruginosa* for 2 hours. They then were washed in saline three times, and incubated in radiolabeled leucine (2 μCi/ml) for 30 minutes at 20° C. They were washed three times, suspended in Aquasol, and counted in scintillation vials.

**Electron Microscopy**

Lenses were incubated in saline or saline with 3 mm sodium salicylate (Fig 1). They then were contaminated with an *S. epidermidis* bacterial suspension as previously described for 4 hours. The soft contact lenses were fixed in 2% buffered glutaraldehyde. A rapid dehydration was performed with graded alcohols. They then were processed by critical point drying. A palladium gold carbonation was used for plating by sputter coating, and the lenses were viewed with a scanning Jeol electron microscope (Mexford, MA).

**Studies Using Salicylate and Sorbic Acid**

Contact lenses were incubated overnight in 24 well plates containing *S. epidermidis* in TSB diluted 1:1 with sterile saline with or without sodium salicylate (3 mm) or sorbic acid (0.015%). They then were rinsed gently with saline and transferred to a 15-ml centrifuge tube and vortexed with 4 ml NaCl vigorously five times for 1 minute. Colony-forming units then were done. In addition, overnight cultures were placed in the wells without lens. The media were removed, and the remaining biofilm was scraped out of the wells and suspended in 1 ml saline. Optical densities were read at 686 μm.

**Statistical Analysis**

Simple linear regression analysis was used to detect dose-related differences between the control, the 1-, and 3-mm groups. One way analysis of variance with a Newman–Keuls comparison test was used to detect differences between the salicylate and sorbic acid group.

**Results**

Inhibition of *S. epidermidis* biofilm was demonstrated on the sides of plastic contact lens cases with 1 and 3 mm sodium salicylate as seen in Figure 2. There was 39% inhibition with 1 mm and 95% inhibition with 3 mm. The difference was significant with *P* < 0.0001. A dose-related decrease in corresponding bacterial counts also was observed. The control lens cases contained $1.2 \times 10^7$ CFU/ml in the dissolved biofilm. There were $9.6 \times 10^6$ CFU/ml in 1 mm and $2.4 \times 10^6$ CFU/ml in 3 mm sodium salicylate. Results of a similar assay performed with *P. aeruginosa* are demonstrated in Figure 3. A dose-related decrease in adherent biofilm was noted. A corresponding
Table 2. Inhibition of Biofilm to Polystyrene Using Radiolabeled Staphylococcus epidermidis

<table>
<thead>
<tr>
<th></th>
<th>Optical Density* (µm)</th>
<th>(%)</th>
<th>Counts/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>0.618</td>
<td>(100)</td>
<td>95,535</td>
</tr>
<tr>
<td>1 mm</td>
<td>0.554</td>
<td>(89)</td>
<td>63,235</td>
</tr>
<tr>
<td>3 mm</td>
<td>0.296</td>
<td>(47)</td>
<td>46,784</td>
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</table>

*Mean of three assays.

decrease in the number of CFU/ml bacteria also was observed.

The results of the studies using S. epidermidis with standardized polyethylene are presented in Table 1. The study was performed in triplicate. There was minimal inhibition noted at 1 mm and 21% at 3 mm ($P < 0.04$). Studies using polystyrene are presented in Table 2. There was a dose-related linear inhibition of biofilm as measured by both optical density ($P < 0.001$) and uptake of radiolabeled sodium acetate. Table 3 gives the results of the studies using contact lenses and S. epidermidis. In these studies, the number of bacteria adhering to contact lenses incubated in contaminated saline was quantitatively measured in two ways. Both the optical density and number of colony-forming units per milliliter bacteria fell in a dose-related manner with increasing concentrations of sodium salicylate. Although there was a dose-related decrease in adherence, it did not achieve statistical significance.

Table 4 lists the results of an assay in which adherence to lenses was studied with P. aeruginosa. Both assays demonstrate a dose-related inhibition of growth (colony-forming units per milliliter); however, it was not statistically significant. The radiolabeling method produced a similar degree of inhibition. In Table 5, adherence of P. aeruginosa to lenses was studied after exposure in contaminated neutralized H$_2$O$_2$. A dose-related decrease in adherence again was noted ($P < 0.003$).

Optical densities of the biofilm scraped out of the plastic wells were 0.788 µm for controls, 0.194 µm for salicylate, and 1.435 µm for sorbic acid (mean trials, 7). There was a highly significant difference among the groups ($P < 0.001$). Of note was the paradoxical increase in biofilm found with sorbic acid. Colony counts done on lenses that were incubated demonstrated $6 \times 10^5$ CFU/ml in the control saline, $1.3 \times 10^4$ CFU/ml with salicylate, and $2.5 \times 10^5$ CFU/ml with sorbic acid. These differences were not significant.

**Discussion**

Despite adequate disinfection, corneal infection continues to be a problem associated with soft contact lens use. The pathophysiology of these infections is complex and probably multifactorial. It seems logical that contamination of lens care cases and solutions is a major risk factor of infection. In addition, recent data from studies of other medical devices and lenses suggest that adherence of bacteria is significantly dependent on biofilm formation. Microorganisms produce a protective polysaccharide film that is resistant to disinfectants and antimicrobial agents.

Our previous work has demonstrated that the salicylates and other nonsteroidal anti-inflammatory agents interfere with bacterial attachment to a number of medical polymers. In addition, we have demonstrated that
the salicylates decrease the amount of *S. epidermidis* extracellular slime. Although experience with biofilm has been most extensive with *P. aeruginosa*, the observed effect has been demonstrated with a variety of other organisms.

In this study, we demonstrated that the addition of low-dose sodium salicylate to saline decreases biofilm formation on contact lens cases. In addition, there was reduced bacterial adherence to lenses exposed to saline contaminated with *P. aeruginosa* and *S. epidermidis* compared with control saline. The concentrations of sodium salicylate used were 0.013% to 0.039%. The concentration of other nonsteroidal anti-inflammatory drugs used in commercially available topical ophthalmic drops is 0.1% to 1.0% or approximately 2.5 to 25 times greater than the highest concentration of sodium salicylate we used. Hence, we are using concentrations that would be otherwise considered subtherapeutic. It is unclear whether other nonsteroidal anti-inflammatory drugs have this property.

Sorific acid currently is used as a preservative in contact lens solutions at a concentration of 0.1%, which is higher than the concentrations that we used. Our studies suggest that it paradoxically may increase biofilm deposition on lenses, opposite of the effect with sodium salicylate.

Previous research has shown that *P. aeruginosa* preferably adheres to focal deposits on contact lenses. The strong association of *Pseudomonas* to contact lens-related bacterial keratitis may be explained by the production of large amount of extracellular slime. This may allow *Pseudomonas* to adhere to the surface of the contact lens. It is unclear whether the addition of a nonsteroidal anti-inflammatory drug to the lens solutions would decrease the rate of corneal infection. However, by decreasing bacterial adhesion to the contact lens, we postulate a decreased risk of inoculation into the cornea. This hopefully would decrease the incidence of bacterial keratitis. It is also possible that decreasing biofilm might decrease the incidence of lens intolerance due to allergic problems. We suggest that further studies on biofilm formation and nonsteroidal anti-inflammatory drugs are indicated, as well as toxicity studies of the clinical effect of low dose nonsteroidal anti-inflammatories on the ocular surface.

<table>
<thead>
<tr>
<th>Vortex Method</th>
<th>Trial 1 CFU/ml</th>
<th>Trial 2 CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>4.0 × 10^6</td>
<td>1.8 × 10^6</td>
</tr>
<tr>
<td>1 mm</td>
<td>1.4 × 10^6</td>
<td>1.2 × 10^6</td>
</tr>
<tr>
<td>3 mm</td>
<td>8.5 × 10^5</td>
<td>8.4 × 10^5</td>
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<table>
<thead>
<tr>
<th>Radiolabel Method</th>
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<tbody>
<tr>
<td>Control</td>
<td>209,434</td>
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<tr>
<td>Salicylate (1 mm)</td>
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CFU = colony-forming units.

<table>
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<th>H₂O₂</th>
<th>Counts/min</th>
<th>Final pH</th>
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<td>H₂O₂</td>
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<tr>
<td>H₂O₂ + 1 mm</td>
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<tr>
<td>H₂O₂ + 3 mm</td>
<td>215,831</td>
<td>6.4</td>
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</table>

**Table 5. Adherence of *Pseudomonas aeruginosa* to Contact Lenses after H₂O₂ Disinfection**

**References**


