Biofilm Formation by *Staphylococcus epidermidis* on Intraocular Lens Material

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**PURPOSE.** To compare biofilm formation by *Staphylococcus epidermidis* on different intraocular lens (IOL) materials.

**METHODS.** The *S. epidermidis* strains, ATCC 12228 (American Type Culture Collection) and ATCC 35984 (biofilm-producer) were used. Biofilms were cultivated on disks of different IOL materials: silicone, PMMA (polymethylmethacrylate), acrylic, or MPC (2-methacryloyloxyethyl phosphorylcholine) surface-modified acrylic. Biofilms were stained with crystal violet (CV) which served as an index of biofilm formation. The bacterial population was enumerated after biofilm homogenization. Biofilms were also examined by scanning electron microscopy (SEM).

**RESULTS.** Among the four materials tested, the least amount of biofilm formed on silicone. Biofilm production was significantly different between acrylic and MPC surface-modified acrylic lenses at 48 hours (*P* < 0.05–0.01). The bacterial populations were significantly different between acrylic and silicone over 72 hours (*P* < 0.05–0.01). The population on acrylic and MPC surface-modified acrylic continued to increase over 72 hours. The biofilm was recognized after a 24-hour incubation. Rates of biofilm-positive SEM fields, which were defined as being occupied by biofilm over at least half of the area, were significantly different between acrylic and silicone at 72 hours (*P* < 0.05–0.01).

**CONCLUSIONS.** *S. epidermidis* formed biofilm most intensely on acrylic among the four IOL materials tested. MPC surface-modified acrylic has a preventive effect on biofilm formation. (Invest Ophthalmol Vis Sci. 2006;47:2971–2975) DOI: 10.1167/iovs.05-1172

Postoperative endophthalmitis is a serious complication of intraocular lens (IOL) implantation. Investigators have reported postoperative endophthalmitis incidence rates of 0.08% to 0.11% after cataract surgery.¹,² Visual outcome after endophthalmitis is generally poor, and only half of the eyes achieve a final best corrected visual acuity of 20/40.¹,² Potential sources of infection include contamination of the ocular surface and lids, irrigating solutions, and medications, surgical instruments involving IOLs, respiratory and skin flora of the surgeon and assistants, and contamination of the air in the operating room.

The binding of bacteria to IOLs during implantation and colonization of IOLs after implantation appear to contribute to the pathogenesis of postoperative endophthalmitis.³,⁴ Bacterial adhesion to and biofilm formation on IOLs has been a subject of recent interest in ophthalmologic literature.⁵–¹³ Biofilms and the communities of attached bacteria¹⁴ have received increased attention as the cause of eye infections, including those that highly disturb refraction.

*Staphylococcus epidermidis* is the most common organism isolated in cases of postoperative endophthalmitis.¹⁵,¹⁶ This organism is normally considered to be of low pathogenicity, yet growing evidence indicates that it is implicated in many ocular infections. *S. epidermidis* is frequently involved in surface colonization of synthetic devices, such as vascular prostheses, artificial hearts, and total-joint replacements, and this organism’s adhesion to the IOL materials has recently been emphasized in the literature.³,⁵–¹³ We have reported biofilm formation by *Enterococcus faecalis* on commercially available IOL materials.¹³ The purpose of this study was to compare the formation of *S. epidermidis* biofilm on various IOL materials, as a step toward enhancing the understanding of the interactions between IOL materials and pathogenic bacteria.

**MATERIALS AND METHODS**

**Intraocular Lenses**

All IOLs were hydrophobic. IOLs made of silicone (AQ310N; Canon-Starr, Tokyo, Japan), polymethylmethacrylate (PMMA; Hoya, Tokyo, Japan), acrylic (Hoya) and MPC (2-methacryloyloxyethyl phosphorylcholine) surface-modified acrylic (Hoya) were used in the study. The haptics were removed from the silicone IOL (AQ310N) for use in the study. All silicone IOLs had identical dioptric power (20 D). PMMA, acrylic, and MPC surface-modified acrylic IOLs did not have any dioptric powers or haptics. Acrylic IOLs contained fluorine, which reduces surface adhesiveness.¹⁷,¹⁸ MPC was synthesized and then used in the preparation of cross-linked polymer membranes with lauryl methacrylate, hydroxypropyl methacrylate, and trimethoxysilylpropyl methacrylate (cross-linker) monomers on the MPC-treated acrylic lenses.¹⁹ The characteristics of the MPC copolymers relevant to the improved blood compatibility is the property of minimizing protein adsorption through an increase in the amount of free water in the MPC hydrogels.²⁰ MPC coating has been found to reduce the levels of adherent bacteria to medical devices.¹⁹ All the sample materials had identical dimensions (6.0 mm diameter × 1.0 mm thickness).

**Bacteria and Media**

The *S. epidermidis* strains ATCC (American Type Culture Collection) 12228 and ATCC 35984 (biofilm-producer) were used. Both strains were purchased from Summit Pharmaceuticals International (Tokyo, Japan). Stock cultures were kept frozen at −75°C in brain–heart infusion (BHI) broth containing 25% glycerol. Before each assay, small quantities of the culture were subcultured on the BHI broth overnight at 37°C to confirm purity and viability. ATCC 35984 is a strongly adherent, slime-producing strain.²¹,²² All *S. epidermidis* were cultivated in trypticase soy broth (TSB) supplemented with 0.25% glucose (Difco Laboratories, Detroit, MI).

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IOL Biofilm Assay

The ability of *S. epidermidis* to form biofilms on abiotic surfaces was quantified essentially as described by O’Toole et al. Briefly, *S. epidermidis* was grown overnight in TSB with 0.25% glucose at 37°C. The IOLs were fixed to the bottom of a 96-well polystyrene microtiter plate (M53096F; Sumitomo-Bakelite, Tokyo, Japan).

*S. epidermidis* cultures were diluted 1:40 in TSB containing 0.25% glucose, and 200 μL of diluted culture was added to each IOL-containing well for incubation at 37°C. Each experiment was performed in five replicate wells. After incubation for 24, 48, or 72 hours, each IOL was gently washed three times with 200 μL phosphate-buffered saline (PBS), dried, and stained with 1% crystal violet for 15 minutes. The IOLs were rinsed again with PBS, and bound crystal violet was solubilized in 200 μL of ethanol-acetone (80:20, vol/vol). The optical density at 620 nm (OD620) was determined using a microplate reader (model 680; Bio-Rad Laboratories, Inc., Hercules, CA).

Enumeration of IOL-Adherent Bacteria

After incubation as just described, each IOL was gently washed three times with phosphate-buffered saline (PBS). Each IOL was transferred to a sterile 1.5-mL microtube (LockFit; Treff, Degersheim, Switzerland) containing 0.3 mL of 1-mm-diameter sterile glass beads (SGMT No. 001; Toshinriko Co. Ltd., Tokyo, Japan) in 1 mL of PBS. The tubes were subsequently vortexed for 1.5 minutes at 2500 rpm to separate the cells from the biofilm matrix. This method was determined experimentally to yield the maximum number of colony-forming units (CFU) with no detectable loss of viability (data not shown). Disaggregated bacteria were then enumerated by quantitative track dilution plating as described previously. Each of the experiments was conducted in triplicate.

Scanning Electron Microscopy

After incubation as described, each IOL was gently washed three times with phosphate-buffered saline (PBS). The IOLs were first fixed with 2.5% (wt/vol) glutaraldehyde in a filter-sterilized 0.1 M phosphate buffer (pH 7.4) at room temperature for 2 hours and then rinsed 3 times for 15 minutes in a 0.1 M sodium cacodylate buffer. Next, a postfixation step was performed for 1 hour with 1% (wt/vol) osmium tetroxide in a 0.1 M sodium cacodylate buffer. This step was followed by a quick rinse in distilled water. The fixed lenses were then dehydrated in successive ethanol-water mixtures with increasing ethanol concentrations of 50%, 70%, 80%, and 95% by volume for 7 minutes each and then two times in pure ethanol for 15 minutes. They were put into an ethanol bath that was allowed to evaporate. The dried samples were stuck onto metal holders with double-sided adhesive tape and finally coated with platinum and palladium in an evaporator. Observations were performed at 15 kV with a scanning electron microscope (model S3500N; Hitachi, Tokyo, Japan). Five fields of view at magnifications from ×500 to ×600 were chosen randomly from the optic surface of each sample. Each experiment was conducted in triplicate.

Three examiners (YO, SK, TT) classified each field as biofilm-positive or negative. A biofilm-positive field was defined as being occupied by biofilm over at least half of the area (Fig. 1). The rates of positive fields were calculated and averaged for each group.

Statistical Analysis

Nonparametric tests (Kruskal-Wallis) and multiple-comparison tests (Scheffe, Tukey, and Newman-Keuls) were performed on computer (Stat-Mate, ver. 3, plug-in software for Microsoft Excel; ATMS, Tokyo, Japan).

RESULTS

Biofilm Formation on IOLs

*S. epidermidis* biofilm formation on each IOL material was initiated with approximately 10⁶ CFU/mL of strains ATCC 12228 and ATCC 35984. The biofilm was then detected by crystal violet staining at 24, 48, or 72 hours of incubation. The IOL materials supported the growth of biofilm over 72 hours. As for the ATCC 12228 strain, stainable biomasses on the four materials increased until 48 hours (Fig. 2A). By 48 hours, biofilm production on PMMA, acrylic, and MPC surface-modified acrylic IOLs had increased significantly compared with that on silicone IOLs (*P < 0.01*). At 72 hours, however, the biomasses on PMMA, acrylic, and MPC surface-modified acrylic IOLs had decreased. There were significant differences in biofilm production between acrylic and MPC surface-modified acrylic at 24 and 48 hours (*P < 0.05–0.01*).

As for the strain ATCC 35984, the amount of biomass was the least on silicone among the four materials tested (*P < 0.05–0.01; Fig. 2B). There were significant differences in biofilm production between acrylic and MPC surface-modified acrylic over 72 hours (*P < 0.05–0.01*). There were no differences between PMMA and acrylic in biomass production over 72 hours. The optical density of the biomass of ATCC 35984 was generally greater than that of ATCC 12228.

Bacterial Enumeration in IOL Biofilms

Homogenization of the biofilm on the IOL, determined via quantitative counting, revealed significant differences in the number of adherent bacteria between acrylic, silicone, and MPC surface-modified acrylic materials after 24-hour incubation (*P < 0.05–0.01*; Fig. 3) for both strains.

As for the strain ATCC 12228, the number of adherent bacteria on silicone IOLs decreased over 72 hours, whereas the number of adherent bacteria on PMMA remained relatively unchanged, and the number of adherent bacteria on both acrylic and MPC surface-modified acrylic increased over 72 hours (Fig. 3A). For ATCC 35984, the number of adherent...
bacteria on acrylic and MPC surface-modified acrylic continued to increase over 72 hours (Fig. 3B). The number of adherent bacteria was significantly different between silicone and other IOL materials over 72 hours ($P < 0.05$). The difference between acrylic and MPC surface-modified acrylic continued to decrease over 72 hours.

**SEM of the Development of *S. epidermidis* Biofilm**

A scanning electron microscope (SEM) was used to examine the biofilm on each IOL material (Fig. 1). The definition of biofilm was the recognition of slime and a multilayer formation of bacteria (Fig. 1). With both strains, the biofilm was recognized at 24 hours of incubation and developed over 48 hours.

Rates of biofilm-positive SEM fields, which were defined as being occupied by biofilm on over at least half of the area, were significantly different between acrylic and silicone materials over 72 hours with both strains ($P < 0.05-0.01$; Fig. 4). As for the strain ATCC 12228, the positive rates were significantly different between acrylic and the other IOL materials at 48 and 72 hours of incubation ($P < 0.05-0.01$). There were also significant differences in the positive rates between silicone, PMMA, and MPC surface-modified acrylic over 72 hours ($P < 0.05-0.01$). For ATCC 35984, between acrylic, silicone, and PMMA, the positive rate was significantly different over 72 hours ($P < 0.05-0.01$). There were also significant differences in the positive rate between silicone and MPC surface-modified acrylic at 48 and 72 hours of incubation ($P < 0.01$).

**DISCUSSION**

Intraocular lens-associated biofilms have been the subject of several recent reports, especially for assessing Staphylococal biofilm formation on IOL materials. We adopted the method of spectrophotometrically quantifying biofilm density by crystal violet staining, a commonly used technique in quantifying biofilms on other systems. In several reports, the sonication method was used for detaching the bacterial cells. However, if an IOL loses fewer bacteria through sonication, the loss results in a smaller number of cells on the agar plates. This means that these bacterial cells were not tightly attached to the IOL surfaces. As a result, this solution put on agar will grow more bacteria. To detect bacterial cells in this study, vortexing with glass beads was used. We also confirmed that this method induced no detectable loss of viability of bacteria.

The results of this study suggest that *S. epidermidis* biofilms form more readily on acrylic IOLs and least on silicone IOL material. These results were in accordance with our previous report. In the case of *E. faecalis* biofilm, the quantity of biofilm formation was not in accordance with the number of bacteria. In this study, however, the results of biofilm formation, bacterial enumeration, and SEM observation had similar tendencies (acrylic > MPC or PMMA > silicone). Though the different scales cannot be compared, the difference between silicone and the other IOL materials was greater in biofilm.
formation than in the other two aspects assessed in this study. The double washing during the crystal-violet-staining process may be a reason for the greater difference between silicone and other IOL materials. Each IOL was gently washed three times before and after crystal violet staining: The first wash was for removing the planktonic bacteria, and the second was for removing excess 1% crystal violet. Without the second wash, it would be impossible to determine precisely the optical density and other IOL materials.

Several researchers have tried to determine which biomaterial has the strongest affinity for bacteria. Schauersberger et al. showed results similar to ours, whereas Kodjikian et al. reported no significant difference among hydrophobic materials. Comparison between these studies (including the current study) is difficult, or even impossible, because of variable conditions such as the bacterial strains, IOL materials (including loop materials), the incubation time, and the quantitative or qualitative methods to determine bacterial adhesion.

Bacterial adherence to the implant surfaces seems to depend on the hydrophobicity or hydrophilicity of the biomaterial. Crystal violet staining and viable IOL-adherent bacterial counts indicate that MPC surface-modified acrylic IOLs result in less biofilm than does acrylic IOL over 48 hours. Nomura et al. showed that the addition of heparin had reduced the formation of biofilm on both silicone elastomer and polyvinyl chloride catheters. Cagavi et al. showed that hydrophilic-coated materials reduced bacterial colonization. Our results and these studies suggest that the reduction of surface hydrophobicity hinders bacterial colonization.

Bacteria in biofilms are more resistant to antiseptics, antibiotics, and host defenses. Bacterial adhesion is the first step in biofilm formation. Therefore refinement of IOL polymer design based on bacterial pathophysiology studies may provide a means of reducing the incidence of intraocular infections.

References


