In Vivo Study of Bacterial Adhesion to Five Types of Intraocular Lenses

Laurent Kodjikian,[1,2] *Carole Burillon,*[1] *Cécile Chanloy,*[3] *Valérie Bostvironnois,*[3] *Gérard Pellon,*[4] *Eliane Mari,*[3] *Jean Freney,*[2] *and Thierry Roger*[3]

Purpose. To determine in vivo behavior of the ability of the *Staphylococcus epidermidis* strain (American Type Culture Collection [ATCC] 14990) to attach to 120 intraocular lenses (IOLs) made of five different biomaterials: fluorine polymethylmethacrylate (PMMA), heparinized PMMA, silicone, hydrophobic acrylic, and hydrogel. The pig was chosen as an animal model of endophthalmitis, after a bibliographical analysis and a personal study of its aqueous humor composition.

Methods. Crystalline lenses from 90 domestic pigs were removed aseptically and replaced with previously infected IOLs. The animals were killed 24 hours, 72 hours, and 1 week after implantation of the IOLs. The extent of bacterial binding was then measured by counting. Results were compared with a two-factor analysis of variance (ANOVA 2), confirmed by the Kruskal-Wallis nonparametric test.

Results. The extent of bacterial binding (expressed as bound bacteria per area unit) was found to range in increasing order from hydrogel, to fluorine PMMA, to hydrophobic acrylic, to heparinized PMMA, to silicone polymer. Comparison of pairs of materials showed statistically significant differences, except between hydrogel and fluorine PMMA.

Conclusions. To the authors' knowledge, no study has been published so far concerning the in vivo evolution of populations of bacteria adhering to different intraocular materials. Bacterial adhesion to the implant surface must therefore depend on the hydrophobicity or hydrophilicity of the biomaterial. Adhesion is also affected by the nature of the surrounding medium. Because of its complexity, the latter appears to be very difficult to model, thus making in vivo studies essential. (*Invest Ophthalmol Vis Sci.* 2002;43:3717–3721)

Postoperative endophthalmitis after intraocular lens (IOL) implantation is still one of the most fearsome complications of cataract surgery. Bacterial adhesion to IOLs during insertion is believed to represent a prominent etiological factor in postoperative endophthalmitis and in pseudophakic chronic intraocular inflammation.[1,2] Thus, reducing adhesion of bacteria to IOLs—mainly of *Staphylococcus epidermidis*, the bacteria[3] most often involved—would decrease the incidence of these diseases.

We previously studied the in vitro adhesion of *S. epidermidis* to IOLs made of different, more or less hydrophilic, biomaterials.[4] The binding was found to be weakest on hydrogel (HEMA, hydroxy-ethyl-methacrylate or PHEMA, poly-HEMA) and strongest on the silicone polymer. The difference between hydrophilic acrylic (acrylate or methacrylate polymers), polymethylmethacrylate (PMMA), and heparinized (HSM or heparin-surface-modified) PMMA was not significant. Bacterial adhesion to the implant surface has thus been shown to depend on the hydrophobicity or hydrophilicity of the biomaterial.

However, the influence of the surrounding medium is essential as well, making it difficult to extrapolate our in vitro results to the clinical situation. In that this medium is very difficult to model because of its complexity, an in vivo study seemed essential.

The purpose of the study was to determine in vivo bacterial adhesion to IOLs made of five different materials. IOLs were infected before being implanted in eyes of pigs, and the in vivo behavior of attached bacteria, or more exactly the evolution of the amount of attached bacteria, was studied.

Materials and Methods

Intraocular Lenses

The present study was performed with 120 IOLs made of five different plastic materials: fluorine or fluorine surface-modified PMMA (24 lenses), HSM PMMA (24 lenses), silicone (24 lenses), hydrophobic acrylic (24 lenses), and hydrogel (HEMA or PHEMA; 24 lenses). These new, sterile inserts were manufactured by various firms in France (Table 1). For each material, 18 IOLs were implanted in pigs' eyes and 6 IOLs were used as the IOL control to count the amount of adhering bacteria that was introduced into each eye for each experiment.

Strain

The microbiology department of Edouard Herriot Hospital (Lyon, France) provided an *S. epidermidis* reference strain (ATCC 14990 American Type Culture Collection, Rockville, MD) producing a great amount of slime. We ascertained that this bacterium could grow in the aqueous humor of the pig (data not shown).

Pigs

The domestic pig was chosen as a suitable animal model of endophthalmitis, after a bibliographical analysis and a personal study of its aqueous humor composition. In a preliminary study, we took a sample of aqueous humor from 25 pigs and analyzed its composition. We compared its composition with that of human aqueous humor[5] (Table 2). In fact, the eye volume, and particularly the anterior chamber volume (300 μL), as well as the aqueous humor composition, were similar in both pigs and humans. Thus, IOLs designed for human surgery could be inserted in the pigs' eyes.

This study involved 135 domestic pigs, 45 of which were used for the preliminary feasibility study. Only one eye of each of the remaining 90 pigs was implanted with infected IOLs, after crystalline lens extraction surgery, according to the guidelines of the Animal Care Committee of the Veterinary Department, Marcy l'Etoile, France.

From the Department of [1]Ophthalmology, Herriot Hospital, Lyon, France; [3]Veterinary Department, Marcy l'Etoile, France; and the Departments of [2]Microbiology and [4]Biochemistry, University of Lyon, Lyon France.

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Corresponding author: Laurent Kodjikian, Edouard Herriot Hospital, Department of Ophthalmology, EA 3090, 5 Place d'Arsonval, Lyon 69003 France; kodjikian.laurent@wanadoo.fr.

TABLE 1. Characteristics of IOLs

Material	Manufacturer	Model Number	Style of Haptics
Fluorine PMMA	Bausch and Lomb, Paris	Centra 60F	1-Piece
Heparinized PMMA	Pharmacia, Paris	811C	1-Piece
Silicone	Allergan, Paris	SI40NB	3-Piece
Hydrophobic Acrylic	Alcon, Paris	MA60BM	3-Piece
Hydrogel	Corneal, Annecy	ISH60P	Plate

IOL Contamination

We knew that identical amounts of bacteria could not be bound to each material, because significant differences had been shown in our prior in vitro study.[4] Therefore, bacterial concentration was adjusted spectrophotometrically to 10^8 colony-forming units (CFU) per milliliter to reach a minimum bacterial count of approximately 10^5 CFU per IOL. We had shown in a preliminary study that it was essential to inoculate the eye with at least 10^5 CFU to induce endophthalmitis in pigs (data not shown).

Complete lenses (including haptics) were incubated in bacterial suspension for 1 hour at 37°C, with continuous shaking, before being washed three times in a phosphate-buffered saline solution (PBS buffer, pH 7.8) to eliminate nonadhering bacteria.

Bacterial Counting

For each of the five lens materials, 18 IOLs were implanted before being removed at different times to measure the amount of remaining bacteria (hereafter termed CFU IOL) and 6 were used to control the precise amount of adhering bacteria before implantation (termed CFU control). Bacterial counting was performed as follows: Lenses were soaked in a PBS buffer, and bound bacteria were dispersed by sonication at 45 kHz for 5 minutes (Branson, Shelton, CT). The resultant suspension was vortexed, diluted, and spread over a nutritive agar plate (Trypticase-Soja; BioMérieux, Marcy l'Etoile, France). This process has been found to remove all adherent bacteria without affecting their viability. Colonies were counted after a 24-hour incubation at 37°C. The number of bacteria was expressed as colony-forming units per milliliter (CFU/mL). Because the area of a lens depends on its diameter, as well as on its haptic shape and dioptric power, IOL manufacturers gave the exact area of all implants. Results are always expressed as $\log_{10}$ CFU per 50 mm^2.

To compare the results accurately, it was essential to bind identical amounts of bacteria to each material and to obtain the same control value for repeated experiments performed with a given material. Because it was not possible to satisfy both conditions, variability factors could be canceled by calculating the ratio (CFU IOL/CFU control) between the amount of remaining bacteria after different times of implantation and the initial amount of bacteria adhering before implantation. Results are expressed as a ratio of the corresponding $\log_{10}$ CFU per 50 mm^2, which shows the in vivo evolution of the amount of adhering bacteria.

Surgery Time

All animal procedures were approved by the Animal Care Committee of the Veterinary Department, Marcy l'Etoile, France, and were conducted according to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research.

After removing crystalline lenses (by manual extracapsular extraction) under aseptic conditions and general anesthesia, previously infected IOLs were implanted into the anterior chamber. For foldable IOLs, folding forceps were used. We consistently used sterile patches and a povidone iodine 5% solution (Betadine, NAPP Laboratories, Cambridge, UK) directly on the eye's surface. To explant the lenses, we first performed the enucleation of the involved eye. After washing with the povidone iodine 5% solution and rinsing with sterile balanced salt solution (BSS), we performed a large (approximately 200°) corneal incision to remove the IOLs with sterile forceps under a laminated flux hood. In the preliminary study, we showed that our surgical procedure avoided any contamination. Sterile IOLs of each of the five materials were implanted into 30 pigs (30 eyes) and then removed according to the same procedure after 72 hours in 10 pigs and after 1 week in 20 pigs. All the IOLs remained sterile, confirming the rigor of the asepsis.

Animals were killed at 24 hours (24H group), 72 hours (72H group) and 1 week (1W group). Endophthalmitis often appeared clinically either during the first day, between the second and the fifth days, or after the sixth day.[6] Six IOLs were implanted for each of the five lens materials at each of the aforementioned times—that is, 18 IOLs were tested per polymer. Bacterial counting was conducted on every IOL removed, using the technique described previously.

Statistical Methods

Results were compared on computer with a two-factor analysis of variance (ANOVA 2, Excel 2000; Microsoft, Redmond, WA), to determine statistical differences relating to materials and/or to the time of surgery. To check the validity of the method, population distribution had to be normal, and the Levene test was made. If the latter test showed variance heteroscedasticity, a nonparametric method (Kruskal-Wallis test) was used to corroborate the results.[7]

RESULTS

Results were expressed as ratios. A positive ratio indicates that bacterial growth took place on the IOL surface, whereas a negative one means that the count of bound bacteria had decreased between IOL implantation and removal. The mean ratios obtained for each of the tested materials at each time with standard errors are shown in Figure 1 and Table 3. Standard deviations were most appreciable after 1 week (Table 3). They showed variability, both between the tested materials and between the times of IOL removal for a given material.

Only two materials, silicone and HSM PMMA, presented a globally positive ratio, showing bacterial growth on their surface. The others had a globally negative ratio, showing a decline of the bacterial population colonizing their surface. Moreover, only hydrogel showed an immediate negative ratio at 24 hours.

At first, results were compared using a two-factor analysis of variance (ANOVA 2, Excel 2000; Microsoft). According to the results, the difference was statistically significant between different materials ($P = 0.007$), but not between different times ($P = 0.57$). Normal distribution was found, but the Levene test showed variance heteroscedasticity (data not shown), meaning that ANOVA 2 was plainly not suitable. Therefore, a nonparametric method (Kruskal-Wallis test, a one-factor analysis of variance test) had to be used to corroborate results.[7] Because the ANOVA 2 did not find statistically significant differences between the results obtained at different times, the material was chosen as the variable in the Kruskal-Wallis test. The latter

TABLE 2. Comparison of Aqueous Humor Composition in the Human and the Pig

	Human Aqueous Humor	Pig Aqueous Humor
Sodium (mEq/L)	140–152	137
Potassium (mEq/L)	3.5–5.3	8.6
Calcium (mM/L)	2.2–2.6	1.39
Glucose (mM/L)	2.77–4.16	2.92
Urea (mM/L)	1.99–5.97	5.65
Creatinine (mg/dL)	4.8–8.8	<5

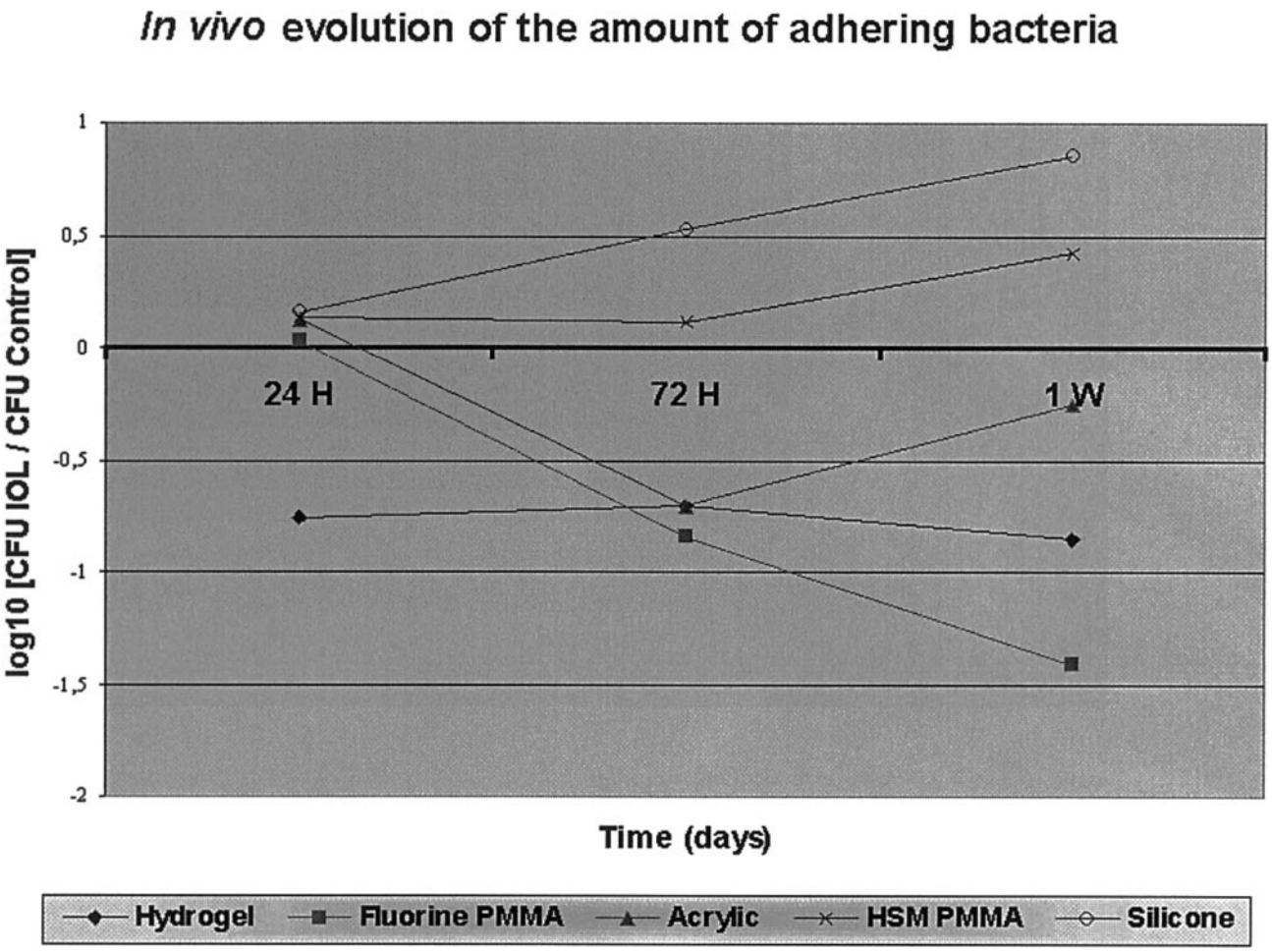

FIGURE 1. In vivo evolution of the amount of bacteria adhering to intraocular lenses (IOLs) in porcine eyes at three different time periods. Results are expressed as $\log_{10}$ CFU IOL/CFU control per 50 mm^2.

showed a significant effect of materials ($P = 0.0018$), fully confirming the ANOVA 2 results.

The ratio of attached bacteria per area unit on the five lens materials increased in the order of hydrogel, fluorine PMMA, acrylic, HSM PMMA, and silicone. Comparing pairs of materials showed statistically significant differences, except between hydrogel and fluorine PMMA.

DISCUSSION

Coagulase-negative staphylococci are currently recognized as important etiological agents of postoperative endophthalmitis after the implantation of IOLs.[8] *S. epidermidis* is part of the normal ocular and periocular surface flora. Bacterial adhesion on IOLs during their insertion is believed to represent a prominent etiological factor of endophthalmitis.[1,2] Because the treatment of this disease is difficult and sometimes inefficient, modification of the polymer lens surface may represent a promising approach intended to alter bacterial adhesion, which is the first step in the colonization of an area.[9]

However, it may be just as important to know the in vivo behavior of attached bacteria on the IOL surface. To our knowledge, no study has been published so far concerning the in vivo evolution of populations of bacteria adhering to different intraocular materials. A biomaterial unsuitable for the in vivo growth of bacteria would be very useful in clinical practice. It could be expected that IOLs made of such a material might prevent the development of endophthalmitis, unlike other biomaterials that allow bacterial growth on their surfaces.

Moreover, many microorganisms colonize IOL surfaces in the form of a biofilm, producing an extracellular, sticky polysaccharide substance called slime.[1,10] The formation of these biofilms is an important strategy used by many bacteria, including *S. epidermidis*, to survive in various environments.[11,12] When bacteria land on an inert surface, the affinity of their interaction and the degree of adherence of the cell to the

TABLE 3. In Vivo evolution of the Amount of Bacteria Adhering to IOLs Inserted in Eyes of Pigs

	Hydrogel	Fluorine PMMA	Acrylic	HSM PMMA	Silicone
24 Hours	−0.76 ± 1.14	0.03 ± 0.46	0.13 ± 1.06	0.14 ± 0.92	0.16 ± 0.43
72 Hours	−0.7 ± 1.05	−0.84 ± 0.46	−0.71 ± 0.97	0.12 ± 0.43	0.53 ± 0.89
1 Week	−0.85 ± 2.08	−1.41 ± 2.57	−0.25 ± 1.74	0.42 ± 0.52	0.85 ± 0.71

Data are mean ratios ± SE. Results are expressed as $\log_{10}$ CFU IOL/CFU control per 50 mm^2

support are governed by the physicochemical properties of both. Later, exopolysaccharides (slime) assist bacteria in firmly adhering to inert surfaces.[11,12] Pathogenic bacteria probably use a similar mechanism to colonize implants such as IOLs.[1,11] The slime matrix formed by exopolysaccharides is not only an adhesive medium, it also affects virulence. Indeed, bacteria in biofilms are more resistant to antiseptics, antibiotics, and host defenses.[11,13–18]

The purpose of this experimental study was to analyze in vivo behavior of bound bacteria on IOLs made of five different materials and implanted into the anterior chambers of eyes in domestic pigs.

Most investigators have concluded that intermediate hydrophobicity is an important factor promoting bacterial binding.[19–22] Bacteria adhere less to IOLs composed of hydrophilic materials such as hydrogel or very hydrophobic ones such as fluorine PMMA than to intermediate hydrophobic ones such as silicone.[22,23] We had found the same results in an in vitro study of *S. epidermidis* adhesion to IOLs.[4] Adhesion was weakest on hydrogel and strongest on the silicone polymer. The differences between hydrophilic acrylic, PMMA, and HSM PMMA were not significant.

The results obtained in this in vivo study were quite similar. Two materials (silicone and HSM PMMA) allowed bacterial growth, confirming that bacteria can adhere easily and strongly to these surfaces. With the three others, we found a decline of the population of bound bacteria, especially with the most hydrophilic biomaterial (hydrogel) and the most hydrophobic one (fluorine PMMA) at the time of IOL removal. As a matter of fact, HSM PMMA is less hydrophilic than hydrogel and hydrophobic acrylic is less hydrophobic than fluorine PMMA and silicone.

Negative ratios could be explained either by a simple decrease in the amount of bacteria or by the release of bound bacteria into aqueous humor. Noticing that all pigs had endophthalmitis at 72 hours and at 1 week does not enable us to choose between the two alternatives. An aqueous humor sample was taken during removal of each IOL to count the amount of suspended bacteria, but analysis was very difficult, because of the presence of the amount of fibrin present, and no conclusion could be drawn.

However, it can be assumed that it is better to harbor bacteria in the anterior chamber than on the IOL surface where they are embedded within a layer of slime. Indeed, host defenses and/or antibiotics are present in aqueous humor but have trouble penetrating the biofilm. Moreover, a biofilm can always be found on the IOL's surface, because it is colonized by either slime-producing or non–slime-producing bacteria.[1] Thus, it is probably easier to kill bacteria suspended in aqueous humor than those bound on an IOL surface.

Hydrogel and fluorine PMMA showed in vitro low bacterial adhesion[4,22] and in vivo bacterial decline. Therefore, fluorine PMMA seems clinically safe for use in inhibiting protein and inflammatory cell response as well as bacterial adhesiveness.[22,24,25] Surface modification of PMMA IOLs with fluorine thus seems to be a better method to reduce bacterial adhesion than coating with heparin. According to some previous studies,[26–28] HSM IOLs provide a highly hydrated surface that modifies some structural fatty acids of *S. epidermidis*, reducing bacterial adhesion. However, the present work showed that HSM PMMA allowed bacterial growth, proving that bacteria adhered rather firmly. This result may relate to the fact that heparin behaves as an adhesion receptor in some *Staphylococcus* species.[29]

Colonization of the IOL surface may lower intrinsic and extrinsic defenses of the eye, making them unable to fully eradicate the adherent bacteria, thus causing an infection. Our results suggest that the risk of endophthalmitis after cataract extraction followed by IOL implantation under antibiotic prophylaxis may be lower with IOLs made of less sticky material, such as hydrogel and fluorine PMMA.

Conclusions

The material of which the IOL is made influences the in vitro adhesion of *S. epidermidis* to its surface. This study shows that, in the same way, IOL materials influence the in vivo behavior of attached bacteria. Silicone and HSM PMMA allowed bacterial growth, whereas hydrogel, fluorine PMMA and hydrophobic acrylic led to bacterial decline. Adhesion is also affected by the nature of the surrounding medium. Because of its complexity, the latter appears to be very difficult to model, thus making an in vivo study essential. Additional in vivo studies are needed to evaluate the clinical impact of all these biomaterials. Moreover, it would have been very interesting to know how much bacteria were released into the aqueous humor. Solutions should be sought to make further experiments possible.

References

1. Cusumano A, Busin M, Spitzanas M. Is chronic intraocular inflammation after lens implantation of bacterial origin? *Ophthalmology.* 1991;98:1703–1710.
2. Dilly PN, Holmes Sellors PJ. Bacterial adhesion to intraocular lenses. *J Cataract Refract Surg.* 1989;15:317–320.
3. Ng EW, Barrett GD, Bowman R. *In vitro* bacterial adhesion to hydrogel and poly(methyl methacrylate) intraocular lenses. *J Cataract Refract Surg.* 1996;22(suppl 2):1331–1335.
4. Burillon C, Kodjikian L, Pellon G, Martra A, Freney J, Renaud FNR. *In vitro* study of bacterial adhesion to different types of intraocular lenses. *Drug Dev Industrial Pharm.* 2002;28:95–99.
5. Ecoffet M, Demailly P, Kopel J. Physiologie de l'humeur aqueuse et de la tension oculaire. In: *Encycl. Méd. Chir.*, Paris: Ophtalmologie, 21020 D^{10}, 11–1985, 12.
6. Salvanet-Bouccara A, Forestier F, Coscas G, Adenis JP, Denis F. Endophtalmies bactériennes: résultats ophtalmologiques d'une enquête prospective multicentrique nationale. *J Fr Ophtalmol.* 1992; 15:669–678.
7. Scherrer B. *Biostatistiques.* Paris: Gaëtan Morin; 1984:850.
8. Jansen B, Peters G. Modern strategies in the prevention of polymer-associated infections. *Hosp Infect.* 1991;19:83–88.
9. Bos R, Van der Mei HC, Busscher HJ. Physico-chemistry of initial microbial adhesive interactions: its mechanisms and methods of study. *FEMS Microbiol. Rev.* 1999;23:179–230.
10. Bayston R, Penny SR. Excessive production of mucoid substance in Staphylococcus SIIA: a possible factor in colonization of Holter shunts. *Dev Med Child. Neurol.* 1972;27(suppl):25–28.
11. Costerton JWK, Cheng KJ, Geesey GC, et al. Bacterial biofilms in nature and disease. *Annu Rev Microbiol.* 1987;41:435–464.
12. Van Loosdrecht MCM, Lyklema J, Norde W, Zehnder AJB. Influence of interfaces on microbial activity. *Microbial Rev.* 1990;54: 75–87.
13. Evans RC, Holmes CJ. Effect of vancomycin hydrochloride on *Staphylococcus epidermidis* biofilm associated with silicone elastomer. *Antimicrob Agents Chemother.* 1987;31:889–894.
14. Sheth NK, Franson TR, Sohnle PG. Influence of bacterial adherence to intravascular catheters on in-vitro antibiotic susceptibility. *Lancet.* 1985;8467:1266–1268.
15. Nickel JC, Ruseka I, Wright JB, Costerton JW. Tobramycin resistance of Pseudomonas aeruginosa cells growing as a biofilm on urinary catheter material. *Antimicrob Agents Chemother.* 1985; 27:619–624.
16. Prosser BLT, Taylor D, Dix BA, Cleeland R. Method of evaluating effects of antibiotics on bacterial biofilms. *Antimicrob Agents Chemother.* 1987;31:1502–1506.
17. Nichols WW, Evans MJ, Slack MPE, Walmsley HL. The penetration

of antibiotics of mucoid and non-mucoid Pseudomonas aeruginosa. *J Gen Microbiol.* 1989;135:1291–1303.

18. Anwar H, Strap JL, Costerton JW. Establishment of aging biofilms: possible mechanism of bacterial resistance to antimicrobial therapy. *Antimicrob Agents Chemother.* 1992;36:1347–1351.
19. Rutter PR, Vincent R. The adhesion of microorganisms to surfaces: physico-chemical aspects. In: Berkeley RCW, Lynch JM, Melling J, Rutter PR, Vincent B, eds. *Microbial Adhesion to Surfaces.* London: Ellis Horwood; 1980:79–91.
20. Magnusson KE. Hydrophobic interaction: a mechanism of bacterial binding. *Scand J Infect Dis.* 1982;33(suppl):32–36.
21. Pascual A, Fleer A, Westerdaal NAC, Verhoef J. Modulation of adhesion of coagulase-negative staphylococci to Teflon catheter in vitro. *Eur J Clin Microbiol.* 1986;5:518–522.
22. Garcia-Saenz MC, Arias-Puente A, Fresnadillo-Martinez MJ, Matilla-Rodriguez A. In vitro adhesion of Staphylococcus epidermidis to intraocular lenses. *J Cataract Refract Surg.* 2000;11:1673–1679.
23. Cusumano A, Busin M, Spitznas M. Bacterial growth is significantly enhanced on foldable intraocular lenses [letter]. *Arch Ophthalmol.* 1994;112:1015–1016.
24. Eloy R, Parrat D, Duc TM, Legeay G, Bechetoille A. In vitro evaluation of inflammatory cell response after CF4 plasma surface modification of poly(methyl methacrylate) intraocular lenses. *J Cataract Refract Surg.* 1993;3:364–370.
25. Thouvenin D, Arne JL, Lesueur L. Comparison of fluorine-surface-modified and unmodified lenses for implantation in pediatric aphakia. *J Cataract Refract Surg.* 1996;9:1226–1231.
26. Portoles M, Refojo MF, Leong FL. Reduced bacterial adhesion to heparin-surface-modified intraocular lenses. *J Cataract Refract Surg.* 1993;19:755–759.
27. Arciola CR, Caramazza R, Pizzoferrato A. In vitro adhesion of Staphylococcus epidermidis on heparin-surface-modified intraocular lenses. *J Cataract Refract Surg.* 1994;20:158–161.
28. Paulsson M, Gouda I, Larm O, Ljungh A. Adherence of coagulase-negative staphylococci to heparin and other glycosaminoglycans immobilized on polymer surfaces. *J Biomed Mater Res.* 1994;28:311–317.
29. Rostand KS, Esko JD. Microbial adherence to and invasion through proteoglycans. *Infect Immun.* 1997;112:1015–1016.